Original Article

Chronic Activation of Liver X Receptor Induces β-Cell Apoptosis Through Hyperactivation of Lipogenesis

Liver X Receptor–Mediated Lipotoxicity in Pancreatic β-Cells

Sung Sik Choe,[1] A Hyun Choi,[1] Joo-Won Lee,[1] Kang Ho Kim,[1] Jun-Jae Chung,[1] Jiyoung Park,[1] Kyeong-Min Lee,[2] Keun-Gyu Park,[3] In-Kyu Lee,[2] and Jae Bum Kim[1]

Liver X receptor (LXR)α and LXRβ play important roles in fatty acid metabolism and cholesterol homeostasis. Although the functional roles of LXR in the liver, intestine, fat, and macrophages are well established, its role in pancreatic β-cells has not been clearly defined. In this study, we revealed that chronic activation of LXR contributes to lipotoxicity-induced β-cell dysfunction. We observed significantly elevated expression of LXR in the islets of diabetic rodent models, including *fa/fa* ZDF rats, OLETF rats, and *db/db* mice. In primary pancreatic islets and INS-1 insulinoma cells, activation of LXR with a synthetic ligand, T0901317, stimulated expression of the lipogenic genes ADD1/SREBP1c, FAS, and ACC and resulted in increased intracellular lipid accumulation. Moreover, chronic LXR activation induced apoptosis in pancreatic islets and INS-1 cells, which was synergistically promoted by high glucose conditions. Taken together, we suggest lipid accumulation caused by chronic activation of LXR in β-cells as a possible cause of β-cell lipotoxicity, a key step in the development of type 2 diabetes. *Diabetes* 56:1534–1543, 2007

Type 2 diabetes is associated with defective insulin secretion, insulin resistance, and elevated hepatic glucose production (1). The accumulation of excess lipid in the pancreatic β-cells of obese subjects (with type 2 diabetes) has been implicated as being one of the main causes of insulin secretory defects, which ultimately results in hyperglycemia—a major characteristic of diabetes (2). Furthermore, mass is reduced as the lipid-laden β-cells undergo apoptosis. As the result of such dysfunctions, β-cells gradually lose their ability to keep up with the prolonged high demand, and overt diabetes appears (3). An interesting observation made in several recent studies is that chronic hyperlipidemia contributes to β-cell dysfunction in an interdependent manner with hyperglycemia (4,5). However, despite considerable effort to decipher the detailed mechanisms behind this phenomenon, it is still unclear how hyperlipidemia induces β-cell failure under hyperglycemic conditions of type 2 diabetes.

The expression of lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) is tightly regulated in the pancreatic β-cells in accordance with nutritional status (6) in a fashion similar to that of fat and liver (7,8). Interestingly, the expression levels of these genes are elevated in the pancreatic β-cells of diabetic animals (9). Recently, it was reported that adipocyte determination and differentiation–dependent factor (ADD) 1/sterol regulatory element binding protein (SREBP) 1c, a key lipogenic transcription factor, is also highly expressed and implicated in stimulation of fatty acid synthesis in islets of diabetic animals (5,9). In β-cells, chronic high-glucose treatment increases the nuclear form of ADD1/SREBP1c, a process involving proteolytic cleavage and nuclear translocation (10,11). In addition, ectopic overexpression of mature ADD1/SREBP1c has been shown to increase intracellular lipid deposition and therefore to hinder glucose-stimulated insulin secretion (GSIS) and stimulate β-cell apoptosis (12,13).

A key transcription factor that regulates the expression of ADD1/SREBP1c is liver X receptor (LXR) (14). The two isoforms of this nuclear hormone receptor, LXRα and LXRβ, play crucial roles in cholesterol and fatty acid metabolism (15,16). As a key modulator of cholesterol homeostasis, LXR controls the expression of cholesterol

From the [1]Department of Biological Sciences, Research Center for Functional Cellulomics, Seoul National University, Seoul, South Korea; the [2]Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu, South Korea; and the [3]Department of Internal Medicine, Keimyung University School of Medicine, Daegu, Republic of Korea.

Address correspondence and reprint requests to Jae Bum Kim, PhD, Department of Biological Sciences, Seoul National University, San 56-1, Sillim-Dong, Kwanak-Gu, Seoul, Korea. E-mail: jaebkim@snu.ac.kr.

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ACC, acetyl-CoA carboxylase; ADD, adipocyte determination and differentiation–dependent factor; DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate; FAS, fatty acid synthase; FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; LXR, liver X receptor; NAC, *N*-acetyl-L-cysteine; ROS, reactive oxygen species; SREBP, sterol regulatory element binding protein; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.

7α-hydroxylase (CYP7A1), ATP-binding cassette (ABC) transporters, and apolipoprotein E, which are required for cholesterol efflux and transport in macrophages, liver, and intestine (17,18). In addition to its effects on cholesterol homeostasis, LXR also directly regulates the expression of lipogenic genes such as ADD1/SREBP1c and FAS in fat and liver (19,20). T0901317, a synthetic agonist for LXR, is well known to show similar efficacy to natural ligands such as oxy-cholesterols, but it is significantly more potent and selectively bound to LXR (21). Thus, activation of LXR with its synthetic ligand, T0901317, lowers plasma cholesterol via cholesterol efflux while simultaneously causing hepatic lipid accumulation and elevation of plasma triglycerides (21).

Regarding the role of LXR in pancreatic β-cells, it has been reported that islets from LXRβ knockout mice display lack of GSIS and increased lipid droplets (22). In addition, several studies demonstrated that T0901317 promotes GSIS in pancreatic islets and β-cells (23,24). These reports suggest that appropriate regulation of LXR activity is important for maintaining proper β-cell function. But, from a different point of view, because LXR activation stimulates lipogenic gene expression in β-cells (23,24), it is also possible that chronic LXR activation might lead to accumulation of lipids and eventually β-cell failure. However, the effects of chronic LXR activation in β-cell dysfunction, linked with type 2 diabetes, are largely unknown. In particular, no studies have fully addressed the functional role of LXR in β-cells under hyperglycemic conditions, which is the hallmark of type 2 diabetic subjects.

In this study, we demonstrate that chronic LXR activation leads to increased lipid accumulation and apoptosis in pancreatic β-cells, which is augmented under high-glucose conditions. The abnormal increase of LXR expression in the pancreatic islets of obese and diabetic animal models and the ability of LXR ligands to induce β-cell dysfunction suggest the involvement of chronic LXR dysregulation in β-cell failure during the progression of type 2 diabetes.

RESEARCH DESIGN AND METHODS

T0901317 was purchased from Calbiochem (San Diego, CA), and GW3965 was kindly provided by Dr. Peter Tontonoz (University of California, Los Angeles, CA). Cerulenin was purchased from Sigma Aldrich (Saint Louis, MO), and *N*-acetyl-L-cysteine (NAC) was purchased from Calbiochem.

