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Effect of Hypertriglyceridemia on Beta Cell Mass and Function in ApoC3 Transgenic Mice*

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Hypertriglyceridemia results from increased production and decreased clearance of triglyceride-rich very low-density lipoproteins, a pathological condition that accounts for heightened risk of ischemic vascular diseases in obesity and type 2 diabetes. Despite its intimate association with insulin resistance, whether hypertriglyceridemia constitutes an independent risk for beta cell dysfunction in diabetes is unknown. Answering this fundamental question is stymied by the fact that hypertriglyceridemia is intertwined with hyperglycemia and insulin resistance in obese and diabetic subjects. To circumvent this limitation, we took advantage of apolipoprotein C3 (ApoC3)-transgenic mice, a model with genetic predisposition to hypertriglyceridemia. We showed that ApoC3-transgenic mice, as opposed to age/sex-matched wild-type littermates, develop hypertriglyceridemia with concomitant elevations in plasma cholesterol and non-esterified fatty acid levels. Anti-insulin and anti-glucagon dual immunohistochemistry in combination with morphometric analysis revealed that ApoC3-transgenic and wild-type littermates had similar beta cell and alpha cell masses as well as islet size and architecture. These effects correlated with similar amplitudes of glucose-stimulated insulin secretion and similar degrees of postprandial glucose excursion in ApoC3-transgenic *versus* wild-type littermates. Oil Red O histology did not visualize lipid infiltration into islets, correlating with the lack of ectopic triglyceride and cholesterol depositions in the pancreata of ApoC3-transgenic *versus* wild-type littermates. ApoC3-transgenic mice, despite persistent hypertriglyceridemia, maintained euglycemia under both fed and fasting conditions without manifestation of insulin resistance and fasting hyperinsulinemia. Thus, hypertriglyceridemia *per se* is not an independent risk factor for beta cell dysfunction in ApoC3 transgenic mice.

Hypertriglyceridemia, characterized by elevated plasma triglyceride (TG)[5] levels, results from increased production and/or decreased clearance of TG-rich very low-density lipoproteins (VLDL-TG) (1, 2). Preclinical and clinical studies characterize hypertriglyceridemia as a major risk factor for ischemic vascular diseases (3–8). Hypertriglyceridemia is a hallmark of diabetic dyslipidemia in insulin-resistant subjects with visceral obesity or type 2 diabetes (7, 9–11). To date, it remains unknown whether hypertriglyceridemia constitutes an independent risk for beta cell dysfunction in obesity and type 2 diabetes.

Critical for plasma TG metabolism is ApoC3, an apolipoprotein (79 amino acids in length) that is produced mainly in the liver and to a lesser extent in the intestine (12). Plasma ApoC3 proteins are present predominantly in TG-rich lipoproteins such as VLDL and chylomicrons (13). ApoC3 exerts its impact on TG metabolism by three distinct mechanisms. First, ApoC3 functions as an inhibitor of hepatic and lipoprotein lipases, key enzymes that catalyze the hydrolysis of TG in VLDL and chylomicrons (14–16). Second, ApoC3 acts to retard ApoE-mediated hepatic uptake of TG-rich lipoproteins (17, 18). Third, ApoC3 serves to facilitate VLDL-TG assembly and secretion from the liver (19–22). As a result, elevated plasma ApoC3 levels are associated with augmented production and retarded clearance of TG-rich particles, characteristic of hypertriglyceridemia. This is evidenced in insulin-resistant subjects with elevated ApoC3 production and altered TG metabolism (23–30). Mice with *apoC3* transgenic production develop hypertriglyceridemia (31). In contrast, mice with *apoC3* gene knock-out manifest hypotriglyceridemia, due to enhanced TG hydrolysis and clearance of TG-rich lipoproteins (32–34). Human subjects with loss-of-function mutations in *APOC3* are associated with significantly decreased plasma TG levels and reduced risk of cardiovascular disease (35–37).

Both hepatic and intestinal *apoC3* expression is inhibited by insulin (25, 38, 39). An impaired ability of insulin to inhibit *apoC3* gene expression results in ApoC3 overproduction, contributing to the pathogenesis of hypertriglyceridemia in insu-

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[5] The abbreviations used are: TG, triglyceride; *glut2*, glucose transporter 2; *ins1*, insulin 1; *ins2*, insulin 2; *mafa*, v-*maf* musculoaponeurotic fibrosarcoma oncogene family, protein A; *neurod*, neurogenic differentiation factor; *pdx1*, pancreatic and duodenal homeobox 1; *foxa2*, Forkhead box A2; STZ, streptozotocin; NEFA, non-esterified fatty acid; HOMA-IR, homeostasis model for insulin resistance.

lin-resistant subjects with obesity and type 2 diabetes (14, 25, 28). Although hypertriglyceridemia constitutes an independent risk for coronary artery disease, it remains unknown whether hypertriglyceridemia *per se* is a predisposing factor for beta cell dysfunction. Due to the intangible association of hypertriglyceridemia with hyperglycemia and insulin resistance in obesity and type 2 diabetes, it has been difficult to separate the effect of hypertriglyceridemia from that of hyperglycemia on beta cell function *in vivo*. Thus, it remains an open question as to whether hypertriglyceridemia is an independent risk factor for beta cell dysfunction in obesity and type 2 diabetes. To circumvent this limitation, we resorted to ApoC3 transgenic mice, an animal model with spontaneous development of hypertriglyceridemia, but without altered glucose metabolism. As a result, ApoC3-transgenic mice offer an ideal model for examining the net effect of hypertriglyceridemia on beta cell function in the absence of hyperglycemia or insulin resistance. In this study, we determined the effect of prevailing hypertriglyceridemia on beta cell mass and function, as well as on basal and glucose-stimulated insulin secretion in correlation with glucose and lipid metabolism in ApoC3-transgenic mice. We hypothesized that chronic hypertriglyceridemia would sabotage beta cell function and perturb glucose-stimulated insulin release in ApoC3-transgenic mice.

Experimental Procedures

Animal Studies—Transgenic mice expressing human *APOC3* in the C57BL/6J background have been described (31, 40, 41). Mice were fed standard rodent chow and water *ad libitum* in sterile cages with a 12-h light/dark cycle in a pathogen-free barrier facility. To determine blood glucose and plasma insulin levels, mice were fasted for 16 h and tail vein blood was sampled. Blood glucose levels were measured, using a Glucometer Elite (Bayer, IN). Plasma insulin levels were determined, using the ultrasensitive mouse insulin enzyme-linked immunosorbent assay (ALPCO, Windham, NH). The homeostasis model for insulin resistance (HOMA-IR) was determined by multiplying fasting blood glucose (mmol/liter) and fasting plasma insulin (μIU/ml) levels, divided by 22.5, as described (42). Plasma levels of TG and cholesterol were determined using Thermo Infinity TG and cholesterol reagents (ThermoFisher Scientific, Middletown, VA). Plasma non-esterified fatty acid (NEFA) levels were determined using the Wako NEFA assay kit (Wako Chemical, Richmond, VA). All procedures were approved by the Institutional Animal Care and Use Committee of University of Pittsburgh.

