Elevated extracellular lipids, such as the free fatty acid palmitate, can induce pancreatic beta cell endoplasmic reticulum (ER) stress and apoptosis, thereby contributing to the initiation and progression of type 2 diabetes. ATP-citrate lyase (ACLY), a key enzyme in cellular lipid production, was identified as a palmitate target in a proteomic screen. We investigated the effects of palmitate on ACLY activity and phosphorylation and its role in beta cell ER stress and apoptosis. We demonstrated that treatment of MIN6 cells, mouse islets and human islets with palmitate reduced ACLY protein levels. These in vitro results were validated by our finding that islets from high fat-fed mice had a significant decrease in ACLY, similar to that previously observed in type 2 diabetic human islets. Palmitate decreased intracellular acetyl-CoA levels to a similar degree as the ACLY inhibitor, SB-204990, suggesting a reduction in ACLY activity. ACLY inhibitors alone were sufficient to induce CCAAT/enhancer-binding protein homologues protein (CHOP)-dependent ER stress and caspase-3-dependent apoptosis. Similarly, even modest shRNA-mediated knockdown of ACLY caused a significant increase in beta cell apoptosis and ER stress. The effects of chemical ACLY inhibition and palmitate were non-additive and therefore potentially mediated by a common mechanism. Indeed, overexpression of ACLY prevented palmitate-induced beta cell death. These observations provide new evidence that ACLY expression and activity can be suppressed by exogenous lipids and demonstrate a critical role for ACLY in pancreatic beta cell survival. These findings add to the emerging body of evidence linking beta cell metabolism with programmed cell death.

Type 2 diabetes is associated with dyslipidemia, hyperglycemia, insulin resistance, and defects in insulin secretion from pancreatic beta cells (1). It is also becoming clear that increased beta cell apoptosis is associated with diabetes in humans and animal models (2–6). The events that cause diabetes remain incompletely understood, but it has been hypothesized that the elevated levels of lipids observed in obese individuals, including increased free fatty acids, might contribute to the pathophysiology of the disease (7). Many studies have shown that chronic high circulating levels of fatty acids are detrimental to beta cell function and survival, both in the presence and absence of elevated glucose (6, 8–11). Therefore, understanding the genome- and proteome-wide changes induced by fatty acids in beta cells and the molecular mechanisms of these changes would contribute to the understanding of the pathogenesis of type 2 diabetes and may open avenues for the development of new therapies. Recently, we reported the effects of elevated palmitate on the beta cell proteome under substimulatory glucose concentrations (6). Among the more interesting findings were significant changes in two gel features identified as ATP-citrate lyase (ACLY)2 (supplemental Fig. S1) (6).

ACLY is a cytosolic protein that catalyzes the formation of acetyl-CoA and oxaloacetate from citrate and coenzyme A in the presence of ATP. Acetyl-CoA is an essential substrate for the biosynthesis of cholesterol and long-chain fatty acids, making ACLY a critical enzyme for de novo synthesis of a wide range of complex cellular lipids (12). The ACLY gene is widely expressed in many tissues, and deletion of the gene in mice leads to embryonic lethality (13). ACLY activity is high in tissues such as fat and liver (~1 unit/g wet weight). ACLY is also expressed and active in pancreatic beta cells (14). ACLY levels and activity are significantly reduced in pancreatic islets from patients with type 2 diabetes (15). Several groups have investigated the role of ACLY in glucose-stimulated insulin release and have obtained conflicting results (16, 17). It has also been proposed that ACLY may promote cell proliferation or survival, especially in the context of cancer (18–20). Notwithstanding, it remains unclear if ACLY plays a role in beta cell survival. There is evidence from other cell types that ACLY activity can be regulated by phosphorylation via kinases, such as PKA, Akt, and GSK-3 (21–24). However, it is controversial whether these phosphorylation events actually alter the enzymatic activity of ACLY and the production of acetyl-CoA (25). Thus, the relationship between ACLY phosphorylation and ACLY activity in pancreatic beta cells also remains unclear.

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** The abbreviations used are: ACLY, ATP-citrate lyase; ER, endoplasmic reticulum; CHOP, CCAAT/enhancer-binding protein homologues protein.
In the present study, building upon our previous proteomic screen (6), we have characterized the time- and dose-dependent effects of palmitate on ACLY protein expression, activity, and phosphorylation status in the presence of basal or high concentrations of glucose. Loss-of-function and gain-of-function approaches provide evidence that ACLY plays a critical role in the prevention of pancreatic beta cell ER stress and apoptosis.

