Adipose differentiation-related protein regulates lipids and insulin in pancreatic islets

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The current epidemic of obesity is feared to increase the prevalence of type 2 diabetes due to its contribution to insulin resistance (38). However, excess adiposity and dyslipidemia commonly seen in obesity may have an additional role in the development of diabetes by directly damaging β-cells (45). Indeed, prolonged exposure to elevated levels of fatty acids impairs insulin secretion in vivo and ex vivo, a phenomenon termed “lipotoxicity” (16, 47, 50, 56). Ceramide generation, an increase in reactive oxygen species, 12/15-lipoxygenase activation, and atypical protein kinase C activation are some of the mechanisms proposed for islet dysfunction and lipid-induced apoptosis (5, 44, 51, 52). On the other hand, lipids serve a nutrient function and are the principal energy source in islets deprived of exogenous nutrients (34, 54). Not only does acute exposure to fatty acids augment insulin secretion ex vivo, but a rise in fatty acids also facilitates glucose-stimulated insulin secretion (GSIS) after fasting in vivo (10). The activation of cell surface fatty acid receptor G protein-coupled receptor 40 (GPR40) plays a significant role in the augmentation of insulin secretion by fatty acids. However, cellular uptake of fatty acids is also believed to contribute to the insulin secretion by provision of lipid metabolites, including long-chain acyl-CoA and diacylglycerides (25, 43, 65). Thus, fatty acids play complex and seemingly contradictory roles in β-cells, which calls for a better understanding of fatty acid uptake, storage, and metabolism in islets.

Lipid droplets are the cellular organelles that enclose neutral lipid depots (triglycerides and cholesterol ester) and exist in virtually all cells, albeit in variable sizes and quantities (14, 42). The largest lipid droplets are found in adipocytes and measure >50 μm, whereas islet β-cells contain submicron droplets that are visible as round lucent areas under electron microscopes (42). Lipid droplets were long considered inert storage for excess lipids. However, recent studies have revealed that lipid droplets play dynamic roles in lipid metabolism that are orchestrated by proteins found on the surface of lipid droplets (14, 35). Adipose differentiation-related protein (ADFP; a unifying nomenclature perilipin 2) belongs to the PAT (perilipin, ADFP, TIP47) family of evolutionarily conserved lipid droplet-coating proteins that are located primarily on the surface of lipid droplets and are critical for their formation (3, 15). Although ADFP’s precise role is not fully understood, its importance in the regulation of lipid metabolism is substantiated from several studies (6, 32). The upregulation of ADFP is often seen at the site of increased lipid accumulation such as fatty liver, atheroma plaque, and mammary gland epithelium (21, 28, 40). Moreover, the reduction of ADFP via gene knockout and antisense oligonucleotide (ASO) reverses fatty liver and alters lipid metabolism (7, 24, 57). Therefore, we hypothesize that ADFP may participate in the regulation of intracellular lipid metabolism in islet β-cells.

In this study, we have demonstrated that ADFP is highly expressed in pancreatic β-cells on nutritional cues. Moreover, we have shown that the reduction of ADFP in insulin-secreting cell lines impairs the acute augmentation of insulin secretion by fatty acids. Collectively, our study indicates that ADFP plays a significant role in fatty acid metabolism in islet β-cells.

MATERIALS AND METHODS

Animal studies. Experiments were performed in accordance with the Institutional Animal Care and Use Committee guidelines of the...
University of Pennsylvania and Eastern Virginia Medical School and with the approval of both institutions. For the high-fat diet experiment, 4-wk-old male C57Bl/6j mice (Jackson Laboratories, Bar Harbor, ME) were housed n = 5/cage in a 12:12-h light-dark cycle at an ambient temperature of 22°C and allowed free access to food and water. Groups of mice were fed regular rodent chow (5001, 4.5 kcal% fat; Lab Diet, Richmond, IN) or a high-fat diet (D12455i, 45 kcal% fat; Research Diets, New Brunswick, NJ) for 12 wk. For the fasting experiment, chow was removed for 24 h from 3-mo-old male C57Bl/6j mice on regular rodent chow. They were allowed free access to drinking water during fasting.

