RESEARCH ARTICLE

Thiazolidinediones (TZDs) enhance insulin secretory response via GPR40 and adenylate cyclase (AC)

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Abstract

Thiazolidinediones are synthetic PPARγ ligands that enhance insulin sensitivity, and that could increase insulin secretion from β-cells. However, the functional role and mechanism(s) of action in pancreatic β-cells have not been investigated in detail.

KEYWORDS

adenylyl cyclase, cAMP, glucolipotoxicity, GPR40, insulin secretion, lobeglitazone

1 | INTRODUCTION

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated nuclear receptor that regulates glucose and lipid metabolism and inflammation in many cells including pancreatic β-cells (P. Ferré. 2004; Semple et al., 2006; Welters et al., 2004). We have also reported that PPARγ was detected in pancreatic islets, and treatment with a PPARγ agonist or overexpression of PPARγ improved glucose-stimulated insulin secretion (GSIS), which was associated with an increased expression of genes involved in β-cell function, including glucose transporter 2 (GLUT2), insulin receptor substrate 2, and pancreatic duodenal homeobox 1 (Kim et al., 2008).

G protein-coupled receptors (GPCRs) are important signaling molecules that regulate a myriad of cellular physiological processes. Among these GPCRs, GPR40, also referred to as FFAR1, couples with a G-protein α-subunit of Gq family to function as a receptor for a range of (medium to long)-chain saturated and unsaturated fatty acids (Briscoe et al., 2003). GPR40 was first detected in pancreatic β-cells, and it increased Gq-mediated phospholipase C activity and intracellular calcium levels in pancreatic islets (Itoh et al., 2003). Recently, TZDs have been shown to bind to PPARγ by binding to GPR40 (Gras et al., 2009; Kotarsky et al., 2003; Mieczkowska et al., 2012; Smith et al., 2009; Stoddart et al., 2007; Zhou C et al., 2010), a cell membrane receptor that is associated with free fatty acid and glucose-induced insulin secretion (Alquier et al., 2009; Itoh et al., 2003). In the present study, we therefore examined the effects of PPARγ agonists on the regulation of GPR40 pathway, focusing on the incretin sensitivity and glucolipotoxicity in pancreatic β-cells. Insulin secretion is also associated with the generation of another second messenger, cAMP, via adenylyl cyclase (AC). The AC family consists of nine G protein–responsive cyclases, which can be activated by calcium, and which are found in pancreatic β-cells (Delmeire et al., 2003; Landa et al., 2005; Yang et al., 1999). The enzyme adenylyl cyclase catalyzes the conversion of ATP to cAMP, an important second messenger involved in

potentiating rather than initiating insulin secretion. The increase in insulin secretion by TZDs is not fully explained by GPR40, and we hypothesized that TZDs might increase insulin secretion by the distinct roles of both GPR40 and AC activation.

2 | METHODS

2.1 | Cell culture and measurement of insulin release

Rat insulinoma cell line, INS-1 cells, were kindly provided by Dr. P. Maechler (Geneva) (Asfari et al., 1992). INS-1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen) including 10% fetal calf serum, and additions were as previously described (Lawrence et al., 2008; Merglen et al., 2004). Cells were

harvested using a nonenzymatic cell dissociation liquid (Invitrogen). Cells were resuspended in the above media and were dissociated before being seeded into 96-well plates (40,000 cells per well). The medium was replaced with defined serum-free medium containing TZDs, and other inhibitors at the designated concentrations. Rosiglitazone was purchased from Sigma-Aldrich, while pioglitazone and lobeglitazone were kind gifts from Chonggundang Co. After 24 h of treatment, cells were washed with glucose-free Krebs (NaCl 140 mM, KCl 3.6 mM, $CaCl_2$ 1.5 mM, $MgCl_2$ 1.19 mM, KH_2PO_4 1.19 mM, $NaHCO_3$ 2 mM, and HEPES (pH 7.4) 10 mM) containing 0.1% insulin-free bovine serum albumin, and then incubated for an additional 60 min in 1 ml of KRBH buffer containing 3.3 or 16.7 mM of glucose. Insulin released in the medium was determined by enzyme-linked immunosorbent assay (Mercodia). Insulin concentration in nanograms per milliliter (ng/ml) was normalized against total protein in micrograms.
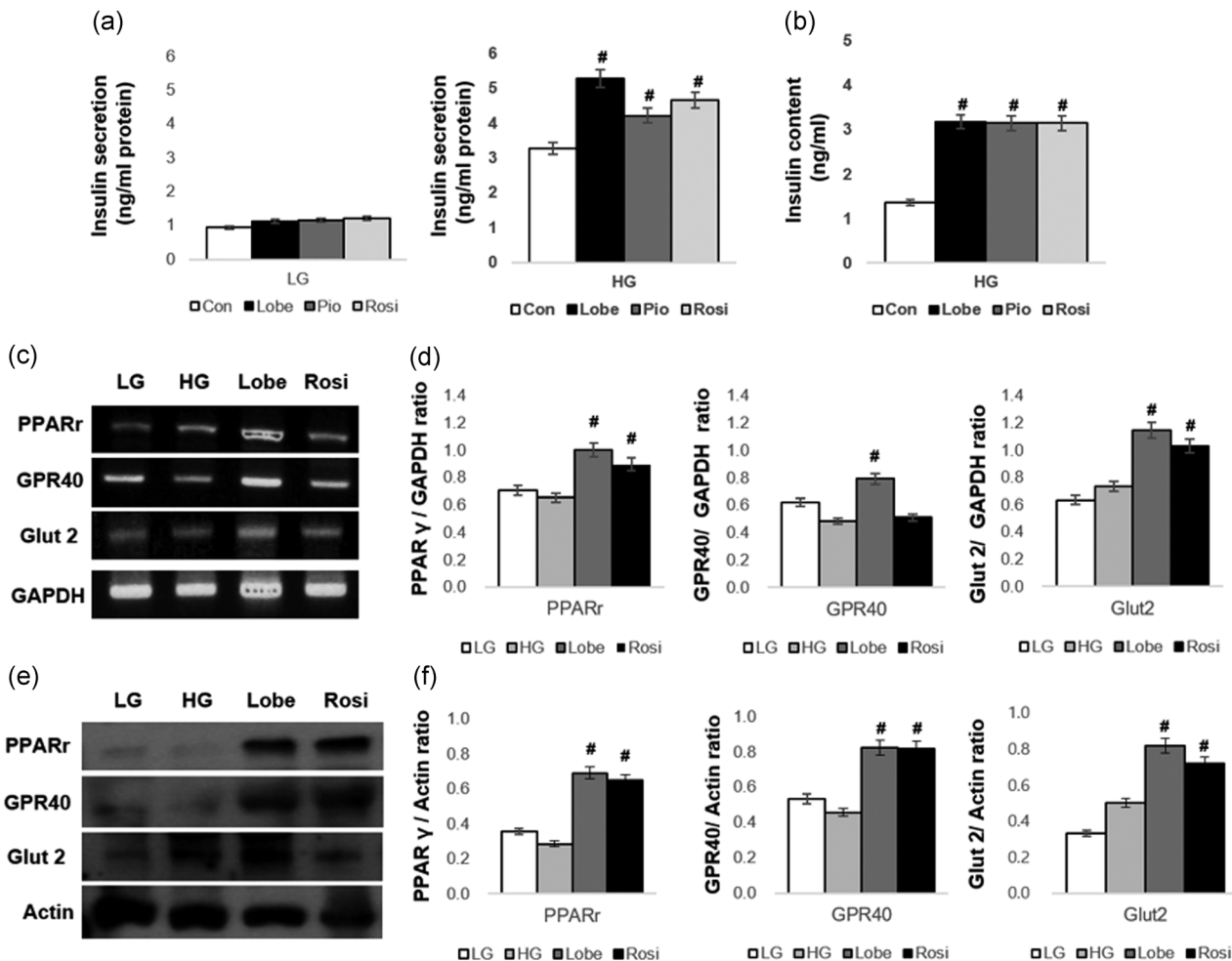