**MATERIALS AND METHODS**

**Reagents**—Reagents were from Sigma unless otherwise indicated. Palmitic acid was dissolved in 65 mM NaOH and complexed with essentially fatty acid-free BSA (20%, w/v) as described previously (6, 26). This complex was added to DMEM or RPMI 1640 medium (Invitrogen) to give a final palmitate concentration of 1500 μM and a palmitate/BSA molar ratio of 6:1 (unbound palmitate concentration is estimated at 300 nM), unless otherwise indicated. A vehicle control was included for each experiment. The blood of diabetic humans can contain a ratio of up to 6:1 total fatty acids to albumin. Under such conditions, the total fatty acid can be >2000 μM, where the unbound fatty acid can be >400 nM (27). We chose a simplified in vitro approach where palmitate is the only fatty acid added to rapidly model chronic prediabetic or diabetic conditions. We have previously demonstrated that these culture conditions lead to programmed cell death that requires active signal transduction, and we have shown that the cell death does not result from nonspecific detergent effects (6, 26). SB-204990 (3R*,5S*)-3-carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic acid was a gift from GlaxoSmithKline (Collegeville, PA). Medica 16 (3,3,14,14-tetramethylhexadecanedioic acid) was from Sigma or Cayman (Ann Arbor, MI). Annexin V conjugated with Alexa Fluor 647 was from Invitrogen. Primary antibodies, anti-total-ACLY, anti-phospho-ACLY (Ser454), anti-β-actin, and anti-cleaved caspase-3 were purchased from Cell Signaling Technology (Danvers, MA). Anti-phospho-ACLY (Thr427/Ser453) was from Epitomics (Burlington, Canada), and anti-CHOP was from Affinity BioReagents (Rockford, IL).

**Experimental Animals**—All animal procedures were approved by the University of British Columbia Animal Care Committee in accordance with guidelines of the Canadian Council on Animal Care. C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, MA). The mice were fed either a high fat diet (58% kcal from fat) or a control diet (11% kcal from fat). Open Source Diets (New Brunswick, NJ) for 4 weeks starting at 4 weeks of age.

**Pancreatic Islet Isolation and Cell Culture**—Pancreatic islets were isolated from 8–12-week-old C57BL/6J mice unless indicated otherwise using a previously reported protocol (29) that involves filtration instead of density centrifugation (30). After isolation, islets were cultured overnight in 35 × 10-mm suspension dishes (Nalge, Rochester, NY) in RPMI medium with penicillin/streptomycin and 10% FBS (Invitrogen). After overnight culture (37 °C, 5% CO2), the islets were washed with PBS and incubated in medium containing the indicated palmitate-BSA complex or BSA only (as the vehicle control) with 5 or 25 mM glucose as indicated. MIN6 insulinoma cells were cultured in DMEM with 10% FBS and penicillin/streptomycin.

**Immunoblot**—Western blots were performed as described previously (31). Briefly, proteins were extracted with lysis buffer (Cell Signaling) containing protease inhibitors. Clarified lysate samples containing 30 μg of protein were subjected to SDS-PAGE with a separating gel containing 8% (w/v) polyacrylamide and then transferred to a PVDF membrane. After blocking with 1-block (Applied Biosystems, Foster City, CA), the membrane was incubated with primary antibodies overnight at 4 °C and then with peroxidase-labeled secondary antibodies for 1 h at room temperature. Protein bands were detected using ECL Plus Western blotting detection reagents and by exposing membranes to autoradiography film. Band intensities were quantified using the histogram function in Photoshop (Adobe Systems, San Jose, CA).

**Imaging**—Staining and live cell imaging protocols were essentially as described (6). To image beta cell death, transfected MIN6 cells were incubated with a solution containing 4 μg/ml propidium iodide and annexin V conjugated with 1:1000 Alexa Fluor® 647 for 30 min. Cells were imaged 24–48 h after transfection with either a Zeiss 200m microscope or an ImageXpress Micro® system (Molecular Devices, Sunnyvale, CA). The percentage of the dead cells among the transfected cells was calculated by counting the cells positive for either propidium iodide or annexin V (depending on the study) and GFP against all of the GFP-positive cells.

