PPARβ/δ activation induces enteroendocrine L cell GLP-1 production.


To cite this version:

HAL Id: inserm-00565808
https://www.hal.inserm.fr/inserm-00565808
Submitted on 14 Feb 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
PPARβ/δ Activation Induces Enteroendocrine L Cell GLP-1 Production

Short Title:
PPARβ/δ activation increases gut GLP-1 production

Mehdi Daoudi1,2,3,4, Nathalie Hennuyer2,4, Michael G. Borland5, Veronique Touche1,2,3,4, Christian Duhem2,4, Barbara Gross1,2,3,4, Robert Caiazzo1,6,7, Julie Kerr-Conte1,6, François Pattou1,6,7, Jeffrey M. Peters5, Bart Staels1,2,3,4† and Sophie Lestavel1,2,3,4

1 Université Lille Nord de France, F-59000, Lille, France
2 Inserm, U1011, F-59000, Lille, France
3 UDSL, F-59000, Lille, France
4 Institut Pasteur de Lille, F-59019, Lille, France
5 Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, PA 16802, United States
6 Inserm, U859, F-59000, Lille, France
7 CHRU Lille, F-59000, Lille, France

Grant Support
This work was supported by EU Grants AMI-DIAB (Fonds de Compétitivité des Entreprises, convention n°06 2 90 6169) and Hepadip (LSHM-CT-2005-018734) with the collaboration of Université Lille 2, Région Nord/Pas-de-Calais, the FEDER and French State. We acknowledge grant support from the ‘Nouvelle Société Française d’Athérosclérose’ (to M. Daoudi).

Abbreviations
BA, Bile Acid; BSA, Bovine Serum Albumin; CMC, Carboxy-Methyl-Cellulose; DCA, DeoxyCholic Acid; DPP-4, DiPeptidyl Peptidase-4; FBS, Fetal Bovine Serum; GLP, Glucagon-Like Peptide; GPBAR1, G Protein Coupled Bile Acid Receptor 1; IPGTT, Intra-Peritoneal Glucose Tolerance Test; LCA, LithoCholic Acid; OGTT, Oral Glucose Tolerance Test; PLN2, Perilipin; PC, Prohormone Convertase, PPARs, Peroxisome Proliferator-Activated Receptor; RT-QPCR, real time-quantitative PCR.

† corresponding author:
Inserm UMR 1011, Université Lille Nord de France
Institut Pasteur de Lille
1 rue du professeur Calmette
BP245 59019 Lille - France
Tel: +33320877388 – Fax: +33320877360 – e-mail: Bart.Staels@pasteur-lille.fr

Disclosures
No conflicts of interest exist

How each author was involved with the manuscript
M. Daoudi : study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis.
N. Hennuyer : study concept and design; acquisition of data; analysis and interpretation of data, statistical analysis; critical revision of the manuscript
M. Borland : acquisition of data; analysis and interpretation of data; statistical analysis.
V. Touche : acquisition of data; analysis and interpretation of data; statistical analysis.
C. Duhem : acquisition of data; analysis and interpretation of data; statistical analysis.
B. Gross : material support, critical revision of the manuscript.
R. Caiazzo : material support
J. Kerr-Conte : material support
F. Pattou : critical revision of the manuscript for important intellectual content, material support,
J. Peters : critical revision of the manuscript for important intellectual content, material support,
B. Staels : study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; obtained funding; study supervision
S. Lestavel: study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; study supervision
Abstract

**Background and Aims:** GLP-1, an intestinal incretin produced by L cells through proglucagon processing, is secreted after nutrient ingestion and acts on endocrine pancreas β-cells to enhance insulin secretion. PPARβ/δ is a nuclear receptor which improves glucose homeostasis and pancreas islet function in diabetic animal models. Here, we investigated whether PPARβ/δ 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, PPARβ/δ-null and ob/ob C57Bl/6 mice treated with the PPARβ/δ synthetic agonists GW501516 or GW0742. **Results:** PPARβ/δ 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 mRNA in the small intestine in wild-type, but not in PPARβ/δ-deficient mice. Treatment of wild-type and ob/ob mice with GW501516 enhanced the increase in plasma GLP-1 after an oral glucose load and improved glucose tolerance. Concomitantly, proglucagon and GLP-1 receptor mRNA increased in the small intestine and pancreas respectively. Finally, PPARβ/δ agonists activate the proglucagon gene transcription by interfering with the β-catenin/TCF-4 pathway. **Conclusions:** Our data show that PPARβ/δ activation potentiates GLP-1 production by the small intestine. Pharmacological targeting of PPARβ/δ is a promising approach in the treatment of type 2 diabetes, especially in combination with DPP-4 inhibitors.