Islet isolation and cell culture. Pancreatic islets were isolated from Otsuka Long-Evans Tokushima Fatty (OLETF) rats (male, 28 weeks old), *fa/fa* Zucker Diabetic Fatty (ZDF) rats (male, 16 weeks old), *db/db* mice (male, 12 weeks old), and Sprague-Dawley (SD) rats (male, 10 weeks old) by collagenase XI (Sigma Aldrich) digestion and were purified using Ficoll gradient solutions (29, 24, and 15% [wt/vol] in Hanks' balanced salt solution). After centrifugation for 30 min, the islets were removed from the layer between the 24 and 15% layers and washed twice with cold Hanks' balanced salt solution. The isolated islets were cultured in suspension in RPMI-1460 medium supplemented with 10% fetal bovine serum (Gibco). Rat insulinoma INS-1 cells were also maintained in RPMI-1460 medium supplemented with 10% fetal bovine serum.

Northern blot analysis and real-time quantitative RT-PCR. Total RNA was isolated with TRIzol reagent (Invitrogen Life Technologies) according to the manufacturer's protocol. After denaturing in formamide and formaldehyde, RNA was separated by electrophoresis on formaldehyde-containing agarose gels. After electrophoresis, RNA was transferred to nylon membranes (Schleicher and Schuell), cross-linked with UV, and hybridized with DNA probes. The DNA probes were labeled by the random priming method using the Klenow fragment of DNA polymerase I (Takara) and [α-^{32}P]dCTP (Amersham Pharmacia). cDNAs used as probes were LXRα, ABCA1, ADD1/SREBP1c, FAS, ACC, PEPCK, glucose-6-phosphatase, UCP2, pro-insulin, and 36B4. For real-time quantitative RT-PCR analysis, cDNAs were synthesized with RevertAid M-MuLV reverse transcriptase (MBI Fermentas) using oligo-dT and subjected to PCR amplification using gene-specific primers by a Cycler real-time PCR detection system (Bio-Rad) using SYBR Green I (BioWhittaker Molecular Applications). The relative abundance of mRNA was calculated after normalization to cyclophilin mRNA. The primer sequences used for real-time PCR analyses are available on request.

Western blot analysis. INS-1 cells were lysed with NETN buffer (100 mmol/l NaCl, 20 mmol/l Tris pH 8.0, 1 mmol/l EDTA, 0.5% NP40, 1 mmol/l phenylmethylsulfonyl fluoride, 100 mmol/l NaF, 1 mmol/l Na_3VO_4, 10 μg/ml aprotinin, 2 μg/ml pepstatin A, and 10 μg/ml leupeptin). The proteins were separated by electrophoresis on SDS-polyacrylamide gels and transferred to PVDF (polyvinylidene difluoride) membranes (Millipore). After transfer, the membranes were blocked with 5% nonfat milk and probed with primary antibodies. Antibodies against LXRα and ADD1/SREBP1c were generated with glutathione S-transferase–fused recombinant proteins (LabFrontier, Korea). GAPDH (glyceraldehyde-3-phosphate dehydrogenase) antibody was purchased from LabFrontier, ACC antibody from Upstate Biotechnology, and pro-insulin antibody from Santa Cruz Biotechnology. The results were visualized with horseradish peroxidase–conjugated secondary antibodies (Sigma Aldrich) and enhanced chemiluminescence.

Measurements of cellular triglycerides, free fatty acids, and cholesterol. The cellular contents of triglycerides and cholesterol in INS-1 cells were measured using triglycerides and cholesterol assay kits (Infinity). The amount of free fatty acids (FFAs) was determined using a nonesterified fatty acid assay kit (Roche). Each analysis was performed according to the manufacturers' protocol.

Measurements of cellular reactive oxygen species level. Cellular reactive oxygen species (ROS) was measured using 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA) and luminol (Molecular Probes). INS-1 cells were washed with PBS and then incubated in the dark for 30 min with DCF-DA (10 μmol/l). The fluorescence of DCF-DA was measured by a fluorescence microscope (Olympus) and an EnVision 2102 multilabel reader (PerkinElmer) at an excitation wavelength of 488 nm and emission at 515–540 nm. Luminol (5 μmol/l) was used for quantitative measurement of cellular ROS. Chemiluminescence of luminol was determined using a luminometer (LB9501; Berthold) for 3 min.

Apoptosis assay. Apoptosis was measured using an in situ cell death detection kit (Roche). Each analysis was performed according to the manufacturer's protocol. Fluorescein labeling images were taken by a fluorescence microscope (Olympus) using an excitation wavelength in the range of ~450–500 nm and a detection wavelength in the range of ~515–565 nm. Peroxidase labeling images were viewed under a microscope, and statistical analyses of the obtained images were performed using LSM510 software (Carl Zeiss).

RESULTS

Expression of LXRα and LXRβ is aberrantly increased in the pancreatic islets of diabetic animal models. Animal models of type 2 diabetes such as *fa/fa* ZDF rats, OLETF rats, and *ob/ob* mice exhibit increased lipogenic gene expression and lipid accumulation in their pancreatic islets as well as in the fat and liver, accompanied by insulin resistance, hyperglycemia, and hyperlipidemia (9). Interestingly, the mRNA levels of both LXRα and LXRβ were remarkably elevated in the pancreatic islets of these diabetic rodents compared with their nondiabetic counterparts (Fig. 1). The mRNA of ADD1/SREBP1c, a well-established target gene of LXR, was also increased in all three animal models, consistent with previous reports (9). On the contrary, the mRNA levels of GLUT2 and pro-insulin were either decreased or remained unaltered, respectively (Fig. 1*B* and *C*). These results suggest the possibility that the elevation of LXR might be involved in the dysfunction of pancreatic β-cells observed in type 2 diabetic subjects.