**Metabolite Measurements**—ACLY activity was inferred from the intracellular levels of its substrate (coenzyme A), its reaction product (acetyl-CoA), and its downstream product (malonyl-CoA) using high performance liquid chromatography (32). MIN6 cells were cultured in 150 × 15-mm tissue culture dishes, treated as indicated, and snap-frozen in liquid nitrogen prior to extraction with perchloric acid (33).

**Quantitative Real-time PCR**—Total RNA was extracted from MIN6 cells after 24-h palmitate treatment using the Qiagen RNeasy minikit (Mississauga, Canada). cDNA was then synthesized from 0.5 μg of RNA using SuperScript III reverse transcriptase (Invitrogen). Real-time PCR was performed for the acly gene (forward primer, 5’-AGGAAATGCACCTCACCAGGT-3’; reverse primer, 5’-CGGATCACAGATGCTGGTCA-3’) using PerfeCTa® SYBR Green (Quanta Biosciences, Gaithersburg, MD) and the StepOnePlus™ system (Applied Biosystems, Foster City, CA).

**ACLY Overexpression and Knockdown**—ACLY was transiently overexpressed in MIN6 cells using the vector, pCMV6-ACLY-GFP, expressing the GFP-tagged open reading frame sequence of ACLY from Origene (Burlington, Canada). Empty vector lacking the ACLY sequence, pCMV6-GFP, was used as control. Knockdown of ACLY in MIN6 cells was accomplished using the shRNA vector tagged with GFP, pGFP-V-RS, targeting mouse ACLY (Origene). A scrambled vector with a GFP tag, pGFP-V-RS, was used as a control. In both overexpression and knockdown experiments, plasmid DNA (1 μg) was transfected using Lipofectamine 2000 (Invitrogen), resulting in robust GFP expression in >10% of cells after 24 h. Following the indicated treatments, GFP-positive cells were sorted by fluorescence-activated cell sorting (BD FACS Vantage SE.DIVA) prior to Western blot analysis as described (6). Apoptosis was imaged using
an ImageXpress Micro® system in single cells as described above.

**Insulin Secretion**—The dynamics of insulin secretion in response to high glucose or direct depolarization with KCl were examined using a perifusion system described previously (6, 30). All experiments were initiated after a 1-h basal wash with Krebs-Ringer/BSA buffer containing 3 mM glucose. The ACLY inhibitor, 100 μM SB-204990, was present throughout the experiment. Fatty acids were not present in the solutions unless otherwise indicated.

**Statistics**—Data are expressed as mean ± S.E. Multiple comparisons between groups were performed using ANOVA followed by Tukey’s post hoc test. Where appropriate, unpaired or paired t tests were used when the difference between two groups was analyzed. A p value of less than 0.05 was considered statistically significant.

**RESULTS**

*Expression of ACLY in Beta Cells*—ACLY was expressed primarily in the cytoplasm of primary mouse beta cells and MIN6 cells (supplemental Fig. S2, A and C). Immunofluorescent staining with an antibody that recognizes ACLY phosphorylated at serine 454 illustrated that ACLY phosphorylation is evident under basal conditions (supplemental Fig. S2, B and D). Quantitative comparison of ACLY protein expression in mouse islets and MIN6 cells demonstrated that ACLY levels were higher in the transformed cell line (supplemental Fig. S2E). The abundance of ACLY phosphorylated at serine 454 was also higher in transformed cells, roughly in proportion to the increased overall ACLY protein expression.