**Keywords**

GLP-1; PPARβ/δ; incretin; diabetes
Introduction

Glucagon-like peptides (GLPs) and glucagon are hormones encoded by the same gene, proglucagon, each having different physiological activities\textsuperscript{1}. Due to alternative post-translational 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\textsuperscript{2-4}. Glucagon stimulates hepatic glucose production to maintain blood glucose levels upon fasting\textsuperscript{5} and inhibits insulin gene expression in pancreatic \(\beta\)-cells\textsuperscript{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\textsuperscript{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\textsuperscript{8}, whereas GLP-1 receptor gene disruption in mice results in glucose intolerance\textsuperscript{9-11}. The insulinotropic activity of GLP-1 is preserved in type 2 diabetes\textsuperscript{12-14}, but is reduced compared to healthy subjects\textsuperscript{14,15}. Treatment of diabetic patients with GLP-1 increases meal-stimulated insulin levels and suppresses postprandial hyperglycaemia without causing hypoglycaemia\textsuperscript{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 diabetes has led to the development of long-acting DPPIV-resistant GLP-1 analogs and orally bioavailable DPP-4 inhibitors, both approaches having proven efficacy in lowering blood glucose levels and HbA\textsubscript{1c} in patients with type 2 diabetes\textsuperscript{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-kb 5\textsuperscript{\prime}-upstream sequence of the transcriptional initiation site\textsuperscript{19}. A number of transcription factors control proglucagon gene expression in a tissue-specific manner allowing physiologically appropriate regulation of the production of the different active peptides\textsuperscript{20}. The transcription factor Pax6 activates proglucagon gene transcription in \(\alpha\)-cells by binding the G1
response element in the proglucagon promoter\textsuperscript{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\textsuperscript{23}. By contrast, the G2 response element of the proglucagon promoter appears involved in the tissue-specific regulation of proglucagon transcription since protein kinase C activation enhances transcription in $\alpha$-cells via this site\textsuperscript{24}, without influencing intestinal expression\textsuperscript{25}. Moreover, insulin and GSK-3β inhibitors, such as lithium, stimulate G2 response element activity specifically in enteroendocrine L cells through the $\beta$-catenin/TCF-4 signaling pathway resulting in increased GLP-1 production\textsuperscript{26,27}.

The Peroxisome Proliferator-Activated Receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily which regulate fatty acid, cholesterol and carbohydrate metabolism\textsuperscript{28}. Whereas PPAR$\alpha$ and PPAR$\gamma$ are the targets of the fibrates and glitazones respectively, the PPAR$\beta/\delta$ isoform appears an interesting pharmacological target for the treatment of disorders associated with the metabolic syndrome\textsuperscript{29,30}. PPAR$\beta/\delta$-deficient mice challenged with a high-fat diet exhibit obesity\textsuperscript{31} and glucose-intolerance\textsuperscript{32}. Furthermore, in vivo PPAR$\beta/\delta$ activation reduces body weight and lipid accumulation in adipose tissue and improves insulin sensitivity through increased skeletal muscle fatty acid oxidation\textsuperscript{31,32}.

In this study, we examined the hypothesis that PPAR$\beta/\delta$ 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 PPAR$\beta/\delta$ in glucose metabolism thus expanding its interest as target for the treatment of type 2 diabetes.
Materials and Methods

**Chemicals and reagents.** See Supplementary Material.

**In Vitro Studies**

**Cell culture and treatment.** The mouse GLUTag L cell model was kindly provided by D.J. Drucker (University of Toronto, Canada). The human NCI-H716 L cell, murine α-TC1/9 α-cells and rat INS1-E β-cells were purchased from ATCC® (CCL-251™ and CRL-2350™ respectively). For details, see Supplementary Material.

**Transient transfection assays.** GLUTag L cells (14x10^4 cells/cm^2) were transfected for 12h using Lipofectamine™2000 reagent (Invitrogen) in serum free culture medium. For details, see Supplementary Material.

**Gene reporter assays.** See Supplementary Material.

**GLP-1 secretion assays.** GLUTag L cells were starved for 30min in glucose-free Krebs-phosphate-buffered medium (120mM NaCl, 5mM KCl, 0.25mM MgCl₂, 0.5mM CaCl₂ and 2.2mM NaHCO₃, pH7.2) supplemented with diprotin-A 100µM and BSA 0.1%. Cells were subsequently stimulated for 30min with Krebs buffer in the presence or not of glucose 5mM or a BA mix (50µM each; LCA and DCA). The cell supernatants were centrifuged at 500 x g at 4°C for 5min and GLP-1 measured with Elisa Kit (EGLP-35K, Millipore) using Mithras technology (Berthold).

**Western blot analysis.** See Supplementary Material.

**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 (gastrojejunal derivation) and enrolled in the ABOS study (ClinGov NCT01129297). For details, see Supplementary Material.
Animal Models and Experimental Protocols

PPARβ/δ-deficient and ob/ob mice. 6-8 week-old male PPARβ/δ-deficient\textsuperscript{55}, obese ob/ob and wild-type mice (Charles River laboratories), fed a chow diet (A03; UAR, France) and maintained in a temperature-controlled room (22°C) on a 12h 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 6h 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 intra-peritoneal glucose tolerance tests (OGTT, IPGTT). Oral (OGTT) and intra-peritoneal (IPGTT) glucose tolerance tests were performed on overnight fasted mice. Sitagliptin was given orally at a dose of 25 mg/kg 45min before the glucose challenge (3g/kg of body weight). For details, see Supplementary Material.

RNA isolation and quantification by real time-quantitative PCR (RT-QPCR). RNA was extracted from cells and intestinal mucosa using the Trizol\textsuperscript{®} reagent (Life Technologies, Gaithersburg, MD, USA). Mouse and human intestinal tissues and pancreas RNA were extracted using the guanidinium thiocyanate (GSCN)/phenol/chloroform (Sigma). RNA extracts were treated with DNase I to eliminate contaminating genomic DNA. For details on RNA quantification, see Supplementary Material.