LXR activation induces lipogenic gene expression in pancreatic β-cells. Because LXR stimulates lipogenesis in hepatocytes, adipocytes, and myotubes (15,20,21,25), we sought to determine whether the same applies to β-cells. As the first step, we examined the mRNA levels of both LXRα and LXRβ in pancreatic islets and rat insulinoma INS-1 cells (supplemental Fig. 1, which can be found in an online appendix at http://dx.doi.org/10.2337/db06-1059). As previously reported (24), we observed that LXRβ is more abundantly expressed than LXRα in pancreatic

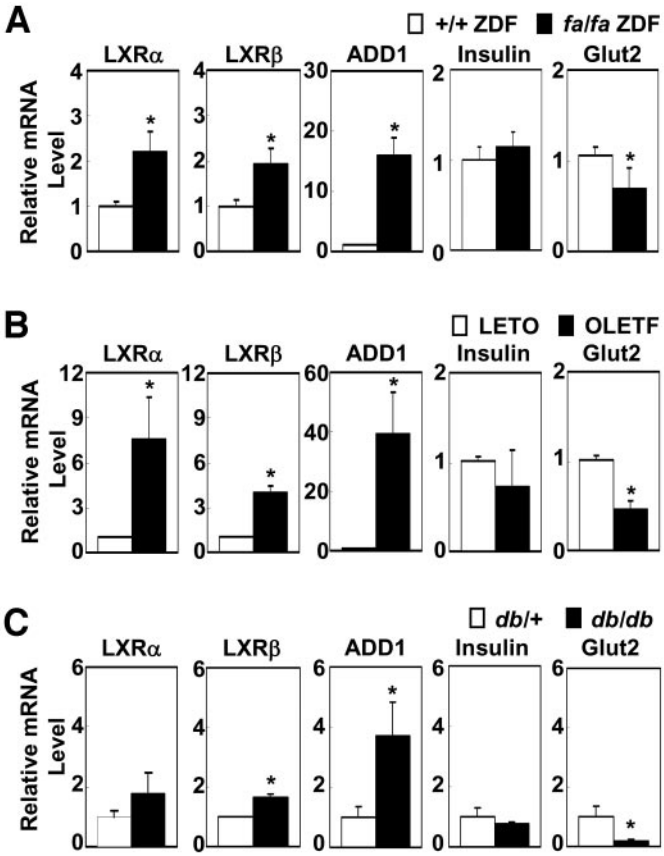

FIG. 1. Gene expression profiles of pancreatic islets from diabetic animals. *A*: Pancreatic islets were isolated from 16-week-old +/+ and *fa/fa* ZDF rats ($n = 3$). The mRNA levels of the indicated genes were analyzed by real-time quantitative RT-PCR and normalized to cyclophilin levels. *$P < 0.05$ versus wild-type control by Student's *t* test. *B* and *C*: mRNA levels of the indicated genes were measured using pancreatic islets isolated from 28-week-old LETO (□) and OLETF (■) rats ($n = 4$) and from 12-week-old *db/+* (□) and *db/db* (■) mice ($n = 4$). *$P < 0.05$ vs. counterpart control, LETO, by Student's *t* test.

islets and INS-1 cells (supplemental Fig. 1*B*). When INS-1 cells were treated with the LXR ligand T0901317, the expression of several LXR target genes linked with lipogenesis, such as ADD1/SREBP1c, ACC, and FAS, was significantly enhanced, whereas the insulin level was not affected (Fig. 2*A*). On the other hand, mRNA levels of the gluconeogenic enzymes PEPCK and glucose-6-phosphatase were downregulated by T0901317 (Fig. 2*A*), which is consistent with a previous report (26). A similar stimulation of lipogenic gene expression by LXR activation was also observed in isolated primary pancreatic islets (Fig. 2*B*). GW3965, a milder agonist of LXR, also promoted the expression of lipogenic genes in INS-1 cells (supplemental Fig. 2). In addition, the LXR ligands T0901317 and GW3965 dose-dependently increased lipogenic gene expression (supplemental Fig. 2). On the contrary, LXR activation did not alter the expression of CPT1 (carnitine palmitoyl transferase 1), ACO (acyl-CoA oxidase), and peroxisome proliferator–activated receptor-α (PPARα), which are involved in mitochondrial and peroxisomal lipid oxidation (Fig. 2*C*). Together, these results explicitly reveal that LXR activation in pancreatic β-cells stimulates lipogenic gene expression as observed in other lipogenic tissues, such as liver and adipose tissue.

LXR activation stimulates lipid accumulation in pancreatic β-cells. To directly assess the effect of LXR activation on lipid metabolism in β-cells, we measured the levels of intracellular triglycerides, FFAs, and cholesterol in INS-1 cells exposed to T0901317. In accordance with the gene expression profiles, cellular triglycerides and FFA levels, but not cholesterol, were significantly elevated by LXR activation (Fig. 3). Oil Red O staining also confirmed that activation of LXR in pancreatic islets increased intracellular lipid accumulation (Fig. 3*D*). Therefore, it is likely that abnormal activation of LXR would provoke lipid dysregulation in β-cells, as frequently found in type 2 diabetic subjects.

Chronic LXR activation induces apoptosis in β-cells. Accumulation of large amounts of lipid metabolites in β-cells has been demonstrated to induce apoptosis (3). Because LXR activation led to increased lipid levels in β-cells (Fig. 3), we next examined whether LXR activation could influence β-cell death. Using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assays, we observed that ~10% of INS-1 cells treated with T0901317 underwent apoptosis, whereas only ~2–3% of cells showed spontaneous cell death (Fig. 4*A* and *B*). Furthermore, the expression of the proapoptotic genes Bax and Caspase-3 was increased in INS-1 cells by LXR stimulation (Fig. 4*C*). Also, apoptosis of primary pancreatic islets was significantly elevated by LXR activa-

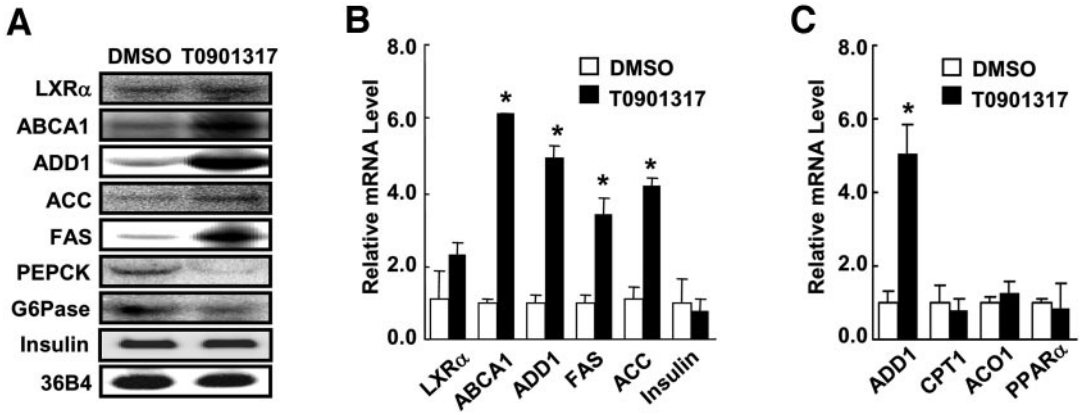

FIG. 2. LXR activation in pancreatic β-cells stimulates the expression of lipogenic genes. *A*: INS-1 cells were treated with the LXR agonist T0901317 (10 μmol/l) for 36 h. Total RNA was isolated and analyzed by Northern blot analysis. 36B4 was used as a loading control. *B* and *C*: cDNAs prepared from primary pancreatic islets (*B*) or INS-1 cells (*C*) treated with T0901317 (10 μmol/l) for 36 h were subjected to real-time quantitative RT-PCR analysis. *$P < 0.01$ versus DMSO control by *t* test. The relative amount of each mRNA was normalized to cyclophilin levels. G6Pase, glucose-6-phosphatase.