*Palmitate and High Fat Diet Reduce ACLY*—It is established that ACLY is a critical enzyme at the interface of carbohydrate and lipid metabolism in all cell types (13, 20). However, the external factors that might modulate the protein expression of ACLY remain enigmatic. In a recent fluorescent two-dimensional gel proteomic screen of MIN6 cells exposed to palmitate in 5 mM glucose for 36 h, we sequenced ACLY protein from two gel features (6), with one ACLY protein spot being decreased while another nearby ACLY spot was increased (supplemental Fig. S1). This pattern suggested that ACLY might undergo a differential post-translational modification in the presence of palmitate. In those earlier proteomic analyses, the total level of ACLY expression and the nature of post-translational modification were not addressed. Thus, in the present study, we examined the effect of palmitate on the total protein levels of ACLY as well as the phosphorylation status at several residues, using Western blot analysis. Indeed, exposure of MIN6 cells to palmitate for 24 h induced a significant loss in ACLY protein (Fig. 1, A and B). After a 24-h treatment with 1500 μM palmitate (6:1 palmitate/BSA ratio) at either 5 or 25 mM glucose, expression of ACLY protein was reduced 50% relative to control (Fig. 1B). Importantly, similar results were observed in mouse (Fig. 1F) and human (Fig. 1D) islets. Total ACLY protein levels were also determined in MIN6 cells (n = 3) (E) and mouse islets (n = 3) (F) following treatment for 24 h in the absence of palmitate but with the indicated concentrations of glucose from 0 to 25 mM. ACLY band intensities were normalized to actin. * values that differ significantly from the corresponding non-palmitate control. Error bars, S.E.
observed in mouse islets and human islets (Fig. 1, C and D). In MIN6 cells, palmitate had a more potent effect on ACLY protein levels in 25 mM glucose. In primary cells, palmitate was more consistently effective at low glucose (Fig. 1). The non-metabolizable analogue of palmitate, bromo-palmitate (6:1), did not reduce ACLY levels (data not shown). Raising the extracellular glucose as high as 25 mM did not lead to changes in expression of ACLY (Fig. 1, E and F), confirming the work of others (34).

Using real-time quantitative PCR, we determined that palmitate decreased ACLY mRNA expression to a degree that partially accounted for the decrease in ACLY protein levels (supplemental Fig. S3). Interestingly, high glucose increased ACLY mRNA, although this apparently did not result in an increase in ACLY protein.

To add physiological context to our in vitro data, we also investigated the effect of a high fat diet on ACLY expression in

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**FIGURE 2.** ACLY protein expression is reduced by a high fat diet. Total ACLY levels in isolated islets from C57BL/6J mice after a 4-week high fat diet or their control littermates fed a low fat diet (n = 4). *, values that differ significantly from the corresponding control. Error bars, S.E.

**FIGURE 3.** Palmitate induces early ACLY phosphorylation that does not impact ACLY activity. Phosphorylation of ACLY at serine 454 (A) and threonine 447/serine 451 (B) were determined following treatment of MIN6 cells for 24 h with palmitate (1500 μM; 6:1 ratio versus BSA) and with 5 or 25 mM glucose as indicated. Shown is the time course of changes in total ACLY (C) and phosphorylated ACLY (serine 454) (D) in MIN6 cells exposed to palmitate (1500 μM, 6:1 ratio versus BSA) in 5 or 25 mM glucose for the indicated time points up to 6 h. *, significant difference from the control of the same glucose condition; **, significant difference between the controls at different glucose conditions. Error bars, S.E.
mouse islets. After a 4-week high fat diet, islet ACLY protein levels were significantly reduced (Fig. 2), to a similar degree as we observed in vitro and as has been reported for type 2 diabetic human islets (15). Together, these data validate our in vitro culture system and demonstrate the physiological relevance of our observations.

Palmitate Decreases ACLY Activity—Some studies have suggested that ACLY activity might be modulated by phosphorylation (21, 24), whereas others revealed no apparent relationship between ACLY phosphorylation and enzymatic activity (25). In the present work, culture of MIN6 cells for 24 h in medium containing palmitate increased ACLY phosphorylation at serine 454 (Fig. 3A) and threonine 447/serine 451 (Fig. 3B). ACLY phosphorylation at serine 454 occurred within 6 h, well before significant changes in ACLY protein levels were observed (Fig. 3, C and D). ACLY phosphorylation on threonine 447/serine 451 was reduced at 2–6 h under 5 mM glucose but unchanged under 25 mM glucose (data not shown).

Given the controversy surrounding the putative link between phosphorylation and activity of ACLY, we assessed ACLY activity more directly based on intracellular levels of its substrates and products. We did not observe effects of acute 15-min palmitate treatment on the levels of the ACLY substrate (coenzyme A; Fig. 4A), its immediate product (acetyl-CoA; Fig. 4B), or its further downstream product (malonyl-CoA; Fig. 4C). This finding agrees with the requirement for prolonged palmitate exposure to reduce ACLY protein levels in beta cells. However, the concentration of acetyl-CoA was significantly reduced by 24-h palmitate treatment. The magnitude of this decrease was similar to that induced by treating cells with a potent and specific inhibitor of ACLY, SB-204990 (35) (Fig. 4E). The cellular concentration of coenzyme A and malonyl-CoA were not affected by chronic palmitate treatment (Fig. 4, D and F). Together, these data suggest that long term exposure to palmitate reduces ACLY activity, despite an early increase in its phosphorylation.