Biochemical analysis. Blood glucose was measured from tail vein using OneTouch Ultra Glucometer (Lifescan; Johnson & Johnson, Milpitas, CA). For additional assays, tail vein blood was centrifuged at 500 g at 4°C for 20 min, and serum was stored at −20°C until analysis. Serum insulin levels were measured with rat insulin ELISA kit using mouse insulin standards from Crystall Chem (Chicago, IL). Serum triglyceride (TG; Stanbio, Boerne, TX), β-hydroxybutyric acid (Biodesign International, Saco, ME), and nonesterified fatty acid (NEFA C; Wako Chemicals USA, Richmond, VA) levels were measured enzymatically according to the manufacturer’s protocols.

Histology. Immunohistochemistry was performed on pancreatic section prepared from paraffin-embedded tissue as described before using the following antibodies (23): 1:2,000 guinea pig anti-ADFP antibody (Fitzgerald, Concord, MA), 1:500 rabbit anti-somatostatin antibody (Bachem America, Torrance, CA), 1:5,000 rabbit anti-glucagon antibody (Biodesign International, Saco, ME), 1:5,000 rabbit anti-pancreatic polypeptide antibody (Linco Diagnostics, St. Charles, MO), 1:200 rabbit anti-insulin antibody (Santa Cruz Biotechnology, Santa Cruz, CA), 1:800 Cy2-conjugated donkey anti-rabbit antibody, and 1:800 Cy3-conjugated donkey anti-guinea pig antibody (Jackson ImmunoResearch, West Grove, PA). Nikon Eclipse E600 light microscope (Nikon Instruments, Melville, NY) or Zeiss 510 laser-scanning confocal microscope (Heidenheim, Germany) was used to capture the images.

Human islet preparation and incubation. Human islets were acquired and sent to us from the Islet Cell Resource Consortium (ICRC) and Juvenile Diabetes Research Foundation (JDRF) Basic Human Islet Distribution Program. Islets were incubated overnight in CMRL-1066 supplemented with 2% human fatty acid-free albumin (Sigma-Aldrich, St. Louis, MO) alone or human albumin coupled with 0.5 mM of fatty acids [oleic acids (OA) or palmitic acids (PA)] at 37°C and 5% CO2. Hand-picked islets were washed with 1× PBS. RNA was extracted as described below. Protein was extracted using CelLytic M (Sigma-Aldrich) with protease inhibitor according to the manufacturer’s instruction.

Mouse islet isolation. Mice were anesthetized with pentobarbital sodium (50 mg/kg ip), and islets were separated using collagenase digestion followed by Ficoll density gradient centrifugation, as has been described previously (17, 27). Thereafter, islets were hand-picked under a dissecting microscope and processed for RNA extraction, protein extraction, and TG measurement.

Cell culture and ADFP transfection. MIN6 cells (passages 29 to 35) were maintained in DMEM growth medium containing 25 mM glucose, 10% FBS, β-mercaptoethanol (final 0.0005%), 50 μl penicillin, and 50 μg/l streptomycin at 37°C in a 5% CO2 humidifier. Chimeric antisense oligonucleotides were synthesized by Isis Pharmaceuticals (Carlsbad, CA) and previously used successfully to reduce ADFP ex vivo and in vivo (24, 57). ADFP-ASO, ISIS 384423 (5′-GGTCTATCTGGCAGCAACAT-3′), is a 20-mer phosphorothioate oligonucleotide complementary to the mRNA for mouse ADFP. A control oligo (Cont), ISIS 141923 (5′-CTTCCCCGAAAGGTCCTCC-3′), contains the same chemical modifications, with no complementarity to known genes, including the ADFP mRNA. Two predesigned siRNA targeting mouse ADFP, s62014 (sense: CAAAG-GAUCAUGUACCAGUAtt) and s62015 (sense: GGAAUUUGAUG-GUUCAGATT), were obtained from Ambion (Austin, TX). One day before the experiment, cells were seeded at 2 × 105/well in a 12-well culture dish in DMEM growth medium without antibiotics. The next day, cells were transfected with 300 nM of Cont or ADFP-ASO using Lipofectamin 2000 (Invitrogen) for 6 h according to the manufacturer’s instructions. Subsequently, cells were incubated overnight with fresh DMEM containing 1% FBS, 3 mM glucose, and 0.0005% β-mercaptoethanol with or without 0.1 mM OA coupled with fatty acid-free BSA (0.5% final). The following day, cells were harvested for protein analysis, RNA extraction, and TG measurements. Alternatively, insulin secretion assay or [3H]OA labeling of cells was performed as described below. Additional experiments were performed by transfecting 4 × 105/well of MIN6 cells in six wells with mixtures of 2.5 nM each of siRNA targeting ADFP (s62014 and S62015 above) or 5 nM of Silencer Negative control no. 1 siRNA (catalog no. 4390843; Ambion) in place of Cont or ADFP-ASO, using Lipofectamin 2000 for 6 h. Thereafter, overnight incubation and analyses of RNA, protein, and insulin secretion followed the same protocol as those used for ADFP-ASO experiments.

