Research Article

PPAR_δ_ Activation Rescues Pancreatic β-Cell Line INS-1E from Palmitate-Induced Endoplasmic Reticulum Stress through Enhanced Fatty Acid Oxidation

Mingming Cao, 1 Yuzhen Tong, 2 Qingguo Lv, 1 Xiang Chen, 3 Yang Long, 3 Li Jiang, 1 Jun Wan, 1 Yuwei Zhang, 1 Fang Zhang, 1 and Nanwei Tong 1

1 Division of Endocrinology, West China Hospital, Sichuan University, 37 Guoxuexiang, Chengdu 610041, China
2 School of Clinical Medicine, West China Hospital, Sichuan University, 37 Guoxuexiang, Chengdu 610041, China
3 Research Laboratory of Endocrine and Metabolic Diseases, West China Hospital, Sichuan University, Chengdu 610041, China

Correspondence should be addressed to Nanwei Tong, buddyjun@hotmail.com

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One of the key factors responsible for the development of type 2 diabetes is the loss of functional pancreatic β cells. This occurs due to a chronic exposure to a high fatty acid environment. ER stress is caused by an accumulation of irreversible misfold or unfold protein; these trigger the death of functional pancreatic β cells. PPAR_δ_ is an orphan nuclear receptor. It plays a pivotal role in regulating the metabolism of dietary lipids and fats. However, the correlation between PPAR_δ_ and ER stress of pancreatic β cells is not quite clear till date. Here, we show that PPAR_δ_ attenuates palmitate-induced ER stress of pancreatic β cells. On the other hand, PPAR_δ_ agonist inhibits both abnormal changes in ER structure and activation of signaling cascade, which is downstream ER stress. Further, we illustrate that PPAR_δ_ attenuates palmitate-induced ER stress by promoting fatty acid oxidation through treatment with etomoxir, an inhibitor of fatty acid oxidation. It dramatically abolishes PPAR_δ_-mediated inhibition of ER stress. Finally, we show that PPAR_δ_ could protect pancreatic β cells from palmitate-induced cell death and dysfunction of insulin secretion. Our work elucidates the protective effect of PPAR_δ_ on the fatty-acid-induced toxicity of pancreatic β cells.

1. Introduction

One of the major risk factors responsible for the development of type 2 diabetes is the loss of functional pancreatic β cells: this is usually associated with hyperlipidemia-induced lipotoxicity. The underlying mechanism of this loss of functional pancreatic β cells is not fully understood till date [1]. Unlike type 1 diabetes, in the progression of type 2 diabetes, the β-cell dysfunction is predominantly associated with persistent hyperglycemia-induced glucotoxicity [2], enhanced level of plasma free fatty acids (FFAs) [3, 4], and increase in circulating cytokines [5] and chronic oxidative stresses [6]. Among these factors, research studies have found that continuous intake of food that is rich in high fatty acids leads to an elevated secretion of insulin from pancreatic β cells. Moreover, chronic exposure to an environment with high level of fatty acids results in desensitization and suppression of insulin production, even leading to apoptosis [7]. Therefore, new pharmacological drugs capable of controlling plasma fatty acids are required to treat obesity and type 2 diabetes.

Unlike unsaturated fatty acids, saturated fatty acids, including palmitate, could cause cellular dysfunction and even cell death in pancreatic β cells [8]. Recent studies suggested that metabolically generated reactive oxygen species (ROS) is required for fatty-acid-induced apoptosis in pancreatic β cells, which could be even aggravated by inhibition of neuronal nitric oxide synthase by using chemical agents [6, 9]. Further, fatty acids synergize with glucose to induce pancreatic β-cell apoptosis by activating GSK-3β [4]. The
specific toxic effects of saturated fatty acids may be related to ceramide formation that an increase in cellular levels of palmitic or stearic acid but not of palmitoleic acid is correlated with de novo synthesis of ceramide, leading to activation of apoptotic signaling pathway [10].