ACLY Inhibition Induces Beta Cell Apoptosis—One goal of the present study was to determine whether ACLY was modulated under proapoptotic conditions. Previous studies have firmly established that the free fatty acid palmitate can induce ER stress and apoptosis in primary pancreatic beta cells and beta cell lines (6, 36). Here, we confirmed that palmitate initiated a robust dose-dependent ER stress response, marked by increased expression of CHOP protein (Fig. 5A). This response was followed by an increase in apoptosis, mediated by elevated cleavage and activation of caspase-3 (Fig. 5B). Because palmitate provoked a reduction in total ACLY protein and activity, we tested whether treatment with two structurally unrelated small molecule inhibitors of ACLY, SB-204990 (35) and Medica 16 (37), would be sufficient to cause beta cell apoptosis and ER stress. Indeed, blocking ACLY activity with either inhibitor caused robust beta cell apoptosis (Fig. 5D and supplemental Fig. S4). In both cases, the observed apoptosis was comparable with that seen with palmitate alone and was not additive to the effects of palmitate, suggesting a common mechanism. Interestingly, in high glucose, the combination of palmitate...
tate and SB-204990 led to less ER stress than when cells were exposed to palmitate or SB-204990 individually (Fig. 5C), suggesting a complex relationship between ACLY and glucolipotoxicity. In order to further confirm the prosurvival role of ACLY, we employed shRNA-mediated knockdown. Consistent with the experiments using ACLY inhibitors, beta cells with even 33% less ACLY protein (Fig. 6A) had increased ER stress (Fig. 6B) and a significantly higher rate of apoptosis (Fig. 6, C and D). Together, these data suggest that ACLY plays a critical role in beta cell survival.

Because a reduction in ACLY translation may contribute to the loss of ACLY in palmitate-treated beta cells and palmitate is known to activate ER stress, we investigated the role of the unfolded protein response. In particular, we examined whether ER stress might play a role in decreasing ACLY protein expression or phosphorylation using thapsigargin, a SERCA inhibitor and well characterized inducer of beta cell ER stress (38). ACLY protein was significantly reduced in the thapsigargin-treated MIN6 cells (Fig. 7). Thus, the reduction in ACLY may be in part downstream of the initiation of ER stress. These data support the concept that ACLY is a component of a vicious circle that contributes to ER stress and apoptosis in palmitate-treated beta cells.

We examined other possible links between ACLY reduction and beta cell apoptosis. It has been shown that cytosolic acetyl-CoA is an important source for acetylation leading to protein modifications and gene transcription (39), which could be reduced by palmitate-induced ACLY loss (Fig. 4E). However, replacing acetyl-CoA with exogenous acetate failed to prevent beta cell death (supplemental Fig. S5). 

**ACLY Overexpression Protects Beta Cells**—In order to test whether the reduction in ACLY was required for the proapoptotic effects of free fatty acids, we sought to rescue MIN6 cells exposed to palmitate by enhancing the expression of ACLY.

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**FIGURE 5.** ER stress and apoptosis induced by palmitate and an ACLY inhibitor, SB-204990. MIN6 cells were treated for 24 h with the indicated ratios of palmitate to BSA in medium containing 5 mM or 25 mM glucose and analyzed by Western blot for CHOP (A) and cleaved caspase-3 (B). CHOP (C) and cleaved caspase-3 (D) levels in MIN6 cells were detected following treatment as above with the addition of the ACLY inhibitor, SB-204990 (100 μM) (n = 3). *, values that differ significantly from the control with the same glucose concentration. Error bars, S.E.
MIN6 cells were transfected with a construct containing GFP-tagged ACLY and then purified by fluorescence-activated cell sorting (FACS). Transfected cells showed significantly higher ACLY expression (2-fold) when compared with cells transfected with a GFP vector control (Fig. 8A). To determine if overexpressing ACLY could protect beta cells from palmitate-induced apoptosis, MIN6 cells were treated with palmitate for 24 h after transfection with constructs containing GFP or GFP-tagged ACLY and then monitored by propidium iodide labeling to assess cell death (Fig. 8, B and C). ACLY overexpression was associated with a significant reduction in the number of propidium iodide-positive cells observed following palmitate (Fig. 8C). To extend this analysis, the palmitate-treated transfected cells were purified by FACS and then examined by Western blotting (Fig. 8, D and E). After exposure to high palmitate for 24 h, cells overexpressing ACLY showed less CHOP protein at 5 mM glucose but not at 25 mM glucose (Fig. 8D) and reduced caspase-3 cleavage in both glucose conditions (Fig. 8E) when compared with the GFP control, most strikingly at 5 mM glucose. Collectively, these data demonstrate a protective role for ACLY in pancreatic beta cells.