Statistical analysis

Statistical analyses were performed by using the unpaired Student’s t test. All data are expressed as mean±SE. Statistically significant differences between treatments are reported to DMSO (control) and CMC (vehicle) for in vitro and in vivo experiments respectively. P values < .05 were considered statistically significant.
Results

**Glucose is a Positive Modulator of Proglucagon and Prohormone Convertase-1/3 (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 1A) and PC-1/3 (Figure 1B) mRNA levels significantly increased upon incubation with glucose compared to lactate. By contrast, PC-2 mRNA was not regulated by glucose within 12h of incubation, while both glucose and lactate tended to increase its expression after 24h (Figure 1C). Expression of GPBAR1 (also known as TGR5), the Gs-coupled membrane receptor that binds BAs to increase GLP-1 secretion\(^{36,38}\), was detected in GLUTag L cells. GPBAR1 mRNA was not regulated by glucose (Figure 1D). The expression of the three PPAR isoforms was analysed in GLUTag L cells. PPAR\(\beta/\delta\) and PPAR\(\gamma\) were expressed at high levels (Ct=24 and Ct=26 respectively), while PPAR\(\alpha\) was barely detectable (Ct\(\geq\)34). Glucose had no major effect on PPAR\(\beta/\delta\) mRNA expression (Figure 1E). By contrast, PPAR\(\gamma\) mRNA levels were significantly lowered by glucose (Figure 1F).

**PPAR\(\beta/\delta\) Activation in GLUTag L Cells Increases Proglucagon Expression and Improves Glucose- and BA-Induced GLP-1 Release.**

Since PPAR\(\beta/\delta\) and PPAR\(\gamma\) are expressed in GLUTag L cells, it was next examined whether activation of these PPARs may regulate GLP-1 production. Surprisingly, PPAR\(\gamma\) activation with rosiglitazone transiently decreased proglucagon mRNA levels (supplementary FigureS1). By contrast, treatment with the PPAR\(\beta/\delta\) agonist GW501516 increased proglucagon mRNA in a time-dependent manner, reaching \(>2\)-fold increase after 24h (Figure 2A). Treatment of GLUTag L cells with GW0742, another PPAR\(\beta/\delta\) agonist, also significantly increased proglucagon mRNA levels (supplementary FigureS2-A). Incubation of GLUTag L cells for 24h with increasing doses of GW501516 resulted in \(>4\)-fold elevated proglucagon mRNA levels at the highest tested dose (Figure 2B). To test whether this effect is
mediated via PPARβ/δ, GLUTag L cells were transfected with PPARβ/δ siRNA before incubation for 24h with GW501516. PPARβ/δ knockdown abolished the induction of proglucagon mRNA levels by GW501516 (Figure 2C). Western blot analysis showed that proglucagon protein levels increased upon treatment for 24h with GW501516, an effect blunted by PPARβ/δ-silencing (Figure 2D). 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 2E. Treatment for 24h with GW501516 enhanced GLP-1 secretion both in basal and upon stimulation with glucose alone or in combination with GPBAR1 agonists (LCA and DCA) (Figure 2F). GLP-1 release also increased in response to glucose upon treatment for 24h with the other PPARβ/δ agonist GW0742 (supplementary Figure S2-B).

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

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

**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-bp 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 an activation of proglucagon promoter activity, an effect enhanced by PPARβ/δ co-transfection (Figure4A). Since lithium activates the −350-bp proglucagon promoter in GLUTag L cells via the β-catenin/TCF-4 signaling pathway and since PPARβ/δ activation has been shown to influence the β-catenin/TCF-4 signaling pathway, 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 (Figure4B). 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 (Figure4C). Therefore, PPARβ/δ transcriptionally regulates enteroendocrine L cell proglucagon expression through stimulation of the β-catenin/TCF-4 pathway (Figure4D).

**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 (Figure5 and supplementary FigureS3 Top). Measured as a marker of PPARβ/δ activity and a well-characterized PPARβ/δ target gene, PLN2 (perilipin2, also called ADRP) intestinal mRNA levels increased upon GW0742 treatment measured (supplementary FigureS3 Bottom). This effect was mediated via PPARβ/δ, since proglucagon mRNA was not increased upon GW0742-treatment of PPARβ/δ-deficient mice (Figure5). A similar, increase of proglucagon mRNA levels was observed in the jejunal mucosa upon GW501516 treatment (Figure6A, left panel). However, proglucagon mRNA levels were not regulated in the pancreas (Figure6A, central panel), nor in αTC-1/9 cells (supplementary FigureS4-A) or isolated human
islets (supplementary FigureS4-B), while PLN2 or CTP-1α, analysed 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 (Figure6A, right panel) and in isolated human islets (supplementary FigureS4-B). This up-regulation may enhance the response to the increased intestinal GLP-1 production. By contrast, treatment of rat INS1-E pancreatic β-cells with GW51516 did not influence RGLP-1 mRNA, whereas CPT-1 mRNA increased (FigureS4, panel 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 (Figure6B) and insulin concentrations (Figure6C) 15min after the oral glucose gavage, associated with an improved glucose tolerance (Figure6D). Indeed, the iAUC_{glucose} was reduced by 25% (P<.05) after PPARβ/δ activation (Figure6D, inset). These results suggest that PPARβ/δ activation may improve glucose homeostasis, at least in part, through enhancing the GLP-1 pathway.