FIGURE 1 Effect of thiazolidinediones (TZDs) applied simultaneously with glucose on (a) insulin secretion, and (b) content, and (c–f) the level of PPARγ and GPR40 in INS-1 cells. (a, b) Effect of TZDs on insulin secretion and contents at 3.3 mM (LG) glucose, or stimulation by 16.7 mM (HG) glucose alone or 16.7 mM glucose and TZDs (Lobe, lobeglitazone; Pio, pioglitazone; Rosi, rosiglitazone). The data presented were obtained in one representative experiment from at least three independent experiments. #$p < .01$ compared to control cells. (c–f) After 24 h of incubation, the levels of individual proteins and RNA were assessed using Western blot analysis and RT-PCR. Actin was included to confirm equal protein loading. Figures 1e and 4d are experimental results from one blot, and the same control of actin bands was used in both Figures 1e and 4d. The data presented were obtained in one representative experiment from at least three independent experiments. PPARγ, proliferator-activated receptor gamma; RT-PCR, reverse transcription polymerase chain reaction. #$p < .01$ compared to HG
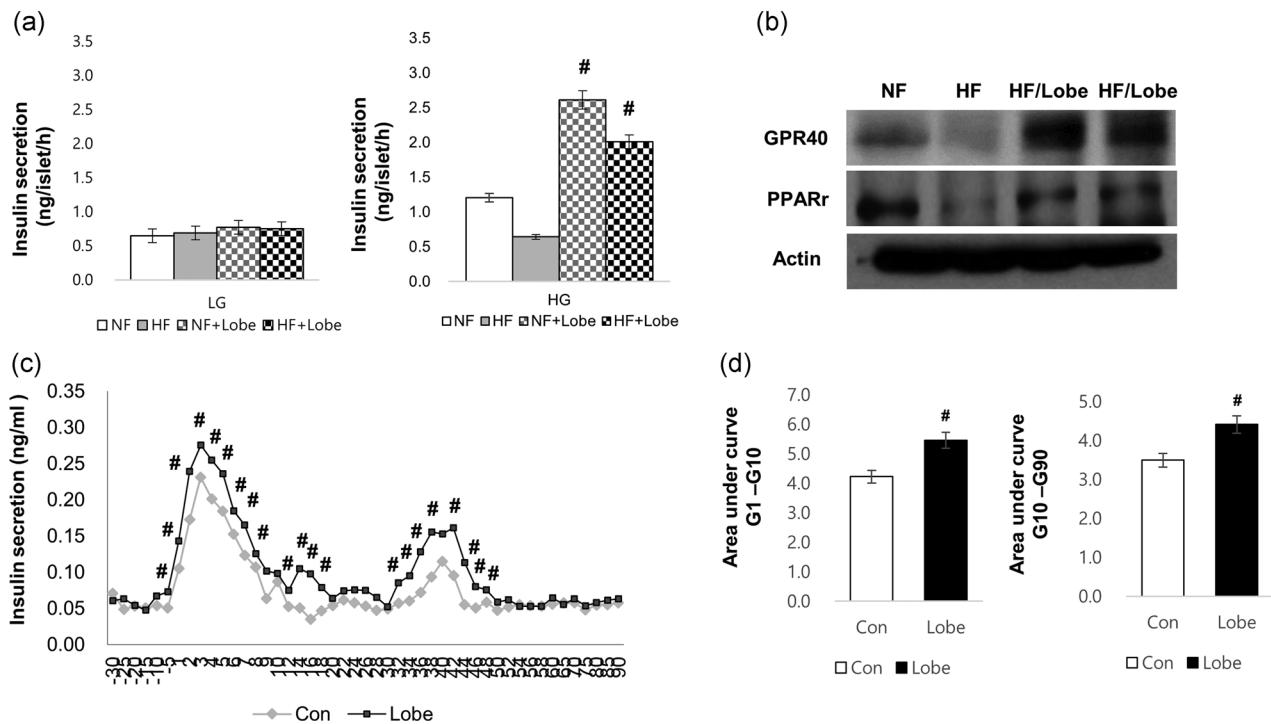
FIGURE 2 Lobeglitazone induced glucose-stimulated insulin secretion in islets. (a) Isolated normal chow-fed mouse and high fat-fed mouse islets were treated with lobeglitazone for 24 h, and insulin level was measured by Insulin ELISA. $^{\#}p < .005$ versus high fat-fed mouse (HF). (b) GPR40 and PPARγ in mouse islets exposed to lobeglitazone and 16.7 mM glucose for 24 h. (c) Islets were perifused with 3.3 mM glucose for 30 min at flow rate of 1 ml/min, followed by 16.7 mM glucose solution. Fractions were collected at 1 min intervals during first peak insulin secretion, and then at 2 min intervals ($^{\#}p < .005$ compared to Con). ELISA, enzyme-linked immunosorbent assay; PPARγ, proliferator-activated receptor gamma

2.2 | Islet perifusion

Islets were isolated by collagenase P digestion, as previously described (Gregory et al., 2007). Islets were then isolated and hand-picked under stereomicroscopy at room temperature. After purification, islets were cultured overnight at 37°C in a humidified atmosphere of 95% air and 5% CO_2 in RPMI 1640 supplemented with 10% fetal bovine serum, 100 unit/ml penicillin, and 0.1 mg/ml streptomycin. All of the procedures were approved by the Institutional Animal Care and Use Committee at the Samsung Biomedical Research Institute. For islet perifusion, batches of 30 islets each were perifused in parallel microchambers (Biovail International, Minneapolis, MN) with oxygenated KRBB medium with (3.3 or 16.7) mM glucose at a rate of 1 mL/min, and the fractions were collected at 1 min intervals during the 1st peak insulin secretion, and thereafter collected at 2 min intervals. Insulin concentration in aliquots of the incubation or perifusion buffers was measured by the Ultrasensitive Rat Insulin EIA kit from ALPCO Diagnostics (Windham).

2.3 | Chromatin immunoprecipitation (ChIP) assay

The chromatin immunoprecipitation (ChIP) assay was carried out using the EZ ChIP™ kit (Millipore), as instructed by the

manufacturer. Soluble chromatin was prepared as described from 2×10^7 INS-1 cells. For each immunoprecipitation reaction, 30 μg of pre-cleared chromatin was incubated with anti-GPR40 or control antiserum overnight at 4°C on a rotator wheel. Following centrifugation, the extracts were incubated with anti-GPR40 (catalog no. SC-3290; Santa Cruz Biotechnology, Inc.), or control mouse IgG at 4°C overnight on a rotator wheel, and the beads were then washed. Precipitated protein-DNA complexes were eluted, and cross-linking was reversed for the purification of DNA. Polymerase chain reaction (PCR) was performed using the primers 5′-TGGGGAAAGTGAAGGGCGTA-3′ and 5′-CTTGTTGTCCAGCACTTGCC-3′ specific for the (−962 to +235) region of the rat GPR40 promoter that contains three potential PPREs. The PCR products were analyzed by electrophoresis on 1.2% agarose gels, stained with ethidium bromide, and quantified by densitometry.