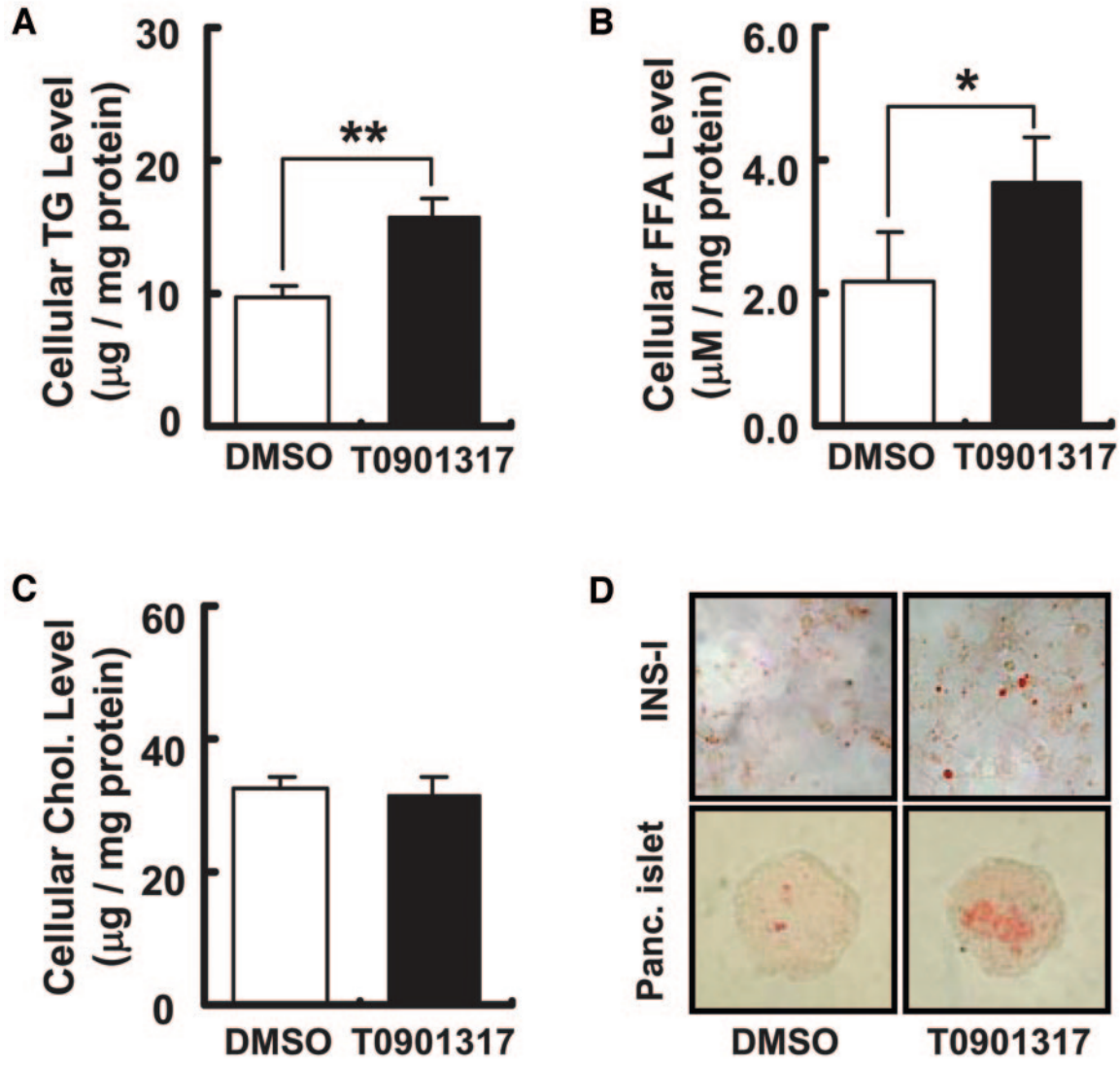

FIG. 3. LXR activation in pancreatic β-cells promotes lipid accumulation. *A–C*: Total cell lysates were collected from INS-1 cells incubated in the absence (□) or presence (■) of T0901317 (10 μmol/l) for 3 days, and cellular triglycerides, FFAs, and cholesterol were measured with them. The data were normalized with protein concentrations. *$P < 0.05$; **$P < 0.01$. *D*: INS-1 cells (*top*) and primary pancreatic islets (*bottom*) incubated with T0901317 for 3 days were stained with Oil Red O. TG, triglyceride.

tion with T0901317 (Fig. 4*D*), even though it took longer for the primary pancreatic islets to undergo apoptosis compared with INS-1 cells. These results indicate that LXR activation leads to increased apoptosis in β-cells, possibly because of increased intracellular lipid accumulation.

LXR activation–induced lipid accumulation in β-cells is synergistically augmented by high glucose levels. That hyperglycemia induces lipogenic gene expression and promotes de novo synthesis of FFAs and triglycerides in β-cells (27,28) prompted us to test whether the effects of LXR activation on β-cells could be differentially modulated by glucose levels. Long-term exposure of INS-1 cells to high glucose conditions increased the expression of ADD1/SREBP1c, ACC, and FAS (Fig. 5*A* and *B*), in accordance with previous reports (27,28). In addition, we observed that the posttranslational processing of ADD1/SREBP1c was increased in response to LXR ligands, hence the increase of the nuclear form of ADD1/SREBP1c (nADD1) (Fig. 5*B*). Notably, such stimulatory effects on lipogenic gene expression and ADD1/SREBP1c protein processing were exacerbated when β-cells were cultured in high-glucose medium along with LXR ligands (Fig. 5*A* and *B*). Indeed, lipid accumulation detected by Oil Red O staining in INS-1 cells was greatly increased by LXR activation and high glucose conditions (Fig. 5*C*). Direct measurement of cellular triglycerides and FFAs also indicated that high glucose conditions greatly enhanced LXR activation–mediated lipid accumulation. (Fig. 5*D* and *E*). On the other hand, neither LXR activation nor high glucose affected the levels of intracellular cholesterol in β-cells (Fig. 5*F*), implying that cholesterol metabolism in β-cells plays a minimal role in LXR-activated β-cell dysfunction.

