Modulation of palmitate-induced endoplasmic reticulum stress and apoptosis in pancreatic β-cells by stearoyl-CoA desaturase and Elovl6

Christopher D. Green and L. Karl Olson

Departments of 1Physiology and 2Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan

Submitted 20 September 2010; accepted in final form 19 January 2011

Green CD, Olson LK. Modulation of palmitate-induced endoplasmic reticulum stress and apoptosis in pancreatic β-cells by stearoyl-CoA desaturase and Elovl6. Am J Physiol Endocrinol Metab 300: E640–E649, 2011. First published January 25, 2011; doi:10.1152/ajpendo.00544.2010.—Induction of endoplasmic reticulum (ER) stress and apoptosis by elevated exogenous saturated fatty acids (FAs) plays a role in the pathogenesis of β-cell dysfunction and loss of islet mass in type 2 diabetes. Regulation of monounsaturated FA (MUFAs) synthesis through FA desaturases and elongases may alter the susceptibility of β-cells to saturated FA-induced ER stress and apoptosis. Herein, stearoyl-CoA desaturase (SCD)1 and SCD2 mRNA expression were shown to be induced in islets from prediabetic hyperinsulinemic Zucker diabetic fatty (ZDF) rats, whereas SCD1, SCD2, and fatty acid elongase 6 (Elovl6) mRNA levels were markedly reduced in diabetic ZDF rat islets. Knockdown of SCD in INS-1 β-cells decreased desaturation of palmitate to MUFA, lowered FA partitioning into complex neutral lipids, and increased palmitate-induced ER stress and apoptosis. Overexpression of SCD2 increased desaturation of palmitate to MUFA and attenuated palmitate-induced ER stress and apoptosis. Knockdown of Elovl6 limited palmitate elongation to stearate, increasing palmitoleate production and attenuating palmitate-induced ER stress and apoptosis, whereas overexpression of Elovl6 increased palmitate elongation to stearate and palmitate-induced ER stress and apoptosis. Overall, these data support the hypothesis that enhanced MUFA synthesis via upregulation of SCD2 activity can protect β-cells from elevated saturated FAs, as occurs in prediabetic states. Overt type 2 diabetes is associated with diminished islet expression of SCD and Elovl6, and this can disrupt desaturation of saturated FAs to MUFA, rendering β-cells more susceptible to saturated FA-induced ER stress and apoptosis.

fatty acid elongase 6; lipotoxicity; fatty acid desaturation; fatty acid elongation; saturated fatty acids; monounsaturated fatty acids

Type 2 diabetes arises from an inability of pancreatic β-cells to compensate for insulin resistance in peripheral tissues. Progressive loss of β-cell compensation is likely due to reduced insulin secretory capacity and/or β-cell mass (8, 33, 53, 68). Elevated levels of plasma nonesterified free fatty acid (FFA), a risk factor for insulin resistance and type 2 diabetes (47, 52), have been associated with the pathogenesis of β-cell dysfunction (51, 58). However, the response of β-cells to long-term elevations in fatty acids (FAs) is dependent largely on the FA composition. Saturated FAs, such as palmitate (16:0), cause diminished insulin secretion and insulin gene expression and the induction of apoptosis through multiple processes, including generation of ceramides, reactive oxygen species, and endoplasmic reticulum (ER) stress (10, 15, 30, 32, 39). Monounsaturated FAs (MUFA), such as palmitoleate (16:1, n-7) and oleate (18:1, n-9), and the polyunsaturated FA (PUFA) eicosapentaenolate (20:5, n-3) can protect β-cells from apoptosis and insulin secretory defects induced by saturated FAs (31, 38, 39).

In addition to exogenous FA structure, evidence indicates that the intracellular capacity to modulate FA fate plays an important role in β-cells.

Alterations in FA metabolism critically affect the response of β-cells to exogenous FAs, particularly the lipotoxicity of saturated FAs. Approaches used to enhance FA oxidation and triacylglyceride (TAG) storage have demonstrated significant alterations in the effects of exogenous saturated FAs on β-cell function (5, 35, 54). Studies have also shown that regulation of FA structure may participate in modulating the effects of FAs on β-cells. Subpopulations of MIN-6 and rat β-cells identified to be resistant to palmitate-induced apoptosis were associated with increased expression of the FA Δ9 desaturase stearoyl-CoA desaturase (SCD)1 and increased conversion of palmitate to MUFA (7, 24). The level of SCD1 gene expression has also been correlated with the susceptibility of β-cell lines and islets to ER stress in vitro and with the severity of diabetes in vivo in a mouse model of obesity (18, 34).