Western blotting. Islets and MIN6 cells were solubilized in lysis buffer containing 50 mM Tris-HCl (pH 7.4), 250 mM mannitol, and 0.5% (w/vol) Triton X-100 supplemented with a complete protein inhibition cocktail tablet from Roche (Penzberg, Germany), and insoluble material was removed by centrifugation at 14,000 g for 15 min at 4°C. Western blot of protein extracts was performed as described previously (24). Antibodies were diluted to the following concentrations: 1:1,000 guinea pig anti-ADFP (Fitzgerald), 1:1,000 rabbit anti-GAPDH (Cell Signaling Technology, Danvers, MA), 1:500 rabbit anti-β-actin (Santa Cruz Biotechnology), 1:1,000 horse-radish peroxidase (HRP)-conjugated goat anti-guinea pig IgG (Santa Cruz Biotechnology), and 1:500 HRP-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology).

RNA extraction and gene expression analysis. RNA was extracted from islets and MIN6 cells using the RNeasy kit (Qiagen, Valencia, CA). cDNA was generated by SuperScript Choice System for cDNA synthesis (Invitrogen, Carlsbad, CA), using 50 ng of RNA as a template. The expression of genes was analyzed by ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA) with commercial primers for the system. The results were normalized using 36B4 gene or β-actin expression as an internal standard.

[3H]OA labeling of MIN6 cells. MIN6 cells transfected with Cont or ADFP-ASO were incubated with 1 μCi/ml [3H]OA (specific activity 10 mCi/mol; NEN, Boston, MA) for 2 h at 37°C in a 5% CO2 humidifier. Cells were washed on ice, placed with PBS, and solubilized in the same lysis buffer described in Western blotting. Aliquot of lysate was measured for [3H] activity by liquid scintillation to obtain cellular uptake of [3H]OA. Twenty microliters of medium was combined with 500 μl of water and 50 μl of 10% BSA. Thereafter, the mixture was combined with 50 μl of 60% perchloric acid and was centrifuged at 12,000 g at room temperature for 15 min. [3H]water recovered in the supernatant was measured using liquid scintillation. The rate of lipolysis was measured from the reduction in [3H]-labeled triglyceride during 4 h of incubation in the presence of acyl-CoA synthetase inhibitor triacsin C at 9.6 μM (Enzo Life Sciences, Plymouth Meeting, PA) (22, 31).

Lipid extraction from islets. MIN6 cells were solubilized in the same lysis buffer described in Western blotting, and aliquots were taken for protein concentration measurement by DC protein assay kits (Bio-Rad, Hercules, CA). Lipids were extracted from the cell lysate using Folch methods (13). In brief, cell lysate was mixed with 5 volumes of Folch buffer [chloroform-methanol (2:1)], with occasional vortexing on ice for 20 min. Then samples were centrifuged at 800 g for 30 min at 4°C. The organic phase at the bottom was transferred to a new tube and incubated with one volume of water, with occasional vortexing on ice for 15 min. Next, samples were centrifuged again at 800 g for 20 min at 4°C. The organic phase was once again transferred to new tubes and then dried under nitrogen. The dried contents were
incubated with reaction buffer for enzymatic colorimetric assay of TG (Stanbio).

**Insulin secretion assay.** MIN6 cells were preincubated with glucose-free Krebs buffer (pH 7.4) containing 2.2 mM Ca\(^{2+}\), 0.25% BSA, and 10 mM HEPES under 5% CO\(_2\) atmosphere at 37°C for 1 h. Then cells were incubated with fresh Krebs buffer with varying concentrations of glucose and PA for 1 h, and insulin secreted in the medium was measured by a radioimmunoassay (Linco Research, St. Charles, MO).

**Statistics.** The data are presented as means ± SE. Differences between the two groups were assessed with unpaired Student’s *t*-test. *P* < 0.05 was considered significant.

