Elovl6 Deficiency Improves Glycemic Control in Diabetic *db/db* Mice by Expanding β-Cell Mass and Increasing Insulin Secretory Capacity

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Dysfunctional fatty acid (FA) metabolism plays an important role in the pathogenesis of β-cell dysfunction and loss of β-cell mass in type 2 diabetes (T2D). Elovl6 is a microsomal enzyme that is responsible for converting C16 saturated and monounsaturated FAs into C18 species. We previously showed that Elovl6 played a critical role in the development of obesity-induced insulin resistance by modifying FA composition. To further define its role in T2D development, we assessed the effects of *Elovl6* deletion in leptin receptor–deficient C57BL/KsJ *db/db* mice, a model of T2D. The *db/db;Elovl6*$^{-/-}$ mice had a markedly increased β-cell mass with increased proliferation and decreased apoptosis, an adaptive increase in insulin, and improved glycemic control. *db/db* islets were characterized by a prominent elevation of oleate (C18:1n-9), cell stress, and inflammation, which was completely suppressed by Elovl6 deletion. As a mechanistic ex vivo experiment, isolated islets from *Elovl6*$^{-/-}$ mice exhibited reduced susceptibility to palmitate-induced inflammation, endoplasmic reticulum stress, and β-cell apoptosis. In contrast, oleate-treated islets resulted in impaired glucose-stimulated insulin secretion with suppressed related genes irrespective of the Elovl6 gene. Taken together, Elovl6 is a fundamental factor linking dysregulated lipid metabolism to β-cell dysfunction, islet inflammation, and β-cell apoptosis in T2D, highlighting oleate as the potential culprit of β-cell lipotoxicity.

The increasing prevalence of obesity worldwide has become an alarming public health concern because of dramatic increases in the incidence of obesity-associated diseases, including type 2 diabetes (T2D) (1,2). Obesity leads to progressive deterioration of the insulin secretory function of pancreatic β-cells and reduced capacity to compensate for increased peripheral insulin resistance (3,4). β-Cell dysfunction and actual decrease in β-cell mass were both implicated in the deterioration of functional β-cell capacity.

Lipid accumulation in nonadipose tissues, a phenomenon known as lipotoxicity, was implicated in both pathologies as a molecular link between obesity and dysregulated glucose homeostasis (5,6). Pancreatic β-cells are highly susceptible to lipotoxicity, and numerous studies showed that saturated fatty acids (SFAs), such as palmitate (C16:0), suppressed insulin gene expression and secretion and ultimately induced β-cell apoptosis and/or dedifferentiation through multiple processes, including generation of ceramides and reactive oxygen species, endoplasmic reticulum (ER) stress, and inflammation (7–12). Monounsaturated

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FAs (MUFAs), such as palmitoleate (C16:1n-7) and oleate (C18:1n-9), and the polyunsaturated FA (PUFA) eicosapentaenoate (C20:5n-3), can protect β-cells from apoptosis and insulin secretory defects induced by SFAs (9,13,14). In addition to exogenous FA composition, evidence indicates that the intracellular capacity to modulate FA composition of lipid species might be another determinant of β-cell lipotoxicity. FA desaturase and elongase enzymes modify FAs by adding a *cis*-double bond or two carbons, respectively, to fatty acyl-CoA (15,16). Unique roles for these enzymes in pancreatic β-cells remain to be defined.

Elovl6 is a microsomal enzyme involved in the elongation of SFA and MUFA with 12, 14, and 16 carbons (17,18). Loss of Elovl6 function reduces stearate (C18:0) and oleate levels and increases palmitate and palmitoleate levels (19,20). In our previous study, we reported that mice with the targeted disruption of Elovl6 (*Elovl6*$^{-/-}$) were protected against the development of hepatic insulin resistance and deterioration of insulin secretory function of pancreatic β-cells in animals fed a high-fat and high-sucrose diet, despite similar levels of hepatosteatosis and obesity between Elovl6-deficient and wild-type mice (19,21). These findings suggested that the vital role of alterations in FA composition by Elovl6 deficiency extended beyond lipid accumulation and affected insulin sensitivity and β-cell function. Therefore, Elovl6 inhibition could be a potential therapeutic approach in T2D treatment. A critical question that needs to be answered is whether inhibition of this elongase confers reduced susceptibility to T2D. In the current study, we investigated the effects of *Elovl6* deletion in leptin receptor–deficient *Lepr*$^{db/db}$ (*db/db*) mice in a model of T2D (22).

RESEARCH DESIGN AND METHODS

Animals

All animal husbandry and animal experiments complied with the guidelines of the University of Tsukuba's regulations of animal experiments and were approved by the University of Tsukuba Animal Experiment Committee. *Elovl6*$^{-/-}$ mice were generated as described previously (19). We obtained *Lepr*$^{db/+}$ (*db*/+) mice on a C57BL/KsJ background from Charles River Japan and crossed with *Elovl6*$^{-/-}$ mice to obtain *db*/+;*Elovl6*$^{+/-}$ mice. *db*/+;*Elovl6*$^{+/-}$ mice were then crossed more than seven generations into the C57BL/KsJ background. Finally, double-heterozygous male and female mice were bred to generate mice with the double mutation of *Lepr* and *Elovl6* (*db/db*;*Elovl6*$^{-/-}$). All animals were housed in a pathogen-free barrier facility with a 12-h light/dark cycle and were given free access to normal chow and water. Age- and sex-matched littermates were used for all experiments. Mice were sacrificed during the light phase after food deprivation for 4 h.

Blood Chemistry

Plasma glucose, insulin, triglyceride (TG), total cholesterol (T-Cho), free FA (FFA), and glycosylated hemoglobin A_{1c} (HbA_{1c}) levels were determined as described previously (19,23).

Islet Morphology and Immunohistochemistry

Hematoxylin and eosin staining and immunohistochemistry for insulin- and glucagon-containing cells were performed as described previously (21,23,24). Pancreatic sections were stained with an anti-BrdU antibody (Abcam, Cambridge, U.K.). TUNEL staining was performed using the DeadEnd Fluorometric TUNEL system (Promega, Madison, WI). All images were acquired using a BZ-X710 microscope (Keyence, Osaka, Japan), and data were analyzed using a BZ-H3 analyzer (Keyence) and Adobe Photoshop software (Adobe Systems, San Jose, CA).

Isolation and Analysis of Mouse Pancreatic Islets

Isolation of islets from mice was performed by Ficoll-Conray density-gradient centrifugation (21,23,24). Insulin secretion, insulin content, TG content, cholesterol (Cho) content, and FA composition of islets were measured as previously described (21,23,24).

Treatment of Islets With Palmitate and Oleate

Islets were treated with FA as described previously (14) with some modifications. Briefly, palmitate and oleate were dissolved in 100 mmol/L in ethanol to prepare stock solutions, which were then diluted in RPMI 1640 supplemented with 1% FBS and 0.5% FA-free BSA to a final concentration of 500 μmol/L.

Assessment of β-Cell Apoptosis and Proliferation in Isolated Islets

Apoptotic β-cells were detected by immunofluorescence staining with antibody to the cleaved form of caspase-3 (Cell Signaling Technology Japan, Tokyo) and insulin (Abcam), followed by detection with secondary antibodies. The cells were counterstained with DAPI. Cell proliferation was assessed by 5-ethynyl-2'-deoxyuridine (EdU) incorporation using the Click-iT EdU Alexa Fluor 488 Imaging Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. EdU-labeling reagent was added to islet culture medium during the last 24 h of culture. All images were acquired using a TCS SP5 confocal microscope (Leica, Wetzlar, Germany), and data were analyzed using ImageJ software.

RNA Extraction and Quantitative Real-Time PCR

Total RNA extraction from isolated islets, cDNA synthesis, and quantitative real-time PCR were performed as previously described (21,23–27). Primer sequences for *Ins1*, *Ins2*, *Gcg*, *Pdgfra*, *Reg3a*, *Reg3b*, *Reg2*, *Aldh1a3*, *Ccr2*, *Ccl21a*, *Glycam1*, *Saa3*, *Xbp1s*, *Ddit3*, *Casp1*, *Atf4*, *Ppp1r15a*, and *Txnip* are presented in Supplementary Table 1. mRNA expression levels were normalized to that of cyclophilin mRNA.

Statistics

Values are expressed as means ± SEM and were analyzed using ANOVA. Differences were considered significant for P of <0.05.

RESULTS

Elovl6 Deficiency in *db/db* Mice Improves Hyperglycemia and Glucose Intolerance

To investigate the role of Elovl6 in T2D development and progression, we first determined the effect of endogenous Elovl6 in leptin receptor–deficient *db/db* mice, which exhibit obesity, insulin resistance, and resultant β-cell failure as a model of T2D, generating *Elovl6*-deficient *db/db* mice (*db/db*;*Elovl6*$^{-/-}$). During 6–16 weeks of age the *db/db*;*Elovl6*$^{+/+}$ and *db/db*;*Elovl6*$^{-/-}$ mice both gradually gained body weight (BW) without any significant differences (Fig. 1*A*). At 40 weeks of age, when emergence of diabetes caused BW loss in *db/db*;*Elovl6*$^{+/+}$ mice, *db/db*;*Elovl6*$^{-/-}$ mice still sustained a gain, with significant difference compared with *db/+*;*Elovl6*$^{+/+}$ controls. There was no significant difference in food intake between the two genotypes (4.5 ± 0.5 g/day in *db/db*;*Elovl6*$^{+/+}$ mice vs. 4.7 ± 0.1 g/day in *db/db*;*Elovl6*$^{-/-}$ mice). Surprisingly, although obesity in *db/db* mice was not improved, *Elovl6* deficiency markedly improved hyperglycemia (Fig. 1*B*) and showed an adaptive increase in insulin (Fig. 1*C*) in *db/db* mice. These metabolic changes also manifested in ameliorated polydipsia, polyuria (Fig. 1*D*), and elevated HbA_{1c} levels in *db/db*;*Elovl6*$^{-/-}$ mice (Fig. 1*E*). Furthermore, improved plasma glucose clearance (Fig. 2*A*) and increased insulin secretion (Fig. 2*B*) were observed in *db/db*;*Elovl6*$^{-/-}$ mice by oral glucose tolerance test, whereas insulin sensitivity, as evidenced by insulin tolerance test, did not differ between the two genotypes (Fig. 2*C* and *D*). These results suggested that *Elovl6* deficiency prevented T2D progression by increasing insulin secretory capacity of pancreatic β-cells in *db/db* mice.