Amounting works have provided evidence linking ER stress with fatty-acids-induced lipotoxicity in β cells [8]. The endoplasmic reticulum (ER) is an indispensable organelle for eukaryotic cells. It is used for protein synthesis, folding, and regulating the concentration of calcium ion in cells [11]. ER operates in conjunction with the protein folding pathways. This process is so selective that even relatively minor perturbations in the efficiency of protein folding, termed as ER stress, lead to the rejection of nascent proteins. This ultimately leads to an accumulation or degradation of these proteins. Bip is an abundant multifunctional protein that binds and inhibits several ER stress transducers, including PKR-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) [12]. Perturbations altering ER homeostasis can accumulate unfolded proteins (UPs). It drives Bip away from these three ER stress transducers, leading to an activation of UPR signaling pathway. The UPR is an integrated intracellular signaling pathway: it triggers transcriptional induction of UPR genes, translational attenuation of global protein synthesis, and ER-associated degradation (ERAD). These provide an adaptive response for survival. Nevertheless, if the protein-folding defect is not corrected, apoptotic signaling pathway mediated by Chop or JNK would be initiated alternatively, thereby leading to cell death [13]. Though the ER stress-initiated prosurvival or proapoptotic signaling cascades were well established, the regulatory network responsible for fatty-acids-mediated ER stress has not been extensively characterized.

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors. These belong to the steroid receptor superfamily. They play a pivotal role in regulating dietary lipid metabolism and fat storage in mammals [14, 15]. Recent studies have elucidated the protective activity of PPARδ against lipotoxicity. An overexpression of PPARδ significantly reduces the plasma level of FFA as well as the consequent lipotoxicity and improves insulin secretion in pancreatic β cells by modulating the oxidation of fatty acids [16–19]. However, the involvement of PPARδ in fatty-acid-mediated ER stress is still not clear.

In this study, we investigated the role of PPARδ in fatty-acid-induced ER stress of pancreatic β cells. We proved that PPARδ protects pancreatic β cell from palmitate-induced ER stress. Our work leads to a better understanding of the protective effect of PPARδ on fatty-acid-induced toxicity in pancreatic β cells.

2. Methods

2.1. Cell Culture and Reagents. It was cultured in RPMI1640 (Gibco): this medium was supplemented with 10% fetal bovine serum, 10 mM HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 100 IU/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere (5% CO₂, 95% air) at a temperature of 37°C.

The following three agents were diluted in DMSO: PPARδ agonist GW501516 (Alexis), and PPARγ antagonist GSK0660 (Sigma) and Etomoxir (Sigma) inhibitor of CPT-1, a key enzyme associated with the β oxidation of fatty acids. The working concentration of these three agents was 100 nM, 1 μM, and 50 μM, respectively.

2.2. Preparation of Palmitate/BSA Solution. 10 mM palmitate/10% BSA solution was prepared as follows: 500 mM palmitate was added to 5 mL of 0.1 M NaOH. Then, it was mixed in a water bath that was maintained at 70°C. The resulting solution was mixed with 45 mL of 10% BSA (5 g FFA-free BSA diluted in 45 mL PBS) in a water bath, whose temperature was maintained at 60°C. This solution was stored after it was cooled at room temperature. Cells were treated with 0.5 mM palmitate/0.5% BSA, which was diluted in serum-free RPMI 1640 medium before being used, as previously reported [20–22].

2.3. Determination of Insulin Secretion of Pancreatic β Cells. INS-1E cells (5 x 10⁵ cells per well) were seeded in standard glucose concentration (11.1 mmol/L) in 6-well dishes. As described by the above procedure, they were treated with corresponding drugs for 48 h. The culture medium was changed every day to ensure that the concentration of the material was kept constant. The preincubation was done for 1 h in a glucose-free Krebs-Ringer bicarbonate buffer (KRBH; 135 mM NaCl, 3.6 mM KCl, 5 mM NaHCO₃, 0.5 mM NaH₂PO₄, 0.5 mM MgCl₂, 1.5 mM CaCl₂, 10 mM HEPES, and 0.1% glucose-free and FFA-free BSA, pH 7.4). The cells were washed once in a glucose-free KRBH and subsequently treated with KRBH containing low (2.8 mmol/L) or stimulatory (16.7 mmol/L) glucose concentrations for 1 h. Subsequently, the supernatants were obtained and frozen at −80°C for determining the concentration of insulin. A rat insulin radioimmunnoassay kit was used to measure the insulin levels (Linco Research, St Louis, MO, USA). The value of insulin secretion was normalized to the total protein of cells.