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

The pathophysiological relevance of the enhanced oral glucose-induced GLP-1 secretion upon 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 (Figure7A). In line, plasma GLP-1 increased 15min after oral, but not intraperitoneal, glucose loading in GW501516-treated ob/ob mice (Figure7B), evidencing the contribution of the incretin axis. Moreover, fasting plasma insulin was lower in GW501516-treated compared to control ob/ob mice (Figure7C), likely a reflection of the previously reported peripheral insulin-sensitizing effects of PPARβ/δ activation^{31,32}. However, the capacity
of β-cells to secrete insulin upon glucose challenge was also improved in GW501516-treated ob/ob mice. A significant increase of plasma insulin was observed in GW501516-treated ob/ob mice 15min after glucose tolerance test, which was more pronounced upon oral versus intraperitoneal glucose loading, thus illustrating the enhanced incretin effect after PPARβ/δ activation. By contrast, no significant changes in plasma insulin were found in control ob/ob mice likely due to the pronounced hyperinsulinemic insulin-resistant phenotype (Figure7C). Finally, glucose excursion in the oral glucose tolerance test was improved in GW501516-treated ob/ob mice (Figure7D) with a significant reduction of iAUC\textsubscript{glucose} compared to 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 BAs. 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 BA-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 small intestine was also observed (data not shown). This observation is consistent with results obtained in pancreatic α-cells in which proglucagon gene transcription is also decreased by rosiglitazone treatment through interfering with the Pax6/G1 enhancer element pathway44,45. We thus speculate that the negative rosiglitazone effect on proglucagon gene expression in enteroendocrine L cell may be due to inhibition of the Pax6 pathway, which has an important role in intestinal proglucagon gene expression23. In line with these observations, it is reasonable to speculate that PPARγ does not enhance GLP-1 biosynthesis.
Since previous reports have demonstrated that GLP-1 receptor agonists inhibit β-cell apoptosis under conditions of glucolipotoxicity\(^{46,47}\) and that PPARβ/δ activation also prevents palmitate-induced β-cell apoptosis\(^{28}\), we speculated that the protective effect of PPARβ/δ on β-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 β-cell GLP-1 responsiveness. Our data demonstrate that PPARβ/δ activation enhances GLP-1 secretion both in response to an oral glucose load in vivo and in presence of GLP-1 secretagogues (glucose and BAs) in enteroendocrine L cells in vitro and ex vivo. These effects correlated with higher proglucagon mRNA in both L cells and the small intestine, whereas in pancreas, proglucagon mRNA remained unaffected. 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 demonstrate 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 demonstrate 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-producing L cells, but not in pancreatic glucagon-producing α-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 α-cells\(^{27}\). Han et al. recently demonstrated 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 cell 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 α-cell line, G2 response element activity remained unaffected27. This mechanism is consistent with the observation that PPARβ/δ activation does not influence proglucagon gene expression in pancreatic α-cells.

Previous studies suggested that PPARβ/δ activation is similar to PPARγ activation in their effects on improving insulin sensitivity32,49,50. 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 triglycerides31,51,52. 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±0.1 vs. 3.1±0.1g ; P<.05) and fasting glycemia (167.8±11.5 vs. 205.8±7.1mg/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 demonstrated 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,
pharmacological 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.

**Acknowledgements**

The authors gratefully acknowledge D.J. Drucker and F. Gribble for providing the GLUTag L cells and A. Billin and T. Willson for providing GW0742. We appreciate the generous gifts of −350bp-proglucagon-Luc plasmid from W. Knepel and pEGFP-β-catenin-S33Y plasmid from D. Bershadsky.
Figure 1. Glucose increases proglucagon and prohormone convertase 1/3 and decreases PPARγ mRNA levels in GLUTag L cells. GLUTag L cells were 12h-starved in glucose-free medium and then stimulated with glucose (5mM) or lactate (10mM) as control for the indicated times. mRNA levels of proglucagon (A), prohormone convertases 1/3 and 2 (B and C respectively), transmembrane BA receptor, GPBAR1 (D), PPARβ/δ and PPARγ (E and F respectively) were quantified by RT-QPCR using specific oligonucleotides. TFIIB expression is used as control. All RT-QPCR experiments were done 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’s unpaired t test: *P<.05 ; **P<.01.

Figure 2. PPARβ/δ activation with GW501516 increases proglucagon mRNA levels and enhances glucose- and BA-induced GLP-1 release in GLUTag L cells. Proglucagon mRNA levels were quantified in cells treated with GW501516 (100nM) or DMSO (control) for 6, 12 and 24h (A) or for 24h with increasing doses (10-10^3nM) (B). (C, D) The cells were overnight transfected with mouse PPARβ/δ siRNA or control and then treated or not for 24h with GW501516 (1µM). Proglucagon mRNA (C) and proteins (D) levels were quantified by RT-QPCR 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 24h with GW501516 (1µM) or control (DMSO) and then stimulated for 30min with glucose (5mM) or a combination of both glucose (5mM) and BA GPBAR1 agonists DCA and LCA (50µM). GLP-1 concentrations in supernatants were normalized to cell protein content53. Student’s unpaired t test: *P<.05 ; **P<.01; ***P<.001.