Because of the development of hepatosteatosis, adipocyte inflammation, and hyperlipidemia accompanied by the obesity model with pancreatic β-cell dysfunction and glucose intolerance, we weighed liver and epididymal white adipose tissue (eWAT) and measured metabolic parameters. Liver weight and hepatic TG and T-Cho content were higher, whereas plasma levels of TG and FFA were lower in *db/db*;*Elovl6*$^{-/-}$ mice than in *db/db*;*Elovl6*$^{+/+}$ mice (Table 1). Analysis of liver histology further confirmed the dramatic

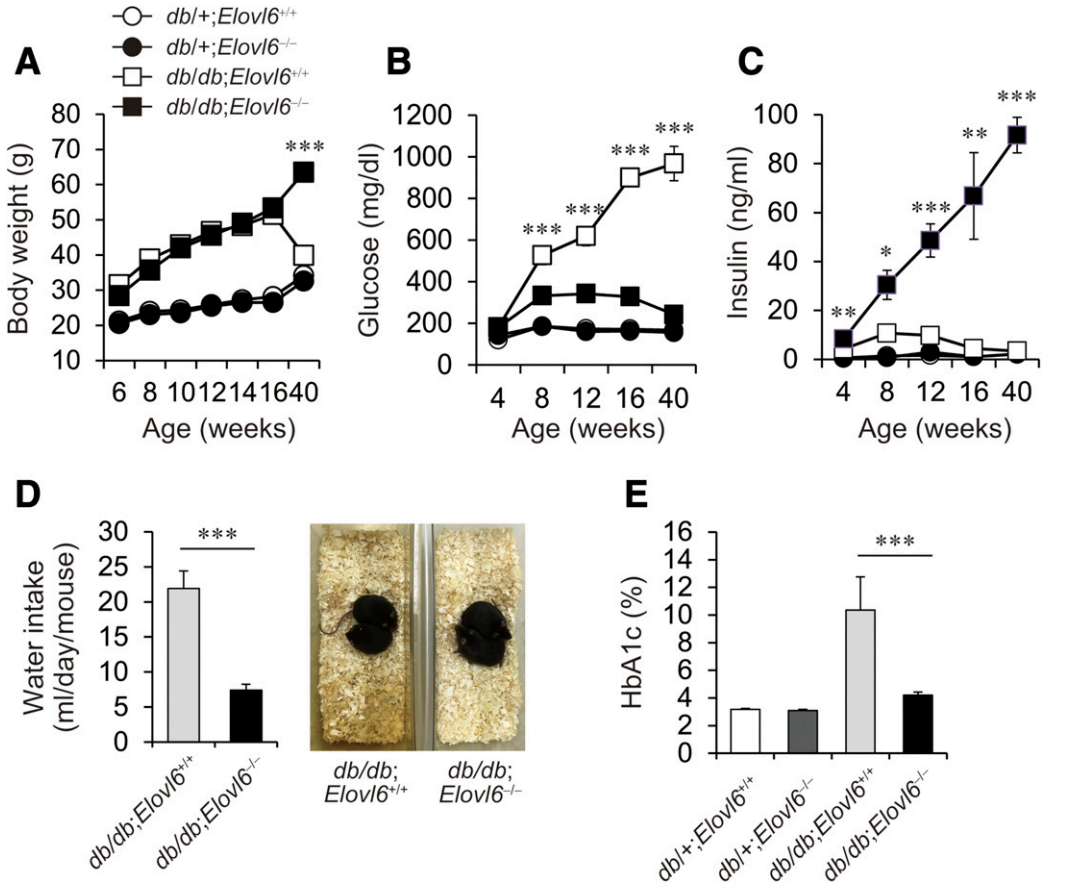

Figure 1—Elovl6 deficiency in *db/db* mice improves hyperglycemia without affecting obesity. *A*: Changes in body weight in *db/+*;*Elovl6*$^{+/+}$, *db/+*;*Elovl6*$^{-/-}$, *db/db*;*Elovl6*$^{+/+}$, and *db/db*;*Elovl6*$^{-/-}$ mice at indicated ages (n = 5–8). Blood glucose (*B*) and plasma insulin (*C*) concentrations in *db/+*;*Elovl6*$^{+/+}$, *db/+*;*Elovl6*$^{-/-}$, *db/db*;*Elovl6*$^{+/+}$, and *db/db*;*Elovl6*$^{-/-}$ mice after 4 h of fasting at indicated ages (n = 10–14). *D*: Water intake (left) and illustrative images of urine output in the cages (right) of *db/db*;*Elovl6*$^{+/+}$ and *db/db*;*Elovl6*$^{-/-}$ mice at 16 weeks of age (n = 4). *E*: HbA_{1c} levels in *db/+*;*Elovl6*$^{+/+}$, *db/+*;*Elovl6*$^{-/-}$, *db/db*;*Elovl6*$^{+/+}$, and *db/db*;*Elovl6*$^{-/-}$ mice at 12 weeks of age (n = 4–11). Data are presented as means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001.

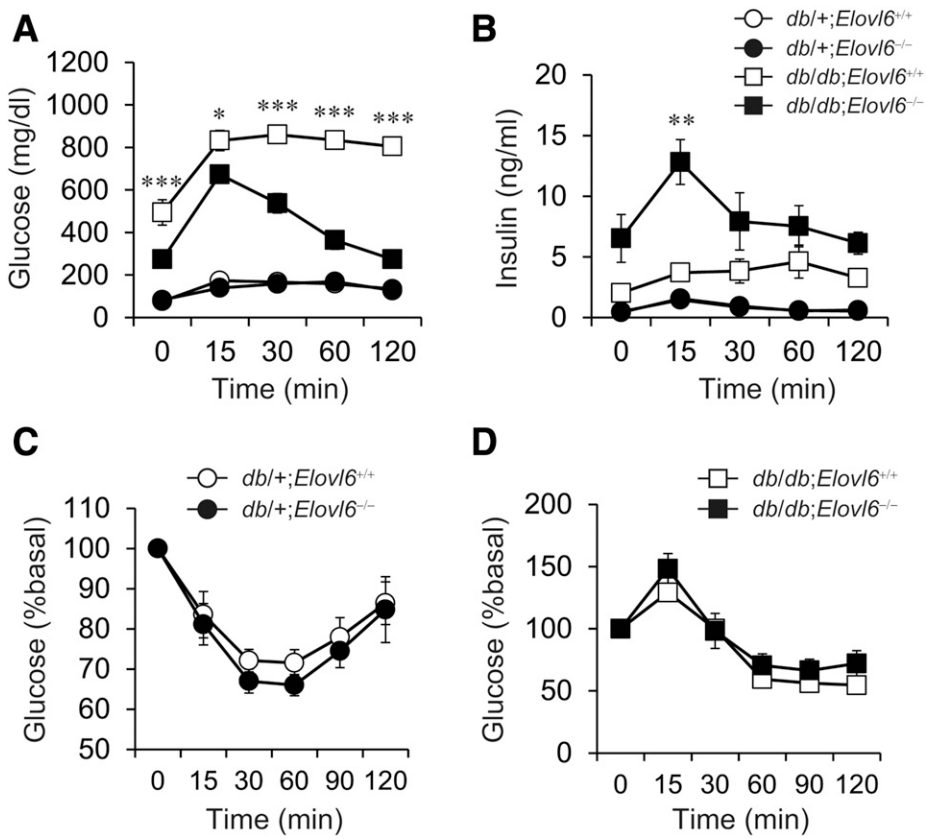

Figure 2—Elovl6 deficiency in *db/db* mice improves glucose intolerance without affecting insulin sensitivity. Oral glucose tolerance tests (1 g/kg) were performed on 12-week-old mice after 16 h of fasting. Blood glucose (*A*) and plasma insulin (*B*) concentrations in *db*/+;*Elovl6*$^{+/+}$, *db*/+;*Elovl6*$^{-/-}$, *db/db*;*Elovl6*$^{+/+}$, and *db/db*;*Elovl6*$^{-/-}$ mice (n = 5–8). *C*: Blood glucose levels in 8-week-old *db*/+;*Elovl6*$^{+/+}$ and *db*/+;*Elovl6*$^{-/-}$ mice administered intraperitoneally with insulin (0.5 units/kg) after 4 h of fasting (n = 8). *D*: Blood glucose concentrations in 8-week-old *db/db*;*Elovl6*$^{+/+}$ and *db/db*;*Elovl6*$^{-/-}$ mice administered intraperitoneally with insulin (2.0 units/kg) after 16 h of fasting (n = 9). Data are presented as means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001.

increase in lipid droplets in *db/db*;*Elovl6*$^{-/-}$ mice compared with *db/db*;*Elovl6*$^{+/+}$ mice (Supplementary Fig. 1*A*). Hepatic gene expression analysis showed a consistent increase in glucokinase (*Gck*) and stearoyl-CoA desaturase 1 (*Scd1*) expression in *db/db*;*Elovl6*$^{-/-}$ mice, indicating increased glycolysis and lipogenesis (Supplementary Fig. 1*B*). In addition, Elovl6 deficiency reduced glucose-6-phosphatase (*G6pc*) mRNA levels in both *db*/+ and *db/db* mice, consistent with a reduction in blood glucose levels (Supplementary Fig. 1*B*). Histological examination of eWAT sections revealed that Elovl6 deficiency in *db/db* mice did not improve adipose tissue inflammation (Supplementary Fig. 2*A*). Real-time PCR analysis showed that expression levels of lipogenic and proinflammatory genes in the eWAT of *db/db*;*Elovl6*$^{+/+}$ and *db/db*;*Elovl6*$^{-/-}$ mice were similar (Supplementary Fig. 2*B*). These results demonstrated that amelioration of T2D progression by Elovl6 deficiency in *db/db* mice was not caused by reduced lipid accumulation and inflammation in liver and WAT. However, as a consequence of the greater steatosis observed in *db/db*;*Elovl6*$^{-/-}$ mice, a more efficient FA storage could improve FA sequestration and reduce β-cell lipotoxicity.