**RESULTS**

**ADFP is highly expressed in pancreatic β-cells and is nutritionally regulated.** ADFP has been shown to play a pivotal role in lipid metabolism in a wide range of cells (6). However, little is known regarding its role in pancreatic islets. As shown in Fig. 1A, ADFP is expressed extensively in pancreatic islets using the antibody previously well characterized for its staining of ADFP (20, 31). In contrast, there was little expression of ADFP in non-β-cells (Fig. 1A). A higher level of ADFP expression in β-cells is also indicated in Fig. 1B, where we observed a large proportion of cells costained with insulin and ADFP. Of note, there was a minority of cells that stained with ADFP but reacted poorly with anti-insulin antibody, making cross-reactivity of anti-ADFP antibody with insulin less likely (Fig. 1B). Higher magnification demonstrated that ADFP distributes widely in β-cells (Fig. 1C). Within the limitation of resolution using confocal microscope, ADFP did not demonstrate the circular pattern typically seen when it encases lipid droplets in other cells, such as fibroblasts and liver (24, 62). This may be due to the small size of lipid droplets in β-cells or the distribution of ADFP in areas outside of lipid droplets (42, 62). Although its precise subcellular localization remains to be determined, ADFP is seen to be enriched in β-cells compared with non-β-cells and nonislet areas in the pancreas.

It has been shown that the cellular levels of ADFP are closely correlated with lipid storage in previous studies of cells such as macrophages and hepatocytes (28, 40). Therefore, we tested whether the ADFP expression in pancreatic islets is altered when islets are exposed to a high influx of lipids in vivo. C57Bl/6J mice placed on a high-fat diet [diet-induced obesity (DIO)] develop obesity, mild hyperglycemia, hyperinsulinemia, and hyperlipidemia and are commonly used as a model of human obesity and type 2 diabetes (Supplemental Fig. S1, A–E; Supplemental Material for this article is available online at the AJP-Endocrinology and Metabolism web site). Additionally, previous studies have indicated that TG content

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**Fig. 1.** Immunofluorescence of pancreatic islet showing adipose differentiation-related protein (ADFP; *A1*), non-β-cells stained with glucagon, somatostatin, and pancreatic polypeptide (*A2*), and a merged picture (*A3*). *A3* shows ADFP in red and non-β-cells in green. Pancreatic islet stained with ADFP (*B1*) and insulin (*B2*) was observed using a Zeiss 510 laser-scanning confocal microscope. *B3* shows the merged picture with ADFP in red and insulin in green. Arrows show cells with prominent ADFP staining without significant insulin signal (*B1* and *B3*). C: higher magnification obtained by the confocal microscope showed the distributions of ADFP (*C1* and red in *C3*) and insulin (*C2* and green in *C3*). Scale bar for *A* and *B*, 10 μm; scale bar for *C*, 1 μm.

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is increased in DIO islets (30, 61, 67). We observed a 3.4-fold increase in islet TGs in DIO mice, although the study did not reach statistical significance ($P = 0.075, n = 4$). A Western blot of pancreatic islets showed that the ADFP protein was increased to 3.5-fold of control in DIO islets ($P < 0.05$; Fig. 2, A and B), confirming its correlation with lipid storage in islets as well.

Next, we studied ADFP expression during fasting since lipids are the principal source of energy in islets deprived of exogenous nutrients (34, 54). C57Bl/6J mice, after 24 h of fasting, lost 13% of their body weight ($P < 0.005$), with a 37% reduction in blood glucose levels ($P < 0.005$) and a 57% reduction in serum insulin levels ($P < 0.01$; Supplemental Fig. S2, A–C). Serum β-hydroxybutyrate was markedly elevated in fasted mice, reflecting increased lipolysis, which provides free fatty acids for energy usage (0.88 ± 0.04 mg/dl in fed mice vs. 4.55 ± 0.87 mg/dl in fasted mice, $P < 0.01, n = 6$; Supplemental Fig. S2D). In islets, ADFP protein was increased to 2.5-fold of control after fasting ($P < 0.01$; Fig. 2, C and D). Since real-time PCR did not show a significant increase in ADFP levels after fasting, the increase in ADFP protein in fasted islets is likely due to a posttranslational stabilization of ADFP by intracellular lipids, which is reported to play a significant role in the regulation of ADFP (Fig. 2E) (37, 64).