2.4. Electron Microscopy. The cell pellet was fixed with 0.3% cacodylate-buffered glutaraldehyde (pH 7.4) for 30 min at 4°C. Then, it was centrifuged at 10000 g for 15 min and refixed with 3% cacodylate-buffered glutaraldehyde for 30 min at 4°C. The cell pellet was postfixed in 1% osmium tetroxide (pH 7.4). It was dehydrated in ethanol and embedded in an Epon-Araldite mixture. Ultrathin sections were stained with uranyl acetate and lead citrate. These sections were then examined with a transmission electron microscope (H-600IV; Hitachi, Japan).

2.5. Real-Time RT-PCR. Total RNA was isolated using Tri-reagent (Invitrogen) according to the manufacturer’s instructions First strand cDNA was reverse-transcribed from 500 ng of total RNA in a final volume of 10 μl using PrimeScript RT reagent Kit (TakaRa, DRR037A), in accordance
with the manufacturer’s instructions. The primers used were as follows: Bip, 5′-acctttggctcactcag-3′ and 5′-agctccagt- 
ttgccacagt-3′; ATF4, 5′-cttcagcagggct-3′ and 5′-tctcgcactcagagttg-3′; XBP-1s, 5′-tctccagggagggctcat-3′ 
and 5′-ttctcgcttccagagaattgc-3′; Chop, 5′-tccagatccta- 
ctcgagtc-3′ and 5′-tctctatctctctgtctcct-3′; CPT-1, 5′- 
cctgctgctgacccatag-3′ and 5′-gtgaggtggggtctgctcctcc-3′; 
ACO, 5′-agatctggaggctggtccccat-3′ and 5′-agaaaccaagagtt- 
ggaacct-3′; GAPDH, 5′-atatcgacctaaacaggag-3′ and 5′- 
atactcagacccata-3′. PCR was performed with SYBR Premix 
Ex Taq II KIT (TakaRa, DRR081A) in a ABI 7300 real-
time PCR system according to manufacturer’s instructions.

2.6. Western Blotting. Cells were lysed using RIPA lysis buffer 
(KeyGEN, China), and the supernatant was collected after being subjected to centrifugation at 14,000 g for 10 min at a 
temperature of 4°C. Protein concentration was quantified by 
the BCA assay (Pierce, no. 23223). Samples were separated 
by 10% SDS-PAGE and transferred to PVDF membranes 
(Amersham Biosciences). The membranes were blocked 
overnight with PBS containing 0.1% Tween 20 in 5% 
(w/v) skimmed milk at 4°C. Then, these were subsequently probed 
by primary antibodies. Blots were incubated with their 
respective primary antibodies for 2 h at room temperature. The primary antibodies included JNK (sc-572, Santa Cruz), 
p-JNK (sc-6254, Santa Cruz), Bip (sc-13968, Santa Cruz), 
Chop (#2895, Cell Signaling Technology), and BCL-2 (sc- 
783, Santa Cruz). After washing three times in TBST, the 
blots were incubated with secondary antibody that was 
conjugated to horseradish peroxidase for 2 h at room temper-

ature. Blots were visualized using enhanced chemilumines-
cence reagents (Amersham Pharmacia Biotech, Piscataway, 
NJ, USA). β-actin was used as an internal control for validating 
intracellular proteins. The relative densitometric value of 
each blot was calculated and analyzed by using Quantity One 
software (Bio-Rad).

2.7. TUNEL Assay. TUNEL staining was performed using 
terminal deoxynucleotidyl transferase (Promega Inc., Madis-
on, WI, USA). Cells were fixed in freshly prepared 4% 
formaldehyde solution in PBS (pH 7.4) for 25 minutes at 4°C. 
These were washed with fresh PBS for 10 minutes at room temperature. These were permeated in 
0.2% Triton-100 solution in PBS for another 5 min. After equilibrating for 10 min, the cells were incubated with 
terminal deoxynucleotidyl transferase and observed through a fluorescence microscope. A 
nucleus with bright green fluorescence staining was recorded as a 
TUNEL-positive event. TUNEL-positive cells were counted by using fluorescence microscope (Olympus Optical 
Co, Hamburg, Germany).