Elovl6 Deficiency Enhances β-Cell Mass Expansion in Response to Increased Insulin Demand in *db/db* Mice

Pancreatic islets isolated from *db/db*;*Elovl6*$^{-/-}$ mice were morphologically compared with those from *db*/+;*Elovl6*$^{+/+}$, *db*/+;*Elovl6*$^{-/-}$, and *db/db*;*Elovl6*$^{+/+}$ mice. The number (Fig. 3*A*) and size (Fig. 3*B*) of islets were both greater in *db/db*;*Elovl6*$^{-/-}$ mice than in *db/db*;*Elovl6*$^{+/+}$ mice. Histological examination indicated that the islets in *db/db*;*Elovl6*$^{-/-}$ mice were markedly enlarged compared with those in *db/db*;*Elovl6*$^{+/+}$ mice (Fig. 3*C*). Immunostaining of pancreatic sections from 16-week-old animals with antibodies to insulin and glucagon revealed that Elovl6 deficiency in *db/db* mice resulted in a significant increase in β-cell mass, whereas β-cell mass was smaller in *db/db* mice than in *db*/+;*Elovl6*$^{+/+}$ mice (Fig. 3*D*). In contrast, we did not detect any apparent differences in scattered α-cell distribution between *db/db*;*Elovl6*$^{+/+}$ and *db/db*;*Elovl6*$^{-/-}$ mice.

To elucidate how *Elovl6* deletion may enlarge β-cell mass, we studied β-cell proliferation and apoptosis. To evaluate β-cell proliferation, mice were administered BrdU. The number of BrdU-positive cells was ∼2.5-fold greater in islets from *db/db*;*Elovl6*$^{-/-}$ mice than in those from

Table 1—Phenotypic comparison of *db/+;Elovl6*$^{+/+}$, *db/+;Elovl6*$^{-/-}$, *db/db;Elovl6*$^{+/+}$, and *db/db;Elovl6*$^{-/-}$ mice at 16 weeks of age

	db/+;Elovl6$^{+/+}$ (n = 8)	*db/+;Elovl6*$^{-/-}$ (n = 10)	*db/db;Elovl6*$^{+/+}$ (n = 11)	*db/db;Elovl6*$^{-/-}$ (n = 13)
BW (g)	32.1 ± 0.8	27.1 ± 0.8	50.6 ± 1.6	51.3 ± 1.0
Liver weight (% BW)	3.5 ± 0.1	3.9 ± 0.1##	6.1 ± 0.5	8.9 ± 0.2***
eWAT weight (% BW)	2.1 ± 0.1	2.0 ± 0.2	5.1 ± 0.3	4.2 ± 0.2**
Plasma glucose				
Fasting (mg/dL)	65.4 ± 5.3	57.3 ± 7.8	470.2 ± 35.4	204.5 ± 13.5***
Refed (mg/dL)	195.2 ± 17.9	192.2 ± 19.2	621.5 ± 38.9	350.8 ± 24.8***
Plasma insulin				
Fasting (ng/mL)	0.37 ± 0.17	0.27 ± 0.11	2.6 ± 0.5	2.7 ± 0.6
Refed (ng/mL)	4.9 ± 0.7	4.5 ± 0.8	16.2 ± 4.0	82.6 ± 9.1***
Plasma				
FFA (mmol/L)	0.81 ± 0.10	0.75 ± 0.04	0.93 ± 0.06	0.74 ± 0.03**
TG (mg/dL)	75.5 ± 11.7	105.8 ± 11.7	114.8 ± 15.2	69.1 ± 4.0**
T-Cho (mg/dL)	126.9 ± 5.5	106.2 ± 18.7	192.4 ± 13.1	204.4 ± 14.3
HDL-Cho (mg/dL)	52.0 ± 4.4	70.0 ± 8.4	48.0 ± 6.0	40.0 ± 2.3
Non–HDL-Cho (mg/dL)	68.8 ± 7.3	36.2 ± 11.3	144.4 ± 17.0	164.4 ± 14.1
Liver				
TG (mg/dL)	23.6 ± 3.0	26.2 ± 5.2	52.1 ± 4.5	76.8 ± 6.6**
T-Cho (mg/dL)	2.8 ± 0.5	2.3 ± 0.1	4.2 ± 0.7	5.3 ± 0.7

Values are presented as means ± SEM (n = 8–13 per group). ##$P < 0.01$ vs. *db/db;Elovl6*$^{+/+}$. **$P < 0.01$ vs. *db/db;Elovl6*$^{+/+}$. ***$P < 0.001$ vs. *db/db;Elovl6*$^{+/+}$.

db/db;Elovl6$^{+/+}$ mice (Fig. 3*E*). TUNEL staining demonstrated that β-cell apoptosis was ~4.3-fold greater in *db/db;Elovl6*$^{+/+}$ mice than in *db/+;Elovl6*$^{+/+}$ mice (Fig. 3*F*). *Elovl6* deletion, however, dramatically reduced β-cell apoptosis in *db/db* mice to levels observed in *db/+;Elovl6*$^{+/+}$ and *db/+; Elovl6*$^{-/-}$ mice. Therefore, islet hyperplasia in *db/db;Elovl6*$^{-/-}$ mice was a consequence of both increased proliferation and reduced apoptosis of β-cells.

Elovl6 Deficiency Improves β-Cell Function in Pancreatic Islets of *db/db* Mice

To evaluate the effects of Elovl6 deficiency on insulin secretion ex vivo, pancreatic islets were isolated from *db/+;Elovl6*$^{+/+}$, *db/+;Elovl6*$^{-/-}$, *db/db;Elovl6*$^{+/+}$, and *db/db;Elovl6*$^{-/-}$ mice. Insulin secretion from *db/db;Elovl6*$^{+/+}$ islets in response to high glucose was blunted (Fig. 4*A*). Elovl6 deficiency enhanced glucose-stimulated insulin secretion (GSIS) in *db/db* islets. Elovl6 deficiency also showed the tendency to enhance KCl-stimulated insulin secretion in both *db/+* and *db/db* islets. The insulin content of *db/db;Elovl6*$^{+/+}$ islets was significantly lower than that of *db/+;Elovl6*$^{+/+}$ islets, whereas the reduction in insulin was prevented by Elovl6 deficiency (Fig. 4*B*). These results suggested that Elovl6 deficiency improved impaired GSIS from *db/db;Elovl6*$^{-/-}$ islets, which contributed to improvement of glucose intolerance observed in *db/db* mice.

Elovl6 Deficiency Alters FA Composition in the Islets of *db/db* Mice

Because excessive lipid accumulation in pancreatic β-cells is known to be associated with lipotoxicity and reduces insulin secretion, we further determined islet TG and Cho contents. The TG content of *db/+;Elovl6*$^{-/-}$ islets was slightly but significantly lower than that of the *db/+;Elovl6*$^{+/+}$ islets (Fig. 4*C*). The TG content of *db/db;Elovl6*$^{+/+}$ islets was markedly higher than that of *db/+;Elovl6*$^{+/+}$ islets. Conversely, the TG content of *db/db;Elovl6*$^{-/-}$ islets was markedly lower than that of *db/db;Elovl6*$^{+/+}$ islets and similar to that of *db/+;Elovl6*$^{+/+}$ and *db/+;Elovl6*$^{-/-}$ islets. No significant difference was found for Cho contents between the four genotypes (Supplementary Fig. 4).

To further estimate the effect of the diabetic state and Elovl6 deficiency on FA profile in pancreatic islets, we analyzed the FA composition of islets isolated from *db/+;Elovl6*$^{+/+}$, *db/+;Elovl6*$^{-/-}$, *db/db;Elovl6*$^{+/+}$, and *db/db;Elovl6*$^{-/-}$ mice (Fig. 4*D*). The difference in FA composition between *db/+;Elovl6*$^{+/+}$ and *db/+;Elovl6*$^{-/-}$ mice was not significant. Compared with *db/+;Elovl6*$^{+/+}$ mice, the islet FA composition in *db/db;Elovl6*$^{+/+}$ mice showed a significant decrease in stearate and an increase in palmitoleate and oleate composition. Compared with *db/db;Elovl6*$^{+/+}$ islets, there was a marked reduction in oleate composition of *db/db;Elovl6*$^{-/-}$ islets, which was similar to that observed in *db/+;Elovl6*$^{+/+}$ and *db/+;Elovl6*$^{-/-}$ controls. The ratio of MUFAs in *db/db;Elovl6*$^{-/-}$ islets was consistently lower than that in *db/db;Elovl6*$^{+/+}$ islets (Fig. 4*D*, inset). We also analyzed the plasma FA composition of *db/+;Elovl6*$^{+/+}$, *db/+;Elovl6*$^{-/-}$, *db/db;Elovl6*$^{+/+}$, and *db/db;Elovl6*$^{-/-}$ mice (Supplementary Fig. 5). We found that the changes in plasma FA composition resulting from Elovl6 deletion in *db/db* mice were different from those in islets: *db/db;Elovl6*$^{-/-}$ mice had decreased levels of stearate and oleate and increased levels of palmitate and palmitoleate in plasma. These results suggested that the modulation of intracellular FA composition in β-cells by Elovl6 was associated with β-cell dysfunction and that Elovl6 deficiency could play a positive role in β-cell mass and function