2.4 | Western blot analysis

After the required incubation period, whole cells were lysed in 10 mM Tris-HCl, 2 mM EDTA, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride, at pH 8.0. Thirty micrograms of proteins per lane were electrophoresed on 10% polyacrylamide gels and electroblotted onto nitrocellulose membranes (Bio-Rad).
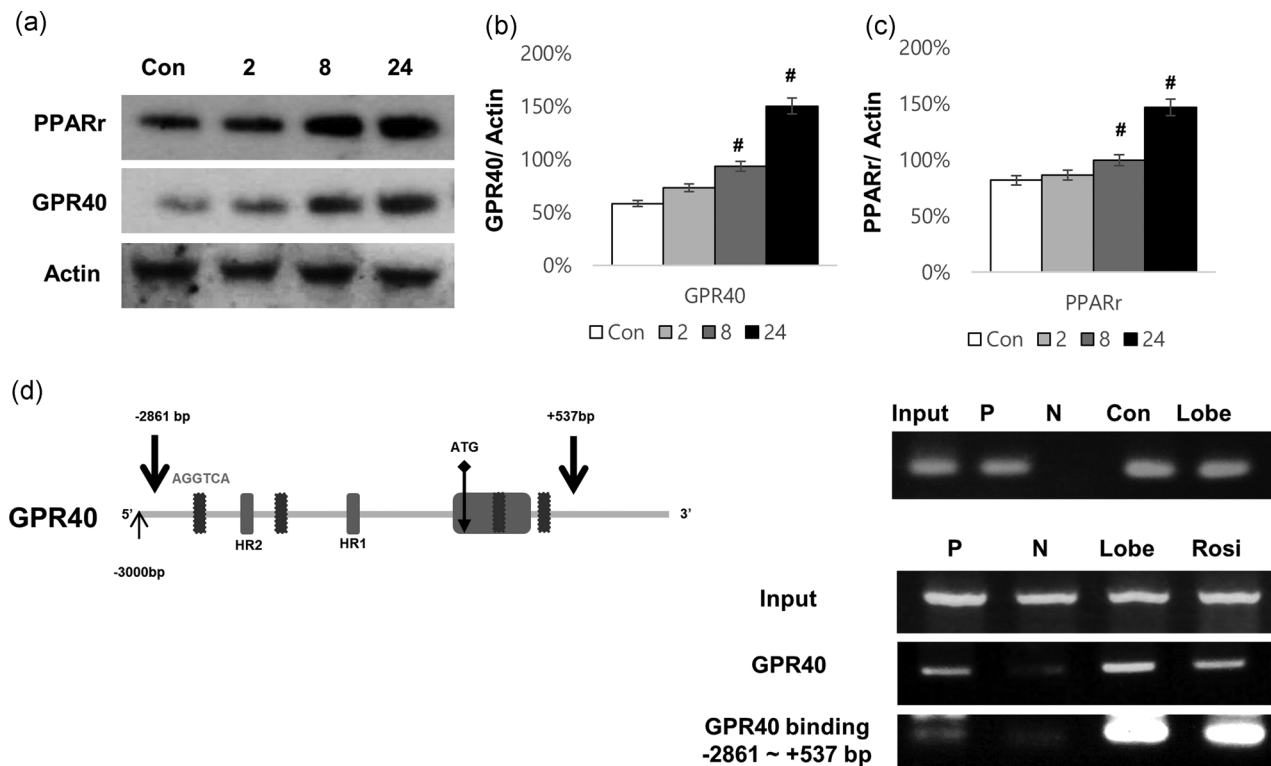
FIGURE 3 (a) Representative immunoblots of GPR40 and PPARγ in INS-1 cells exposed at indicated time to 16.7 mM glucose and lobeglitazone. (b, c) Quantification of PPARγ and GPR40 expression levels. The data represent mean ± *SEM* ($N = 3$; $^\#p < .05$ compared to control group). (d) Chromatin immunoprecipitation assay to assess PPAR binding to the putative PPRE on the GPR40 gene. Results show the expected 567-bp PCR product with lobeglitazone and input DNA, but not the control. (P, anti-RNA Polymerase II antibody; N, normal rabbit IgG; antibody, PPARγ). IgG, immunoglobulin G; PCR, polymerase chian reaction; PPARγ, proliferator-activated receptor gamma

Blots were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h and incubated overnight at 4°C with the primary antibodies. The monoclonal antibody against β-actin (Santa Cruz Biotechnology, Inc.) was used as an internal control to show equal protein loading.

2.5 | Small-interfering RNA experiments

INS-1 cells were transfected with specific small interfering RNA (siRNA) oligomers against PPARγ or GPR40, using TransIT-X2® Dynamic Delivery System transfection reagent (Mirus), according to the manufacturer's instructions. Negative control siRNA oligomers were used as a negative control. After transfection for 24 h, cells were exposed to PPARγ and GPR40. The silencing of target genes was validated by western blot and real-time PCR.

2.6 | Statistics

The data were presented as the mean ± *SD*. The Mann–Whitney tests were performed to compare differences between two independent groups. One-way analysis of variance with post hoc analysis was used to compare differences between several groups. *p* values less than 0.05 were considered statistically significant (PRISM; Graphpad Software Corp.).

3 | RESULTS

3.1 | Effect of TZDs on insulin secretion

Ins-1 cells were treated with 50 ng/mL of TZDs for 24 h, and insulin secretion and contents were measured. TZD treatment for 24 h in the presence of elevated glucose significantly increased GSIS (Figure 1a,b), and also increased GPR40 expression (Figure 1c–f).

3.2 | Lobeglitazone stimulated GSIS in islets

Lobeglitazone was tested at 50 ng/ml in an islet perifusion system in the presence of either (3.3 or 16.7) mM glucose. In the presence of 16.7 mM glucose, lobeglitazone increased insulin release from islets (Figures 2a, c, and d). In addition, lobeglitazone reversed the decreased expressions of GPR40 and PPARγ
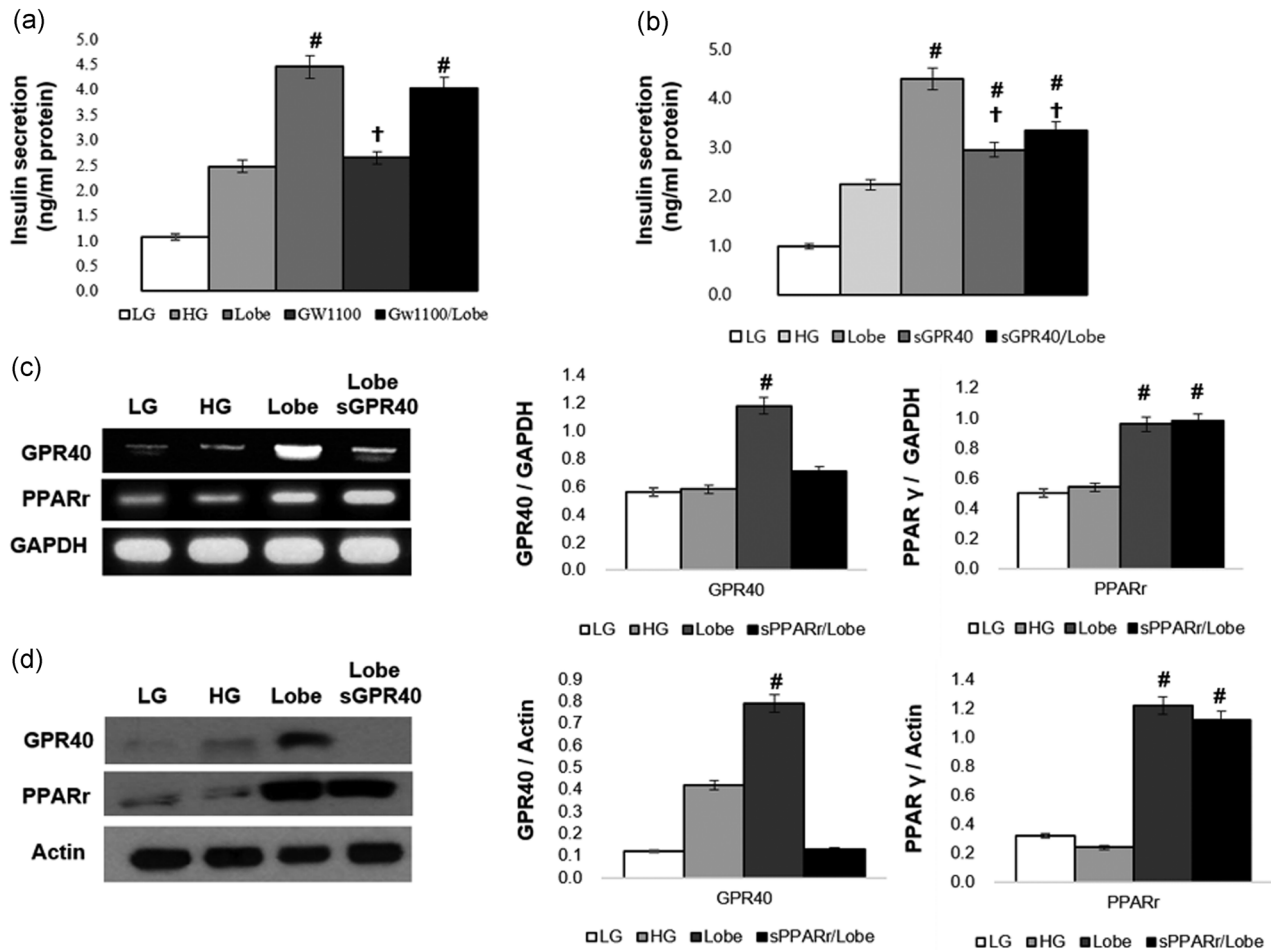
FIGURE 4 Suppression of GPR40 mRNA and protein levels in INS-1 cells with (a) GPR40 antagonist GW1100 or (b) GPR40 siRNA for 24 h. ($^\#p$ < .005 compared to HG group, ^+p < .005 compared to lobeglitazone group) (c) RT-PCR and (d) western blot experiments illustrating PPARγ and GPR40 expression in INS-1 cells with GW1100 or siRNA. Quantification of PPARγ and GPR40 expression levels. Figures 1e and 4d are experimental results from one blot, the same control of actin bands was used in both Figures 1e and 4d. The data represent mean ± SEM (N = 3; $^\#p$ < .005 compared to high control group). mRNA, messenger RNA; PPARγ, proliferator-activated receptor gamma; siRNA. small interfering RNA