FA desaturase and elongase enzymes modify FAs by adding a cis-double bond or two carbons, respectively, to a fatty acyl-CoA. These activities are essential for a variety of cellular functions, including maintenance of membrane FA composition and generation of signaling molecules. The desaturase subtypes in mammals include Δ5 desaturase (Δ5D), Δ6 desaturase (Δ6D), and SCD. Isoforms of SCD include two in rat (SCD1 and -2) (41) and three in human (SCD1, 2, and 5) (60, 70, 71). FA elongase (Elovl) subtypes range from Elovl1 to Elovl7 in mouse, rat, and human (www.ensembl.org). Synthesis of PUFA from essential dietary FAs occurs through the desaturases Δ5D and Δ6D and the elongases Elovl2 and Elovl5 (27, 44, 55). SCD, the rate-limiting enzyme in C16 and C18 MUFA synthesis, and Elovl6 synthesize the MUFA oleate, palmitoleate, and vaccinate (18:1, n-7) (42, 44). Elovl5 can also elongate palmitoleate to vaccinate (21, 27). Elovl1, -3, and -4 elongate a broad array of very long-chain FAs (>20) and are involved in sphingolipid synthesis and brown adipose, skin barrier function, and retinal degeneration, respectively (1, 45, 57, 65, 66, 69). However, unique roles for these enzymes in pancreatic β-cells remain to be defined.

In this study, FA desaturase and elongase gene expression were characterized in insulin secreting (INS)-1 β-cells, Sprague-Dawley rat islets, and prediabetic and diabetic Zucker diabetic fatty (ZDF) rat islets. SCD1 and SCD2 mRNA levels were increased in prediabetic ZDF islets and, along with Elovl6, reduced in diabetic ZDF islets undergoing islet failure. To examine the potential role of SCD and Elovl6 in modulating susceptibility of β-cells to exogenous palmitate, SCD and Elovl6 were selectively knocked down or overexpressed in INS-1 β-cells. Overall, results show that regulation of MUFA synthesis by...
SCD and Elovl6 modulates the susceptibility of β-cells to palmitate-induced ER stress and apoptosis.

MATERIALS AND METHODS

Materials. Palmitic acid was from Nu-Chek Prep (Elysian, MN), and [1-¹⁴C]palmitic acid (57.5 mCi/mmol) was from PerkinElmer (Shelton, CT). FA-free BSA was from Cell Line (Kankakee, IL). Phospho-JNK (pSAPK/JNK; 9255), total JNK (SAPK/JNK; 9252), and cleaved caspase-9 (9507) antibodies were from Cell Signaling Technology (Beverly, MA). GADD153/CHOP (sc-7531) and actin- 

horseradish peroxidase-conjugated (HRP; sc-1615-HRP) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Goat anti-rabbit-HRP and goat anti-mouse-HRP secondary antibodies were from Vector Laboratories (Burlingame, CA) and Bio-Rad Laboratories (Hercules, CA), respectively. Cell Death Detection ELISA kit was from Roche Diagnostics (Indianapolis, IN).

Rats and islet isolation. The Institutional Animal Care and Use Committee at Michigan State University approved all animal procedures. All animals were kept on a 12:12-h light-dark cycle with food and water ad libitum. Sprague-Dawley rats were from Charles River Laboratories and fed Harlan-Teklad laboratory chow (no. 8640) prior and water ad libitum. Sprague-Dawley rats were from Charles River Laboratories and maintained on Purina 5008 diet. Body weights and blood glucose levels were measured weekly. At 6 and 13 wk of age, ZDF rats were euthanized for islet isolation, and blood was collected from the abdominal aorta. Islets were dissociated from pancreatic tissue by collagenase digestion and isolated by hand. Plasma insulin levels were measured using a Rat Insulin Radioimmunoassay kit (Millipore, Billerica, MA).

INS-1 cell culture. INS-1 cells were maintained in RPMI-1640 medium containing 11.1 mM glucose (Invitrogen, Carlsbad, CA) and supplemented with 10% FBS, 1 mM pyruvate, 10 mM HEPES, 50 μM 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin (INS-1 medium). Cells were seeded at a density of 0.2 × 10⁶ cells/cm² for each experiment. For FA treatments, cells were cultured for the indicated lengths of time in a modified INS-1 medium containing 0.5% FBS and palmitate complexed to BSA.

FA preparation. Palmitic acid was bound to FA-free BSA, as described previously (12). Briefly, stock solutions of 100 mM palmitic acid dissolved in 0.1 M sodium hydroxide and 5% FA-free BSA dissolved in glucose-free RPMI-1640 were heated to 70 and 55°C, respectively. The 100 mM palmitic acid solution was then added dropwise into the 5% BSA solution to make a final stock of 5 mM palmitate and 4.75% BSA. The solution was kept at 55°C for 10 min, mixed, and brought to room temperature. The 5 mM palmitate, 4.75% BSA stock was then diluted into culture medium and adjusted with BSA to give the final indicated concentrations.