These results imply that the formation of lipid droplets is increased in fasted islets when energy status favors lipolysis. To further characterize the metabolic status during fasting in islets, real-time PCR compared genes involved in fatty acid metabolism between islets of fasted and control animals (Fig. 2E). Key proteins and enzymes associated with fatty acid oxidation such as peroxisome proliferator-activated receptor-α ($P < 0.05$), acyl-CoA dehydrogenase, long chain ($P < 0.05$), acyl-CoA thioesterase 2 ($P < 0.05$), and carnitine palmitoyltransferase 1a ($P < 0.005$) were significantly upregulated in fasted islets. Marked increase in the expression of pyruvate dehydrogenase kinase, isozyme 4 ($P < 0.01$), 3-hydroxy-3-methylglutaryl-CoA synthase 2 ($P < 0.005$), and uncoupling protein 2 ($P < 0.01$) is also compatible with the active metabolism of fatty acids in fasted islets. Diacylglycerol O-acyltransferase (DGAT2), the enzyme involved in the final stage of TG synthesis, was significantly increased as well ($P < 0.05$; Fig. 2E). The isozyme DGAT1 was also expressed in islets, but the level of expression was not altered by fasting (data not shown). Thus, fasting islets showed upregulation in a multitude of pathways governing lipid metabolism, including oxidation, ketogenesis, and TG synthesis. Therefore, the increase in ADFP in fasted islets may reflect active utilization of lipid during fasting and implies its role beyond passive storage of excess lipids. Regulation of islet ADFP by lipids was also shown in human islets. Overnight incubation with fatty acids increased ADFP expression in both mRNA and protein levels (Fig. 3, A–C). Western blot showed that ADFP in human islets was increased 2.9-fold in response to 0.5 mM OA and 1.8-fold in response to 0.5 mM PA (Fig. 3C).

ADFP-ASO decreases TG content, lipolysis, and insulin secretion in insulin-secreting cells. To better understand the functional role of ADFP in β-cells, we downregulated the ADFP expression using ASO in MIN6 cells (24). Overnight incubation of MIN6 cells with OA resulted in robust expression of ADFP, indicating that fatty acids are the major regulator of ADFP in MIN6 cells (Fig. 4, A–C). A similar upregulation of ADFP in response to incubation with lipids is reported in non-insulin-secreting cells such as macrophages, fibroblasts, and liver cells (9, 12, 60). ADFP-ASO decreased both the basal and fatty acid-stimulated ADFP levels in MIN6 cells at both the mRNA and protein levels (Fig. 4, A–C). Overall, we achieved significant suppression of ADFP gene expression (25% of control without OA and 23% of control with OA, $P < 0.005$ for both). There was no compensatory rise in TIP47, another member of PAT lipid droplet protein expressed ubiquitously (40% of control in the absence of OA and 70% of control in the presence of OA, $P < 0.01$ and $P < 0.05$, respectively; Fig. 4C). Mild reduction in the expression of TIP47 may be secondary to the decrease in TG content and fatty acid utilization in MIN6 cells after ADFP-ASO treatment, as shown below (Fig. 5, A–D). The reduction of ADFP protein by ASO was also significant but was somewhat smaller compared with that achieved in mRNA levels, especially in the presence of OA (48% of control in the absence of OA and 59%
CONTROL oligo (Fig. 5A). This is most likely due to the previously reported posttranslational stabilization of ADFP by lipids (37, 64).

As shown in Fig. 4C, ADFP-ASO also reduced the expression of genes involved in both lipogenesis and β-oxidation. A similar reduction of genes involved in lipid metabolism was observed previously in liver treated with ADFP-ASO (24, 57).

Since the best-defined role of ADFP in other cells is to increase intracellular lipid storage, we sought to determine the effect of ADFP-ASO on the TG content of MIN6 cells (6). As has been reported previously in insulin-secreting cells and islets, OA increased TG content to 189% of control in MIN6 cells treated with control oligo (P < 0.005; Fig. 5A). On the other hand, the reduction of ADFP by ADFP-ASO in MIN6 cells was associated with lower TG content (54% of control in the absence of OA and 56% of control in the presence of OA, P < 0.005 and P < 0.05, respectively; Fig. 5A).