LXR activation increases ROS accumulation in conjunction with high glucose levels. Elevated FFA levels contribute to the pathophysiology of type 2 diabetes via the generation of ROS in metabolic organs, including pancreatic β-cells (29). Thus, we investigated whether increased lipid accumulation by LXR activation would lead to an increase of ROS levels in β-cells. As shown in Fig. 6*A*, ROS levels were elevated in INS-1 cells treated with T0901317, and longer LXR activation resulted in more ROS generation. This effect on cellular ROS production by LXR

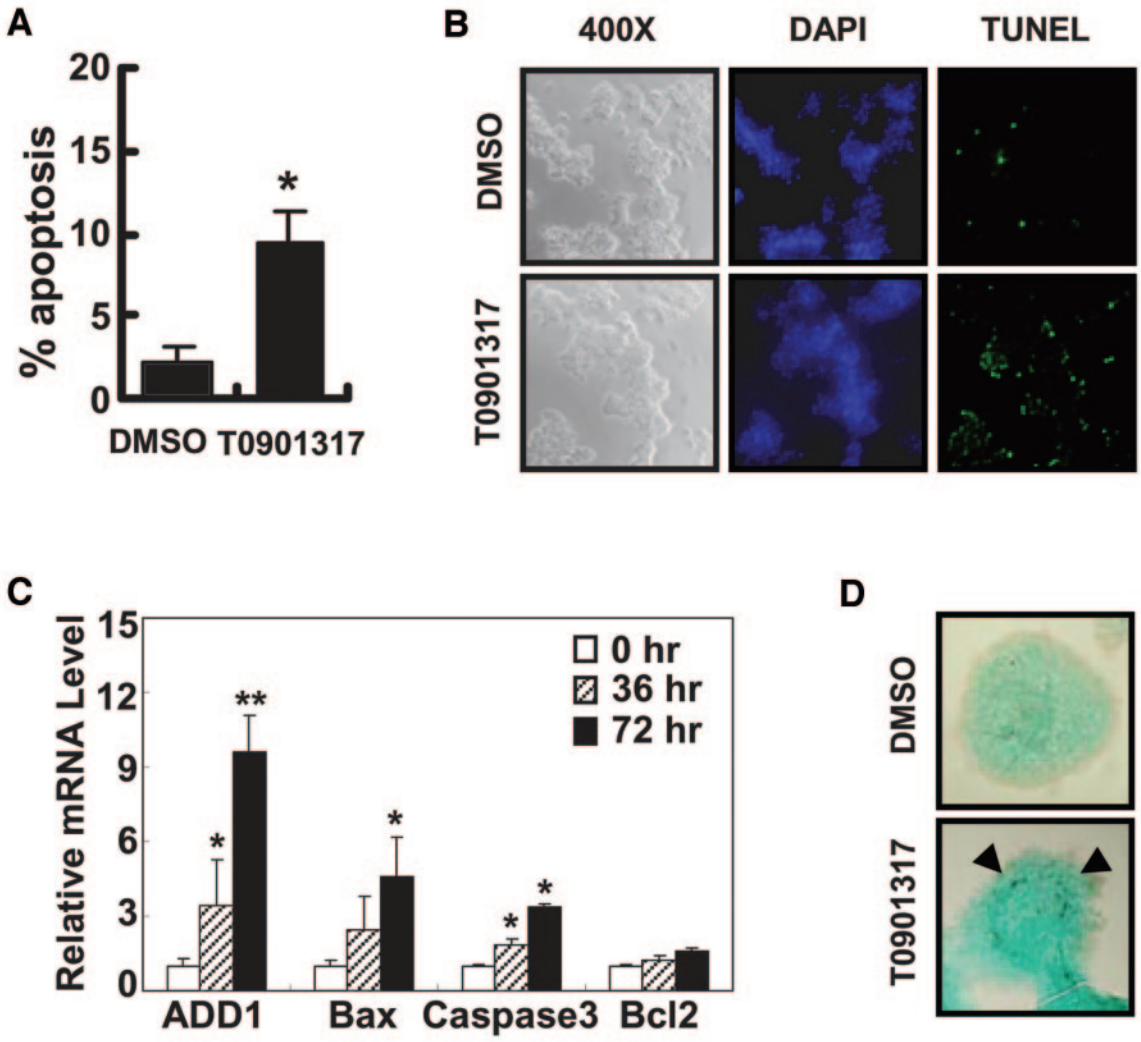

FIG. 4. LXR activation induces β-cell apoptosis. *A* and *B*: TUNEL assays were conducted with INS-1 cells treated with or without T0901317 (10 μmol/l) for 3 days to measure the degree of apoptosis and were viewed under a fluorescent microscope. *$P < 0.01$ versus DMSO control by *t* test. *C*: cDNAs isolated from INS-1 cells treated with or without T0901317 for the indicated time points were subjected to real-time quantitative RT-PCR analysis. The relative amount of each mRNA was normalized to cyclophilin levels. *$P < 0.05$, **$P < 0.01$ versus DMSO control. *D*: TUNEL assays were performed with primary pancreatic islets treated with or without T0901317 for 6 days. The apoptosis-progressing cells (arrowhead) were determined from microscopic images. DAPI, 4′6-diamidino-2-phenylindole.

activation in β-cells was further increased when the cells were incubated in high glucose medium (Fig. 6*B*). Interestingly, an inhibitor of FAS, cerulenin, dramatically abolished the ability of T0901317 to induce lipid accumulation and ROS generation in INS-1 cells (Fig. 6*C* and *D*). Moreover, the inhibitory effects of cerulenin on lipogenesis and ROS generation in β-cells were clearly observed even under high glucose conditions (Fig. 6*C* and *D*). These results strongly suggest that ROS production could be stimulated in the β-cells of diabetic subjects by the anomalous activation of lipogenic LXR target gene expression and the resulting buildup of lipid metabolites.

High glucose aggravates the detrimental effects of chronic LXR activation in β-cells. The synergism between LXR activation and high glucose was also observed in β-cell apoptosis. When INS-1 cells were subjected to T0901317 or high glucose separately, only a moderate portion of the cells were TUNEL positive (~10 and ~ 8%, respectively). However, the fraction of cells undergoing apoptosis (~43%) was greatly increased when β-cells were subjected to both chronic LXR activation and high glucose conditions (Fig. 7*A* and *B*). Similar results were also obtained using primary pancreatic islets (Fig. 7*C* and supplemental Fig. 3). Importantly, β-cell apoptosis induced by LXR activation and high glucose was prevented to a significant extent by cerulenin and NAC, a well-known antioxidant (Fig. 7*D*). This clearly indicates that β-cell apoptosis induced by LXR activation occurs through the stimulation of lipogenic activity and ROS generation. Taken together, these data demonstrate that β-cells exposed to high glucose levels would be more sensitive to the lipotoxic effects of LXR activation and suggest that the hyperglycemic conditions found in type 2 diabetic patients would aggravate β-cell dysfunction and apoptosis induced by the lipogenic activity of LXR.