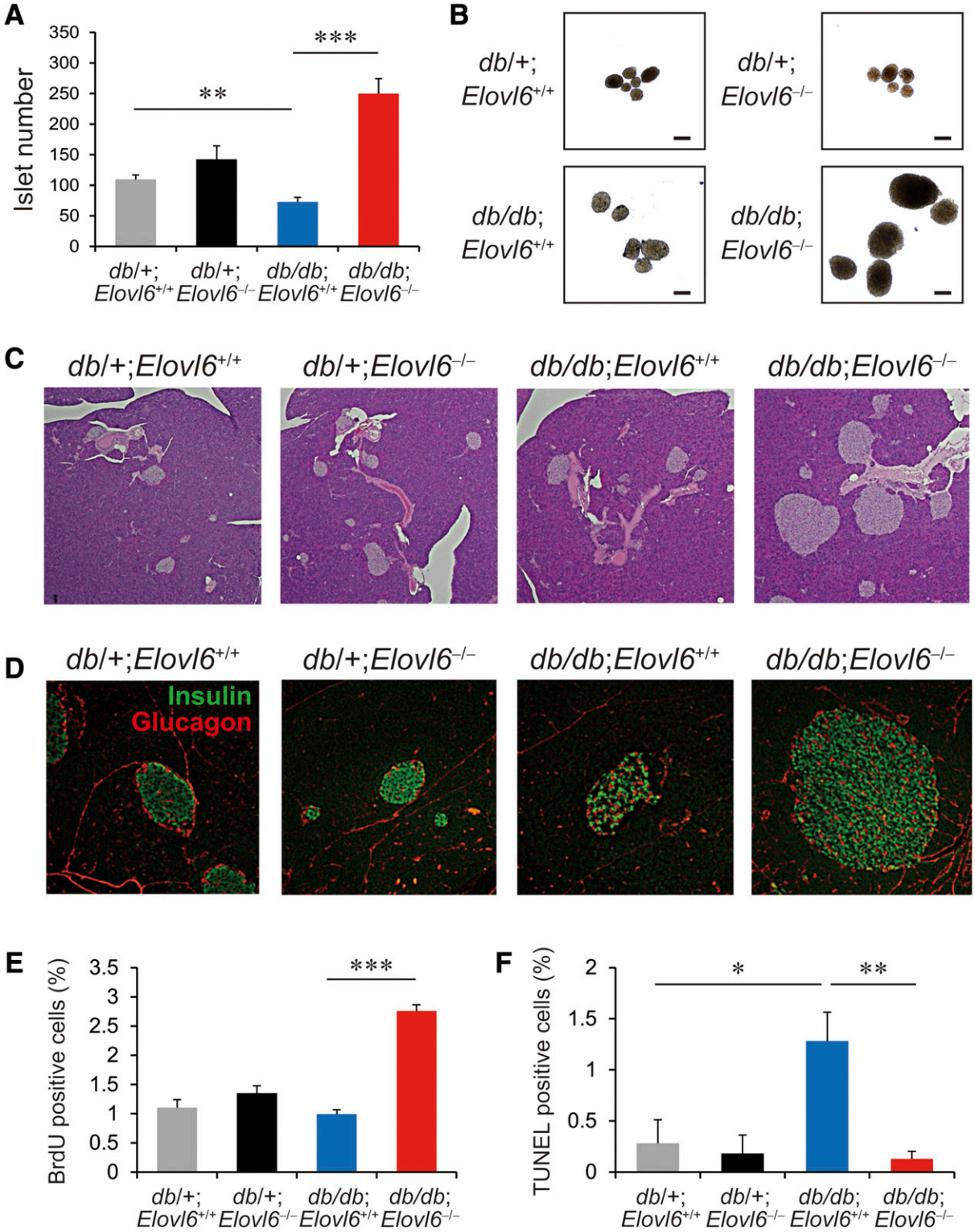

Figure 3—Elovl6 deficiency increases pancreatic islet numbers and expands β-cell mass in *db/db* mice. *A*: Pancreatic islets were isolated from *db/+;Elovl6*$^{+/+}$, *db/+;Elovl6*$^{-/-}$, *db/db;Elovl6*$^{+/+}$, and *db/db;Elovl6*$^{-/-}$ mice at 12 weeks of age and counted manually (n = 12–14). *B*: Representative images of isolated islets that were visualized by microscopy. Scale bars: 100 μm. Representative images of pancreatic sections from 16-week-old *db/+;Elovl6*$^{+/+}$, *db/+;Elovl6*$^{-/-}$, *db/db;Elovl6*$^{+/+}$, and *db/db;Elovl6*$^{-/-}$ mice that were stained with hematoxylin and eosin (*C*) or antibodies to insulin (green) and glucagon (red) (*D*). *E*: BrdU-positive cells within islet areas were detected by immunohistochemistry, and the proportion of BrdU-positive proliferating cells is shown as the percentage of the total number of insulin-positive cells in sections. For continuous BrdU labeling in mice, BrdU was diluted in drinking water to a concentration of 1.0 mg/mL and administered for consumption ad libitum for 5 days. *F*: TUNEL staining was performed, and the proportion of TUNEL-positive cells is shown as the percentage of the total number of insulin-positive cells in sections. More than 100 islets were counted in each mouse for panels *E* and *F*. Data are presented as means ± SEM. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

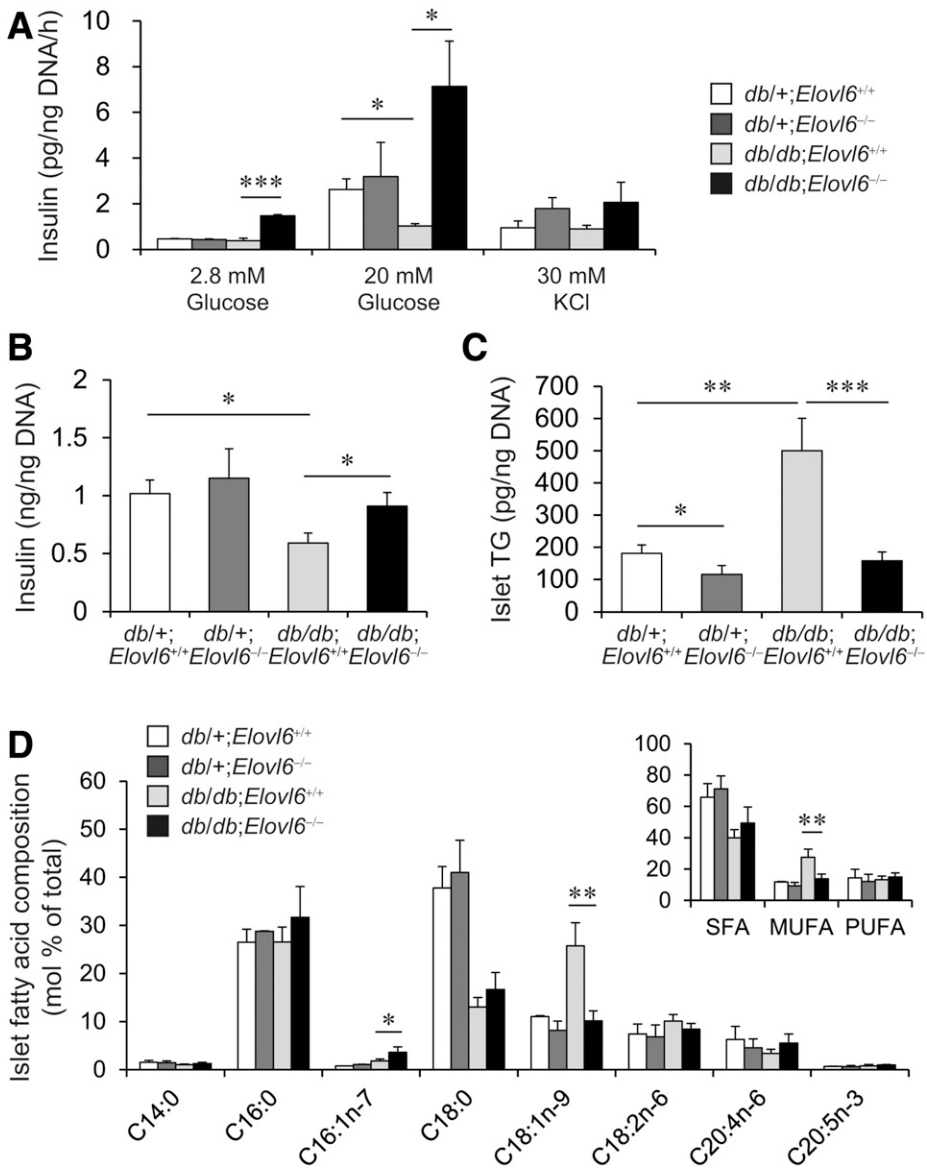

Figure 4—Elovl6 deficiency in *db/db* mice enhances insulin secretion, increases insulin content, and decreases TG and oleic acid levels in pancreatic islets. *A*: Glucose- or KCl-stimulated insulin secretion in islets isolated from *db/+;Elovl6*$^{+/+}$, *db/+;Elovl6*$^{-/-}$, *db/db;Elovl6*$^{+/+}$, and *db/db;Elovl6*$^{-/-}$ mice at 12 weeks of age. Ten isolated islets from each group (six batches in each genotype) were incubated in Krebs-Ringer bicarbonate HEPES buffer containing 1% BSA and 2.8 mmol/L glucose, 20 mmol/L glucose, or 2.8 mmol/L glucose with 30 mmol/L KCl for 30 min, and insulin secretion per DNA was measured (n = 7–8). *B*: Insulin content in isolated islets from *db/+;Elovl6*$^{+/+}$, *db/+;Elovl6*$^{-/-}$, *db/db;Elovl6*$^{+/+}$, and *db/db;Elovl6*$^{-/-}$ mice at 12 weeks of age (n = 12). *C*: TG content in isolated islets from *db/+;Elovl6*$^{+/+}$, *db/+;Elovl6*$^{-/-}$, *db/db;Elovl6*$^{+/+}$, and *db/db;Elovl6*$^{-/-}$ mice at 12 weeks of age (n = 12). *D*: FA composition in isolated islets from *db/+;Elovl6*$^{+/+}$ (n = 3), *db/+;Elovl6*$^{-/-}$ (n = 3), *db/db;Elovl6*$^{+/+}$ (n = 4), and *db/db;Elovl6*$^{-/-}$ (n = 4) mice at 12 weeks of age (n = 3–4). Data are presented as means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001.

through a reduction of oleate and TG content under diabetic conditions.