Figure 3. PPARβ/δ activation increases proglucagon expression and enhances glucose- and BA-induced GLP-1 release in human enteroendocrine L cells. Differentiated NCI-H716 L cells were treated for 24h with GW501516 (1µM) or DMSO (control). Proglucagon mRNA (A) and proteins (B) levels were quantified by RT-QPCR and western blot analysis respectively. (C) GLP-1 secretion assay in differentiated NCI-H716 L cells treated for 24h with
GW501516 (1µM) or DMSO (control) and thereafter stimulated for 30min with glucose (5mM) or a combination of both glucose (5mM) and BA GPBAR1 agonists DCA and LCA (50µM). (D and E) Fresh human jejunum tissue was exposed ex vivo for 24h to GW501516 (1µM) or DMSO (control). Proglucagon mRNA levels (D) and GLP-1 glucose- and BA-induced GLP-1 release (E) was measured by RT-QPCR and GLP-1 secretion assay respectively. Student’s unpaired t test: *P<.05 ; **P<.01.

**Figure 4. PPARβ/δ Activation Induces Proglucagon Promoter Activity through the β-catenin/TCF-4 Signaling Pathway in GLUTag L cells.** (A) Cells were co-transfected with -350Proglucagon-luc and pSG5-PPARβ/δ or empty pSG5 plasmids and then treated or not with the GW501516 or GW0742 (1µM) during 24h. The values are normalized to pSG5-empty control and the average of at least three independent experiments is presented. (B, C) Proglucagon mRNA levels were quantified in cells transfected with mouse TCF-4 siRNA vs. control siRNA (B) or with mouse β-catenin siRNA vs. control siRNA or with the pEGFP-β-catenin-S33Y plasmid encoding non-degradable human β-catenin and then treated or not for 24h with GW501516 (1µM). All RT-QPCR experiments were done in triplicate. Statistically significant differences are reported to control. Student’s 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 5. The increase of intestinal proglucagon mRNA levels by GW0742 is PPARβ/δ dependent.** Wild-type (PPARβ/δ+/+) and PPARβ/δ-deficient (PPARβ/δ−/−) 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 quantified by RT-QPCR. Student’s unpaired t test: *P<.05.

**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, 6h-fasted treated mice were sacrificed and
mRNA levels of proglucagon in jejunal mucosa of small intestine (left panel) and pancreas (central panel) or GLP-1 receptor in pancreas (right panel) were quantified by RT-QPCR. (B-D) OGTT was performed on overnight fasted 2 week treated mice receiving the DPP-4 inhibitor Sitagliptin (25 mg/kg) 45min before glucose loading. (B) Plasma GLP-1 levels were measured 15min after glucose loading. (C) Plasma insulin levels were quantified before and 15min after glucose loading. (D) Glucose excursion curves during OGTT. The inset graph represents the glycemic integrative area under the curve (iAUC). Student’s unpaired t test: *P<.05 ; **P<.01 ; ***P<.001.

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 6h-fasted 3 week ob/ob treated mice were quantified by RT-QPCR. (B-D) IPGTT and OGTT were performed in 2 week-treated overnight fasted ob/ob mice which received the DPP-4 inhibitor Sitagliptin (25 mg/kg) 45min before glucose challenge. (B) Plasma GLP-1 levels were measured 15min after glucose challenge. (C) Plasma insulin levels were quantified before and 15min after glucose loading. (D) Glucose excursion curves during OGTT. The inset bar graph represents the glycemic integrative area under the curve (iAUC). Student’s unpaired t test: *P<.05 ; **P<.01 ; ***P<.001.
References

mRNA levels normalized to TFIIB (relative to T0)

- Lactate
- Glucose

** A **
Proglucagon

** B **
Prohormone Convertase 1/3

** C **
Prohormone Convertase 2

** D **
GPBAR1

** E **
PPARβ/δ

** F **
PPARγ

Daoudi M. et al. Figure 1
**Figure 2**

**A**

Proglucagon/TFIIB mRNA (relative to T0) over time (h) for control and GW501516 groups.

**B**

Proglucagon/TFIIB mRNA (relative to control) plotted against logarithm of GW501516 concentration (nM).

**C**

Proglucagon/TFIIB mRNA levels comparing control and GW501516 groups with different si-RNA treatments.

**D**

Western blot showing Proglucagon and TFIIB protein levels.

**E**

Experimental setup for GLP-1 secretion assay, including cell culture, activation, washing, and GLP-1 secretion assay in Krebs buffer.

**F**

GLP-1 secretion (pmol/µg cell protein) in response to glucose and GPBAR1 agonists.