Whether ACLY is required for glucose-stimulated insulin secretion remains a controversial subject. To test the hypothesis that ACLY plays a role in palmitate- or glucose-stimulated insulin secretion, we examined the effects of a specific ACLY inhibitor, SB-204990, in an islet perifusion system. In these experiments, SB-204990 did not inhibit insulin secretion induced by palmitate, glucose, or KCl in perifused mouse islets (supplemental Fig. S6). These data suggest that ACLY is dispensable for glucose-stimulated insulin release in mouse islets under these conditions. This study comes with the caveat that it is not possible to simultaneously assess the activity of the drug during the perifusion experiment.

**DISCUSSION**

The goal of the present study was to investigate the role of ACLY in palmitate-induced beta cell ER stress and apoptosis. Here, we present four major new findings. First, our data demonstrate that ACLY, a rate-limiting and non-redundant enzyme required for de novo lipid synthesis, is strikingly downregulated by prolonged exposure to the free fatty acid palmitate. Second, we provide evidence that the apparent activity of ACLY is not directly correlated with the phosphorylation of at least two sites in beta cells. Third, we find that loss of ACLY is sufficient to induce beta cell ER stress and apoptosis, whereas acute inhibition of this enzyme does not impair glucose-stimulated insulin secretion.
insulin release in our hands. Finally, we demonstrate that overexpression of ACLY can reverse palmitate-induced beta cell death. Together, these investigations point to an important and novel role for ACLY in pancreatic beta cell survival.

In our previous proteomic screen, it was shown that palmitate induced changes in a number of proteins in pancreatic beta cells, including ACLY (6). In the present report, we show clearly that ACLY protein levels and activity are reduced by palmitate. MacDonald and colleagues (15) also reported that ACLY mRNA was reduced in islets from type 2 diabetic patients. ACLY plays an important role in de novo lipid synthesis; therefore, it has been suggested as a therapeutic target for lipid reduction in obese individuals and obesity-related diseases, such as coronary heart disease (40). As such, several pharmaceutical companies have invested in the development of ACLY inhibitors. Most previous studies have focused on the roles of ACLY and its blockade in cells with high lipogenic capacity, such as adipocytes (41) and hepatocytes (42), and consequential effects on the plasma triglyceride and lipoprotein levels (35). Our results suggest that this class of inhibitors might not be an ideal therapeutic approach for type 2 diabetes, since they induce beta cell death.

ACLY is overexpressed in many tumors and immortalized cell lines (18, 20). ACLY inhibitors have been proposed as cancer therapeutics due to their ability to prevent proliferation and stimulate apoptosis in cancer cells that display high rates of de novo lipogenesis (18, 19). Pancreatic beta cells rely critically on glycolysis for glucose sensing, and previous studies have demonstrated lipogenesis in beta cells (34). ACLY is also expressed at a high level in pancreatic beta cells and at an even higher level in the MIN6 beta cell line. In fact, our recent gene array data suggest that mRNA encoding ACLY is the most highly