Elovl6 Deficiency Reduces Expression of Genes for Inflammation and Cell Death in Pancreatic Islets of *db/db* Mice

To provide insight into how Elovl6 deficiency might alter the gene expression profile to preserve β-cell function and mass, we analyzed isolated islets for genes encoding proteins with functions in FA and glucose metabolism, insulin production, cell proliferation, apoptosis, inflammation, and ER stress (Fig. 5). Expression levels of lipogenic genes, including *Elovl6*, *Scd1*, and *Scd2*, were significantly lower in *db/db;Elovl6*$^{+/+}$ islets than in control *db/+;Elovl6*$^{+/+}$ islets (Fig. 5*A*). Expression levels of *Scd1* and *Scd2* were similarly decreased in *db/db;Elovl6*$^{-/-}$ islets, whereas the expression of sterol regulatory element binding protein 1c (*Srebf1c*) and its target gene uncoupling protein-2 (*Ucp2*) tended to decrease in *db/db;Elovl6*$^{-/-}$ islets compared with *db/db;Elovl6*$^{+/+}$ islets. Expression of peroxisome proliferator

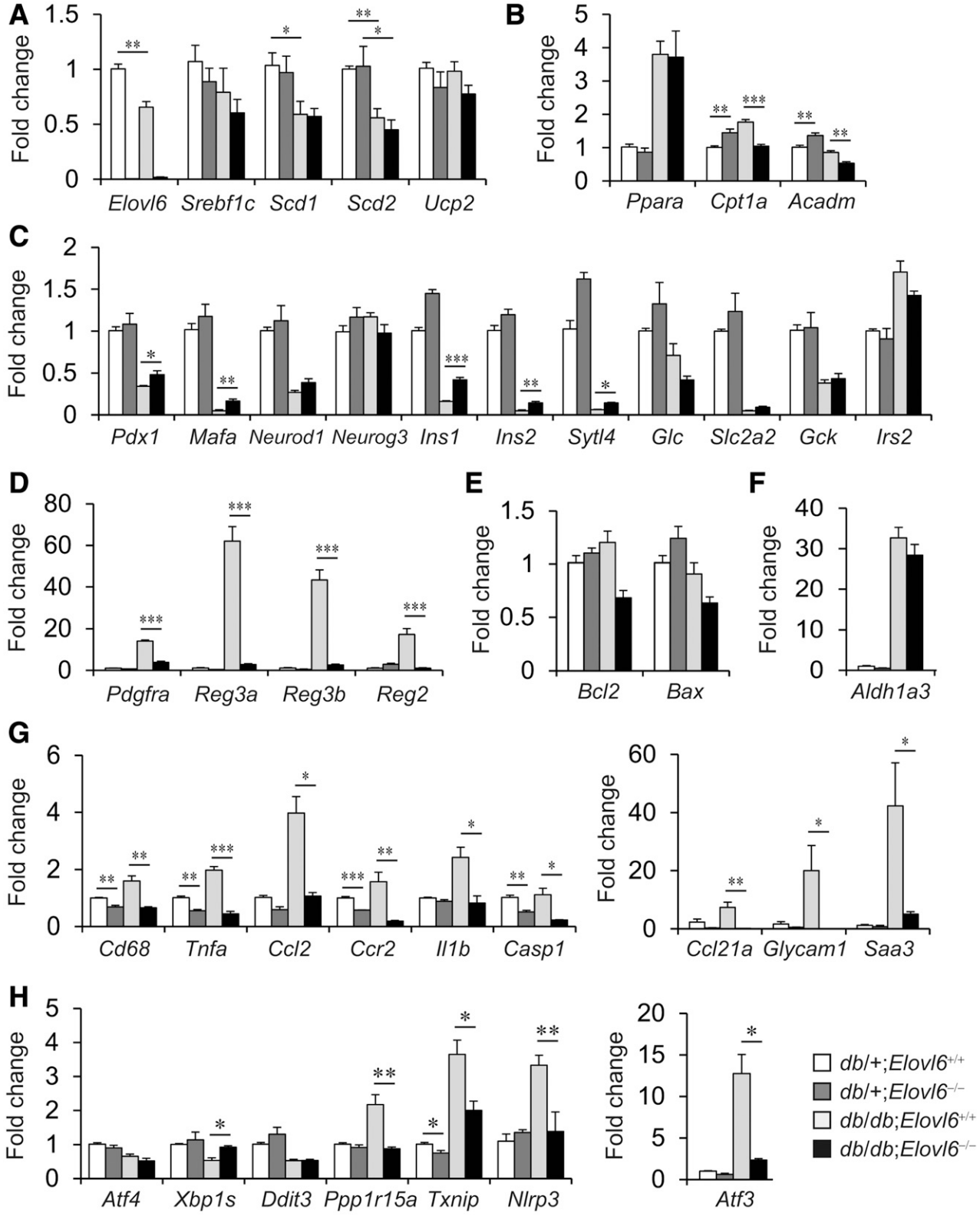

Figure 5—Effect of Elovl6 deficiency on islet gene expression in *db/db* mice. Real-time RT-PCR analysis to measure mRNA levels of genes involved in FA metabolism (*A*), β-cell oxidation (*B*), β-cell function (*C*), β-cell proliferation and islet regeneration (*D*), apoptosis (*E*), dedifferentiation (*F*), inflammation (*G*), and stress response (*H*) in isolated islets from *db*/+;*Elovl6*$^{+/+}$, *db*/+;*Elovl6*$^{-/-}$, *db/db*;*Elovl6*$^{+/+}$, and *db/db*;*Elovl6*$^{-/-}$ mice at 12 weeks of age (n = 7–8). Expression values were normalized to mRNA levels of cyclophilin and are presented as fold-induction compared with control *db*/+;*Elovl6*$^{+/+}$ islets. Data are presented as means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001.

activated receptor-α (*Ppara*), a key regulator of FA oxidation, was elevated similarly in both *db/db*;*Elovl6*$^{+/+}$ and *db/db*;*Elovl6*$^{-/-}$ islets (Fig. 5*B*). However, expression levels of PPARα target genes involved in β-oxidation, such as carnitine palmitoyltransferase 1a (*Cpt1a*) and acyl-CoA dehydrogenase, medium chain (*Acadm*), were significantly decreased in *db/db*;*Elovl6*$^{-/-}$ islets compared with *db/db*;*Elovl6*$^{+/+}$ islets, suggesting the difference in TG accumulation between *db/db*;*Elovl6*$^{+/+}$ islet and *db/db*;*Elovl6*$^{-/-}$ islet was not caused by the difference in the capacity for islet FA oxidation.

Expression levels of master transcription factors regulating insulin gene expression and GSIS in mature β-cells, such as pancreatic and duodenal homeobox 1 (*Pdx1*), musculoaponeurotic fibrosarcoma oncogene family A (*MafA*), and neurogenic differentiation 1 (*Neurod1*), as well as insulin 1 (*Ins1*), *Ins2*, and synaptotagmin-like 4 (*Sytl4*), a crucial component of the insulin secretion, were coordinately repressed in *db/db*;*Elovl6*$^{+/+}$ islets compared with those in *db/+*;*Elovl6*$^{+/+}$ islets (Fig. 5*C*). Conversely, expression of these genes was not completely but was significantly restored in *db/db*;*Elovl6*$^{-/-}$ islets compared with *db/db*;*Elovl6*$^{+/+}$ islets, although the expression level of neurogenin 3 (*Neurog3*), the islet-defining factor, is similar in all groups. Expression levels of glucose transporter 2 (*Slc2a2*), *Gck*, and insulin receptor substrate 2 (*Irs2*), genes important for glucose metabolism and β-cell mass expansion, were similar between *db/db*;*Elovl6*$^{+/+}$ and *db/db*;*Elovl6*$^{-/-}$ islets (Fig. 5*C*).

Expression levels of genes involved in β-cell proliferation and islet regeneration, such as platelet-derived growth factor receptor, α-polypeptide (*Pdgfra*), regenerating islet-derived 3-α (*Reg3a*), *Reg3b*, and *Reg2*, were markedly higher in *db/db*;*Elovl6*$^{+/+}$ islets than in *db/+*;*Elovl6*$^{+/+}$ islets (Fig. 5*D*). These increases in *db/db*;*Elovl6*$^{+/+}$ islets were cancelled in *db/db*;*Elovl6*$^{-/-}$ islets. Expression levels of apoptosis-related genes, such as B-cell leukemia/lymphoma 2 (*Bcl2*) and BCL2-associated X protein (*Bax*), were similar in all groups (Fig. 5*E*). Recent animal and human studies show that β-cells become dedifferentiated in diabetes, reverting to a progenitor-like stage and partly converting to other endocrine cell types (28,29), and that aldehyde dehydrogenase 1A3 (Aldh1a3) is a newly identified biomarker of dysfunctional β-cells (30). We tested the expression of *Aldh1a3* and found that it was elevated similarly in both *db/db*;*Elovl6*$^{+/+}$ and *db/db*;*Elovl6*$^{-/-}$ islets (Fig. 5*F*), indicating Elovl6 deficiency did not protect from β-cell dedifferentiation in *db/db* mice.

Expression levels of the macrophage marker CD68 antigen (*Cd68*), inflammatory cytokines and their receptors, such as tumor necrosis factor-α (*Tnfα*), *Ccl2*, chemokine (C-C motif) receptor 2 (*Ccr2*), interleukin 1β (*Il1β*), chemokine (C-C motif) ligand 21A (*Ccl21a*), the L-selectin ligand glycosylation–dependent cell adhesion molecule 1 (*Glycam1*), and the acute-phase protein serum amyloid A 3 (*Saa3*), were higher in *db/db*;*Elovl6*$^{+/+}$ islets than in control *db/+*;*Elovl6*$^{+/+}$ islets (Fig. 5*G*). However, Elovl6 deficiency significantly decreased the expression of these proinflammatory genes in both *db/+* and *db/db* islets. Moreover, Elovl6 deficiency significantly decreased the expression of caspase-1 (*Casp1*), a gene involved in inflammation and cell death, in both *db/+* and *db/db* islets (Fig. 5*G*). Decreased pancreatic islet inflammation in *db/db*;*Elovl6*$^{-/-}$ mice was further examined by immunohistochemistry (Supplementary Fig. 6). The data indicated that the number of infiltrating Mac3-positive macrophages was markedly greater in *db/db*;*Elovl6*$^{+/+}$ mice than in *db/+*;*Elovl6*$^{+/+}$ mice, whereas macrophage infiltration was protected in pancreatic islets of *db/db*;*Elovl6*$^{-/-}$ mice.