by high fat feeding (HF) (Figure 2b). These results suggest that lobeglitazone stimulated insulin secretion through the increase in GPR40 expression.

3.3 | Induction of GPR40 expression by lobeglitazone is transcriptional, and requires intact PPARγ binding site in the HR region of the promoter

Figure 3a–c show there was an increase in GPR40 expression by lobeglitazone treatment, suggesting a transcriptional level of regulation. We identified a functional PPRE in the mouse GPR40 promoter from Gene Bank Blast, which showed a 100% identity with the rat GPR40 promoter at positions (−2381 to +537) from the transcription start site (Gene ID: 266607). PPARγ binding was determined with the chromatin immunoprecipitation assay. Flanking primer pairs for a 1158 bp PCR product that included the GPR40 PPRE (schema in Figure 3d) with input

DNA PPARγ antibody–precipitated DNA generated the correctly-sized PCR band; whereas, with nonimmune serum, only faint bands were observed.

3.4 | Insulin secretion and GPR40 in INS cells

PPARγ regulation of GPR40 expression was tested using siRNA against GPR40 in INS-1 cells, which showed 85% decrease in insulin secretion. Cells were treated with siRNA GPR40 or vehicle for 24 h (Figure 4a,b). GPR40 messenger RNA and protein band intensities were markedly decreased in the GPR40 siRNA cells (Figure 4c,d). In control cells lobeglitazone doubled it; whereas when lobeglitazone and the GPR40 siRNA duplexes were used together, the increase was eliminated. When using GPR40 antagonist GW1100, this phenomenon was also confirmed.
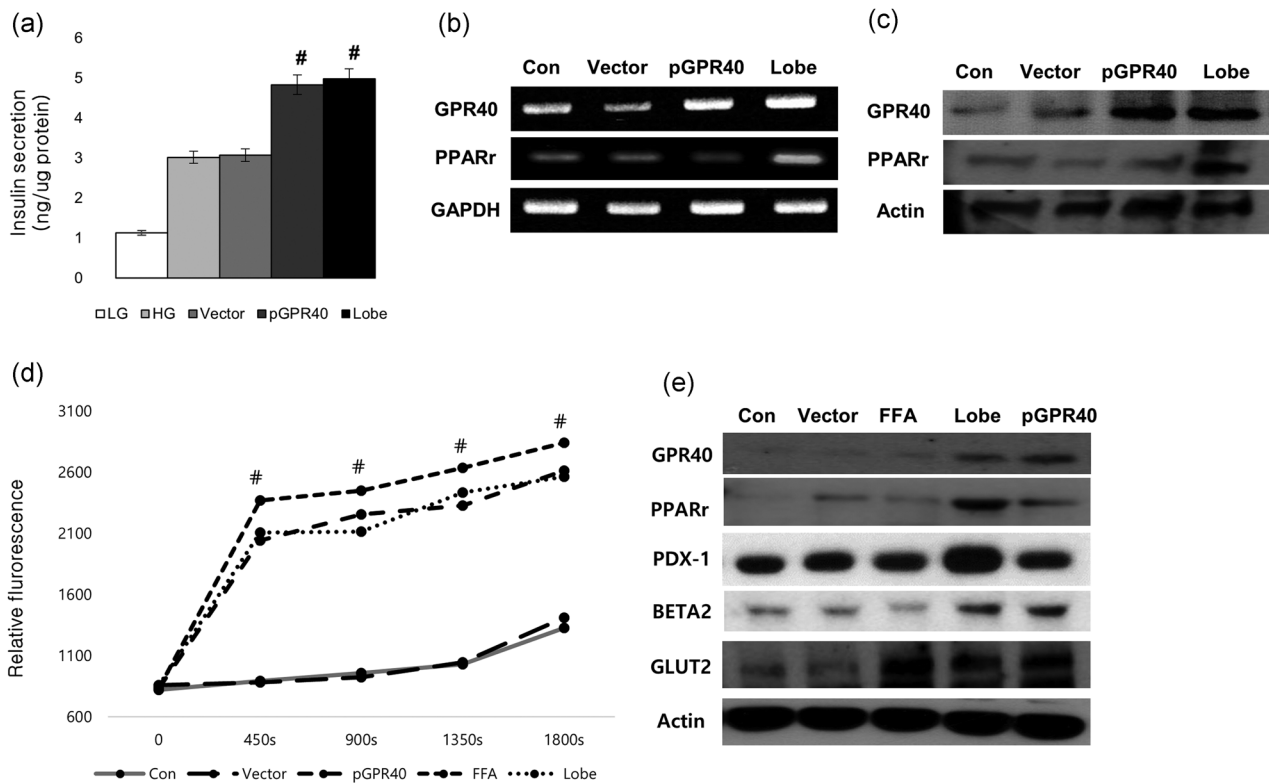
FIGURE 5 (a) Insulin release was significantly increased after pGPR40 treatment at 16.7 mM glucose concentration (#p < .005 compared to control group). INS-1 cells were cultured for 24 h in medium with GPR40 overexpression vector (pGPR40) or empty vector (Con), and then cell lysates underwent (b) GPR40 RT PCR, and (c) immunoblotting. (d) GPR40 activity increased both GPR40 overexpression and TZDs in INS-1 cells (#p < .005 compared to control group). (E) INS-1 cells were treated with lobeglitazone or GPR40 overexpression (pGPR40) for 24 h, followed by measurement of PPARγ target genes by western blot. PCR, polymerase chain reaction; PPARγ, proliferator-activated receptor gamma; TZD, thiazolidinedione

GPR40 overexpression (pGPR40) significantly increased insulin secretion and GPR40 activity similar to cells treated with lobeglitazone (Figure 5a–d). In addition, we could confirm the increase of PDX-1, BETA2, and GLUT2 as in our previous study, such as increase of PPARγ and GPR40 by lobeglitazone, but these results were not accompanied by pGPR40 or FFA (Figure 5e). These results demonstrate that lobeglitazone stimulated insulin secretion in association with the increase in expression of GPR40 in β-cells.

3.5 | Protective effects against the palmitate-induced inhibition of insulin secretion

To determine the effect of lobeglitazone on glucolipotoxicity, Ins-1 cells were treated with 25 mM glucose or 0.5 mM palmitate + 50 ng lobeglitazone at 5 mM glucose for 48 h. Treatment with high glucose and/or fatty acids significantly decreased glucose-stimulated insulin secretion, which lobeglitazone treatment restored (Figure 6a). Lobeglitazone treatment also attenuated glucolipotoxicity, with a significant increase of GPR40, PPARγ, and GLUT2 levels (Figure 6b). GPR40 siRNA (Figure 6c) reduced

insulin secretion from lobeglitazone but significantly increased insulin secretion on pGPR40 (Figure 6d). These results demonstrate that GPR40 is necessary for the protective effects against glucolipotoxicity in pancreatic β-cell.