**Legend**

- Control
- GW501516
- si-control
- si-PPARβδ
- *: p < 0.05
- **: p < 0.01
- ***: p < 0.001
- ns: not significant

**Graphical Data**

- **A**: Control vs. GW501516 with significant differences.
- **B**: Logarithmic concentration response with high correlation (R² = 0.9922).
- **C**: si-RNA effects showing control and GW501516 comparisons.
- **D**: Protein levels with different bands for Proglucagon and TFIIB.
- **E**: Time points for cell culture, activation, and secretion assay.
- **F**: GLP-1 secretion showing treatment effects.
Daoudi M. et al. Figure 4

A) Proglucagon promoter activity (relative to pSG5-empty control)

B) Proglucagon/TFII B mRNA (relative to control)

C) Proglucagon/TFII B mRNA (relative to pSG5-empty control)

D) Diagram showing the interaction between PPARβ/δ agonist, GSK-3β, β-Catenin, TCF-4, and Proglucagon.
Proglucagon/TFIIB mRNA (relative to PPARβ/δ+/-, vehicle)

- Vehicle
- GW0742

PPARβ/δ+/-
PPARβ/δ-/-
Daoudi M. et al. Figure 6

**A**
Proglucagon/TFIIB mRNA (relative to vehicle)
- Small Intestine
  - GW501516

**B**
Plasma GLP-1 (pM) 15 min after OGTT
- GW501516

**C**
Plasma insulin (µg/l) 0-15 min after OGTT
- GW501516
- Vehicle

**D**
Glycemia (mg/dl) Stagliuin
- Sitagliptin
- iAUC

**Proglucagon/TFIIB mRNA (relative to vehicle)**
- Small Intestine
  - GW501516

**Proglucagon/TFIIB mRNA (relative to vehicle)**
- Pancreas

**GLP-1 receptor/TFIIB mRNA (relative to vehicle)**
- Pancreas
Figure 7

A. mRNA levels (relative to vehicle)

- Proglucagon
- PLN2

B. Plasma GLP-1 (pM)

- IPGTT
- OGTT

C. Plasma Insulin (µg/l)

- T0
- 15 min after IPGTT
- 15 min after OGTT

D. Glycemia (mg/dl)

- OGTT
- IPGTT

- Sitagliptin

** p < 0.01
* p < 0.05
*** p < 0.001
Supplementary Material

**Chemicals and reagents.** The DPP-4 inhibitors diprotin A and sitagliptin were purchased from Sigma and MSD respectively. BSA, Deoxycholic acid (DCA) and lithocholic acid (LCA) was purchased from Sigma-aldrich. BSA was dissolved in demineralised water. DCA and LCA were dissolved in DMSO. The synthetic PPARβ/δ agonist GW501516 was a generous gift of Genfit (Parc Eurasanté, Loos F-59120, France). The synthetic PPARβ/δ agonist GW0742 was synthesized by GlaxoSmithKline (Research Triangle Park, NC). GW501516 and GW0742 were dissolved in dimethylsulfoxide (DMSO, Sigma-aldrich) and 0.5% Carboxy-Methyl-Cellulose (CMC, low viscosity, Sigma) for in vitro and in vivo experiments respectively. These PPARβ/δ agonists pharmacologically activate both murine and human PPARβ/δ with similar and potent EC$_{50}$ values in the nanomolar range for PPARβ/δ activity and with $>1000$-fold selectivity for PPARβ/δ over the other PPAR receptor subtypes$^{1,2}$.

**RNA quantification by real-time PCR.** Total RNA (1-2 µg) was reverse transcribed using the SuperScript First-strand Synthesis System for Reverse transcription (Life Technologies, Gaithersburg, MD, USA). Reverse transcription reactions without reverse transcriptase were performed as negative controls for subsequent PCR reactions. Reverse transcribed cDNAs were quantified by SYBR® green based real-time PCR using specific oligonucleotides (listed in Supplemental Table S1) on an Mx3000 apparatus (Stratagene, La Jolla, CA) after 1/5 and 1/2.5 dilution of RT products for the in vitro and in vivo samples respectively. mRNA levels were normalized to TFIIB or cyclophilin as internal control.

**In vitro studies**

**Cell culture and treatment.** GLUTag L cells were grown and maintained in DMEM + GlutaMAX$^TM$-1 with glucose 1 g/l (Cat. No. 21885-025, Invitrogen) supplemented with 10% FBS, glutamine 2mM, penicilin 10$^4$ IU/ml and streptomycin 10 mg/ml. For the experiments,
GLUTag L cells were trypsinized, seeded at a density of $14 \times 10^4$ cells/cm$^2$ and cultured during 42h. The NCI-H716 L cells proliferate in suspension in RPMI-1640 Medium with glucose 4.5 g/l (Cat. No. A10491-01, Invitrogen) supplemented with 10% FBS, 2mM glutamine, penicillin $10^4$ IU/ml and streptomycin 10 mg/ml. For the experiments, NCI-H716 L cells ($14 \times 10^4$ cells/cm$^2$) were differentiated during 48h in DMEM + GlutaMAX™-1 with glucose 4.5 g/l (Cat. No. 31966-021, Invitrogen) supplemented with 10% FBS, glutamine 2mM, penicillin $10^4$ IU/ml and streptomycin 10 mg/ml in BD Matrigel™ Basement Membrane Matrix pre-coated plates according to the manufacturer’s instructions (Cat. No. 356234, BD Biosciences). The murine pancreatic $\alpha$-cell line $\alpha$-TC1 clone 9 ($\alpha$-TC1/9) were cultured in DMEM (Cat. No. 11966025, Invitrogen) supplemented with glucose 3g/l, 2mM Glutamine, sodium Pyruvate 1mM, Hepes 15mM, nonessential amino acids 0.1mM, BSA 0.02%, 10% FBS, penicillin $10^4$ IU/ml and streptomycin 10 mg/ml. The rat pancreatic $\beta$-cell line INS1-E was cultured as previously described. All cells were treated with DMSO (control) or the PPAR$\beta/\delta$ synthetic agonists (GW501516 and GW0742) in serum-free medium.