Modulation of SCD and Elovl6 activities with small interfering RNA. siGENOME SMARTpool small interfering (si)RNA against rat Elovl6 (M-089126) and SCD2 (M-099222) and siCONTROL Non-Targeting siRNA (D-001206–130) were from Thermo Fisher Scientific (Waltham, MA). The sequences of Elovl6 siRNA were 5’-GCU-GUACGCGCUCCUUUA-3’, 5’-GCAAUAGGUAGUACGAU-3’, 5’-UUAAACCUCCUGGUCCUA-3’, and 5’-AUUCUGGCC- 

UUACGGCUUU-3’, whereas the sequences of SCD2 siRNA were 5’-GAUUGUUCGCCUAAAUUA-3’, 5’-GGCUUCACCCCUGCU- 

UU-3’, 5’-GCAUUUUGACGUACGUAA-3’, and 5’-CCGCCC- 

CCACCUAAUGGUA-3’. INS-1 cells (2 × 10⁶) were electroporated in 100 μl of electroporation buffer (7 mM ATP, 11.6 mM MgCl₂, 6H₂O, 68 mM K₂HPO₄, 14 mM NaHCO₃, and 2.2 mM glucose) containing 0.1 nmol siRNA using a Amaxa Nucleofector (program D-026). Cells were cultured for 24 h in INS-1 medium and subsequently cultured for the indicated lengths of time in modified INS-1 medium containing increasing concentrations of palmitic acid. Cells were then used for analysis of mRNA and protein expression and apoptosis.

Overexpression of SCD2 and Elovl6 with recombinant adenosviruses. Construction of recombinant adenosviruses that express SCD2 or Elovl6 has been described previously (21, 61). All experiments were controlled using an adenosvirus that expresses luciferase (received from Dr. Christopher J. Rhodes, University of Chicago, Chicago, IL). Adenosviruses were amplified in human embryonic kidney-293 cells and titered using the Adeno-X Rapid Titer kit (Clontech, Mountain- 

view, CA). For gene transduction, 90% confluent cell cultures were infected for 2 h with five plaque-forming units (pfu)/cell of Elovl6- or 10 pfu/cell of SCD2-expressing adenosvirus and then cultured for an additional 24 h in INS-1 medium. Cells were then cultured for the indicated lengths of time in modified INS-1 medium containing increasing concentrations of palmitic acid. Cells were then used for analysis of palmitate fate, protein expression, and apoptosis.

Complex lipid analysis and FA profiling. Cells electroporated with siRNA or infected with adenosviruses were incubated for 24 h in INS-1 medium. Cells were then incubated for 12 h in modified INS-1 medium containing 400 μM palmitate and 1 μCi [1-¹⁴C]palmitic acid, after which cells were extracted with 40% methanol. Methanol extracts were acidified, and total lipids were extracted with chloroform- methanol (2:2) containing butyalted hydroxytoluene, as described by Pawar et al. (49). Total lipid extracts were fractionated by TLC, and incorporation of ¹⁴C-labeled palmitate into complex lipids [e.g., TAG, diacylglyceride (DAG), cholesterol esters (CE), etc.] was quantified on a PhospholImager 820 (Molecular Dynamics, Sunnyvale, CA). Total lipid extracts were also used to determine the conversion of ¹⁴C-labeled palmitate into other FA species. For this purpose, total lipid extracts were saponified (0.5 N KOH in 80% methanol, 1 h at 10°C), neutralized, extracted in diethyl ether, dried, and dissolved in methanol and 0.1 mM butylated hydroxytoluene, as described previously (20, 61). FAs were fractionated by reverse-phase HPLC using a J’sphere ODS-H80 column (YMCK-Waters, Milford, MA), and ¹⁴C-labeled FAs were quantified by flow-through scintillation counting (IN/US Systems, Brandon, FL).

RNA analysis. Levels of mRNA in INS-1 cells and rat islets were determined by quantitative real-time RT-PCR, as described previously.

Fig. 1. Fatty acid (FA) elongase (Elovl) and desaturase mRNA levels in rat islets and insulin-secreting (INS-1) cells. Total RNA was isolated from Sprague-Dawley rat islets (A) and INS-1 cells (B) and analyzed for Elovl1–7 (E1–E7), stearoyl-CoA desaturase (SCD1), SCD2, Δ9-desaturase (DS5), and Δ6-desaturase (DS6) mRNA expression. Levels of mRNA are relative to ribosomal protein L32 (RPL32). Values are means ± SE for 3 independent experiments. Elovl and desaturase mRNA levels are graphed using different scales.
Table 1. Physiological parameters of 6- and 13-wk-old control (fa/?) and ZDF (fa/fa) rats

<table>
<thead>
<tr>
<th></th>
<th>Control (fa/?)</th>
<th>6 wk</th>
<th>13 wk</th>
<th>ZDF (fa/fa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, g</strong></td>
<td>173.8 ± 9.0</td>
<td>330.4 ± 5.8#</td>
<td>179.3 ± 4.0</td>
<td>353.5 ± 11.1#</td>
</tr>
<tr>
<td><strong>Insulin, ng/ml</strong></td>
<td>2.0 ± 0.3</td>
<td>3.6 ± 1.1</td>
<td>9.2 ± 1.4#</td>
<td>2.6 ± 0.5#</td>
</tr>
<tr>
<td><strong>Glucose, mg/dl</strong></td>
<td>83.0 ± 3.5</td>
<td>80.6 ± 4.0</td>
<td>117.0 ± 5.0*</td>
<td>316.7 ± 25*#</td>
</tr>
</tbody>
</table>

Data are means ± SE. ZDF, Zucker diabetic fatty. Glucose values are fed blood glucose. *P < 0.01 when compared with age-matched control. #P < 0.01 when compared with 6-wk-old rats with same phenotype.