3.6 | Lobeglitazone increased incretin sensitivity through GPR40

To examine the relationship between GPR40 and incretin sensitivity, Ins-1 cells was treated with (0, 10, 50, and 100) nM exendin-4 with lobeglitazone (Figure 7a). Lobeglitazone increased insulin secretion at every exendin-4 concentration, and siRNA GPR40 and GPR40 overexpression verified incretin sensitivity (Figure 7b,c).

3.7 | Lobeglitazone and adenylyl cyclase (AC) in β-cells increase insulin secretion

Glucose metabolism also induces the generation of another second messenger, cAMP, via adenylyl cyclases in β-cells (Dyachok et al., 2008), and transcripts and proteins of the β-cell specific subtype of
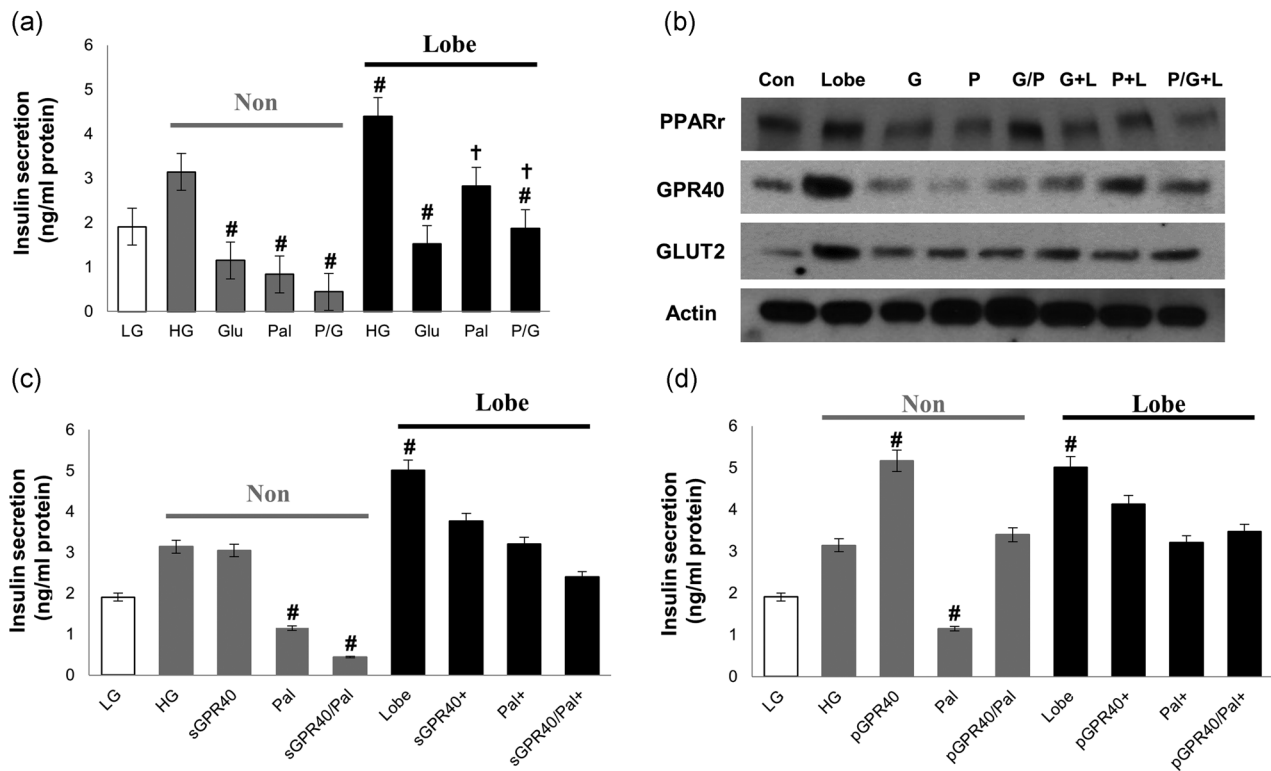
FIGURE 6 (a) Effects of glucotoxicity (Glu; 25 mM glucose) and lipotoxicity (Pal; 0.5 mM palmitate) on insulin secretion from INS-1 cells that have been exposed to 50 ng lobeglitazone at 16.7 mM glucose. ($^{#}p < .005$ compared to HG group, $^{+}p < .005$ compared to toxicity group). (b) Effects of toxicity on the expression of PPARγ, GPR40, and GLUT2 in INS-1 cells detecting by western blot analysis. (c) Effect of reduced GPR40 levels in INS-1 cells using siRNA and scrambled siRNA after 24 h incubation with lobeglitazone. Insulin release was significantly decreased after GPR40 siRNA treatment at high glucose concentration ($^{#}p < .005$ compared to HG group, $^{+}p < .005$ compared to lobeglitazone group). (d) Effect of increased GPR40 levels in INS-1 cells using GPR40 overexpression and empty vector after 24 h incubation with lobeglitazone. Insulin release was significantly increased after GPR40 overexpression treatment at high glucose concentration ($^{#}p < .005$ compared to HG group, $^{+}p < .005$ compared to lobeglitazone group). PPARγ, proliferator-activated receptor gamma; siRNA, small interfering RNA

AC were strongly downregulated in rat and human islets exposed to elevated glucose concentrations (Roger et al., 2011). To obtain insight into the role of AC, we investigated its function in INS-1 cells. Ins-1 cells were treated with siRNA PPARγ and siRNA GPR40 (Figure 8a), and lobeglitazone did not change cAMP level in cells treated with siRNA PPARγ. AC expression was increased by lobeglitazone (Figure 8b,c), and could also be observed by immunofluorescence staining (Figure 8d). Given that lobeglitazone could increase the cAMP concentration, GSIS would also increase in association with the AC at least in part. In contrast to the widely known AC signaling, this process is independent of GPR40 and can account for insulin secretion.

4 | DISCUSSION

We have previously demonstrated that rosiglitazone could stimulate GPR40 expression in pancreatic β-cells (Divakaruni et al., 2013). In this study, we showed that lobeglitazone has the same effect that was mediated by PPARγ, but was also associated with the GPR40 activation, which could increase the incretin sensitivity, and could also