2.8. Caspase-3 Activity Assay. The activity of caspase-3 was 
determined using the Caspase-3 Activity Assay Kit (Bey-
otime, China). Cells were harvested and washed with PBS 
twice and then removed. The supernatant material was 
separated by centrifugation at 10,000 g for 1 min at 4°C. Cells 
were lysed on ice with lysis buffer for 15 min after being 
subjected to centrifugation at 16,000 g for 15 min at 4°C. 
80 μL analysis buffer, 10 μL caspase-3 substrate (Ac-DEVD- 
pNA) (2 mM), and 10 μL cell lystate were mixed in a 96-
well plate. Then, they were subjected to incubation at 37°C 
for 2 h. The absorbance of samples was measured using a 
spectrophotometer at a wavelength of 405 nm.

2.9. Data Analysis and Statistics. All the quantitative data 
were recorded in terms of mean ± SD. Comparisons between 
two groups were performed by Student’s t-test. Comparisons 
among multiple groups were performed by one-way 
ANOVA.

3. Results

3.1. PPARδ Attenuates Palmitate-Induced ER Stress in Pan-
creatic β Cells. We initially examined the role of PPARδ 
in palmitate-induced ER stress. The structure of ER from INS-
1E cells treated by palmitate or the combination of palmitate 
and GW501516, a chemical activator of PPARδ, was visu-
ialized by TEM. As shown, treatment of palmitate caused 
severe morphologic changes in the ER structure, including 
abnormal extension and swelling, which was significantly 
blocked by treating with GW501516 (Figure1(a)). These 
results suggest that activation of PPARδ inhibits palmitate-
induced ER stress.

It is reported that Bip saves the cell from apoptosis by 
repressing ER stress [23]. We wondered whether Bip 
was involved in PPARδ-mediated inhibition of ER stress 
under palmitate treatment. However, our results indicate 
that activation of PPARδ had no impact on the expression 
level of Bip (Figure1(b)). Notably, we found that palmitate-
induced expression of ATF-4 and XBP-1s was attenuated by 
GW501516 (Figure1(b)). We had a keen interest in exam-
ining the activation status of Chop and JNK, which were 
upstream effectors of PERK-eIF2-ATF4-Chop and IRE-1-
XBP1s-p-JNK signaling pathway, respectively. As expected, 
GW501516 treatment inhibited either the overexpression of 
Chop or phosphorylation of JNK (Figure1(c)). These 
results indicate that the inhibitory effect of PPARδ on ER 
stress might be associated with ATF-4 and XBP-1s-mediated signaling pathway.

3.2. PPARδ Attenuates Palmitate-Induced ER Stress by Pro-
moting Fatty Acid Oxidation. PPARδ is an activator of fatty 
acid oxidation, and fatty acid oxidation can be attenuated 
with the help of palmitate-induced ER stress [24]. To 
determine whether lipid oxidation was involved in PPARδ-
mediated inhibition of ER stress, the expression of two key 
enzymes involved in lipid oxidation, CPT-1 and ACO was 
examined. As shown in Figure 2(a), the expression level of 
either CPT-1 or ACO was only slightly increased in response 
to palmitate solo treatment. Strikingly, PPARδ activation 
notably elevated the expression of both CPT-1 and ACO 
under palmitate treatment.

To further elucidate if lipid oxidation has a role in 
PPARδ-mediated inhibition of ER stress, etomoxir, an 
inhibitor of CPT-1, was used to block fatty acid oxidation. As 
shown, etomoxir markedly abolished GW501516-mediated 
inhibition of Chop through treatment of palmitate, at either
transcriptional or translational level, though no significant changes were found in the level of Bip (Figures 2(b) and 2(c)). These results indicate that PPARδ attenuates palmitate-induced ER stress by promoting fatty acid oxidation.