Glucose Tolerance Test—Mice were fasted for 16 h, followed intraperitoneal injection of glucose (2 g/kg). Blood glucose levels were determined before and at different times after glucose administration.

Glucose-stimulated Insulin Secretion—Aliquots (25 μl) of tail vein blood were sampled at 0, 15, and 30 min after intraperitoneal injection of glucose (2 g/kg) to 16-h fasted mice for determining plasma insulin levels at basal and glucose-stimulated conditions, using the ultrasensitive insulin ELISA (ALPCO, Windham, NH).

Insulin Tolerance Test—Mice were injected intraperitoneally with regular human insulin (1 IU/kg, Eli Lilly and Co., Indianapolis, IN), followed by determination of blood glucose levels.

Islet Isolation and ex Vivo Glucose-stimulated Insulin Secretion—Mice were euthanized, followed by pancreatic intraductal infusion of 3 ml of cold Hank's buffer containing 1.95 mg/ml of collagenase-V (Sigma). The pancreas was procured for islet isolation, as described (43). To determine *ex vivo* glucose-stimulated insulin secretion, islets were cultured in RPMI 1640 medium overnight, followed by incubation in Kreb buffer containing 2.8 mM glucose for 30 min. Islets were induced by shifting culture medium from 2.8 to 20 mM glucose concentrations. Aliquots (50 μl) of culture medium were collected at 0 and 30 min for determining insulin concentrations, as described (44).

RNA Isolation and Real-time Quantitative RT-PCR—Total RNA was prepared from the pancreas using the TRIzol Reagent (Invitrogen). Real-time quantitative RT-PCR was used for quantifying mRNA concentrations using the Roche LightCycler-RNA amplification kit (Roche Diagnostics, Indianapolis, IN), as described (45). As tabulated in Table 1, all primers were obtained commercially from Integrated DNA Technologies (Coralville, IA).

Immunofluorescent Microscopy—Mice were euthanized after a 16-h fast. The pancreas was retrieved and fixed in 4% paraformaldehyde for 16 h at 4 °C, followed by incubation in 30% sucrose overnight at 4 °C. Cyrosections (6 μm) were cut. Random sections of 100-μm distance were preincubated with normal donkey serum, and then immunostained with guinea pig anti-insulin (DAKO, 1:300) and mouse anti-glucagon (Sigma number G2654, 1:500). After washing with PBS buffer containing 0.5% Tween 20, sections were incubated with Cy2-conjugated donkey anti-guinea pig IgG or Cy3-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories). The nuclei of cells were stained with 4′,6-diamidino-2-phenylindole (DAPI, Sigma), prior to visualization in the Aviovert 200 fluorescent microscope (Zeiss, Oberkochen, Germany).

Determination of Beta Cell and Alpha Cell Masses—Digital images of pancreas sections immunostained by anti-insulin or anti-glucagon antibody were subjected to morphometric analysis using MetaMorph Image Analysis software (Molecular Devices, Downingtown, PA). Beta cell area out of total pancreas area per section was determined in 3–5 non-consecutive sections for determining beta cell mass, as described (46). Likewise, alpha cell mass was determined and compared between ApoC3-tg and WT groups.

Oil Red O Staining—Pancreas tissues were embedded in the Histoprep tissue-embedding media and snap frozen. Frozen sections (6 μm in thickness) were cut and stained with Oil Red O, followed by counterstaining with hematoxylin.

FPLC Fractionation of Lipoproteins—Aliquots (400 μl) of plasma pooled from ApoC3-transgenic mice and control littermates were applied to two head-to-tail-linked Tricorn high performance Superose S-6 10/300GL columns using an FPLC system (GE Healthcare), followed by elution with PBS at a constant flow rate of 0.3 ml/min. Fractions (400 μl) were eluted for determining TG levels, as described (25, 45). Aliquots (50 μl) of samples corresponding to the peak fractions of VLDL-TG particles

TABLE 1

Primers used for real-time quantitative RT-PCR assay

All nucleotide sequences are in 5′ to 3′ orientation and purchased from Integrated DNA Technologies (Coralville, IA).

Name	Nucleotide sequence
INS-1 forward	CTTGCCCTCTGGGAGCCCA
INS-1 reverse	TGAAGGTCCCCGGGGCTTC
INS-2 forward	CTTCCTCTGGGAGTCCCAC
INS-2 reverse	CACCTGCTCCCGGGCCTCCA
GK forward	TGGATGACAGAGCCAGGATGG
GK reverse	ACTTCTGAGCCTTCTGGGGTG
Glut2 forward	TCAGAAGACAAGATCACCGG
Glut2 reverse	GTGAGCAGATCCTTCAGTCT
MafA forward	AGGAGGAGGTCATCCGACTG
MafA reverse	CTTCTCGCTCTCCAGAATGTG
PDX-1 forward	AGCAGTACTACGCGGCCACA
PDX-1 reverse	GCACTTCGTATGGGGAGATG
NeuroD forward	GATCGTCACTATTCAGAACC
NeuroD reverse	CCTCTAGATCCTCATCTTCC
FoxA2 reverse	CTGGGAGCCGTGAAGATGGA
FoxA2 reverse	TGAGCCGCTCATGCCCGCCA
IRS-1 forward	GCGAGCCCTCCGGATACC
IRS-1 reverse	GTGTAGAGAGCCACCAGGTGC
IRS-2 forward	AGCAGAAGCACGGCCACAA
IRS-2 reverse	TGCTCGTTCTCCGCCGCTA
18S RNA forward	AAACGGCTACCACATCCAAG
18S RNA reverse	CCTCCAATGGATCCTCGTTA