protect against glucolipotoxicity. The effect of PPARγ to increase insulin secretion was not observed without GPR40. GPR40−/− mice have shown reduced incretin and insulin secretions in response to fat, and were not protected from insulin resistance induced by a high-fat diet (Lan et al., 2008). This is consistent with our study showing that increased GPR40 by lobeglitazone protects against lipotoxicity. GPR40 is a Gq-coupled family A GPCR that is specifically expressed in the pancreatic β-cell (Bartoov-Shifman et al., 2007; Tomita et al., 2006), and a sensor of (medium- to long) -chain fatty acids, and has drawn increased attention in recent years for its potential as a novel therapeutic target for the metabolic syndrome (Burant, 2013; Hara et al., 2011; Winzell & Ahrén, 2007). Robust effects of GPR40 agonists on increasing insulin secretion and lowering blood glucose have been shown in rodent models of type 2 diabetes (Burant, 2013). Crosstalk between GPR40 and PPARγ was demonstrated in human endothelial cells (Wang et al., 2015). Rosiglitazone was found to require GPR40 activation with downstream p38 MAPK phosphorylation to optimally propagate PPAR nuclear signaling (Anetta et al., 2007; Sugawara et al., 2007). We observed that lobeglitazone in β-cells leads to the increased expression as well as the activation, of GPR40. PPARγ binding was determined by chromatin immunoprecipitation assay. This finding
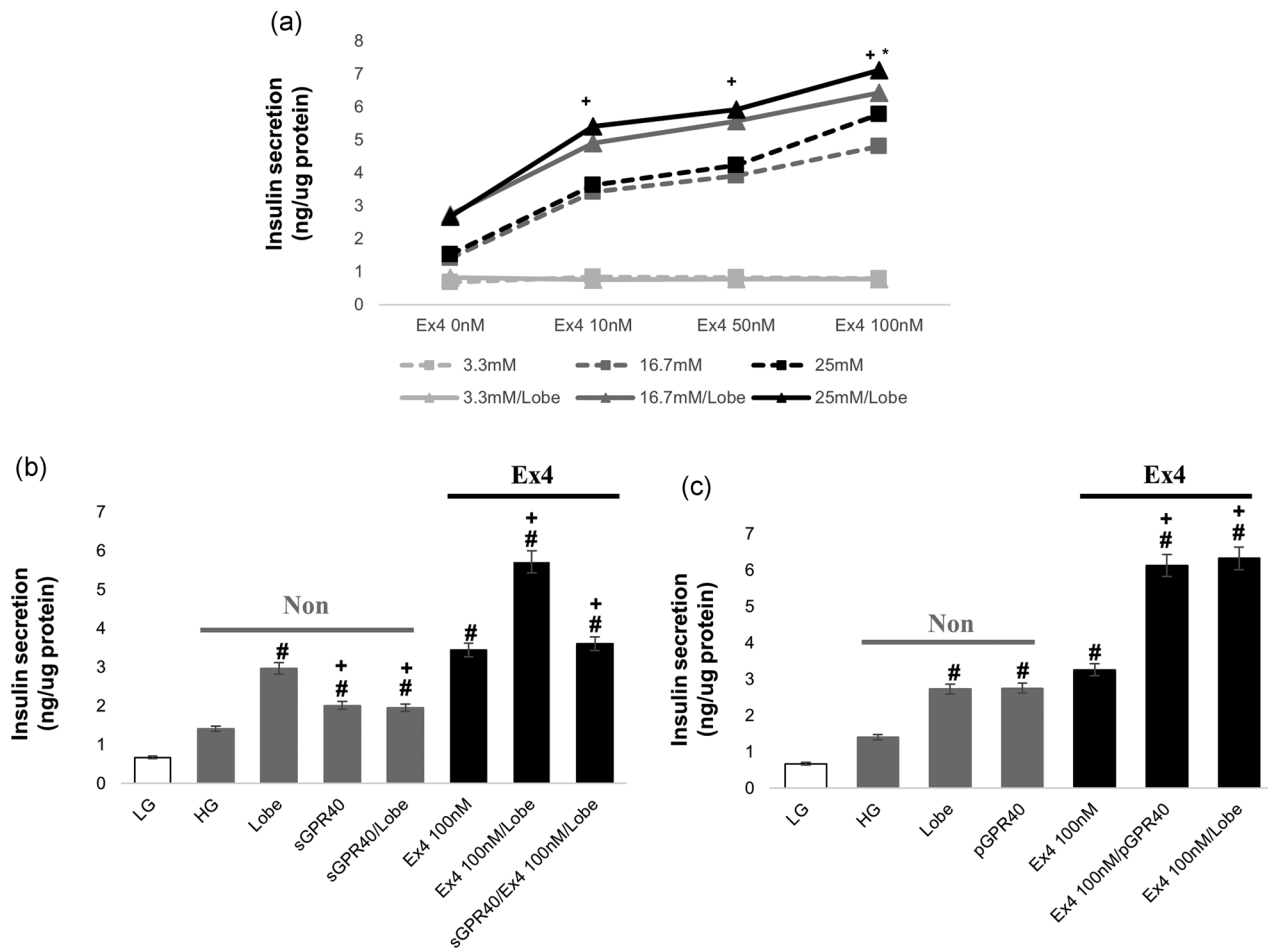
FIGURE 7 (a) INS-1 cells were treated with vehicle (dashed lines) or lobeglitazone (solid lines), and then treated with various doses of exendin-4 for 1 h ($^+p < .005$ compared to vehicle group). (b) Insulin release was significantly increased after exendin-4 treatment at lobeglitazone ($^\#p < .005$ compared to HG group, $^+p < .005$ compared to lobeglitazone group). (c) Insulin release was significantly increased after GPR40 overexpression treatment at Exdin-4 treatment ($^\#p < .005$ compared to HG group, $^+p < .005$ compared to lobeglitazone)

is consistent with recent reports that TZDs bind to and activate GPR40 in bronchial epithelial cells (Gras et al., 2009), osteocytes (Mieczkowska et al., 2012), and GPR40-transfected HEK293 cells (Smith et al., 2009; Sugawara et al., 2007), causing the rapid phosphorylation of p38 MAPK. However, in our study, it was very interesting to see that TZDs significantly increased insulin secretion only after 24 h of stimulation, and GSIS was not increased during short-term incubation with TZDs. We have reported that the increase in Ca^{2+} and insulin secretion stimulated by rosiglitazone in pancreatic β-cells was mediated via GPR40 expression, thus delaying membrane repolarization, and enhancing Ca^{2+} influx (Kim et al., 2013). Increased GPR40 by lobeglitazone in our study could lead to an increase in Ca^{2+} influx, and thus a rise in cAMP. Insulin secretion was also associated with another second messenger, cAMP, which is produced via AC. AC converts ATP to cAMP, an important messenger that regulates biological function throughout the body. Desch et al. (2010) described a second indirect mechanism through which PPARγ stimulates renin gene expression in kidney cells; this involves a multiplication of the cAMP upon simultaneous AC agonist treatment by inducing AC6

gene. Because cAMP stimulates renin gene expression, the potentiation of cAMP level by rosiglitazone could potentially result in dramatic increase in renin gene expression. AC6 is the second gene next to hRen (Todorov et al., 2007), identified as being regulated by PPARγ through a Pal3 site, and not through established PPRE. Calcium may also regulate cAMP level through AC1 or AC8, as both are stimulated by Ca^{2+}/calmodulin, and are expressed in β-cells as well (Delmeire et al., 2003; Roger et al., 2011). AC8 is significantly reduced in islets from diabetic rats (Dolz et al., 2011), or in β-cells at glucotoxicity, suggesting that the reduction of AC8 might be associated with the impaired insulin secretory response of β-cells in diabetes. It is known that AC8 is important for the generation of cAMP, and could play a critical role in GSIS (Dou et al., 2015; Roger et al., 2011). There are also incretin and glitazone-related mechanisms that involve the increase in cAMP level via AC8 (Dolz et al., 2011; Dou et al., 2015; Kong et al., 2019; Roger et al., 2011), suggesting that there are other potential mechanisms to increase insulin secretion through lobeglitazone. Despite this important role of insulinotropic effects in the regulation of insulin secretion, little is known about which of the
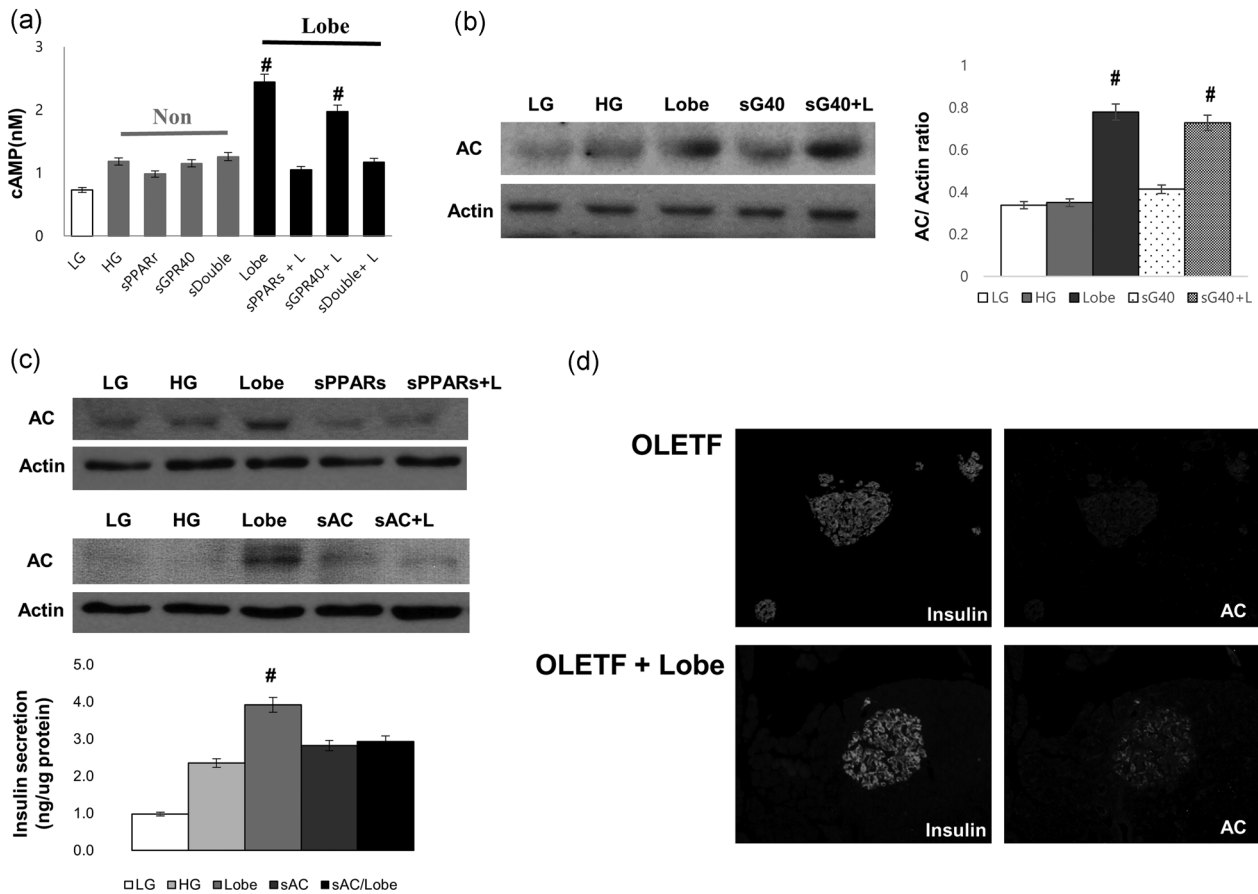
FIGURE 8 (a) INS-1 cells were treated with PPARγ siRNA or GPR40 siRNA, and then cAMP level was measured. ($^{#}p < .005$ compared to vehicle group). (b, c) Effect of increased AC expression in INS-1 cells using GPR40 siRNA, PPARγ siRNA, and AC siRNA after 24 h incubation with lobeglitazone ($^{#}p < .005$ compared to HG). (d) The pancreatic islets from OLETF and OLETF with lobeglitazone, were examined by immunofluorescence for AC and insulin. OLETF, Otsuka Long-Evans Tokushima Fatty; PPARγ, proliferator-activated receptor gamma; siRNA, small interfering RNA