RESULTS

Elovl and desaturase gene expression in rat islets and INS-1 cells. Elovl and desaturase mRNA levels were determined in Spague-Dawley rat islets and INS-1 cells under nonstimulatory glucose conditions. Rat islets and INS-1 cells expressed Elovl1, Elovl2, Elovl4, Elovl5, Elovl6, Elovl7, Δ5D, Δ6D, SCD1, and SCD2 (Fig. 1). The general pattern of gene expression was similar between islets and INS-1 cells, with the exception that Elovl2, Δ5D, and Δ6D expression were elevated in INS-1 cells. SCD2 mRNA levels were approximately five-fold higher than SCD1 mRNA in both rat islets and INS-1 cells. In liver, SCD1 was abundantly expressed, whereas SCD2 mRNA was nearly undetectable (data not shown).

FA desaturase and elongase gene expression in prediabetic and diabetic ZDF rat islets. FA desaturase and elongase mRNA levels were quantified in islets isolated from 6- or 13-wk-old control (+/?) and ZDF (fa/fa) rats. ZDF rats younger than 10 wk of age are considered prediabetic, displaying obesity and insulin resistance while maintaining nearly normal glucose levels (36). Six-week-old ZDF rats had slightly elevated fed blood glucose levels and were profoundly hyperinsulinemic relative to controls (Table 1). The increased plasma insulin levels in 6-wk-old ZDF rats coincided with an approximately twofold increase in islet insulin mRNA expression (Fig. 2A). SCD1, SCD2, Elovl5, and Δ6D mRNA levels were also increased from 1.4- to 2.3-fold (Fig. 2, B and C). At 13 wk of age, ZDF rats had profound hyperglycemia, and both plasma insulin and insulin mRNA levels were diminished compared with 6-wk-old ZDF rats (Table 1 and Fig. 2A), suggesting islet failure. SCD1, SCD2, Elovl6, and Elovl2 mRNA levels were also markedly decreased (Fig. 2, B and C). In both control and ZDF islets, there was an age-dependent decrease in Δ5D and Δ6D mRNA levels. This age-dependent decrease in Δ5D and Δ6D expression was more profound in the ZDF rat islets. These results show that expression of FA desaturases and elongases in pancreatic islets is affected by age and metabolic status and raise the issue as to whether FA desaturase or elongase activities modulate β-cell survival during metabolic stresses such as hyperglycemia or hyperlipidemia.

Effect of reduced SCD or Elovl6 expression on palmitate desaturation and elongation and incorporation into complex neutral lipids. To study the impact of reduced expression of SCD or Elovl6 on palmitate desaturation and elongation and incorporation into neutral lipids, siRNA targeting SCD and Elovl6 genes were electroporated into INS-1 cells. SCD siRNA reduced mRNA levels of both SCD1 and SCD2, whereas it increased Elovl6 mRNA (Fig. 3A). The increase in Elovl6 mRNA was selective because SCD siRNA had no effect on Elovl5 mRNA. Elovl6 siRNA reduced Elovl6 gene expression but did not affect SCD1, SCD2, or Elovl5 mRNA.

INS-1 cells electroporated with SCD2 or Elovl6 siRNA were incubated for 12 h with 400 μM palmitate and [1-14C]palmitic.
Decreased expression of SCD significantly reduced $^{14}$C-labeling of 16:1, n-7, 18:1, n-7, and 18:1, n-9 and led to a buildup of $^{14}$C-labeled 16:0, consistent with reduced desaturation of palmitate to MUFA. In contrast, decreased expression of Elovl6 reduced $^{14}$C-labeling of 18:0 and 18:1, n-9, whereas it increased labeled 16:1, n-7, consistent with reduced elongation of 16:0 and 16:1, n-7. These results show that knockdown of SCD or Elovl6 effectively decreases the desaturation and elongation, respectively, of palmitate and alters MUFA synthesis.

INS-1 cells treated with SCD siRNA also had a 25 and 100% reduction of palmitate incorporation into TAG or CE, respectively, and this coincided with a 2.4-fold increase in $^{14}$C-labeled DAG (Fig. 3C). In contrast, Elovl6 siRNA did not affect palmitate incorporation into TAG but caused an ~20% decrease in $^{14}$C-labeled DAG.

Impact of reduced SCD or Elovl6 activity on palmitate-induced ER stress. Loss of β-cell function upon exposure to exogenous FFAs, particularly saturated FAs, involves activation of ER stress response pathways, including inositol-requiring ER-to-nucleus signal kinase (IRE)1. RNA-dependent protein kinase-like ER kinase (PERK), and, to a lesser extent, activating transcription factor (ATF), (14). To test whether reduced SCD or Elovl6 expression affects the response of β-cells to exogenous palmitate, INS-1 cells treated siRNA were cultured for 9 h with 0, 200, or 400 μM palmitate and examined for induction of ER stress. ATF3 and CCAAT/enhancer-binding protein homologous protein (CHOP) expression were assessed as indices of PERK pathway activation, whereas splicing of X-box-binding protein 1 (Xbp1) mRNA to Xbp1s mRNA was assessed as an index of IRE1 pathway activation. In control cells, 400 μM palmitate was required to activate the IRE1 and PERK pathways, as indicated by a 2.3- to fourfold increase Xbp1s, ATF3, and CHOP mRNA levels (Fig. 4A) and a 5.5-fold increase in CHOP protein (Fig. 4B). Knockdown of SCD activity increased the sensitivity of the cells to palmitate such that 200 μM palmitate elevated Xbp1s, ATF3, and CHOP mRNA levels by approximately twofold (Fig. 4A). Decreased SCD activity also increased the extent to which 400 μM palmitate induced Xbp1s, ATF3, and CHOP mRNA levels and CHOP protein levels (Fig. 4, A and B). In contrast, decreased Elovl6 activity reduced Xbp1s splicing by induced 400 μM palmitate but had no significant effect on ATF3 or CHOP mRNA levels or CHOP protein levels.