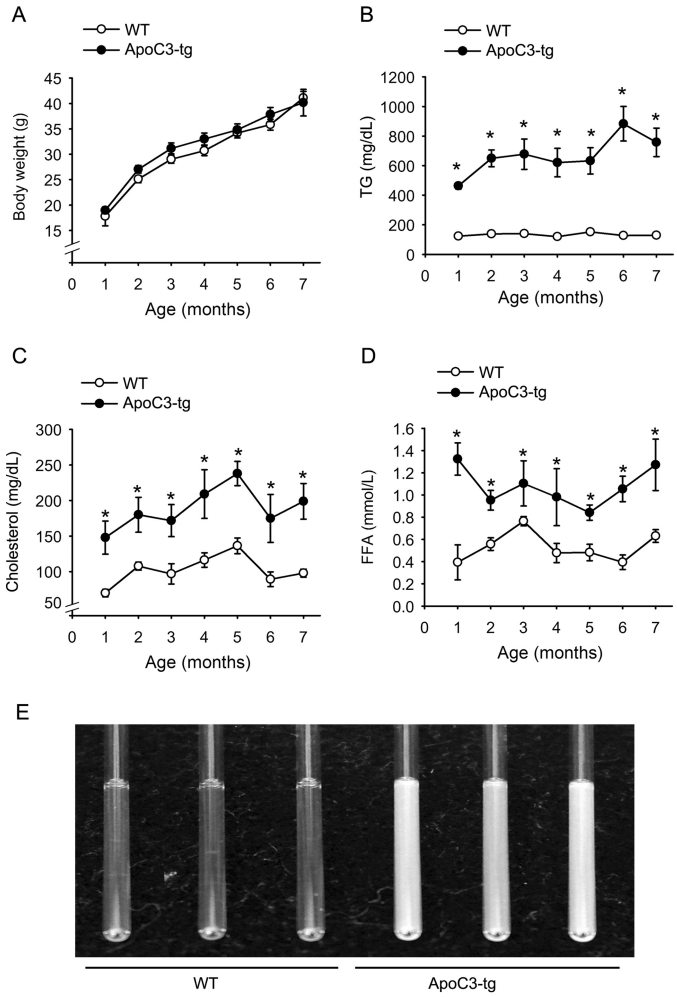


FIGURE 1. **ApoC3-transgenic mice develop hypertriglyceridemia.** ApoC3-transgenic and WT littermates (male, $n = 8$/group) were monitored for weight gain for 7 months. At different months, mice were fasted for 16 h for the determination of plasma TG, cholesterol, and NEFA levels. *A,* body weight. *B,* plasma TG levels. *C,* plasma cholesterol levels. *D,* plasma NEFA levels. *E,* sera of ApoC3-tg and WT littermates. Sera in capillary tubes were visualized under light. *, $p < 0.005$ *versus* WT.

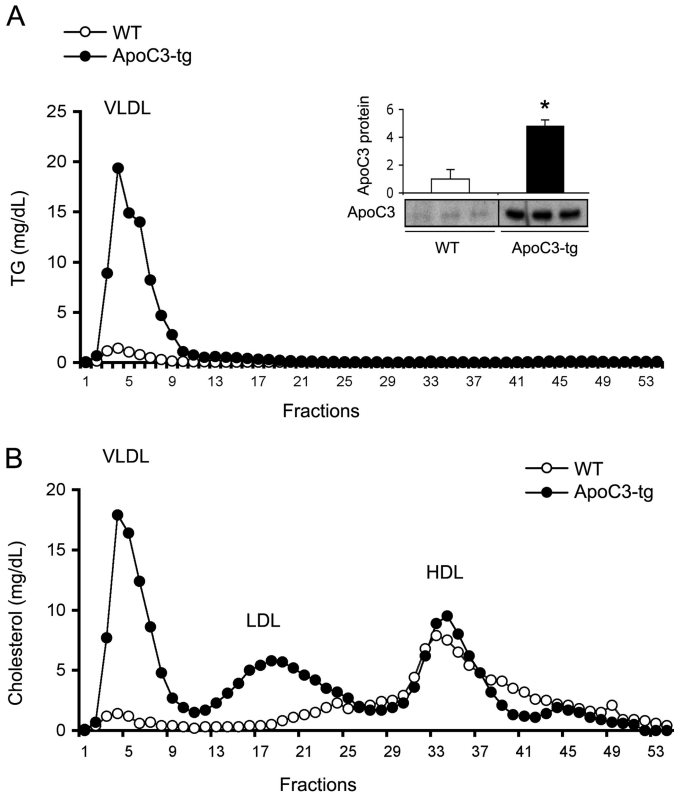


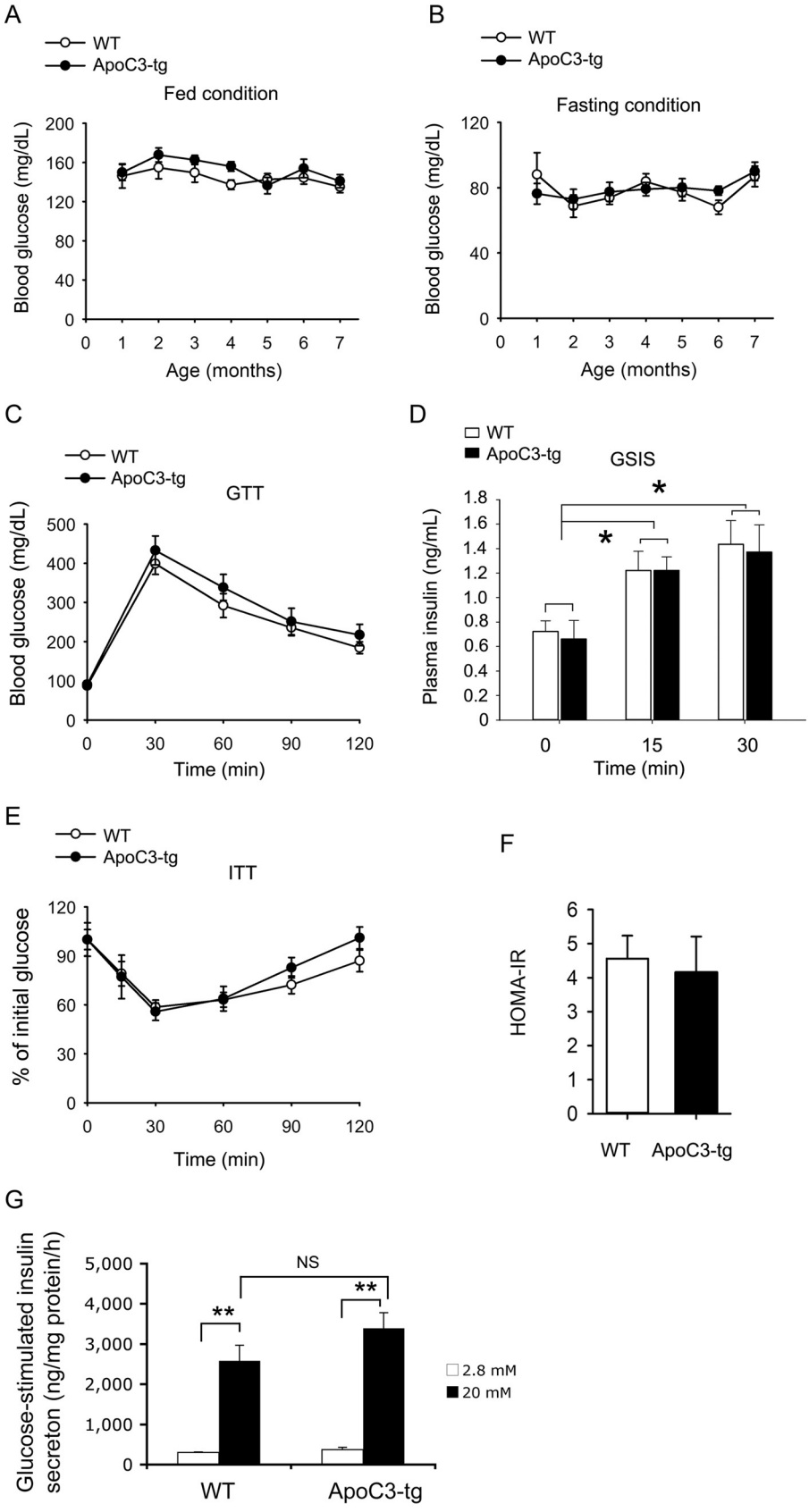
FIGURE 2. **ApoC3-transgenic mice exhibit abnormal VLDL-TG and VLDL-cholesterol metabolism.** Aliquots (400 μl) of plasma pooled from ApoC3-transgenic and WT littermates (male, $n = 8$/group, 7 months old) were fractionated by gel filtration in a FPLC system. Fractions (400 μl) were eluted for the determination of TG levels (*A*) and cholesterol levels (*B*). Aliquots of VLDL-TG peak fractions (50 μl) were subjected to immunoblot analysis for determining ApoC3 protein levels, as shown in the *inset* in *A*. *, $p < 0.005$ *versus* WT.