multiple subtypes of AC are expressed in β-cells. However, we could confirm that AC is increased by lobeglitazone using AC siRNA, which increases insulin secretion. Although in this study we could not show the AC-specific molecular site of interaction between lobeglitazone and insulin secretion, we did show that TZDs could induce GPR40 and AC expression, which were associated with the increased incretin sensitivity and protection against lipotoxicity. There might be other possible mechanisms associated with AC and insulin secretion, and further study is needed to examine the molecular mechanisms of interaction between lobeglitazone and AC in β-cells.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Mina Hwang, and Moon-Kyu Lee are the primary authors and contributed equally to this study. Mina Hwang, Hyo-Sup Kim, Sang-Man Jin, Kyoo-Yeon Hur, Jae-Hyeon Kim, and Moon-Kyu Lee designed the study. Mina Hwang, and Moon-Kyu Le performed experiments and data analysis. Mina Hwang, and Moon-Kyu Le wrote the manuscript. Moon-Kyu Lee is the guarantor of this study and, such as had full access to all data in the study and takes full responsibility for it.

DATA AVAILABILITY STATEMENT

The data that support the finding of this study are available from the authors on reasonable request, see author contributions for specific data sets.

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REFERENCES

Alquier, T., Peyot, M. L., Latour, M. G., Kebede, M., Sorensen, C. M., Gesta, S., Ronald Kahn, C., Smith, R. D., Jetton, T. L., Metz, T. O., Prentki, M., & Poitout, V. (2009). Deletion of GPR40 impairs glucose-induced insulin secretion in vivo in mice without affecting intracellular fuel metabolism in islets. *Diabetes, 58*, 2607–2615.

Anetta, P., Shuibang, W., Jianhua, Z., Robert, A. W., & Robert, L. D. (2007). Nitric oxide activation of peroxisome proliferator-activated receptor gamma through a p38 MAPK signaling pathway. *FASEB Journal*, 21(3), 950–961.

Asfari, M., Janjic, D., Meda, P., Li, G., Halban, P. A., & Wollheim, C. B. (1992). Establishment of 2-mercaptoethanol-dependent differentiated insulin secreting cell line. *Endocrinology*, 130, 167–178.

Bartoov-Shifman, R., Ridner, G., Bahar, K., Rubins, N., & Walker, M. D. (2007). Regulation of the gene encoding GPR40, a fatty acid receptor expressed selectively in pancreatic beta cells. *Journal of Biological Chemistry*, 282, 23561–23571.

Briscoe, C. P., Tadayyon, M., Andrews, J. L, Benson, W. G., Chambers, J. K., Eilert, M. M., Ellis, C., Elshourbagy, N. A., Goetz, A. S., Minnick, D. T., Murdock, P. R., Sauls, H. R., Jr., Shabon, U., Spinage, L. D., Strum, J. C., Szekeres, P. G., Tan, K. B., Way, J. M., Ignar, D. M., ... Muir, A. I. (2003). The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *Journal of Biological Chemistry*, 278, 11303–11311.

Burant, C. F. (2013). Activation of GPR40 as a therapeutic target for the treatment of type 2 diabetes. *Diabetes Care*, 36, S175–S179.

Delmeire, D., Flamez, D., Hinke, S. A., Cali, J. J., Pipeleers, D., & Schuit, F. (2003). Type VIII adenylyl cyclase in rat cells: Coincidence signal detector/generator for glucose and GLP-1. *Diabetologia*, 46, 1383–1393.

Desch, M., Schubert, T., Schreiber, A., Mayer, S., Friedrich, B., Artunc, F., & Todorov, V. T. (2010). PPAR-γ dependent regulation of adenylate cyclase 6 amplifies the stimulatory effect of cAMP on renin gene expression. *Molecular Endocrinology*, 24, 2139–2151.

Divakaruni, A. S., Wiley, S. E., Rogers, G. W., Andreyev, A. Y., Petrosyan, S., Loviscach, M., Wall, E. A., Yadava, N., Heuck, A. P., Ferrick, D. A., Henry, R. R., McDonald, W. G., Colca, J. R., Simon, M. I., Ciaraldi, T. P., & Murphy, A. N. (2013). Thiazolidinediones are acute, specific inhibitors of the mitochondrial pyruvate carrier. *Proceedings of the National Academy of Sciences of the United States of America*, 2 110(14), 5422–5427.

Dolz, M., Movassat, J., Bailbé, D., Le Stunff, H., Giroix, M. H., Fradet, M., Kergoat, M., & Portha, B. (2011). cAMP-secretion coupling is impaired in diabetic GK/Par rat β-cells: A defect counteracted by GLP-1. *American Journal of Physiology, Endocrinology and Metabolism*, 301(5), E797–E806.

Dou, H., Wang, C., Wu, X., Yao, L., Zhang, X., Teng, S., Xu, H., Liu, B., Wu, Q., Zhang, Q., Hu, M., Wang, Y., Wang, L., Wu, Y., Shang, S., Kang, X., Zheng, L., Zhang, J., Raoux, M., ... Zhou, Z. (2015). Calcium influx activates adenylyl cyclase 8 for sustained insulin secretion in rat pancreatic beta cells. *Diabetologia*, 58(2), 324–333.

Dyachok, O., Idevall-Hagren, O., Sågetorp, J., Tian, G., Wuttke, A., Arrieumerlou, C., Akusjärvi, G., Gylfe, E., & Tengholm, A. (2008). Glucose-induced cyclic AMP oscillations regulate pulsatile insulin secretion. *Cell Metabolism*, 8, 26–37.

Ferré, P. (2004). The biology of peroxisome proliferator-activated receptors: Relationship with lipid metabolism and insulin sensitivity. *Diabetes*, 53(Suppl 1), S43–S50.