The IRE1 pathway also mediates activation of JNK (59), which can lead to enhanced CHOP expression and apoptosis (14). Because JNK phosphorylation/activation occurs prior to induction of CHOP expression in β-cells exposed to elevated FFAs (40), the impact of reduced SCD activity on palmitate-induced JNK activation was tested. INS-1 cells treated for 6 h with 400 μM palmitate displayed increased JNK phosphorylation, and this was increased twofold by decreased SCD activity (Fig. 4C). These findings demonstrate that susceptibility to palmitate-induced ER stress is enhanced in β-cells with a reduced capacity to synthesize MUFA.

Impact of reduced SCD or Elovl6 activity on palmitate-induced apoptosis. INS-1 cells with decreased SCD and Elovl6 activity were then assessed for their susceptibility to palmitate-induced apoptosis. Control cells treated for 24 h with 300 or 400 μM palmitate had increased caspase-9 cleavage and DNA fragmentation, markers of apoptosis (Fig. 5). Knockdown of
SCD activity increased the sensitivity of the cells to palmitate such that 200 μM palmitate increased caspase-9 cleavage and DNA fragmentation. Reduced SCD activity also exacerbated caspase-9 cleavage and DNA fragmentation at 300 and 400 μM palmitate. In contrast, knockdown of Elovl6 activity did not affect palmitate-induced caspase-9 cleavage or DNA fragmentation.

Effect of increased SCD2 or Elovl6 expression on palmitate desaturation and elongation. Based on mRNA levels, SCD2 is the predominately expressed SCD isoform in rat islets and β-cells (Figs. 1 and 2) (24). To study the impact of increased SCD2 or Elovl6 expression on palmitate desaturation and elongation, recombinant adenoviruses were used to overexpress the respective genes in INS-1 cells. Cells were then incubated for 12 h with 400 μM palmitate and [1-14C]palmitic acid, after which 14C incorporation into FA was analyzed. In control cells infected with an adenovirus expressing luciferase, 71.5, 10.6, 10, 2.5, and 5.3% of the 14C-labeled FAs were into palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1,n-7), vaccenic acid (18:1,n-7), and oleic acid (18:1,n-9), respectively (Fig. 6A). Increased expression of SCD2 significantly increased 14C labeling of 16:1,n-7, 18:1,n-7, and 18:1,n-9 and led to a reduction in 14C-labeled 16:0 and 18:0, consistent with an increase in conversion of palmitate to MUFA. In contrast, elevated expression of Elovl6 increased 14C-labeling of 18:0 and 18:1,n-9, whereas it decreased labeled 16:0, 16:1,n-7, and 18:1,n-7. These results show that overexpression of SCD2 preferentially drives synthesis of n-7 MUFA, whereas overexpression of Elovl6 drives synthesis of stearate (18:0) and oleate (18:1,n-9).

Impact of increased SCD2 or Elovl6 activity on palmitate-induced ER stress. INS-1 cells overexpressing either SCD2 or Elovl6 were treated with 400 μM palmitate and examined for activation of ER stress. Treatment of control cells overexpressing luciferase for 9 h with 400 μM palmitate increased CHOP protein levels and JNK phosphorylation (Fig. 6, B and C). Increased SCD2 activity reduced palmitate-induced CHOP expression and JNK phosphorylation by ∼50%. In contrast, increased Elovl6 activity significantly increased palmitate-induced JNK phosphorylation and showed a trend toward increased CHOP protein expression. Together, these data show that enhanced SCD2 activity protects INS-1 cells from palmitate-induced ER stress, whereas palmitate-induced ER stress is potentiated by enhanced Elovl6 activity.

Effect of increased SCD2 or Elovl6 activity on palmitate-induced apoptosis. INS-1 cells with elevated SCD2 and Elovl6 activity were then treated for 24 h with increasing concentrations of palmitate and monitored for apoptosis. Control cells overexpressing luciferase exhibited significant caspase-9 cleavage at 400 μM palmitate and a dose-dependent increase in DNA fragmentation at 200, 300, and 400 μM palmitate (Fig. 7, A and B). Increased SCD2 activity reduced caspase-9 cleavage at 400 μM palmitate and significantly reduced DNA fragmentation at each FA concentration tested. In contrast, increased Elovl6 activity increased caspase-9 cleavage and DNA fragmentation at 200 and 300 μM palmitate but not at 400 μM.