DISCUSSION

Lipid overloading in pancreatic β-cells causes serious β-cell dysfunction and apoptosis, a significant process in the development of type 2 diabetes (3–5). ADD1/SREBP1c is a key factor involved in the dysfunction of β-cells in

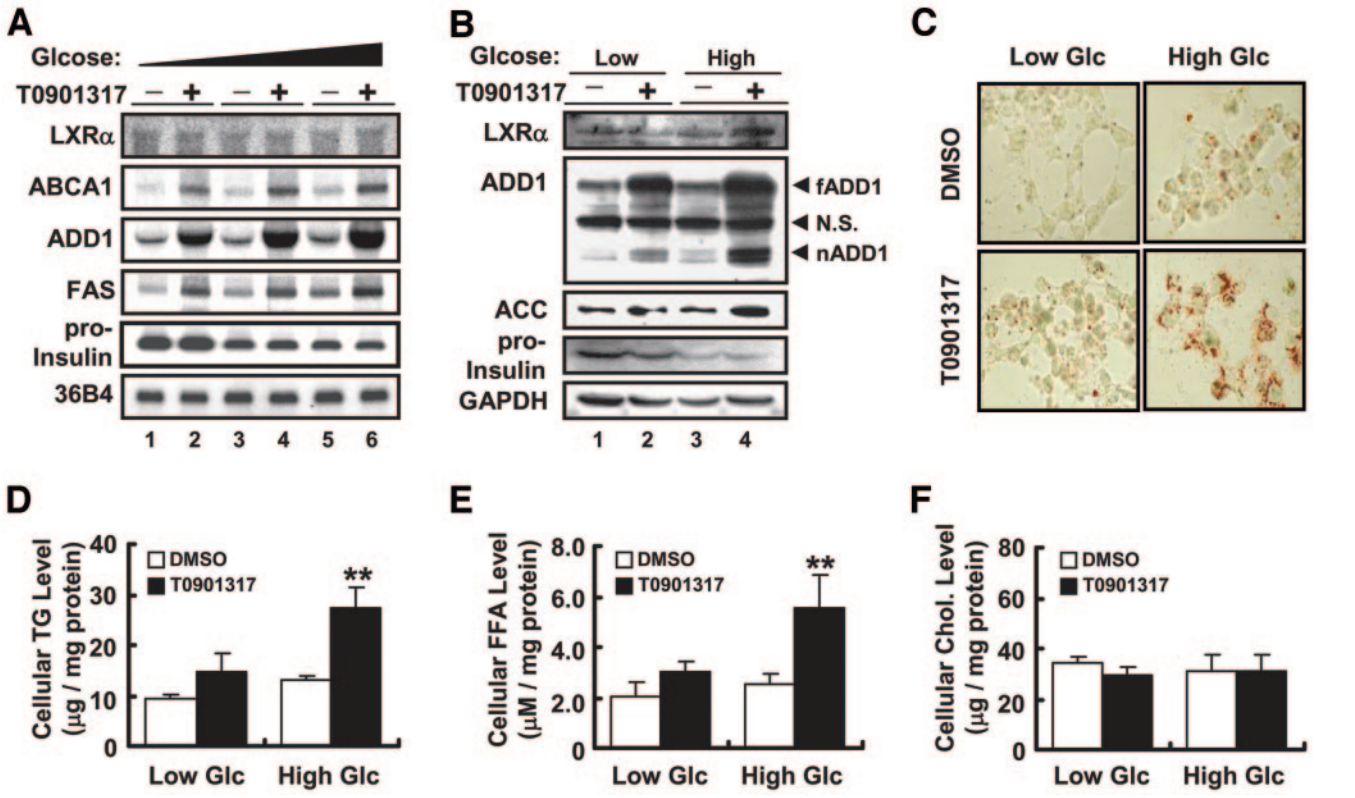

FIG. 5. High glucose conditions accelerate LXR-induced lipogenesis in pancreatic β-cells. *A* and *B*: INS-1 cells were incubated in the absence or presence of T0901317 (10 μmol/l) for 36 h in a medium containing different levels of glucose. Total RNA (*A*) or protein (*B*) was prepared and analyzed by Northern blot or Western blot analyses, respectively. *A*: 11.2 mmol/l (*lanes 1* and *2*), 21.2 mmol/l (*lanes 3* and *4*), and 31.2 mmol/l (*lanes 5* and *6*) glucose. *B*: 11.2 mmol/l (*lanes 1* and *2*) and 31.2 mmol/l (*lanes 3* and *4*) glucose. *C–F*: INS-1 cells were incubated for 3 days in the presence or absence of T0901317 in a low-glucose (11.2 mmol/l) or high-glucose medium (31.2 mmol/l) and subjected to Oil Red O staining (*C*). In INS-1 cells, total cellular triglycerides (*D*), FFAs (*E*), or cholesterol (*F*) were determined. **$P < 0.01$ compared with INS-1 cells treated with T0901317 under low-glucose conditions. fADD1, full-length form of ADD1/SREBP1c; Glc, glucose; nADD1, nuclear form of ADD1/SREBP1c; N.S, nonspecific band.

obese and/or diabetic subjects owing to its ability to activate the expression of lipogenic genes (12,13). That LXR controls the expression of ADD1/SREBP1c (14) led us to investigate whether aberrant regulation of LXR could be associated with β-cell dysfunction. Our data indicate that chronic LXR activation would stimulate β-cell dysfunction, primarily via an increase in lipogenic activity and ROS production.

Previous studies have demonstrated that lipid overloading in pancreatic β-cells stimulates several deleterious pathways, such as ceramide production, nitric oxide formation, protein kinase C activation, endoplasmic reticulum stress activation, and ROS generation, to mediate lipoapoptosis (3,30–32). It is not yet completely clear which pathways are directly involved in the induction of β-cell apoptosis caused by LXR activation. However, the near-complete inhibition of apoptosis by pretreatment of the antioxidant NAC (Fig. 7*D*) suggests that ROS generation is probably one of the key factors mediating this effect.

The stimulation of lipid accumulation by LXR activation that we observed seems contradictory to the report by Gerin et al. (22), in which they demonstrated enhanced lipid accumulation in pancreatic islets of β-cell–specific LXRβ knockout mice. However, this discrepancy would be explained by the difference in the composition of the lipids accumulated in the β-cells. In the report by Gerin et al. (22), they suggest that the increased lipid droplets are composed mainly of cholesterol esters because of the reduced expression of cholesterol transporters. In contrast, we observed that although triglycerides and FFA levels were increased in LXR-activated β-cells, there was little change in cholesterol levels (Figs. 3 and 5). These two different observations imply that whereas endogenous LXR is important for maintaining normal β-cell function, abnormal activation of LXR could induce lipotoxicity.