ER stress has been proposed as a mechanism for β-cell dysfunction and death in T2D (3,31–34). Chan et al. (35) reported that the expression of adaptive unfolded protein response (UPR) genes progressively declined in *db/db* islets and that the maintenance of the adaptive UPR is associated with β-cell compensation in obese mice. Consistent with this report, the expression levels of genes for ER stress, such as activating transcription factor 4 (*Atf4*) and DNA-damage inducible transcript 3 (*Ddit3*), were significantly decreased in both *db/db*;*Elovl6*$^{+/+}$ and *db/db*;*Elovl6*$^{-/-}$ islets compared with *db/+*;*Elovl6*$^{+/+}$ and *db/+*;*Elovl6*$^{-/-}$ islets (Fig. 5*H*). Expression of the spliced form of X-box binding protein 1 (*Xbp1s*), which controls the development and maintenance of ER, was also significantly decreased in *db/db*;*Elovl6*$^{+/+}$ islets yet was maintained in islets from *db/db*;*Elovl6*$^{-/-}$ mice. In contrast, the expression levels of genes for proapoptotic and terminal UPR, such as protein phosphatase 1 regulatory subunit 15a (*Ppp1r15a*), thioredoxin interacting protein (*Txnip*), and NLR family pyrin domain containing 3 (*Nlrp3*), and activating transcription factor 3 (*Atf3*), a stress-inducible transcription factor involved in modulating proinflammatory responses and apoptosis, were higher in *db/db*;*Elovl6*$^{+/+}$ islets than in control *db/+*;*Elovl6*$^{+/+}$ islets. Strikingly, the expression of these genes was markedly suppressed in *db/db*;*Elovl6*$^{-/-}$ islets compared with *db/db*;*Elovl6*$^{+/+}$ islets. These results demonstrate that Elovl6 deficiency partially recovers the adaptive UPR and suppresses the terminal UPR in *db/db* islets. Together, these results indicated that Elovl6 regulated both β-cell mass and insulin production potentially thorough modulation of the islet inflammation and ER stress in obese diabetic mice.

Oleate Impairs GSIS and Elovl6 Plays an Important Role in Palmitate-Induced Lipotoxicity in Pancreatic Islets

To test whether islet and β-cell function could be altered by changes in cellular FAs regulated by Elovl6, we evaluated the effects of palmitate and oleate on islets isolated from *Elovl6*$^{+/+}$ and *Elovl6*$^{-/-}$ mice. Consistent with many previous studies, *Elovl6*$^{+/+}$ islets treated with palmitate had increased cleaved caspase-3–positive cells, a marker of apoptosis (Fig. 6*A* and Supplementary Fig. 7*A*). However, this effect was significantly suppressed in *Elovl6*$^{-/-}$ islets. Meanwhile, oleate led to a mild increase in apoptosis in both *Elovl6*$^{+/+}$ and *Elovl6*$^{-/-}$ islets. This result is different from previous studies indicating that oleate did not induce β-cell apoptosis (9,13). The discrepancy is presumably because we used a much lower serum concentration (1%, in contrast to

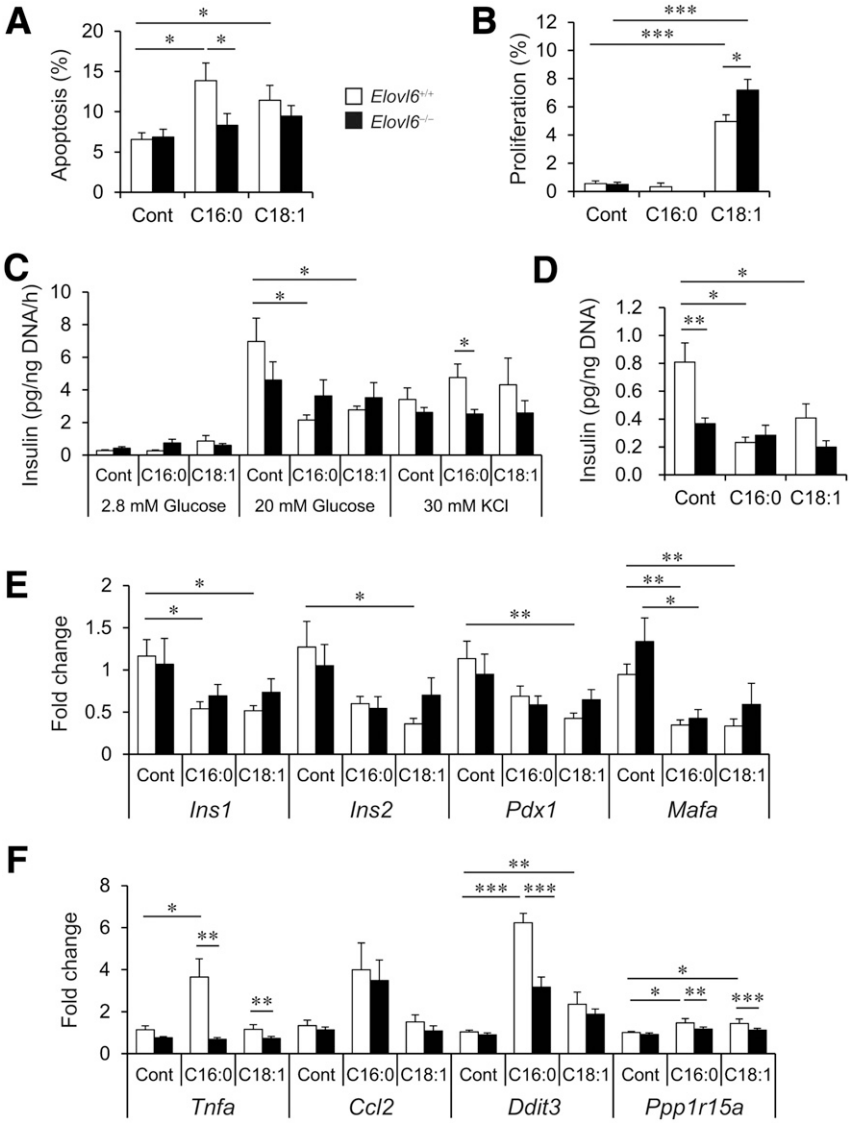

Figure 6—Oleate impairs β-cell function, and Elovl6 deficiency protects islets against palmitate-induced lipotoxicity. Effects of palmitate and oleate on apoptosis (*A*), proliferation (*B*), insulin secretion (*C*), insulin content (*D*), and mRNA expression (*E*) and inflammation and ER stress (*F*) in islets isolated from *Elovl6*$^{+/+}$ and *Elovl6*$^{-/-}$ mice treated without (control [Cont]) or with palmitate or oleate (500 μmol/L) for 48 h. *A*: β-Cell apoptosis revealed by double-staining with antibody to the cleaved form of caspase-3 and insulin. Percentage of cleaved caspase-3^{+} insulin^{+} cells over insulin^{+} cells was determined (n = 6–10). *B*: β-Cell proliferation assessed by staining for EdU, insulin, and DAPI. EdU incorporation in islet β-cells was visualized using a confocal microscope, and the percentage of proliferating β-cells (EdU^{+} insulin^{+} cells over insulin^{+} cells) was quantitated (n = 4–7). *C*: Glucose- or KCl-stimulated insulin secretion in islets isolated from *Elovl6*$^{+/+}$ and *Elovl6*$^{-/-}$ mice treated without (control) or with palmitate or oleate (500 μmol/L) for 48 h. Ten isolated islets from each group (six batches in each genotype) were incubated in Krebs-Ringer bicarbonate HEPES buffer containing 1% BSA and 2.8 mmol/L glucose, 20 mmol/L glucose, or 2.8 mmol/L glucose with 30 mmol/L KCl for 30 min, and insulin secretion per DNA was measured (n = 5–9). *D*: Insulin content in isolated islets from *Elovl6*$^{+/+}$ and *Elovl6*$^{-/-}$ mice without (control) or with palmitate or oleate (500 μmol/L) for 48 h (n = 6–7). mRNA expression of genes for β-cell function (*E*) and inflammation and ER stress (*F*) in isolated islets from *Elovl6*$^{+/+}$ and *Elovl6*$^{-/-}$ mice without (control) or with palmitate or oleate (500 μmol/L) for 48 h (n = 11–16). Data are shown as mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001.

10% in previous studies) in culture medium. β-Cell proliferation was not affected by palmitate but was strongly stimulated by oleate (Fig. 6*B* and Supplementary Fig. 7*B*). Consistent with this result, Moullé et al. (36) recently reported that lipid highly enriched in oleate stimulates β-cell proliferation both in vivo and ex vivo. Moreover, oleate-induced β-cell proliferation was accelerated by *Elovl6* deficiency. This effect could somehow mask the lipotoxic side of oleate in previous studies. Further studies will be required to identify the mechanism whereby oleate promotes β-cell proliferation.

We also evaluated the effects of palmitate and oleate on the insulin secretion of islets isolated from *Elovl6*$^{+/+}$ and *Elovl6*$^{-/-}$ mice. Basal insulin secretion was not affected by FAs and Elovl6 deficiency; however, GSIS was inhibited by the addition of palmitate or oleate in *Elovl6*$^{+/+}$ islets (Fig. 6*C*). Although the GSIS of *Elovl6*$^{-/-}$ islets tended to be

lower than that of *Elovl6*$^{+/+}$ islets, the suppressed GSIS by palmitate or oleate was not observed in *Elovl6*$^{-/-}$ islets. Insulin contents were also decreased by the addition of palmitate or oleate in *Elovl6*$^{+/+}$ islets (Fig. 6*D*). Insulin content of *Elovl6*$^{-/-}$ islets was significantly lower than that of *Elovl6*$^{+/+}$ islets. Insulin content of *Elovl6*$^{-/-}$ islets treated with palmitate or oleate was similar to that of *Elovl6*$^{+/+}$ islets.

Next, the effects of palmitate and oleate on mRNA expression in islets isolated from *Elovl6*$^{+/+}$ and *Elovl6*$^{-/-}$ mice were determined. *Elovl6*$^{+/+}$ islets treated with palmitate exhibited downregulation of *Ins1*, *Ins2*, *Pdx1*, and *Mafa* compared with control islets (Fig. 6*E*). Oleate treatment also led to a significant decrease in the expression of these β-cell–specific genes, indicating β-cell dysfunction was induced by both palmitate and oleate. *Elovl6*$^{-/-}$ islets treated with palmitate or oleate showed a significant decrease in the expression of these β-cell–specific genes to a similar extent as *Elovl6*$^{+/+}$ islets, indicating that protection of β-cell dysfunction by Elovl6 absence is not mediated through expression of these genes.