3.3. PPARδ Protects Pancreatic β Cells from Palmitate-Induced Lipotoxicity. ER stress plays a crucial role in palmitate-induced cell death. Therefore, we examined how PPARδ decreases palmitate-induced lipotoxicity in pancreatic β cells. As shown in Figure 3(a), exposure to palmitate-induced apoptosis occurs in INS-1E cells. Notably, the number of apoptotic cells under palmitate treatment was markedly reduced in the presence of GW501516, whereas the inhibition of PPARδ by GSK0660 enhanced palmitate-induced apoptosis. We also examine the impact of PPARδ on the expression level of Bcl-2 and caspase-3, since both Bcl-2 and caspase-3 are crucial factors in apoptotic signaling pathway and abnormal expression of Bcl-2 and caspase-3 is previously reported during ER stress [25, 26]. As a result, activation of PPARδ by GW501516 markedly induced expression of Bcl-2, an anti-apoptotic protein, in response to palmitate.
On the other hand, GSK0660-induced PPARδ inhibition slightly reduced the expression of Bcl-2 (Figure 3(b)). Correlative, inhibition of fatty acid oxidation by etomoxir blocked substantially GW501516-mediated activation of Bcl-2 (Figure 3(b)). These observations were further supported by caspase-3 activity assay (Figure 3(c)). Palmitate-induced activation of caspase-3 was inhibited by GW501516, but it increased by treating with GSK0660 along or in combination with GW501516 and etomoxir (Figure 3(c)).

Further, the role of PPARδ in regulation of insulin secretion was examined by measuring BIS and GSIS. As shown in Figure 3(d), INS-1E cells treated with palmitate
Figure 3: PPARδ protects pancreatic β cells from palmitate-induced lipotoxicity. (a) INS-1E cells were treated with 0.5 mM palmitate alone, combination of 0.5 mM palmitate and 100 nM GW501516, combination of 0.5 mM palmitate and 1 μM GSK0660, or combination of 0.5 mM palmitate, 100 nM GW501516, and 50 μM Etomoxir 48 h. Apoptosis was measured by TUNEL assay. (b) Expression of Bcl-2 was examined by Western blot. Upper panel: representative image of immunoblot; bottom panel: statistical analyses of relative densitometric value. (c) INS-1E cells were treated with 0.5 mM palmitate alone, combination of 0.5 mM palmitate, 100 nM GW501516, and 1 μM GSK0660, or combination of 0.5 mM palmitate, 100 nM GW501516, and 50 μM Etomoxir for 48 h. Activity of caspase-3 was examined. (d) INS-1E cells were treated with 0.5 mM palmitate alone, combination of 0.5 mM palmitate, and 100 nM GW501516, or combination of 0.5 mM palmitate and 1 μM GSK0660 for 48 h. BIS and GSIS from INS-1E cells were examined. All the data were from three independent experiments (n = 3). *P < 0.05: versus untreated group; #P < 0.05: versus palmitate-treated group; **P < 0.05: versus the group treated with a combination of palmitate and GW501516.
exhibited increased BIS but decreased GSIS compared to untreated control. These phenomena were even aggravated in the presence of GSK0660. Notably, activation of PPARδ by GW501516 substantially reversed palmitate-induced BIS elevation and GSIS decrease. These data suggest a protective role of PPARδ against lipotoxicity in pancreatic β cells.

4. Discussion

PPARδ is one of the members of the nuclear receptor superfamily. Given its effective transcription ability, it could be used in the synthesis of novel drug for treating obesity, hyperlipidemia, and type 2 diabetes [15]. Although the functional role of PPARα and PPARγ in regulating gene transcription and lipid metabolism was extensively documented, the functional role of another family member, namely, PPARδ, was relatively limited. In this study, we illustrated that PPARδ protected pancreatic β cells from palmitate-induced ER stress. We showed that exposure to palmitate triggered ER stress in INS-1E cells. Notably, the activation of PPARδ by GW501516 significantly reduces the palmitate-induced extension and swelling of ER. Although, no difference was found in palmitate-induced expression of Bip in presence or absence of PPARδ. PPARδ could abolish the overexpression or phosphorylation of server key signaling transducer downstream ER stress, including ATF4, XBP1s, Chop, and JNK. This is the first paper elucidating the inhibitory effect of PPARδ on palmitate-induced ER stress. Considering the usage of RPMI 1640 medium (11 mM glucose) in our in vitro experimental model that palmitate-induced lipotoxicity might be subsequently aggravated due to the high glucose concentrations, further work is still needed to characterize the palmitate-induced ER stress with or without glucotoxicity.