were subjected to immunoblot analysis, using rabbit anti-ApoC3 antibody, as described (41).

Pancreatic TG Content—Pancreatic tissues (20 mg) were homogenized in 400 μl of HPLC-grade acetone. After incubation with agitation at room temperature overnight, aliquots (50 μl) of acetone-extracted lipid suspension were used for the determination of triglyceride concentrations using the Infinity triglyceride reagent (ThermoFisher Scientific). Pancreatic fat content was defined as milligrams of triglyceride per g of total pancreatic proteins. Similarly, freshly isolated islets ($n = 100$ islets per mouse) from ApoC3-transgenic and WT mice were used for measuring intra-islet TG content.

Pancreatic Cholesterol Content—Pancreatic tissues (20 mg) were homogenized in 400 μl of hexane:isopropyl alcohol (3:2 in volume). Aliquots (50 μl) of hexane:isopropyl alcohol-extracted cholesterol suspension were used for determining cholesterol concentration using the Infinity cholesterol reagent (ThermoFisher Scientific). Hepatic cholesterol content was defined as milligrams of cholesterol per g of total pancreatic proteins.

Low-dose Streptozotocin Treatment—Mice were intraperitoneally injected daily with streptozotocin (STZ) at a low dose of 50 mg/kg for 5 consecutive days. Prior to and after STZ treatment, body weight and blood glucose levels were measured under non-fasting conditions.


A
WT
ApoC3-tg
Fed condition
Blood glucose (mg/dL)
Age (months)
B
WT
ApoC3-tg
Fasting condition
Blood glucose (mg/dL)
Age (months)
C
WT
ApoC3-tg
GTT
Blood glucose (mg/dL)
Time (min)
D
WT
ApoC3-tg
GSIS
Plasma insulin (ng/mL)
Time (min)
E
WT
ApoC3-tg
ITT
% of initial glucose
Time (min)
F
HOMA-IR
WT
ApoC3-tg
G
Glucose-stimulated insulin secreton (ng/mg protein/h)
NS
2.8 mM
20 mM
WT
ApoC3-tg

Pancreatic Insulin Content—Mice were euthanized for the procurement of the pancreas. The pancreas was homogenized in 800 μl of acid ethanol (0.15 M HCl in 70% ethanol) in 2-ml microtubes, followed by incubation at 4 °C overnight to extract insulin. After centrifugation at 14,000 rpm in a microcentrifuge for 10 min, the supernatants were used for the determination of insulin, using the ultrasensitive mouse insulin enzyme-linked immunosorbent assay (ALPCO, Windham, NH).

Statistics—Statistics of data were analyzed by Student's *t* test and were further validated by analysis of variance using the JMP statistics software (Cary, NC). Dunnett's post hoc tests were performed to determine the significance between ApoC3-transgenic and WT groups. Data are expressed as mean ± S.E. *p* values <0.05 were considered statistically significant.

Results

Characterization of Lipid and Lipoprotein Metabolism in ApoC3-transgenic Mice—ApoC3-transgenic mice (ApoC3-tg, male, $n = 8$) and age/sex-matched wild-type littermates (WT, $n = 8$) grew with a similar weight gain (Fig. 1*A*). Compared with WT controls, ApoC3-transgenic mice developed hypertriglyceridemia after weaning. This effect culminated in about 5-fold increases in plasma TG levels (Fig. 1*B*), with concomitant elevations in plasma levels of cholesterols (Fig. 1*C*) and NEFA (Fig. 1*D*). As a result, sera from ApoC3-transgenic mice appeared milky (Fig. 1*E*).

We then subjected sera from ApoC3-transgenic and WT littermates to gel filtration column chromatography for the fractionation of lipoproteins. ApoC3-transgenic mice had markedly higher levels of VLDL-TG particles, correlating with a 5-fold enrichment of ApoC3 proteins in VLDL-TG particles (Fig. 2*A*). Furthermore, ApoC3-transgenic mice had higher VLDL-cholesterol and LDL-cholesterol levels (Fig. 2*B*), characteristic of pro-atherogenic lipoprotein profiles. In contrast, HDL-cholesterol levels remained unchanged in ApoC3-transgenic *versus* WT littermates (Fig. 2*B*).

Effect of Hypertriglyceridemia on Glucose Metabolism and Insulin Sensitivity—To determine the impact of ApoC3-transgenic production on glucose metabolism, we measured blood glucose levels of ApoC3-transgenic and WT littermates (male, $n = 8$) during a 7-month period. No significant differences in blood glucose levels at both fed and fasting conditions were detected between ApoC3-transgenic and WT littermates (Fig. 3, *A* and *B*). We performed an intraperitoneal glucose tolerance test, demonstrating that ApoC3-transgenic and WT littermates exhibited similar blood glucose profiles in response to glucose challenge (Fig. 3*C*). This effect was reproducible at 3, 4, 5, 6, and 7 months of age. During the glucose tolerance test, aliquots of blood (25 μl) were sampled for determining plasma insulin levels at 0, 15, and 30 min after glucose injection. ApoC3-transgenic and WT littermates had similar basal plasma insulin levels and glucose-stimulated insulin secretion profiles (Fig. 3*D*). Likewise, we performed intraperitoneal insulin tolerance test (ITT). No significant differences in blood glucose profiles were detectable between ApoC3-transgenic and WT groups during the insulin tolerance tests (Fig. 3*E*). This effect correlated with a lack of changes in whole body insulin sensitivity, as indexed by HOMA-IR (Fig. 3*F*). We then isolated islets from ApoC3-transgenic and WT mice, followed by determining the ability of islets to secrete insulin in the presence of low (2.8 mM) and high glucose (20 mM) concentrations in culture. ApoC3-transgenic and WT islets had similar amplitudes of glucose-stimulated insulin secretion (Fig. 3*G*).