To determine whether the downregulation of ADFP alters the handling of exogenous fatty acids, MIN6 cells transfected with control oligo or ADFP-ASO were incubated in the presence or absence of 0.1 mM OA overnight. Thereafter, cells were incubated with [3H]OA added to the medium for 2 h, and the amounts of [3H]OA retained in the cells and of [3H]water in the culture medium were obtained. Overnight incubation with OA did not alter the cellular uptake of [3H]OA, but it did increase the production of [3H]water in control cells, indicating that fatty acid oxidation is upregulated by OA (P < 0.005; Fig. 5, B and C). The reduction of ADFP with ASO resulted in a modest but statistically significant reduction in both the cellular uptake of [3H]OA and the production of [3H]water. In ASO-treated cells, the cellular uptake was 83% of control without preceding OA incubation and 82% of control after OA incubation (P < 0.01; Fig. 5B). [3H]water release was also reduced in ASO-treated cells to 81% of control both with and without preceding OA incubation (P < 0.01; Fig. 5C). Moreover, lipolysis was reduced to 38% of control after ADFP-ASO treatment (Fig. 5D). The results indicate that reduced TG content seen after the downregulation of ADFP is likely from impaired fatty acid uptake and TG synthesis rather than from increased lipolysis or fatty acid oxidation.

We then sought to determine the effects of ADFP-ASO on GSIS and the acute augmentation of insulin secretion by PA. PA was used here since it is a potent enhancer of insulin secretion compared with OA (53). The reduction of ADFP by ASO slightly decreased GSIS in MIN6 cells (88% of control at 25 mM glucose, P < 0.05). As has been reported previously, acute administration of PA (1 h) enhanced insulin secretion in control cells (152% at 25 mM glucose in control oligo-treated cells, P < 0.005). However, MIN6 cells pretreated with ADFP-ASO showed markedly reduced GSIS in the presence of 0.5 mM PA (47% at 25 mM glucose vs. control oligo-treated cells, P < 0.005; Fig. 5E), indicating that ADFP plays a role in acute augmentation of insulin secretion by fatty acid. To rule out off-target effects of ADFP-ASO on insulin secretion, the expression of ADFP was also downregulated using siRNA, targeting different regions of ADFP (Supplemental Fig. S3, A and B). The GSIS in the presence of PA was reduced to 55% of control at 25 mM glucose after ADFP-siRNA treatment, indicating that ADFP indeed plays a role in the regulation of insulin secretion in the presence of PA (P < 0.05; Supplemental Fig. S3C).

DISCUSSION

Our study showed that ADFP is highly expressed in β-cells in mice islets. Moreover, its expression is increased under two conditions when islets are exposed to a high flux of lipids: in a fasting state and on a high-fat diet. ADFP was also expressed in human islets and increased in response to fatty acids. This prompted us to study the role of islet ADFP in the regulation of lipid metabolism. We found that the downregulation of ADFP in insulin-secreting cells decreased TG content, fatty acid utilization, including lipolysis, and the augmentation of insulin secretion by PA. Collectively, our study indicates that ADFP plays an active role in the regulation of lipid metabolism in insulin-secreting cells. ADFP levels were elevated in pancreatic islets after both a high-fat diet and an overnight fast. One of the roles proposed for ADFP is to store lipids as a sequestered depot (6, 33). Indeed, the upregulation of ADFP is commonly associated with cellular lipid accumulation (21, 33, 40). Moreover, ADFP seems to be indispensable for intracellular lipid storage in a variety of cells. The downregulation of ADFP reduces TG contents in a wide range of systems, including insulin-secreting cells in the current study (24, 28, 37, 64).
A high-fat diet for obesity-prone C57Bl/6J mice leads to an accumulation of excess TG in adipose and nonadipose tissues, including liver, muscle, and pancreatic islets (30, 57, 61, 67). Therefore, the increase in islet ADFP in DIO supports the notion that ADFP is a marker of intracellular lipids in nonadipose tissue (3).

However, the role of ADFP and lipid droplets may go beyond the passive storage of excess energy. Previously, fasting has been documented to elevate TG and ADFP levels in the liver (9). This has been attributed to the unique metabolic capacity of the liver that esterifies exogenous fatty acids into TG and secretes VLDL (26). Indeed, fatty liver is seen commonly in starvation and anorexia nervosa when the lipolysis of adipose tissue increases circulating lipids (39, 49). Interestingly, we observed that ADFP protein levels in islets are elevated during fasting when the energy status does not favor lipid storage. Indeed, multiple enzymes involved in fatty acid oxidation were elevated, which indicates the active oxidation of fatty acids during fasting in islets (Fig. 2E). Although it has yet to be determined whether TG content is increased during fasting in islets, the increase in islet ADFP during fasting raises the possibility that lipid droplets are not a simple depot of excess lipids but rather are the obligatory state of lipids during high flux and active utilization of fatty acids. In support of this notion, DGAT2, the enzyme involved in the last step of TG synthesis, was also increased during fasting in islets (Fig. 2E). Therefore, esterification of fatty acids may precede β-oxidation. The functional correlation between lipid oxidation and TG accumulation is also suggested by another lipid droplet protein, OXPAT, which is highly expressed in tissues with an elevated rate of lipid oxidation and promotes both β-oxidation and TG accumulation (63).