Previous studies focusing on the role of fatty acid oxidation in palmitate-induced lipotoxicity come to labyrinthic observations. It has long been proposed that enhanced fatty acid oxidation attenuated β-cell lipotoxicity by reducing the intracellular level of fatty acids; however, the hydrogen peroxide generated from fatty acids peroxisomal metabolism was also considered as important mediator of β-cell toxicity [9, 15]. Moreover, it is reported that stimulation of both lipogenesis and oxidation of fatty acid oxidation protected against palmitate-induced INS-1 cell death, suggesting the protective role of fatty acid metabolism in β-cell lipotoxicity [27]. In our data, activation of PPARδ resulted in a similar protective effect of INS-1E cells under ER stress. PPARδ activation elevated fatty acid oxidation in pancreatic β cells. These were treated with palmitate that PPARδ agonist significantly upregulated several enzymes of fatty acid oxidation, including CPT-1 and ACO. More importantly, we also proved that increased fatty acid oxidation is indispensable for PPARδ-mediated ER stress inhibition. PPARδ-mediated inhibition of Chop could be restored by inhibitor of fatty acid oxidation with etomoxir, though no significant differences were found in the level of Bip. Our results were consistent with previous works showing that activation of LXR by chemical agents rescued INS-1 cells that underwent palmitate-induced apoptosis [27]. This may be due to the predominant role of either PPARδ or LXR as important activator of fatty acid metabolism.

Prolonged ER stress results in cell death [28]. Having establishing the inhibitory role of PPARδ in palmitate-induced ER stress, in this study, we also estimated the protective role of PPARδ against palmitate-induced lipotoxicity. We showed that palmitate-induced apoptotic cell was markedly reduced in the presence of GW501516 whilst inhibition of PPARδ by GSK0660 enhanced palmitate-induced apoptosis. Moreover, PPARδ could modulate the expression level or/and activity of Bcl-2 and caspase-3, both of which were key factors involved in the apoptotic pathway. Interestingly, we also found that activation of PPARδ restored the insulin secretion by reversing palmitate-induced BIS elevation and GSIS decrease. Our results were in line with previous reports that activation of PPARδ improved both vitality and function of pancreatic β cells in either in vitro model or diabetic db/db mice model [1, 29].

In this study, we have investigated the intrinsic link between PPARδ and palmitate-induced lipotoxicity. We have also proved that PPARδ has a protective role in saving pancreatic β cells under palmitate treatment as they inhibit the ER stress. Our work might lead to a better understanding of the role of PPARδ as a potential therapeutic target in treating hyperlipidemia and type 2 diabetes.

Authors’ Contribution

Mingming Cao and Yuzhen Tong contributed equally to this study.

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References

[1] J. Wan, L. Jiang, Q. Lü, L. Ke, X. Li, and N. Tong, “Activation of PPARδ up-regulates fatty acid oxidation and energy uncoupling genes of mitochondria and reduces palmitate-induced apoptosis in pancreatic β-cells,” Biochemical and Biophysical Research Communications, vol. 391, no. 3, pp. 1567–1572, 2010.
[2] M. Nakata, N. Shintani, H. Hashimoto, A. Baba, and T. Yada, “Intra-islet PACAP protects pancreatic β-cells against glucotoxicity and lipotoxicity,” Journal of Molecular Neuroscience, vol. 42, no. 3, pp. 404–410, 2010.
[3] N. Omae, M. Ito, S. Hase et al., “Suppression of FoxO1/cell death-inducing DNA fragmentation factor α-like effector A, (Cidea) axis protects mouse β-cells against palmitic acid-induced apoptosis,” Molecular and Cellular Endocrinology, vol. 348, no. 1, pp. 297–304, 2011.
[4] K. Tanabe, Y. Liu, S. D. Hasan et al., “Glucose and fatty acids synergize to promote B-cell apoptosis through activation of glycogen synthase kinase 3β independent of JNK activation,” PLoS ONE, vol. 6, no. 4, Article ID e18146, 2011.
[5] C. Wang, Y. Guan, and J. Yang, “Cytokines in the progression of pancreatic β-cell dysfunction,” International Journal of Endocrinology, vol. 2010, Article ID 515136, 10 pages, 2010.