**Transient transfection assays.** For the luciferase gene reporter assays, cells were co-transfected with 1µg of the $-350$bp-proglucagon-Luc (from W. Kneipel, University of Göttingen, Germany) and pSG5-mPPAR$\beta/\delta$ or control pSG5-empty plasmids. For RNA interference experiments, 40 pmoles of PPAR$\beta/\delta$, TCF-4, $\beta$-catenin or non-target siRNA as control (Dharmacon, Chicago) were used. For overexpression of constitutively active mutant $\beta$-catenin-S33Y, the cells were transfected with 1µg of pEGFP-$\beta$-catenin-S33Y plasmid (from Alexander D. Bershadsky, Wolfson Building for Biological Research, Israel).

**Gene reporter assays.** Transfected GLUTag L cells were cultured 24h in 96 well plates before treatment with PPAR$\beta/\delta$ agonists (GW501516 and GW0742) at 1µM during 16h in serum free medium and then lysed by addition of ¼ v/v of Luciferase Assay Reagent II (Dual-Luciferase™, Promega). Relative Luc activities were calculated as $–$fold induction relative to control GLUTag cell transfected with empty plasmid.
Western blot analysis. Total cellular extracts from GLUTag and NCI-H716 L cells were obtained after treatment with SDS-lysis buffer (Nonidet P401%, Tris/HCl (pH8.0) 20mM, glycerol 10%, NaCl 150mM, and EDTA 1mM) supplemented with protease inhibitor cocktail (Complete™ Mini, Roche Diagnostics). Cellular proteins (50µg) were separated on 12% SDS-PAGE and transferred to nitrocellulose membrane (Hybond™, ECL™ Nitrocellulose membrane; Amersham Biosciences). After blocking non-specific binding sites with 5% BSA in TBST (20mM Tris HCl, 137mM NaCl, pH7.6, 0.1% Tween 20), the blots were probed with monoclonal antibodies raised against proglucagon (11E2, sc-80603) and TFIIB (sc-225) as control (Santa Cruz Biotechnology, INC.) Horseradish peroxidase-conjugated secondary antibodies were used for detection using the enhanced chemiluminescence detection kit (ECL, Amersham Biosciences) and autoradiography (Camera Gbox, Ozyme).

In vivo Models and Experimental Protocols

Oral and Intra-Peritoneal Glucose Tolerance Tests (OGTT, IPGTT). Blood samples were collected from the tail vein at 0, 15, 30, 45, 60 and 90min and glycemia was measured using a Glucometer (Accu-Check active®, Roche Applied Science). Retro-orbital blood samples were collected in diprotin A (100µM) 15min after the glucose load following immediate centrifugation at 4°C. Plasma Insulin and GLP-1 were measured using Rat insulin Elisa kit (Mercodia, Uppsala, Sweden) and Glucagon-Like peptide-1 (Active) Elisa kit (EG LP-35K, Millipore) respectively.

Human jejunum tissue isolation. The pieces of human jejunum tissue freshly isolated (about 2 cm²) were cultured for 24h in RPMI + GlutaMAX™-1 with glucose 1 g/l (Cat. No. 61870-010, Invitrogen) supplemented with 10% FBS, penicillin 10⁴ IU/ml and streptomycin 10 mg/ml in presence of PPARβ/δ agonist GW501516 (1µM) or DMSO (control).
Supplementary Data

Figure S1. PPARγ activation decreases proglucagon mRNA levels in GLUTag L cells. GLUTag L cells were stimulated for Rosiglitazone (10µM) or DMSO (control) in serum-free medium. Proglucagon mRNA levels were quantified by real-time PCR. All values are expressed as means±SE and statistically significant differences between Rosiglitazone and DMSO treatments are indicated. Student's unpaired t test: *P<.05.

Figure S2. PPARβ/δ activation increases proglucagon mRNA levels and enhances GLP-1 release in GLUTag L cells. GLUTag L cells were stimulated for 24h with GW0742 (1µM) or DMSO (control) in serum-free medium. (A) Proglucagon mRNA levels were quantified by real-time PCR. (B) GLP-1 release was analysed after 30min of stimulation with glucose (5mM). GLP-1 concentrations in supernatants were normalized to cell protein content. All values are expressed as means ± SE and statistically significant differences between GW0742 and DMSO treatments are indicated. Student's unpaired t test: *P<.05 ; **P<.01.

Figure S3. In vivo PPARβ/δ activation increases proglucagon mRNA levels in different parts of the small intestine. Wild-type C57BL/6 male mice (n=8-10 animals per group) were treated or not with GW0742 (10 mg/kg twice a day) during 3 weeks. mRNA was isolated from small intestine and used for real-time PCR measurement of proglucagon and PLN2 mRNA. Values are expressed as means±SE and statistically significant differences are reported to untreated mice. Student’s unpaired t test: *P<.05 ; **P<.01 ; ***P<.001.