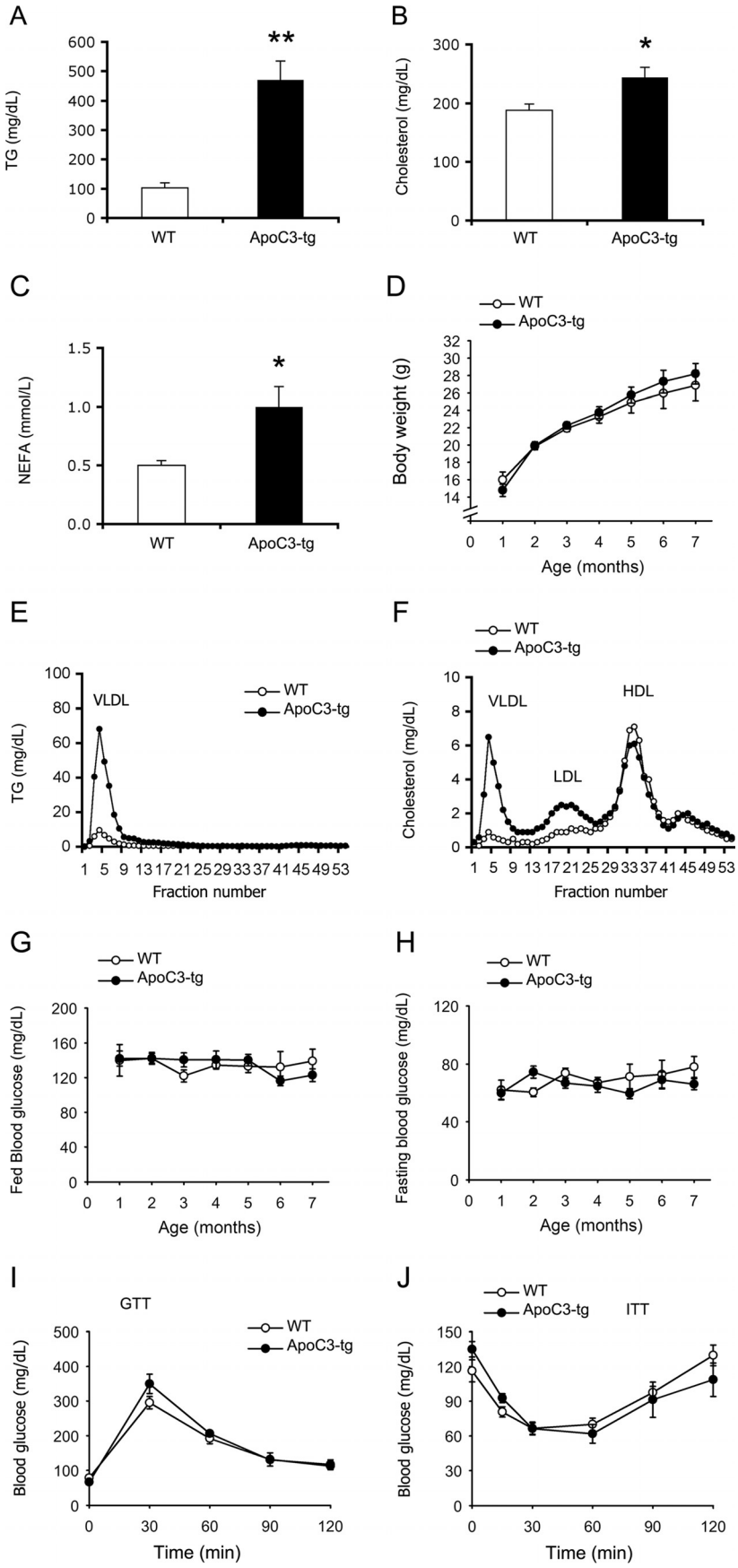
Blood Glucose and Lipid Metabolism in Female ApoC3-transgenic Mice—We recapitulated the above findings in female ApoC3-transgenic mice. Compared with age/sex-matched WT littermates, female ApoC3-transgenic mice exhibited significantly higher plasma levels of TG (Fig. 4*A*), cholesterol (Fig. 4*B*), and NEFA (Fig. 4*C*), independently of weight gain (Fig. 4*D*). These effects were accompanied by the induction of VLDL-TG levels (Fig. 4*E*), as well as VLDL-cholesterol and LDL-cholesterol levels, without alterations in HDL-cholesterol levels (Fig. 4*F*).

Female ApoC3-transgenic mice maintained normal blood glucose levels under fed and fasting conditions (Fig. 4, *G* and *H*). Furthermore, no differences in blood glucose profiles were detectable in female ApoC3-transgenic *versus* WT littermates during both glucose and insulin tolerance tests (Fig. 4, *I* and *J*). Despite the development of hypertriglyceridemia, ApoC3-transgenic mice maintained normal glucose homeostasis irrespective of the differences in sex. We then focused on male mice to determine the effect of prevailing hypertriglyceridemia on beta cell mass and function.

Effect of Hypertriglyceridemia on Beta Cell Mass and Function—To determine the effect of hypertriglyceridemia on beta cell mass, we performed insulin immunohistochemistry on the pancreas, using anti-insulin and anti-glucagon antibodies (Fig. 5, *A* and *B*). ApoC3-transgenic and WT littermates had similar islet size (Fig. 5*C*). Both beta cell and alpha cell masses remained unchanged in ApoC3-transgenic versus WT littermates (Fig. 5, *D* and *E*). Likewise, no significant differences in pancreas weight were seen in ApoC3-transgenic *versus* WT littermates (Fig. 5*F*).

To determine the potential impact of persistent hypertriglyceridemia on beta cell function, we profiled the expression of beta cell genes, whose functions are paramount for beta cell glucose sensing, and insulin synthesis and secretion in 7-month old ApoC3-transgenic and WT littermates (Fig. 5*G*). We did not detect significant differences in beta cell expression of insu-

FIGURE 3. **Impact of ApoC3 transgenic production on glucose metabolism.** *A,* fed blood glucose levels. Fed blood glucose levels were determined in mice under *ad libitum* conditions. *B,* fasting blood glucose levels. Fasting blood glucose levels were determined after 16 h fasting. *C,* glucose tolerance test. Mice were fasted for 16 h, followed by intraperitoneal injection of 2 g/kg of glucose. *D,* basal and glucose-stimulated insulin secretion. During the glucose tolerance test, aliquots of blood (25 μl) were sampled at 0, 15, and 30 min after glucose injection for determining basal and glucose-stimulated insulin release. *E,* insulin tolerance test. Mice were intraperitoneally injected with 1 IU/kg of insulin, followed by the determination of blood glucose profiles. *F,* HOMA-IR index. The HOMA-IR was determined by multiplying fasting blood glucose (mmol/liter) and fasting plasma insulin (μIU/ml) levels, divided by 22.5. Data were obtained from ApoC3-transgenic and WT littermates (male, $n = 8$) at 7 months of age. *G,* glucose-stimulated insulin secretion *ex vivo*. Aliquots of freshly isolated islets ($n = 50$ per mouse) from ApoC3-transgenic and WT mice (male, 4-month old, $n = 3$) were assayed for their ability to secrete insulin in the presence of low (2.8 mM) *versus* high (20 mM) glucose concentrations in culture. *, $p < 0.05$ and **, $p < 0.001$ *versus* WT. *NS*, not significant.