One of the more interesting observations was that the stimulation of lipogenic activity by LXR activation was greatly augmented when β-cells were exposed to high glucose conditions. Notably, we observed that the expression levels of LXR target genes, such as ADD1/SREBP1c, FAS, ACC, and ABCA1, were increased in β-cells even when the cells were subjected only to high glucose conditions, implying that the transcriptional activity of LXR in β-cells could be regulated by glucose levels independent of LXR ligands. Indeed, the transcriptional activity of LXRα was increased by higher glucose concentrations in INS-1 cells (supplemental Fig. 4). Two recent reports suggest that glucose modulates LXR activity by regulating its subcellular localization (33) and by acting as a direct agonist of LXR (34). Meanwhile, we revealed that high glucose levels stimulated the maturation of ADD1/SREBP1c in β-cells. Such actions of glucose on LXR and ADD1/SREBP1c could work in concert with LXR ligands to synergistically increase the accumulation of intracellular lipids (10,12). Most likely because of the dramatic increase in intracellular lipids, ROS generation and β-cell apoptosis were also greatly stimulated when the cells were exposed to high glucose and chronic LXR

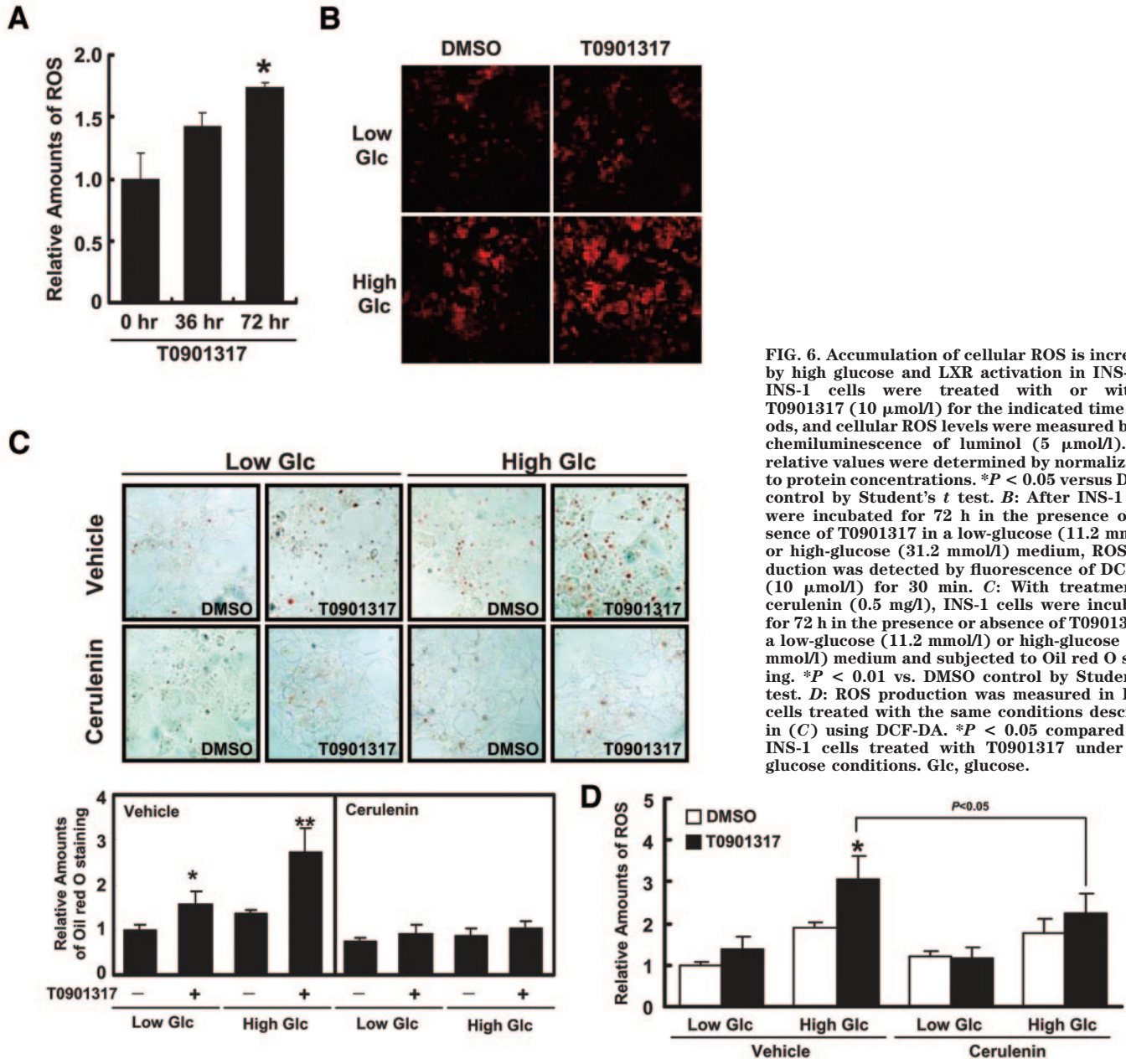

FIG. 6. Accumulation of cellular ROS is increased by high glucose and LXR activation in INS-1. *A*: INS-1 cells were treated with or without T0901317 (10 μmol/l) for the indicated time periods, and cellular ROS levels were measured by the chemiluminescence of luminol (5 μmol/l). The relative values were determined by normalization to protein concentrations. *$P < 0.05$ versus DMSO control by Student's *t* test. *B*: After INS-1 cells were incubated for 72 h in the presence or absence of T0901317 in a low-glucose (11.2 mmol/l) or high-glucose (31.2 mmol/l) medium, ROS production was detected by fluorescence of DCF-DA (10 μmol/l) for 30 min. *C*: With treatment of cerulenin (0.5 mg/l), INS-1 cells were incubated for 72 h in the presence or absence of T0901317 in a low-glucose (11.2 mmol/l) or high-glucose (31.2 mmol/l) medium and subjected to Oil red O staining. *$P < 0.01$ vs. DMSO control by Student's *t* test. *D*: ROS production was measured in INS-1 cells treated with the same conditions described in (*C*) using DCF-DA. *$P < 0.05$ compared with INS-1 cells treated with T0901317 under low-glucose conditions. Glc, glucose.

activation. Because LXR levels are increased in the pancreatic islets of diabetic rodent models (Fig. 1), it is possible to speculate that hyperglycemia and abnormally high LXR activity in diabetic subjects would act in concert to cause lipotoxicity and β-cell dysfunction.

Recently, many studies have reported that LXR plays a role in glucose metabolism as well as lipid metabolism. For example, treatment of LXR ligands in diabetic rodent models decreases blood glucose levels and hepatic glucose production while increasing blood triglyceride levels and hepatic lipid accumulation (15,26). LXR also appears to mediate insulin-dependent gene expression to coordinate both lipid and glucose metabolism in liver (26,35). Moreover, it has been demonstrated that LXR activation acutely increases GSIS in β-cells (23,24). When we recapitulated similar experiments using INS-1 cells to examine the effect of LXR activation on GSIS, we consistently observed that acute activation of LXR enhanced GSIS, whereas long-term LXR stimulation did not have any evident effects on GSIS (data not shown). On the contrary, it appears that chronic LXR activation would induce β-cell dysfunction by inducing intracellular lipid and ROS accumulation. While we were preparing this manuscript, Wente et al. (36) reported that activation of both LXR and RXR (retinoid X receptor) elevates β-cell apoptosis. From these results, it is possible to suggest that LXR can exert two different effects in β-cells depending on the duration of activation and/or other environmental conditions. Acute activation of LXR seems to have a positive effect on insulin secretion, whereas chronic activation of LXR in β-cells provokes lipid dysregulation and concomitant apoptosis. More importantly, the high blood glucose levels of diabetic subjects would aggravate the lipotoxic effects of chronic LXR activation.