FIGURE 8. Overexpression of ACLY protects beta cells from palmitate-induced apoptosis. MIN6 cells were transiently transected with GFP or ACLY-GFP for 24 h. A, total ACLY protein levels in unsorted MIN6 cells following GFP or ACLY-GFP transfection. B, following transfection, cells were then incubated with palmitate (1500 μM, 6:1 ratio) for 24 h. Nuclei of the dead cells stain red with propidium iodide (PI), whereas the nuclei of live cells stain blue with Hoechst 33342. The arrows indicate transfected cells (green) co-stained with propidium iodide. Scale bar, 10 μm. C, the number of dead transfected cells expressed as a percentage of all cells (n = 4). D and E, transfected cells were FACS-purified after treatment for 24 h with palmitate as above and subjected to Western blot analysis to detect CHOP (D) and cleaved caspase-3 (E) (n = 3). Asterisks denote values that differ significantly from the GFP control at the same glucose concentration. **, significant differences between GFP- and ACLY-GFP-transfected cells with palmitate treatment at 5 mM glucose. Error bars, S.E.
expressed enzyme mRNA in MIN6 cells. Here we demonstrate that ACLY plays a critical role in the induction of apoptosis in pancreatic beta cells exposed to palmitate. Whether other pro-apoptotic stimuli suppress ACLY expression remains to be fully investigated. Interestingly, one genomic screen showed that ACLY was one of the many genes down-regulated in INS-1E beta cells treated with proapoptotic cytokines for 24 h (43). Thus, ACLY may be an essential factor for beta cell survival under multiple conditions. Whether ACLY plays the same role in other tissues is unclear, although it has to be suggested that the effects of ACLY on apoptosis might be tissue-specific because knockdown of ACLY in some tumor cells did not induce apoptosis (44). Nevertheless, homozygous ACLY knock-out is lethal in mice, indicating the generalized importance of this enzyme (13).

Recently, another important role for ACLY has emerged. ACLY was shown to be the major source for acetyl-CoA required for histone acetylation and the control of DNA accessibility for gene transcription (39). The effects of ACLY knockdown on gene transcription were completely reversed by acetate, which provides cells with an ACLY-independent source for acetyl-CoA (39). It will undoubtedly be of interest in the future to determine whether specific genes might be altered in pancreatic beta cells as a result of ACLY inhibition by palmitate. Although our data support the hypothesis that ACLY normally performs vital functions in the beta cells, results from our experiments suggested that acetate could not rescue beta cells from the deleterious effects of palmitate (supplemental Fig. S5). This suggests that palmitate-induced beta cell apoptosis is not critically dependent upon changes in acetyl-CoA availability and, by extension, upon histone acetylation, under these experimental conditions. The exact mechanisms linking ACLY to caspase-3 activation remain to be elucidated.

Palmitate is known to trigger ER stress and apoptosis in beta cells, and a number of mechanisms have been proposed (6, 36, 45–53). Our current results suggest that ACLY is another important player in the complex network controlling ER stress and programmed cell death in response to free fatty acids. ER stress accounts for a significant proportion of palmitate-induced beta cell apoptosis, and our results suggest that the loss of ACLY can be induced by ER stress.

Importantly, palmitate-induced beta cell apoptosis at basal glucose was completely reversed by ACLY overexpression, whereas there was an inability to completely rescue ER stress, apoptosis, and cell death at high glucose. We interpret these data as suggesting distinct mechanisms of cell death that differentially involve ACLY. This work adds to a growing body of evidence that suggests that the mechanisms by which palmitate induces programmed cell death in beta cells differ in low versus high glucose (6, 26, 48). The process of autophagy, which has specific energy dependence, is emerging as a modulator of programmed cell death in beta cells (54, 55).

Whether ACLY activity plays a net positive or negative role in diabetes cannot be inferred with any certainty from the studies conducted to date. On one hand, elevated cellular lipid synthesis might be expected to have deleterious effects over the long term. For example, reducing ACLY activity in liver was recently shown to protect against hepatic steatosis (56). On the other hand, de novo lipid synthesis is essential for many cellular functions. In pancreatic beta cells, cellular lipids have been implicated as signals for glucose-stimulated insulin secretion. Some studies have shown that ACLY participates in glucose-stimulated insulin secretion (16, 57), whereas others have failed to show a critical role for this enzyme (14, 17). INS-1 cells with very substantial (~90%) reduction in ACLY expression showed no change in glucose-stimulated insulin release (14). The reasons for the discrepancies in the literature remain unclear but may be related to differences in conditions of cell treatments, especially glucose concentrations and the availability of palmitate. In our hands, for example, using minimal Krebs solution containing 20 mM glucose, we were unable to observe effects of ACLY inhibition on mouse islets insulin secretion.

In summary, our data suggest a model whereby elevated levels of circulating free fatty acids suppress ACLY activity, leading to lipotoxicity in pancreatic beta cells. Although the suppression of lipogenesis by ACLY inhibitors would be expected to reduce the toxic load on beta cells, loss of ACLY activity might also cause adverse direct effects on beta cells. Strategies designed to prevent the loss of ACLY in beta cells while retaining its function in the major sites of lipogenesis might be therapeutically useful.

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