DISCUSSION

Chronic elevations in FFAs are associated with loss of β-cell function and the risk of developing type 2 diabetes (51, 58).
Modulation of intracellular FA metabolism in β-cells is essential for preventing the toxic effects of FFAs and maintaining proper function. This study examined whether alterations in FA desaturase and elongase gene expression could contribute to β-cell compensation and failure in response to lipotoxicity. Rat islets and INS-1/β-cells were found to express the FA desaturases SCD1, SCD2, SCD5, and SCD6 and the FA elongases Elovl1, Elovl2, and Elovl4–7. In agreement with Hellemans et al. (24), SCD2 was shown to be the predominant SCD isoform expressed in rat islets and INS-1/β-cells. This is in stark contrast to liver and adipose tissue, which predominantly express SCD1.

Islets from hyperinsulinemic prediabetic ZDF rats expressed significantly higher levels of SCD1, SCD2, SCD5, and Elovl5 mRNA than control islets. Similar increases in SCD2 and SCD1 mRNA have been reported in islets of Zucker fatty (ZF; fa/fa) rats (24), which are also hyperinsulinemic yet generally do not transition to diabetes. SCD1, SCD5, and Elovl5 are also elevated in livers of ZDF rats, ZF rats, and ob/ob mice (29, 56, 62). The mechanism for increased expression of SCD and Elovl5 in prediabetic ZDF islets is unknown but might be due to the lack of leptin signaling, since leptin has been shown to suppress SCD1 and Elovl5 expression (11, 19). Alternatively, increased expression of SCD1 and Elovl5 could be mediated by insulin as shown in liver (62, 63) or by palmitate as shown for SCD1 expression in INS 832/13 and INS-1 cells (16) (data not shown). Irrespective of the mechanism, increased SCD1, SCD2, SCD5, and Elovl5 expression in ZDF islets likely compensates for the FFA load that gradually increases in ZDF rats prior to the onset of overt diabetes (36). Increased expression

---

**Fig. 5.** Susceptibility to palmitate-induced apoptosis is increased by SCD knockdown. INS-1 cells electroporated with siRNAs for C, S, or E were incubated for 24 h in increasing palmitate concentrations, after which cells were harvested for Western blot analysis or assayed for DNA fragmentation. A: activation of caspase-9 as indicated by formation of 38- and 17-kDa caspase-9 cleavage products. Representative Western blots from 6 independent experiments are shown. B: DNA fragmentation. Data are normalized to cells incubated without palmitate. Values are means ± SE for 6 independent experiments. *P < 0.02 compared with siCTL cells incubated without palmitate. #P < 0.04 compared with siCTL cells incubated in the same palmitate concentration.

**Fig. 6.** Effect of SCD2 and Elovl6 overexpression on monounsaturated FA synthesis and palmitate-induced ER stress. A: incorporation of [1-14C]palmitate into FA species of INS-1 cells overexpressing luciferase (Luc), SCD2, or Elovl6. INS-1 cells infected with adenoviruses that express Luc (L), SCD2 (S), or Elovl6 (E) were cultured for 12 h in INS-1 medium containing 400 μM palmitate and [1-14C]palmitic acid. Conversion of [14C]palmitate into other FA species was determined as described in MATERIALS AND METHODS. Data are reported as %total labeled FA species. Values are the mean ± SE for 3 independent experiments. B: CHOP protein levels. Values are means ± SE for 6 independent experiments. C: levels of pJNK and JNKt protein. Data are means ± SE for 3 independent experiments. For B and C, INS-1 cells infected with adenoviruses that express Luc, SCD2, or Elovl6 were incubated for 9 or 6 h without or with 400 μM palmitate, respectively. CHOP, JNKt, and pJNK levels were determined by Western blot. * and #P < 0.03 compared with control (Luc) cells treated without or with 400 μM palmitate, respectively. Representative Western blots shown.
of SCDs and Elovl5 in prediabetic ZDF rat islets would be expected to increase MUFA synthesis and limit the susceptibility of β-cells to lipotoxicity as proposed for ZF rat islets (24). Simultaneously, elevated Δ6Δ and Elovl5 might function to limit de novo lipogenesis in the ZDF islet by increasing PUFA, which can inhibit transcriptional activity of sterol regulatory element-binding protein-1c, a key regulator of lipogenesis (6, 43).