The functional role of LXR activation in β-cells suggested by previous studies and ours seems to be somewhat contradictory. However, the results from previous reports and ours may represent processes occurring at

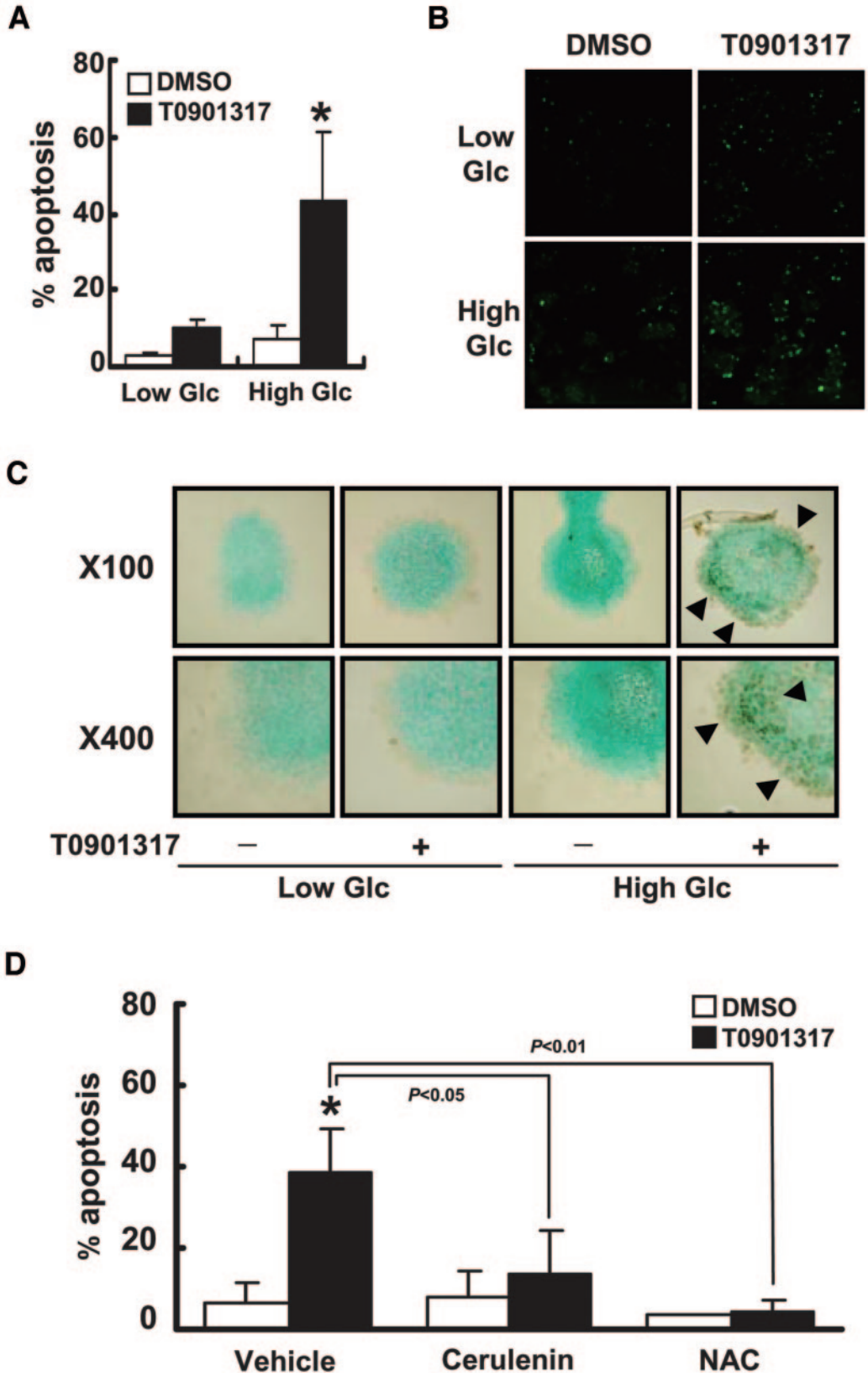

FIG. 7. High-glucose conditions accelerate LXR-induced β-cell apoptosis. *A* and *B*: After INS-1 cells were incubated for 3 days in the presence or absence of T0901317 (10 μmol/l) in a low-glucose (11.2 mmol/l) or high-glucose (31.2 mmol/l) medium, TUNEL assays were conducted to measure apoptosis. TUNEL assay images were examined using fluorescence microscopy. *$P < 0.05$ compared with INS-1 cells treated with T0901317 under low glucose conditions. *C*: TUNEL assays were performed with primary pancreatic islets subjected to the same conditions described in *A*. Apoptotic cells were detected by TUNEL and peroxidase staining. The arrowheads indicate apoptotic cells. *D*: After pretreatment of cerulenin (0.5 mg/l) or NAC (1 mmol/l), INS-1 cells were incubated in the presence or absence of T0901317 with high-glucose medium (31.2 mmol/l) for TUNEL assay. *$P < 0.05$ vs. DMSO control by Student's *t* test. Glc, glucose.

different stages of type 2 diabetes. Acute activation of LXR in β-cells enhances GSIS on rapid increase of certain fatty acids, consistent with previous reports that short-term treatment of fatty acids to β-cells elevates GSIS by stimulating protein kinase C (37). This is analogous to the "β-cell compensatory response" that occurs when insulin demand is increased during the earlier stages of diabetes. However, when the β-cells are subjected to intolerable chronic lipid stimulus, such as the case of β-cells found in the later stages of type 2 diabetic subjects, they appear to exhibit lipid and ROS accumulation, eventually undergoing apoptosis. Without doubt, further examination is required to clarify the discrepancy in the outcomes of LXR activation in β-cells. To address this issue, we tried to knock down LXR levels in β-cells using siRNA. However, insufficient reduction of LXR (~60–70% of control) prevented us from clearly verifying that chronic LXR activation is sufficient for β-cell dysfunction.

Although future studies will reveal whether LXR dysregulation in β-cells contributes to β-cell lipotoxicity in vivo, the data presented here suggest a potential target for the development of a successful method of regulating lipid metabolism in the pancreatic β-cells of obese and diabetic patients.

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