Figure S4. PPARβ/δ activation does not regulate proglucagon gene expression in pancreatic α-cells. (A) αTC-1/9 cells were stimulated for 24h with GW501516 or GW0742 (1µM each) or DMSO (control) in serum-free medium. (B) Freshly isolated human islets were stimulated 48h with GW0742 (1µM) or DMSO (control) in culture medium. (C) Rat INS1-E β-cells were treated or not with GW501516 (1µM) for 48h in serum-free medium. mRNA
levels were quantified by RT-PCR using specific oligonucleotides. TFIIIB (panel A and B) or cyclophilin (panel C) expressions used as control. Relative mRNA levels were calculated as fold-induction relative to the corresponding control. All values are expressed as means ± SE and statistically significant differences between PPARβ/δ agonists and DMSO treatments are indicated. Student's unpaired t test: *P<.05 ; **P<.01 ; ***P<.001.
References


Daoudi M. et al. Figure S1

Proglucagon/cyclophilin mRNA (relative to control)

Time (hrs)

Control
Rosiglitazone

0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4

*
Daoudi M. et al. Figure S2

Proglucagon/TFIIB mRNA (relative to control)

GLP-1 secretion (pM/µg protein)

GW0742
Control

*

#
Daoudi M. et al. Figure S3

Proglucagon/TFIIB mRNA (relative to control)

Duodenum

Jejunum

Ileum

PLN2/TFIIB mRNA (relative to control)

Control  GW0742
Figure S4

A

mRNA levels (relative to vehicle)

Proglucagon    PLN 2

GW501516

GW0742

Control

B

mRNA levels (relative to vehicle)

Proglucagon    RGLP-1    CPT-1α

GW501516

GW0742

C

mRNA levels (relative to vehicle)

RGLP1    CPT-1

GW501516

GW0742
Table S1. Oligonucleotide sequences used for Real Time PCR analysis

<table>
<thead>
<tr>
<th>species</th>
<th>gene name</th>
<th>type</th>
<th>sequence (5' to 3')</th>
<th>final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>L cells</td>
<td>small intestine α-cells or Islets</td>
</tr>
<tr>
<td>Mouse</td>
<td>Proglucagon</td>
<td>F</td>
<td>ATT GCC AAA CGT CAT GAT GA R GGC GAC TTC TTC TGG GAA GT</td>
<td>100 nM 500 nM</td>
</tr>
<tr>
<td>PPARβ/δ</td>
<td></td>
<td>F</td>
<td>ACT TGG CGT GGC GCC TGC R AGC GGT GTG GTG ATG CGC A</td>
<td>100 nM 300 nM</td>
</tr>
<tr>
<td>PPARγ</td>
<td></td>
<td>F</td>
<td>CCG TGA TGG AAG ACC ACT CG R AGG CCT GTG GAG CTG GGT C</td>
<td>100 nM 300 nM</td>
</tr>
<tr>
<td>PPARα</td>
<td></td>
<td>F</td>
<td>ATC GGC TAC GGC AAT GGC TTT A R TAA TTT GTG GTG GAG ATC GGC CTG</td>
<td>300 nM 300 nM</td>
</tr>
<tr>
<td>GPBAR1</td>
<td></td>
<td>F</td>
<td>AAC GCT ACA TGG CAG TGT TG R GGA GGC CAT AAC TTC CAG GTA GA</td>
<td>300 nM 300 nM</td>
</tr>
<tr>
<td>PLN2</td>
<td></td>
<td>F</td>
<td>ATC CAG AAG CTG GAG CCA CAA A R CCA CAT CCT CAG CCC CAG TTA</td>
<td>100 nM</td>
</tr>
<tr>
<td>RGLP-1</td>
<td></td>
<td>F</td>
<td>CTG AAG CGA TGA CCA AGG CGG R CGG AGT GTG AAG AGT CTA AGC G</td>
<td>300 nM</td>
</tr>
<tr>
<td>Human</td>
<td>Proglucagon</td>
<td>F</td>
<td>GCA CAT TCA CCA GTG ACT ACA GCA R TGG CAG GTG GGT CTT CCA AAT A</td>
<td>100 nM 500 nM</td>
</tr>
<tr>
<td>RGLP-1</td>
<td></td>
<td>F</td>
<td>TCC TCC TCG GCT TCA GAC ACC TGC A R CCA CTT CAG GCC TGC GTC CTT GAT G</td>
<td>300 nM</td>
</tr>
<tr>
<td>CPT-1α</td>
<td></td>
<td>F</td>
<td>TCT GGC TGG ACA TGC AGT TGG C R TAT CGT GGT GGG CGT GAT G</td>
<td>100 nM</td>
</tr>
<tr>
<td>Rat</td>
<td>RGLP-1</td>
<td>F</td>
<td>ACA TCC ACT TGA GGG CAG CG R CAG ACA CTG GCA CTG CAC CAG G</td>
<td>300 nM</td>
</tr>
<tr>
<td>CPT-1</td>
<td></td>
<td>F</td>
<td>TCC AAA CAT CAC TGC CCA AG R GAA TTG TGG CTT GCA CAC TG</td>
<td>300 nM</td>
</tr>
<tr>
<td>Mouse/Human</td>
<td>TFIIB</td>
<td>F</td>
<td>ACC AGC CGT TTG GAT GCT C R CCC ACA TCA ATA ACC CGG TC</td>
<td>100 nM 100 nM</td>
</tr>
<tr>
<td>Cyclophilin</td>
<td></td>
<td>F</td>
<td>GCA TAC GGG TCC TGG CAT CTT GTC C R ATG GTG ATC TTC TCC CTG GTC CTT GTC C</td>
<td>100 nM</td>
</tr>
</tbody>
</table>

*a F. forward primer; R. reverse primer.