In comparison with 6-wk-old prediabetic ZDF islets, expression of SCD1, SCD2, Δ6Δ, Δ5Δ, Elovl2, and Elovl-6 was decreased significantly in 13-wk-old diabetic ZDF islets. This decrease in expression coincided with a large decrease in islet insulin gene expression and plasma insulin, consistent with islet failure. The decreased expression of SCDs, Δ6Δ, and Elovl6 might have resulted from reduced plasma insulin levels and diminished insulin signaling in islets. Although these in vivo findings do not indicate whether reduced SCD expression causes islet failure, decreased SCD expression would be expected to contribute to islet failure through increased accumulation of saturated FAs. Conversely, reduced Elovl6 expression may have a protective role by decreasing elongation of palmitate to stearate, thus allowing palmitate to be desaturated to palmitoleate, a potentially less lipotoxic FA. The reduction in Δ6Δ, Δ5Δ, and Elovl2 gene expression in diabetic ZDF islets would be predicted to limit production of PUFAs from dietary essential FAs. How this would impact β-cell function and survival is uncertain since essential FA deficiency increases insulin release in response to glucose (2, 25), whereas an increase in cellular n-3 PUFA at the expense of n-6 PUFAs enhances fuel-induced insulin release and protects against cytokine-induced cell death (64). The impact of decreased Δ6Δ and Δ5Δ expression in diabetic ZDF rats is also difficult to interpret because it is relatively small compared with the age-dependent decrease observed in both control and ZDF rat islets. The age-dependent decrease in Δ6Δ and Δ5Δ expression that occurred in control and ZDF islets is consistent with decreased PUFA desaturase activity that occurs in aging tissues (4, 26).

To examine whether altered expression of SCD or Elovl6 influences β-cell survival by modulating desaturation of palmitate to MUFA, SCD and Elovl6 were knocked down or overexpressed in INS-1 cells and assessed for palmitate-induced ER stress and apoptosis. ER stress results in activation of the unfolded protein response pathways IRE1, PERK, and ATF6 (14). IRE1 induces genes important for ER expansion and reduces protein load by splicing and, in turn, activating the transcriptional activator Xbp1 (9). PERK phosphorylates eukaryotic translation initiation factor 2α (eIF2α) to inhibit general protein translation while enhancing translation of proteins such as ATF4 (28). Sustained PERK-eIF2α activation induces the proapoptotic genes ATF3 and CHOP (28, 50). Active ATF6 induces ER protein chaperones to aid in protein folding (46). In β-cells, exogenous saturated FAs largely activate the IRE1 and PERK pathways, increasing Xbp1 splicing, eIF2α phosphorylation, ATF4 protein, and mRNA and protein levels of ATF3 and CHOP (13, 30).

Treatment of INS-1 cells with siRNA targeting SCD decreased SCD1 and SCD2 mRNA levels by >80% and blocked the desaturation of exogenous palmitate to all species of MUFA (16:1,n-7, 18:1,n-7, and 18:1,n-9), dramatically increasing the ratio of saturated FAs to MUFA. The SCD siRNA treatment had a similar effect on limiting de novo synthesis of MUFA species from acetate or glucose (data not shown) (21). The effectiveness of the SCD siRNA to profoundly lower desaturase activity likely resulted from rapid turnover of SCD1 and SCD2 protein due to NH2-terminal and COOH-terminal degradation sites that limit protein stability (23, 71). Consistent with a role of SCD in TAG synthesis, knockdown of SCD led to a reduction in palmitate incorporation into TAG and CE, whereas DAG was increased. Overexpression of SCD2 in INS-1 cells selectively increased the conversion of palmitate to n-7 MUFAs, including palmitoleate (16:1,n-7) and vaccinate (18:1,n-7), but not to the n-9 MUFA oleate (18:1,n-9). Overexpression of SCD2 also increased de novo synthesis of palmitoleate (16:1,n-7) and vaccinate (18:1,n-7), whereas stearate (18:0) synthesis was limited (21). The increase in Δ9 desaturation in SCD2-overexpressing cells was similar to that achieved after treatment of INS-1 cells with the liver X receptor (LXR) agonist T0901317 (20). The conversion of exogenous palmitate to palmitoleate (16:1,n-7) and vaccinate (18:1,n-7) in SCD2-overexpressing cells raises the possibility that SCD2 has preferential access to palmitate or favors palmitate over stearate as a substrate. In contrast, cells that overexpress SCD1 are reported to have increased oleate (18:1,n-9) (37). Although knockdown of both SCD1 and SCD2

---

**Fig. 7.** Effect of SCD2 and Elovl6 overexpression on palmitate-induced apoptosis. INS-1 cells infected with L, S, or E were incubated for 24 h with increasing palmitate concentrations, after which cells were harvested for Western blot analysis or assayed for DNA fragmentation. A: activation of caspase-9 as indicated by formation of 38- and 17-kDa caspase-9 cleavage products. Representative Western blots from 6 independent experiments are shown. B: DNA fragmentation. Data are normalized to cells incubated without palmitate. Values are means ± SE for 6 independent experiments. *P < 0.03 compared with control (Luc) cell incubated without palmitate. #P < 0.03 and $P < 0.05 compared with control (Luc) cells incubated in the same palmitate concentration.
reduced incorporation of palmitate into TAG and CE, overexpression of SCD2 surprisingly did not change incorporation of palmitate into either TAG or CE (data not shown).