Because loss of β-cells was accompanied with increased inflammation and terminal UPR, we analyzed the expression of genes for inflammation and UPR (Fig. 6*F*). In *Elovl6*$^{+/+}$ islets, palmitate treatment significantly increased the expression of genes for inflammation (*Tnfa*, *Ccl2*) and UPR (*Ddit3*, *Ppp1r15a*). Oleate treatment, however, led only to a modest increase in *Ddit3* gene expression in *Elovl6*$^{+/+}$ islets, suggesting that exogenous oleate does not cause islet inflammation and cell death. These effects of palmitate were significantly attenuated in *Elovl6*$^{-/-}$ islets except for *Ccl2*. Oleate-treated *Elovl6*$^{-/-}$ islets showed a slight but significant decrease of *Tnfa* and *Ppp1r15a* expression compared with *Elovl6*$^{+/+}$ islets. Administration with palmitate or oleate also impaired β-cell functions of MIN6 cells, accompanied by decreased *Pdx1*, *Mafa*, and *Ins1* expression and increased *Ccl2*, *Ddit3*, and *Atf3* expression (Supplementary Fig. 8), indicating other evidence that oleate was more harmful than palmitate in β-cells. Collectively, these findings demonstrate that oleate contributes to impaired GSIS and palmitate contributes to β-cell dysfunction, inflammation, and cellular stress responses and that palmitate lipotoxicity can be partly modified by Elovl6 activity. These results suggest that susceptibility to FA-induced inflammation and ER stress is reduced in islets with a reduced Elovl6 activity.

DISCUSSION

In the current study, we demonstrated that *Elovl6* deletion in *db/db* mice significantly reduced circulating blood glucose levels without affecting obesity or insulin resistance. We observed that compared with *db/db*;*Elovl6*$^{+/+}$ mice, *db/db*;*Elovl6*$^{-/-}$ mice had improved glucose tolerance, enhanced GSIS, an adaptive increase in insulin, and markedly increased β-cell mass associated with increased proliferation and decreased apoptosis. Phenotypic changes in *db/db*;*Elovl6*$^{-/-}$ mice involve attenuated islet inflammation accompanied with decreased islet oleate and TG levels. As far as we know, this is the first study to test the effect of Elovl6 deficiency of function in a T2D model of islet failure, and our findings indicated that Elovl6-mediated modulation of intracellular FA metabolism in β-cells was essential in preventing the toxic effects of FAs and preserving proper β-cell function, which might contribute to the expansion of β-cell mass in obesity.

Elucidation of the factors triggering islet inflammation in T2D is of emerging importance. Previous studies demonstrated that macrophage recruitment and inflammation in pancreatic islets played a key role in β-cell dysfunction in patients with T2D and animal T2D models (37–39). β-Cells respond to endogenous stimuli, including FAs, by initiating macrophage recruitment via production of chemokines, and subsequent interactions between β-cells and macrophages perpetuate inflammatory processes within islets that lead to β-cell dysfunction (3,39,40). Importantly, *db/db*;*Elovl6*$^{-/-}$ islets displayed a marked reduction in oleate and MUFA content without increasing SFA compared with *db/db*;*Elovl6*$^{+/+}$ islets. Oleate-induced β-cell dysfunction was observed in β-cell lines and pancreatic islets that exhibited impaired GSIS after oleate exposure (41–43). Busch et al. (40) performed a microarray analysis of palmitate- and oleate-exposed MIN6 cells and found that the expression of proinflammatory genes encoding chemokines and mediators of the acute phase response were increased by both FAs. In line with these previous reports, we confirmed that oleate contributed to β-cell dysfunction within islets and MIN6 cells, suggesting that the combination of oleate with various stresses, such as high glucose, oxidative stress, and mitochondrial dysfunction, worsens β-cell function and death by cytokine production and promotion of inflammation.

Whether the effect of oleate on β-cell dysfunction is mediated by oleate itself or via intermediate products of oleate metabolism remains to be determined. The underlying mechanism of oleate-associated toxic effects in β-cells remains unknown, but Elovl6-mediated compositional changes in specific FAs may affect cellular functions important for T2D development. Previous studies showed that TG content in pancreatic islets was associated with impaired β-cell secretory capacity and dysfunction (5,6). A potential mechanism of reduction in islet TG in *db/db*;*Elovl6*$^{-/-}$ mice is suppression of TG synthesis secondary to decreased oleate levels in the islets. Because oleate is a good substrate for diacylglycerol acyltransferase, the rate-limiting step in TG synthesis, excessive TG accumulation with oleate might contribute to β-cell dysfunction. The inhibition of conversion from palmitate to stearate resulting in a reduction in oleate levels in β-cells is likely a major contributor to decreased islet TG content and resolved inflammation in the current study.

Chronic exposure of palmitate to islets or β-cell lines has long been known to cause lipotoxicity leading to β-cell apoptosis (14,31,44). Our current studies clearly demonstrate that Elovl6 deficiency did not cause accumulation of

palmitate in islets, but significantly decreased the sensitivity and extent to which palmitate induced inflammation and ER stress, suggesting that FA synthesized by Elovl6, rather than palmitate itself, exerts inflammation and terminal UPR. Consistent with these results, Green et al. (45) reported that increased Elovl6 activity in INS-1 cells significantly increased palmitate-induced ER stress and apoptosis. The underlying mechanism whereby long-chain FAs more than C18 or longer could exert a more toxic effect than C16 is currently unknown. The data from exogenous treatment with oleate in isolated islets and MIN6 cells suggest that lipotoxicity by oleate is not very simple and that the intracellular trafficking of FA or the process by Elovl6 might be important. Because previous studies have shown that increased ceramide formation plays a key role in lipotoxicity-induced apoptosis and loss of function of pancreatic β-cells (7,46,47), Elovl6 may lead to inflammation and terminal UPR through the ceramide signaling pathway.

The current study implicates multiple pathways in long-chain FA–mediated β-cell lipotoxicity. β-Cell dysfunction is mediated by palmitate or oleate through impaired gene expression involving insulin secretion, which highlights underrecognized oleate and TGs lipotoxicity, and is partly ameliorated by Elovl6 absence as estimated by decreases in islet oleate and TGs. Meanwhile, inflammation and ER stress leading to β-cell mass loss involving apoptosis and cell proliferation is essentially cancelled by Elovl6 absence (Fig. 7). These results at least partly explain previously described complex effects of long-chain FAs on β-cells. It can be concluded that endogenous conversion of palmitate to stearate by Elovl6 and presumably subsequent reaction is critical for palmitate-mediated lipotoxic action.

Our results support the hypothesis that *Elovl6* deletion protects against β-cell failure in *db/db* mice as a genetic model of T2D. However, because *Elovl6* deletion was not targeted to the pancreas and was achieved in all tissues, the possibility remains that *Elovl6* deletion might affect the FA profile in other tissues to affect overall organismal metabolism and, therefore, β-cell function. Indeed, we previously

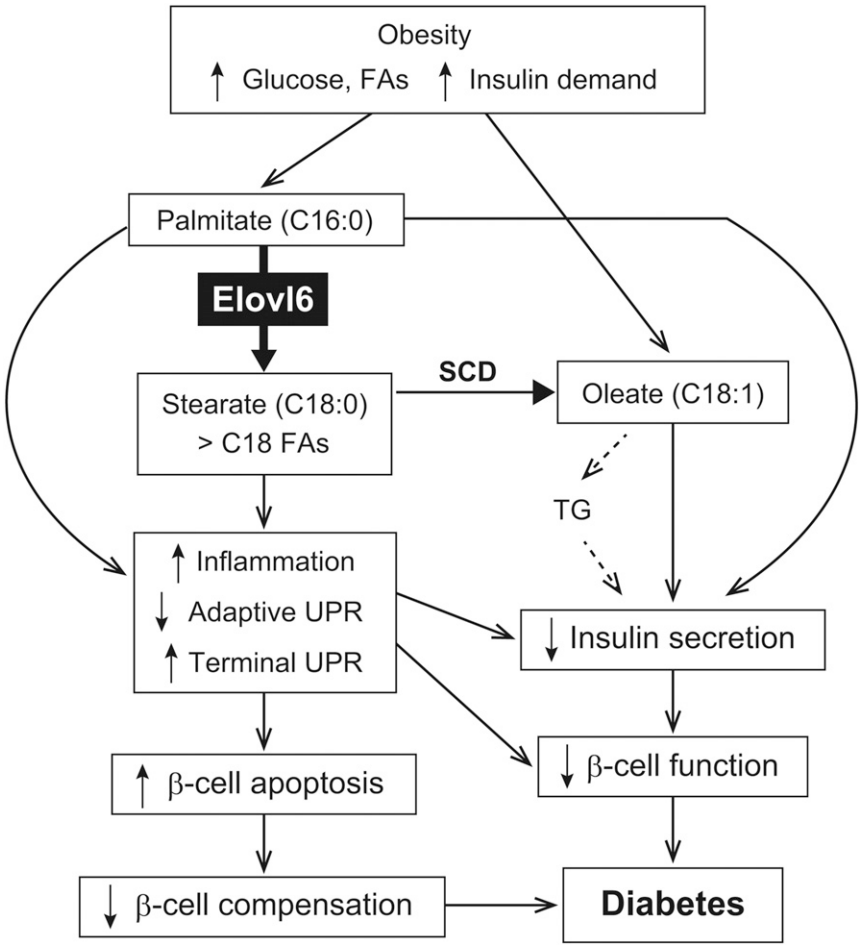

Figure 7—Proposed mechanisms by which Elovl6 modulates β-cell lipotoxicity during progression to T2D. Obesity and its associated metabolic changes, including glucose intolerance, insulin resistance, and increased FFA delivery, lead to palmitate- and oleate-induced β-cell dysfunction, inflammation, ER stress, and apoptosis in pancreatic islets. The loss of Elovl6 function inhibits the elongation of palmitate and synthesis of oleate, resulting in protection against impaired insulin secretion, inflammation, terminal UPR, and β-cell apoptosis in pancreatic islets; maintenance of β-cell compensation; and prevention of T2D.

showed that Elovl6 deficiency altered FA composition in serum and various organs and affected a wide range of cellular functions and the disease progression (19,21,48–50). We assume the changes of plasma FA composition might influence the FA composition of islets; however, changes in FA composition of *db/db*;*Elovl6*$^{-/-}$ islets were different from changes observed in plasma: a marked reduction in oleate component without a reduction in stearate and an increase in palmitate, compared with *db/db*;*Elovl6*$^{+/+}$ islets. Thus, these results suggested that the Elovl6-regulated FA chain elongation from C16 to C18 and subsequent SCD-mediated C18 desaturation in β-cells plays a pivotal role for maintaining proper function and preventing the toxic effects of FAs. Future studies in mice with conditional deletion of *Elovl6* in pancreatic β-cells and other cell types are under way and necessary to further delineate its role in T2D.