Gras, D., Chanez, P., Urbach, V., Vachier, I., Godard, P., & Bonnans, C. (2009). Thiazolidinediones induce proliferation of human bronchial epithelial cells through the GPR40 receptor. *American Journal of Physiology: Lung Cellular and Molecular Physiology*, 296, L970–L978. https://pubmed.ncbi.nlm.nih.gov/19346435/

Gregory, L. S., Pavel, K., & Bluestone, J. A. Murine pancreatic islet isolation. 255. Visualized Experiment. 2007.

Hara, T., Hirasawa, A., Ichimura, A., Kimura, I., & Tsujimoto, G. (2011). Free fatty acid receptors FFAR1 and GPR120 as novel therapeutic targets for metabolic disorders. *Journal of Pharmaceutical Sciences*, 100, 3594–3601.

Itoh, Y., Kawamata, Y., Harada, M., Kobayashi, M., Fujii, R., Fukusumi, S., Ogi, K., Hosoya, M., Tanaka, Y., Uejima, H., Tanaka, H., Maruyama, M., Satoh, R.,

Okubo, S., Kizawa, H., Komatsu, H., Matsumura, F., Noguchi, Y., Shinohara, T., ... Fujino, M. (2003). Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40. *Nature*, 422, 173–176.

Kim, H. S., Hwang, Y. C., Koo, S. H., Park, K. S., Lee, M. S., Kim, K. W., & Lee, M. K. (2013). PPAR-γ activation increases insulin secretion through the up-regulation of the free fatty acid receptor GPR40 in pancreatic β-cells. *PLOS One*, 8, e50128.

Kim, H. S., Noh, J. H., Hong, S. H., Hwang, Y. C., Yang, T. Y., Lee, M. S., Kim, K. W., & Lee, M. K. (2008). Rosiglitazone stimulates the release and synthesis of insulin by enhancing GLUT-2, glucokinase and BETA2/NeuroD expression. *Biochemical and Biophysical Research Communications*, 367, 623–629.

Kong, X., Li, B., Deng, Y., & Ma, X. (2019). FXR mediates adenylyl cyclase 8 expression in pancreatic β-cells. *Journal of Diabetes Research*, 14, 8915818.

Kotarsky, K., Nilsson, N. E., Flodgren, E., Owman, C., & Olde, B. (2003). A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. *Biochemical and Biophysical Research Communications*, 301, 406–410.

Lan, H., Hoos, L. M., Liu, L., Tetzloff, G., Hu, W., Abbondanzo, S. J., Vassileva, G., Gustafson, E. L., Hedrick, J. A., & Davis, H. R. (2008). Lack of FFAR1/GPR40 does not protect mice from high-fat diet-induced metabolic disease. *Diabetes*, 57, 2999–3006.

Landa, L. R., Jr., Harbeck, M., Kaihara, K., Chepurny, O., Kitiphongspattana, K., Graf, O., Nikolaev, V. O., Lohse, M. J., Holz, G. G., & Roe, M. W. (2005). Interplay of Ca2 + and cAMP signaling in the insulin-secreting MIN6 cell line. *Journal of Biological Chemistry*, 280, 31294–31302.

Lawrence, M. C., McGlynn, K., Shao, L., Duan, L., Naziruddin, B., Levy, M. F., & Cobb, M. H. (2008). Chromatin-bound mitogen-activated protein kinases transmit dynamic signals in transcription complexes in beta-cells. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 13315–13320.

Merglen, A., Theander, S., Rubi, B., Chaffard, G., Wollheim, C. B., & Maechler, P. (2004). Glucose sensitivity and metabolism-secretion coupling studied during two-year continuous culture in INS-1E insulinoma cells. *Endocrinology*, 145, 667–678.

Mieczkowska, A., Basle´, M. F., Chappard, D., & Mabilleau, G. (2012). Thiazolidinediones induce osteocyte apoptosis by a G protein-coupled receptor 40-dependent mechanism. *Journal of Biological Chemistry*, 287, 23517–23526.

Roger, B., Papin, J., Vacher, P., Raoux, M., Mulot, A., Dubois, M., Kerr-Conte, J., Voy, B. H., Pattou, F., Charpentier, G., Jonas, J. C., Moustaïd-Moussa, N., & Lang, J. (2011). Adenylyl cyclase 8 is central to glucagon-like peptide 1 signalling and effects of chronically elevated glucose in rat and human pancreatic beta cells. *Diabetologia*, 54, 390–402.

Semple, R. K., Chatterjee, V. K., & O'Rahilly, S. (2006). PPAR gamma and human metabolic disease. *Journal of Clinical Investigation*, 116, 581–589.

Smith, N. J., Stoddart, L. A., Devine, N. M., Jenkins, L., & Milligan, G. (2009). The action and mode of binding of thiazolidinedione ligands at free fatty acid receptor 1. *Journal of Biological Chemistry*, 284, 17527–17539.

Stoddart, L. A., Brown, A. J., & Milligan, G. (2007). Uncovering the pharmacology of the G protein-coupled receptor GPR40: high apparent constitutive activity in guanosine 5-O-(3-[35S] thio) triphosphate binding studies reflects binding of an endogenous agonist. *Molecular Pharmacology*, 71, 994–1005.

Sugawara, Y., Nishii, H., Takahashi, T., Yamauchi, J., Mizuno, N., Tago, K., & Itoh, H. (2007). The lipid raft proteins flotillins/reggies interact with G q and are involved in Gq-mediated p38 mitogen-activated protein kinase activation through tyrosine kinase. *Cellular Signalling*, 19, 1301–1308.

Todorov, V. T., Desch, M., Schmitt-Nilson, N., Todorova, A., & Kurtz, A. (2007). Peroxisome proliferator-activated receptor-gamma is involved in the control of renin gene expression. *Hypertension*, *50*, 939–944.

Tomita, T., Masuzaki, H., Iwakura, H., Fujikura, J., Noguchi, M., Tanaka, T., Ebihara, K., Kawamura, J., Komoto, I., Kawaguchi, Y., Fujimoto, K., Doi, R., Shimada, Y., Hosoda, K., Imamura, M., & Nakao, K. (2006). Expression of the gene for a membrane-bound fatty acid receptor in the pancreas and islet cell tumours in humans: evidence for GPR40 expression in pancreatic beta cells and implications for insulin secretion. *Diabetologia*, *49*, 962–968.

Wang, S., Awad, K. S., Elinoff, J. M., Dougherty, E. J., Ferreyra, G. A., Wang, J. Y., Cai, R., Sun, J., Ptasinska, A., & Danner, R. L. (2015). G protein-coupled receptor 40 (GPR40) and peroxisome proliferator-activated receptor gamma (PPAR gamma): An integrated two-receptor signaling pathway. *Journal of Biological Chemistry*, *290*(32), 19544–19557.

Welters, H. J., McBain, S. C., Tadayyon, M., Scarpello, J. H., Smith, S. A., & Morgan, N. G. (2004). Expression and functional activity of PPAR-r in pancreatic beta-cells. *British Journal of Pharmacology*, *142*, 162–170.

Winzell, M. S., & Ahrén, B. (2007). G-protein-coupled receptors and islet function implications for treatment of type 2 diabetes. *Pharmacology and Therapeutics*, *116*, 437–448.

Yang, B., He, B., Abdel-Halim, S. M., Tibell, A., Brendel, M. D., Bretzel, R. G., Efendic, S., & Hillert, J. (1999). Molecular cloning of a full-length cDNA for human type 3 adenylyl cyclase and its expression in human islets. *Biochemical and Biophysical Research Communications*, *254*, 548–551.

Zhou, C., Tang, C., Chang, E., Ge, M., Lin, S., Cline, E., Tan, C. P., Feng, Y., Zhou, Y. P., Eiermann, G. J., Petrov, A., Salituro, G., Meinke, P., Mosley, R., Akiyama, T. E., Einstein, M., Kumar, S., Berger, J., Howard, A. D., … Yang, L. (2010). Discovery of 5-aryloxy-2,4-thiazolidinediones as potent GPR40 agonists. *Bioorganic & Medicinal Chemistry Letters*, *20*, 1298–1301.

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