A
TG (mg/dL)
600
500
400
300
200
100
0
**
WT
ApoC3-tg
B
Cholesterol (mg/dL)
300
200
100
0
*
WT
ApoC3-tg
C
NEFA (mmol/L)
1.5
1.0
0.5
0.0
*
WT
ApoC3-tg
D
WT
ApoC3-tg
Body weight (g)
32
30
28
26
24
22
20
18
16
14
0 1 2 3 4 5 6 7
Age (months)
E
TG (mg/dL)
100
80
60
40
20
0
VLDL
WT
ApoC3-tg
1 5 9 13 17 21 25 29 33 37 41 45 49 53
Fraction number
F
WT
ApoC3-tg
Cholesterol (mg/dL)
10
8
6
4
2
0
VLDL
LDL
HDL
1 5 9 13 17 21 25 29 33 37 41 45 49 53
Fraction number
G
WT
ApoC3-tg
Fed Blood glucose (mg/dL)
200
160
120
80
40
0
0 1 2 3 4 5 6 7
Age (months)
H
WT
ApoC3-tg
Fasting blood glucose (mg/dL)
120
80
40
0
0 1 2 3 4 5 6 7
Age (months)
I
GTT
WT
ApoC3-tg
Blood glucose (mg/dL)
500
400
300
200
100
0
0 30 60 90 120
Time (min)
J
WT
ApoC3-tg
ITT
Blood glucose (mg/dL)
150
120
90
60
30
0
0 30 60 90 120
Time (min)

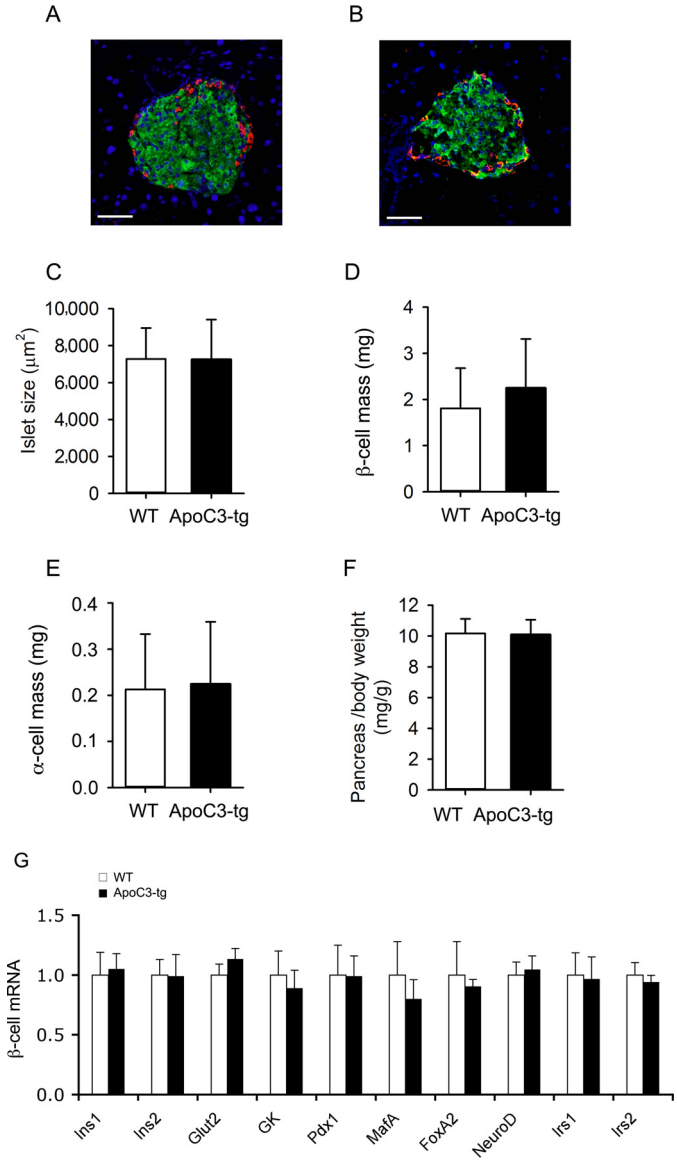


FIGURE 5. **Effect of hypertriglyceridemia on beta cell and function in ApoC3-transgenic mice.** Mice (male, 7 months old, $n = 8$/group) were sacrificed after 16 h fasting at 7 months of age. Pancreas tissues were procured and subjected to anti-insulin and anti-glucagon immunohistochemistry (*A* and *B*). *C,* islet size. *D,* beta cell mass. *E,* alpha cell mass. *F,* pancreas weight. *G,* beta cell mRNA profiles. Beta cell-specific mRNA levels of key genes involved in insulin synthesis and secretion as well as beta cell glucose sensing and insulin signaling were determined by real-time RT-PCR analysis, using 18S RNA as control. *Bar*, 50 μm.

lin gene 1 (*ins1*) and gene 2 (*ins2*), correlating with the lack of changes in pancreatic and duodenal homeobox 1 (*pdx1*), neurogenic differentiation factor (*neuroD*), forkhead box A2 (*foxa2*), and v-*maf* musculoaponeurotic fibrosarcoma oncogene family protein A (*mafa*), four key transcription factors involved in insulin synthesis and secretion. In keeping with the lack of changes in glucose-stimulated insulin release in ApoC3-transgenic *versus* WT mice (Fig. 3*D*), no significant differences were detected in beta cell expression of glucokinase (*gck*) and glucose transporter 2 (*glut2*) (Fig. 5*G*), two components instrumental for beta cell glucose sensing (47, 48).

Effect of Hypertriglyceridemia on Islet Fat Content—To address whether hypertriglyceridemia causes fat infiltration in islets, we performed Oil Red O staining on pancreas tissues from 7-month-old ApoC3-transgenic and WT littermates. No visible lipid droplets were detectable in islets (Fig. 6, *A* and *B*). To corroborate these studies, we determined pancreatic fat content, demonstrating that ApoC3-transgenic mice had normal TG and cholesterol levels (Fig. 6, *C* and *D*). We reproduced these results in both male and female ApoC3-transgenic mice. As an additional control, we isolated islets from ApoC3-transgenic and WT littermates, followed by quantification of the intra-islet lipid content. Intra-islet TG content was similar in ApoC3-transgenic *versus* WT mice (Fig. 6*E*).