[6] E. Bachar, Y. Ariav, E. Cerasi, N. Kaiser, and G. Leibowitz, “Neuronal nitric oxide synthase protects the pancreatic beta cell from glucolipotoxicity-induced endoplasmic reticulum stress and apoptosis,” Diabetologia, vol. 53, no. 10, pp. 2177–2187, 2010.

[7] G. Patané, M. Anello, S. Piro, R. Vigneri, F. Purrello, and A. M. Rabuazzo, “Role of ATP production and uncoupling protein-2 in the insulin secretory defect induced by chronic exposure to high glucose or free fatty acids and effects of peroxisome proliferator-activated receptor-γ inhibition,” Diabetes, vol. 51, no. 9, pp. 2749–2756, 2002.

[8] K. S. Gwiazda, T. L. B. Yang, Y. Lin, and J. D. Johnson, “Ectopic-generated reactive oxygen species for lipotoxicity in pancreatic β-cells,” American Journal of Physiology, vol. 296, no. 4, pp. E690–E701, 2009.

[9] W. Gehrmann, M. Elsner, and S. Lenzen, “Role of metabolically generated reactive oxygen species for lipotoxicity in pancreatic β-cells,” Diabetes, Obesity and Metabolism, vol. 12, no. 2, pp. 149–158, 2010.

[10] K. Maedler, J. Oberholzer, P. Righini, G. A. Spinas, and A. M. Rabuazzo, “Role of ATP production and uncoupling protein-2 in the insulin secretory defect induced by chronic exposure to high glucose or free fatty acids and effects of peroxisome proliferator-activated receptor-γ inhibition,” Diabetologia, vol. 51, no. 9, pp. 2749–2756, 2002.

[11] F. Chai, R. Luo, Y. Li et al., “Down-regulation of GRP78 in human glaucomatous trabecular meshwork cells,” Molecular Vision, vol. 16, pp. 1122–1131, 2010.

[12] M. Wang, S. Wey, Y. Zhang, R. Ye, and A. S. Lee, “Role of the unfolded protein response regulator GRP78/Bip in development, cancer, and neurological disorders,” Antioxidants and Redox Signaling, vol. 11, no. 9, pp. 2307–2316, 2009.

[13] D. T. Rutkowski and R. I. Kaufman, “A trip to the ER: coping with stress,” Trends in Cell Biology, vol. 14, no. 1, pp. 20–28, 2004.

[14] M. J. Watt, R. J. Southgate, A. G. Holmes, and M. A. Febbraio, “Suppression of plasma free fatty acids upregulates peroxisome proliferator-activated receptor (PPAR) α and δ and PPAR coactivator 1α in human skeletal muscle, but not lipid regulatory genes,” Journal of Molecular Endocrinology, vol. 33, no. 2, pp. 533–544, 2004.

[15] P. Arck, B. Toth, A. Pestka, and U. Jeschke, “Nuclear receptors of the peroxisome proliferator-activated receptor (PPAR) family in gestational diabetes: from animal models to clinical trials,” Biology of Reproduction, vol. 83, no. 2, pp. 168–176, 2010.

[16] S. C. Martinez, K. Tanabe, C. Cras-Meneur, N. A. Abumrad, E. Bernal-Mizrachi, and M. A. Permutt, “Inhibition of foxo1 protects pancreatic islet β-cells against fatty acid and endoplasmic reticulum stress-induced apoptosis,” Diabetes, vol. 57, no. 4, pp. 846–859, 2008.

[17] A. Y. Jeong, M. Y. Lee, S. H. Lee, J. H. Park, and H. J. Han, “PPARδ agonist-mediated ROS stimulates mouse embryonic stem cell proliferation through cooperation of p38 MAPK and Wnt/βcatenin,” Cell Cycle, vol. 8, no. 4, pp. 611–619, 2009.

[18] K. Ravnkjaer, F. Frigerio, M. Boergesen, T. Nielsen, P. Maechler, and S. Mandrup, “PPARδ is a fatty acid sensor that enhances mitochondrial oxidation in insulin-secreting cells and protects against fatty acid-induced dysfunction,” Journal of Lipid Research, vol. 51, no. 6, pp. 1370–1379, 2010.