In summary, we provide evidence showing the beneficial effect of Elovl6 loss in *db/db* mice supporting previously not fully appreciated β-cell toxic effect of oleate. The results of the current study, combined with our previous findings demonstrating that Elovl6 deficiency improved obesity-induced peripheral insulin resistance, suggest that limiting Elovl6 expression or activity in individuals during early diabetes or in those with metabolic syndrome might be beneficial for T2D prevention and treatment.

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Author Contributions. T.M. and H.Sh. designed the project. H.Z., T.M., Y.Nakan., K.M., and N.T. performed experiments. H.Z., T.M., Y.Nakan., T.Y., Y.O., S.-i.H., Y.T., Y.A., H.I., S.Y., H.Su., M.S., N.Yah., Y.Nakag., H.So., N.Yam., and H.Sh. analyzed and interpreted data. H.Z., T.M., and H.Sh. prepared the manuscript. All authors reviewed the manuscript. T.M. and H.Sh. are guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Hill JO. Understanding and addressing the epidemic of obesity: an energy balance perspective. Endocr Rev 2006;27:750–761

2. Roglic G, Unwin N, Bennett PH, et al. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. Diabetes Care 2005;28:2130–2135

3. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006; 116:1802–1812

4. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444:840–846

5. Unger RH. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. Diabetes 1995;44:863–870

6. van Herpen NA, Schrauwen-Hinderling VB. Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol Behav 2008;94:231–241

7. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proc Natl Acad Sci U S A 1998;95: 2498–2502

8. Maedler K, Spinas GA, Dyntar D, Moritz W, Kaiser N, Donath MY. Distinct effects of saturated and monounsaturated fatty acids on beta-cell turnover and function. Diabetes 2001;50:69–76

9. Maedler K, Oberholzer J, Bucher P, Spinas GA, Donath MY. Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. Diabetes 2003;52:726–733

10. Guo J, Zhu JX, Deng XH, et al. Palmitate-induced inhibition of insulin gene expression in rat islet β-cells involves the ceramide transport protein. Cell Physiol Biochem 2010;26:717–728

11. Boslem E, MacIntosh G, Preston AM, et al. A lipidomic screen of palmitate-treated MIN6 β-cells links sphingolipid metabolites with endoplasmic reticulum (ER) stress and impaired protein trafficking. Biochem J 2011;435:267–276

12. Manukyan L, Ubhayasekera SJ, Bergquist J, Sargsyan E, Bergsten P. Palmitate-induced impairments of β-cell function are linked with generation of specific ceramide species via acylation of sphingosine. Endocrinology 2015;156:802–812

13. Busch AK, Gurisik E, Cordery DV, et al. Increased fatty acid desaturation and enhanced expression of stearoyl coenzyme A desaturase protects pancreatic beta-cells from lipoapoptosis. Diabetes 2005;54:2917–2924

14. Kato T, Shimano H, Yamamoto T, et al. Palmitate impairs and eicosapentaenoate restores insulin secretion through regulation of SREBP-1c in pancreatic islets. Diabetes 2008;57:2382–2392

15. Jakobsson A, Westerberg R, Jacobsson A. Fatty acid elongases in mammals: their regulation and roles in metabolism. Prog Lipid Res 2006;45:237–249

16. Guillou H, Zadravec D, Martin PG, Jacobsson A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. Prog Lipid Res 2010;49:186–199

17. Moon YA, Shah NA, Mohapatra S, Warrington JA, Horton JD. Identification of a mammalian long chain fatty acyl elongase regulated by sterol regulatory element-binding proteins. J Biol Chem 2001;276:45358–45366

18. Matsuzaka T, Shimano H, Yahagi N, et al. Cloning and characterization of a mammalian fatty acyl-CoA elongase as a lipogenic enzyme regulated by SREBPs. J Lipid Res 2002;43:911–920

19. Matsuzaka T, Shimano H, Yahagi N, et al. Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. Nat Med 2007;13:1193–1202

20. Moon YA, Ochoa CR, Mitsche MA, Hammer RE, Horton JD. Deletion of ELOVL6 blocks the synthesis of oleic acid but does not prevent the development of fatty liver or insulin resistance. J Lipid Res 2014;55:2597–2605

21. Tang N, Matsuzaka T, Suzuki M, et al. Ablation of Elovl6 protects pancreatic islets from high-fat diet-induced impairment of insulin secretion. Biochem Biophys Res Commun 2014;450:318–323

22. Chen H, Charlat O, Tartaglia LA, et al. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell 1996;84:491–495

23. Ishikawa M, Iwasaki Y, Yatoh S, et al. Cholesterol accumulation and diabetes in pancreatic beta-cell-specific SREBP-2 transgenic mice: a new model for lipotoxicity. J Lipid Res 2008;49:2524–2534

24. Takahashi A, Motomura K, Kato T, et al. Transgenic mice overexpressing nuclear SREBP-1c in pancreatic beta-cells. Diabetes 2005;54:492–499

25. Ishikawa M, Okajima F, Inoue N, et al. Distinct effects of pravastatin, atorvastatin, and simvastatin on insulin secretion from a beta-cell line, MIN6 cells. J Atheroscler Thromb 2006;13:329–335

26. Matsuzaka T, Atsumi A, Matsumori R, et al. Elovl6 promotes nonalcoholic steatohepatitis. Hepatology 2012;56:2199–2208

27. Kuba M, Matsuzaka T, Matsumori R, et al. Absence of Elovl6 attenuates steatohepatitis but promotes gallstone formation in a lithogenic diet-fed Ldlr(-/-) mouse model. Sci Rep 2015;5:17604

28. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell 2012;150:1223–1234

29. Cinti F, Bouchi R, Kim-Muller JY, et al. Evidence of β-cell dedifferentiation in human type 2 diabetes. J Clin Endocrinol Metab 2016;101:1044–1054
30. Kim-Muller JY, Fan J, Kim YJ, et al. Aldehyde dehydrogenase 1a3 defines a subset of failing pancreatic β cells in diabetic mice. Nat Commun 2016;7:12631
31. Laybutt DR, Preston AM, Akerfeldt MC, et al. Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. Diabetologia 2007;50:752–763
32. Song B, Scheuner D, Ron D, Pennathur S, Kaufman RJ. Chop deletion reduces oxidative stress, improves beta cell function, and promotes cell survival in multiple mouse models of diabetes. J Clin Invest 2008;118:3378–3389
33. Matsuda T, Kido Y, Asahara S, et al. Ablation of C/EBPbeta alleviates ER stress and pancreatic beta cell failure through the GRP78 chaperone in mice. J Clin Invest 2010;120:115–126
34. Lerner AG, Upton JP, Praveen PV, et al. IRE1α induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. Cell Metab 2012;16:250–264
35. Chan JY, Luzuriaga J, Bensellam M, Biden TJ, Laybutt DR. Failure of the adaptive unfolded protein response in islets of obese mice is linked with abnormalities in β-cell gene expression and progression to diabetes. Diabetes 2013;62:1557–1568
36. Moullé VS, Vivot K, Tremblay C, Zarrouki B, Ghislain J, Poitout V. Glucose and fatty acids synergistically and reversibly promote beta cell proliferation in rats. Diabetologia 2017;60:879–888
37. Ehses JA, Perren A, Eppler E, et al. Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 2007;56:2356–2370
38. Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG. Islet-associated macrophages in type 2 diabetes. Diabetologia 2009;52:1686–1688
39. Eguchi K, Manabe I, Oishi-Tanaka Y, et al. Saturated fatty acid and TLR signaling link β cell dysfunction and islet inflammation. Cell Metab 2012;15:518–533
40. Busch AK, Cordery D, Denyer GS, Biden TJ. Expression profiling of palmitate- and oleate-regulated genes provides novel insights into the effects of chronic lipid exposure on pancreatic beta-cell function. Diabetes 2002;51:977–987
41. Maestre I, Jordán J, Calvo S, et al. Mitochondrial dysfunction is involved in apoptosis induced by serum withdrawal and fatty acids in the beta-cell line INS-1. Endocrinology 2003;144:335–345
42. Wang Y, Wang PY, Takashi K. Chronic effects of different non-esterified fatty acids on pancreatic islets of rats. Endocrine 2006;29:169–173
43. Santos LR, Rebelato E, Graciano MF, Abdulkader F, Curi R, Carpinelli AR. Oleic acid modulates metabolic substrate channeling during glucose-stimulated insulin secretion via NAD(P)H oxidase. Endocrinology 2011;152:3614–3621
44. Igoillo-Esteve M, Marselli L, Cunha DA, et al. Palmitate induces a pro-inflammatory response in human pancreatic islets that mimics CCL2 expression by beta cells in type 2 diabetes. Diabetologia 2010;53:1395–1405
45. 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 2011;300:E640–E649
46. Janikiewicz J, Hanzelka K, Kozinski K, Kolczynska K, Dobrzyn A. Islet β-cell failure in type 2 diabetes–within the network of toxic lipids. Biochem Biophys Res Commun 2015;460:491–496
47. Chaurasia B, Summers SA. Ceramides – lipotoxic inducers of metabolic disorders. Trends Endocrinol Metab 2015;26:538–550
48. Saito R, Matsuzaka T, Karasawa T, et al. Macrophage Elovl6 deficiency ameliorates foam cell formation and reduces atherosclerosis in low-density lipoprotein receptor-deficient mice. Arterioscler Thromb Vasc Biol 2011;31:1973–1979
49. Sunaga H, Matsui H, Ueno M, et al. Deranged fatty acid composition causes pulmonary fibrosis in Elovl6-deficient mice. Nat Commun 2013;4:2563
50. Kikuchi M, Shimada M, Matsuzaka T, et al. Crucial role of Elovl6 in chondrocyte growth and differentiation during growth plate development in mice. PLoS One 2016;11:e0159375