Contribution of Hypertriglyceridemia to Low-dose STZ-elicited Diabetes in ApoC3-transgenic Mice—To address the hypothesis that the prevailing hypertriglyceridemia would aggravate the deleterious effect of hyperglycemia on beta cell function and accelerate the development of diabetes, we treated ApoC3-transgenic and sex/age-matched WT littermates (12-week-old, $n = 8$) with once daily intraperitoneal injection of STZ (50 mg/kg) for 5 days, a low-dose STZ regimen that serves to elicit partial beta cell destruction and induce moderate hyperglycemia in mice. We monitored body weight and blood glucose levels in STZ-treated mice for up to 30 days. Although both groups of mice exhibited significantly higher blood glucose levels secondary to insulin deficiency at day 8 post-STZ treatment, the degree of hyperglycemia over time was indistinguishable between ApoC3-transgenic and WT mice (Fig. 7*A*). Likewise, no differences were detectable in body weight, plasma insulin levels, residual β-cell mass, and pancreas weight between ApoC3-transgenic and WT mice (Fig. 7, *B–E*). These effects ensued despite the presence of severe hypertriglyceridemia in ApoC3-transgenic *versus* WT mice (Fig. 7*F*). These results defied our hypothesis that hypertriglyceridemia is a confounding factor in the development of diabetes in insulin-deficient ApoC3-transgenic mice following low-dose STZ treatment.

Discussion

Hypertriglyceridemia is the most common lipid disorder in subjects with metabolic syndrome. Hypertriglyceridemia along with its metabolic sequelae of the accumulation of TG-rich lipoprotein remnants is atherogenic, accounting for increased risk and progression of coronary artery disease (3–8). Although hypertriglyceridemia is closely associated with obesity and type

FIGURE 4. **Blood glucose and lipid metabolism in female ApoC3-transgenic mice.** ApoC3-transgenic and WT littermates (female, $n = 5$/group) were monitored for weight gain, glucose, and lipid metabolism for 7 months. *A,* plasma TG levels. *B,* plasma cholesterol levels. *C,* plasma NEFA levels. Plasma TG, cholesterol, and NEFA levels were determined under fasting conditions at 7 months of age. *D,* body weight. *E,* TG levels in VLDL fraction. *F,* cholesterol levels in VLDL, LDL, and HDL fractions. Aliquots (400 μl) of plasma pooled from female ApoC3-transgenic and WT littermates ($n = 5$, 7 months old) were fractionated by gel filtration in a FPLC system. Fractions (400 μl) were eluted for the determination of TG and cholesterol levels in VLDL, LDL, and HDL fractions. *G,* fed blood glucose levels. *H,* fasting blood glucose levels. *I,* glucose tolerance test. Mice were fasted for 16 h, followed by intraperitoneal injection of 2 g/kg of glucose. *J,* insulin tolerance test. Mice were intraperitoneally injected with 1 IU/kg of insulin, followed by the determination of blood glucose profiles. Both glucose tolerance test and insulin tolerance test were performed at 7 months of age. *, $p < 0.05$ and **, $p < 0.005$ *versus* WT.

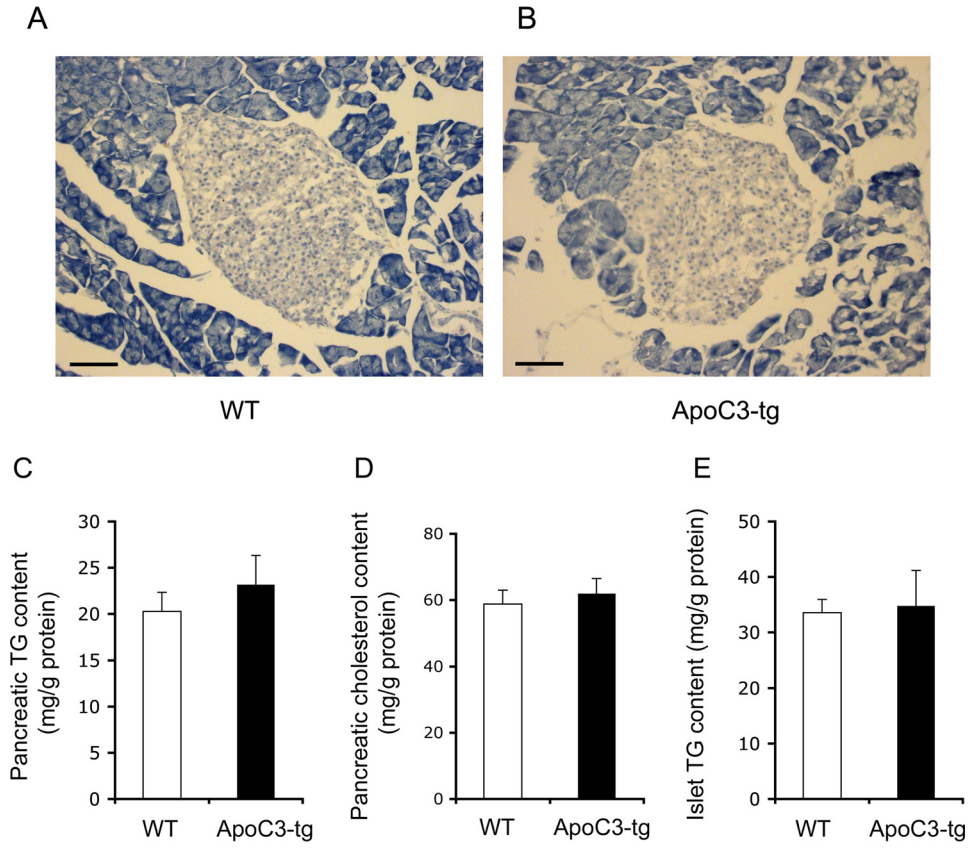


FIGURE 6. **Pancreatic histology and fat content in ApoC3-tg mice.** ApoC3-tg and WT littermates (male, $n = 8$) were sacrificed at 7 months old. Pancreas tissues were procured. Frozen sections (6 μm in thickness) of the pancreas from WT (*A*) and ApoC3-tg (*B*) mice were cut and stained with Oil Red O, followed by counterstaining with hematoxylin. *C*, pancreatic TG content. *D*, pancreatic cholesterol content. *E*, intra-islet TG content. *Bar*, 50 μm.

2 diabetes, it remains unclear whether hypertriglyceridemia *per se* is a causative factor for beta cell dysfunction. Answering this fundamental question is challenged by the fact that hypertriglyceridemia is commonly intertwined with hyperglycemia and insulin resistance in obesity and type 2 diabetes. To overcome this limitation, we took advantage of ApoC3-transgenic mice with genetic predisposition to hypertriglyceridemia without altered glucose metabolism. Therefore this model allowed us to determine the role of hypertriglyceridemia in regulating beta cell mass and function in the absence of the confounding factors, namely hyperglycemia and insulin resistance.