INS-1 cells treated with 400 μM palmitate exhibited increased Xbp1 mRNA splicing, ATF3, and CHOP mRNA levels and CHOP protein levels. Targeted knockdown of SCD1 and SCD2 activity significantly increased the sensitivity and extent to which palmitate induced each of these ER stress markers. Conversely, overexpression of SCD2 reduced palmitate induction of CHOP protein. CHOP protein levels are also regulated by IRE1 activation of the cJun/c-Fos pathway through phosphorylation of JNK (14). INS-1 cells exposed to elevated palmitate had increased JNK phosphorylation, and this was significantly enhanced and reduced by knockdown or overexpression of SCDs, respectively. In addition to activation of proapoptotic ER stress proteins, accumulation of endogenous palmitate causes release of ER Ca\(^{2+}\) stores, which can activate the intrinsic apoptosis pathways (13, 14, 20, 22). As observed for ER stress markers, knockdown of SCDs in INS-1 cells increased the sensitivity to palmitate-induced caspase-9 cleavage and DNA fragmentation, hallmarks of apoptosis. Consistent with a role of SCD to protect β-cells from palmitate-induced ER stress, SCD2 overexpression significantly reduced both markers of apoptosis. Overall, these data confirm that SCD knockdown increases susceptibility of β-cells to damage from prolonged metabolic challenges (18, 24) and demonstrates for the first time that SCD2 can protect against palmitate-induced ER stress and apoptosis. Activation of LXR or PPARα in β-cells has been shown to increase SCD1 and SCD2 gene expression (20, 24) and protect against lipotoxicity (24). Our results suggest that increased expression of SCD2, the predominant Δ9 desaturase in β-cells, alone is sufficient to account for the protective effect of LXR or PPARα against lipotoxicity.

Protection from lipotoxicity in cells with elevated expression of SCD1 has been proposed to involve enhanced palmitate incorporation into neutral lipids (37). Surprisingly, SCD2 overexpression did not enhance storage of exogenous palmitate into TAG or CE (data not shown). In a similar manner, MIN6 cells selected for palmitate resistance have increased expression of SCD1 and oleate-to-palmitate ratio without increased palmitate incorporation in TAG (7). Although palmitate-resistant MIN6 cells had increased incorporation of palmitate into CE, only inhibition of SCD, not acyl-CoA:cholesterol acyltransferase, blocked incorporation of palmitate into neutral lipids and rendered cells susceptible to palmitate-induced apoptosis (7). The absence of an effect of overexpression of SCD2 on neutral lipid synthesis could be due to enhanced glycerolipid/FA cycling or FA oxidation. Alternatively, SCD2 could alter the saturation state of membrane phospholipids, which has recently been shown to impact unfolded protein response pathways (3). Although overexpression of SCD2 had little impact on incorporation of exogenous palmitate into neutral lipids, knockdown of SCD1 and SCD2 in INS-1 cells decreased palmitate incorporation into TAG and CE and led to a buildup of DAG. How these changes in neutral lipid storage exacerbate palmitate-induced ER stress and apoptosis is uncertain. One possibility is that the loss of neutral lipid storage could augment palmitate-induced release of ER calcium into the cytosol (13, 22). An increase in cytosolic calcium along with accumulation of DAG might also activate Ca\(^{2+}\)-dependent protein kinase C or protein kinase D. The loss of CE storage might also increase free cholesterol levels, which is cytotoxic to the ER (17) and proposed to increase Xbp1 splicing (48).

In contrast to SCD, knockdown of Elovl6 expression decreased Xbp1 splicing and tended to lower CHOP protein levels, JNK phosphorylation, and caspase-9 cleavage induced by elevated palmitate. This slightly protective effect of reduced Elovl6 on lipotoxicity was associated with reduced elongation of palmitate to stearate (18:0) and a small increase in MUFA (predominantly 16:1, n-7). Knockdown of Elovl6 also led to similar effects on de novo FA synthesis (21). Conversely, overexpression of Elovl6 increased palmitate-induced JNK phosphorylation, caspase-9 cleavage, and DNA fragmentation. This was associated with increased elongation of palmitate (16:0) to stearate (18:0) and a reduction in total MUFA synthesis (predominantly 16:1, n-7 and 18:1, n-7). Interestingly, overexpression of Elovl6 led to increased desaturation of stearate (18:0) to oleate (18:1, n-9), but this was insufficient in protecting cells from lipotoxicity. In aggregate, the impact of modulation of SCD or Elovl6 on palmitate-induced ER stress and apoptosis was associated with the ratio of saturated FAs to MUFA, particularly the n-7 MUFA species.

In conclusion, altered SCD and Elovl6 expression in INS-1 cells modulates MUFA synthesis and susceptibility to palmitate-induced β-cell lipotoxicity. On the basis of these findings, it is proposed that increased SCD expression that occurs in islets of prediabetic ZDF rats and ZF rats protects β-cells from lipotoxicity by desaturating palmitate to MUFA. On the other hand, the transition from prediabetes to diabetes is associated with decreased SCD expression in islets, and this can exacerbate islet failure through increased palmitate-induced ER stress and apoptosis.

ACKNOWLEDGMENTS

We thank Dr. Donald B. Jump from Oregon State University for providing the Elovl6-expressing recombinant adenovirus.

GRANTS

This work was supported by an American Diabetes Association grant (7-06-RA-103) to L. K. Olson.

DISCLOSURES

The authors have nothing to disclose.

REFERENCES


31. Hotta K, Hotta K, Akazawa R, Negishi M, Mori K. Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in tran-