The downregulation of ADFP decreased TG content in insulin-secreting cells in the current study (Fig. 5A). Fatty acid uptake and the expression of key enzymes involved in TG synthesis were decreased in MIN6 cells after ADFP-ASO treatment, which at least partly explains the decrease in TG content (Figs. 2E and 5B). Importantly, lipolysis and β-oxidation were also reduced after the downregulation of ADFP in our study. Thus, decrease in TG content after ADFP-ASO treatment cannot be explained by an increase in its degradation (Fig. 5, C and D). Collectively, ADFP seems to be indispensable for the efficient utilization of fatty acid in insulin-secreting cells. However, the function of ADFP in lipid metabolism may vary depending on the metabolic status and type of cells. The simultaneous downregulation of ADFP and TIP47 decreased TG content but increased lipolysis in AML12 cells (1). Additionally, mice lacking full-length, functional ADFP are protected from TG accumulation in the liver (7). However, this was not accompanied by a change in fatty acid uptake or in lipolysis, but it was associated with TG accumulation in the microsomal fraction (7). Evidently, further studies are required to understand the complexity of ADFP function in lipid metabolism. It especially needs to be established whether ADFP interacts with hormone-sensitive lipase and other lipases, considering there are publications in support of and against it (31, 58).

The downregulation of ADFP in insulin-secreting cells reduced the augmentation of insulin secretion upon acute exposure to fatty acids, with small effects on insulin secretion in the absence of fatty acids (Fig. 5E). Previous studies have shown that the binding of fatty acids to cell surface GPR40 is...
It is well accepted that prolonged exposure to fatty acids decreases insulin secretion and causes β-cell apoptosis ex vivo (4, 11, 51, 59). This “lipotoxicity” is deemed as one of the culprits for the development of type 2 diabetes (56). However, it remains controversial whether the accumulation of intracellular TG itself promotes or prevents lipotoxicity. Briaud et al. (4) reported that the TG accumulation and esterification of fatty acids into neutral lipids precede lipotoxicity. On the other hand, the detrimental effects of fatty acids on β-cells depend on the properties of the fatty acids, especially the extent of saturation, and inversely correlate with their ability to increase islet TG content (8). Recently, Borg et al. (2) used INS-1 cells to overexpress perilipin, another lipid droplet protein that is highly expressed in adipocytes and steroidogenic cells but also found in limited quantities in other tissues, including islets. Overexpression of perilipin increased the TG content of INS-1 confirming that lipid droplet proteins have profound effects on lipid storage. Additionally, perilipin was reduced in these cells in the absence of PA compared with control cells (2). However, GSIS was better preserved in perilipin overexpressing cells when they were exposed to PA for an extended period. They propose that perilipin may protect islets from adverse effects from PA. Future studies should address whether ADFP has a unique role in the prevention of lipotoxicity in islets. Although both perilipin and ADFP are located primarily on lipid droplets and share structural similarities, functional distinction is well documented in adipocytes, cells enriched with perilipin (18, 62). Perilipin limits access of lipases at the basal state, whereas it recruits hormone-sensitive lipase during hormone-activated lipolysis (36, 55). In contrast, ADFP is found in small lipid droplets produced after lipolysis and during the early stages of adipocyte differentiation (19). Therefore, it is plausible that ADFP has a distinct position in the development of lipotoxicity as a lipid droplet protein that is abundantly expressed in a nutritionally regulated manner in vivo.

In summary, our current study indicates that islet ADFP actively participates in the regulation of lipid metabolism and modulates the acute action of fatty acids on insulin secretion. Further studies of ADFP will increase our understanding of lipid metabolism in pancreatic islets, which is critical in targeting lipotoxicity while preserving the nutritional role of lipids.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
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