We showed that ApoC3-transgenic mice, as opposed to WT littermates, exhibited markedly higher plasma triglyceride levels, accompanied by significant elevations in plasma cholesterol and NEFA levels. These effects ensued independently of body weight and glucose metabolism, as both ApoC3-transgenic and WT littermates maintained similar weight gain and euglycemia under both fed and fasting conditions. In response to the glucose challenge, both ApoC3-transgenic and WT littermates had similar blood glucose profiles, correlating with similar amplitudes of glucose-stimulated insulin secretion. Likewise, both groups of mice maintained the same levels of insulin sensitivity, as determined by a insulin tolerance test and HOMA-IR. We recapitulated these findings in both male and female ApoC3-transgenic *versus* WT littermates. Furthermore, we showed that ApoC3-transgenic mice had similar islet size as well as beta cell and alpha cell masses, as determined by anti-insulin and anti-glucagon immunohistochemistry. These effects correlated with the lack of changes in beta cell expression of key factors involved in beta cell glucose sensing (*gck* and *glut2*), insulin signaling (*irs1* and *irs2*), and insulin synthesis/secretion (*pdx1*, *mafa*, *neurod*, and *foxa2*) in ApoC3-transgenic *versus* WT littermates. Thus, despite the persistence of severe hypertriglyceridemia, ApoC3-transgenic mice maintained normal beta cell mass and function with normal glucose metabolism and insulin sensitivity.

In keeping with our findings, Kozlitina *et al.* (49) reported that there is a lack of association between hypertriglyceridemia and insulin resistance in human subjects with genetic *APOC3* variants. Although this study did not assess beta cell mass and function, fasting levels of blood glucose and plasma insulin as well as HOMA-IR index were normal in the cohort with *APOC3* variants. Reaven *et al.* (50) showed that hypertriglyceridemic ApoC3-transgenic mice are neither insulin resistant nor hyperinsulinemic. These results together with our data argue against the notion that hypertriglyceridemia is an independent risk factor for beta cell dysfunction.

Our studies are at variance with Avall *et al.* (51), who reported that adenovirus-mediated ectopic ApoC3 production resulted in beta cell apoptosis and dysfunction in MIN6 cells and islets. However, the physiological significance of this study is uncertain, as islet cells are not the cell type responsible for endogenous ApoC3 production. On the other hand, the major findings derived from this study are contradicted by Størling *et*

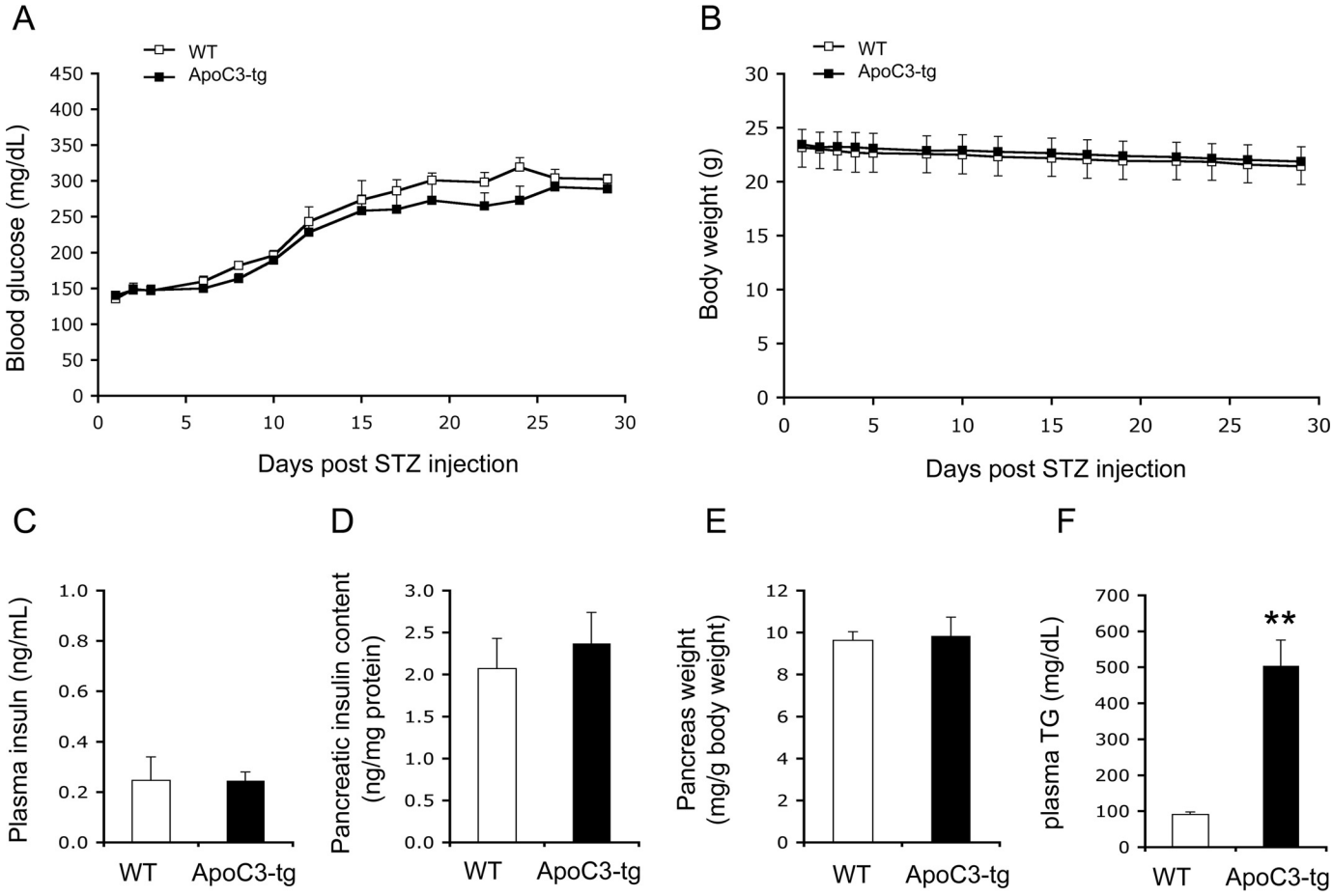


FIGURE 7. **Impact of hypertriglyceridemia on the course of low-dose STZ-induced diabetes in ApoC3-transgenic mice.** ApoC3-transgenic ($n = 8$, 5 males and 3 females, 12 weeks old) and sex/age-matched WT littermates ($n = 8$, 4 males and 4 females) were intraperitoneally injected daily with STZ (50 mg/kg) for 5 days. Body weight and blood glucose levels were determined post STZ injection. *A,* blood glucose levels. Morning blood glucose levels were determined at the non-fasting condition. *B,* body weight. *C,* plasma insulin levels. *D,* pancreatic insulin content. *E,* pancreas weight relative to body weight. *F,* plasma TG levels. Data in *panels C–F* were obtained at day 30 after STZ treatment. **, $p < 0.001$ *versus* WT.

al. (52), who showed that supplementation of ApoC3 proteins somewhat attenuated proinflammatory cytokine-induced beta cell apoptosis in primary rat islets in culture.

In conclusion, we demonstrated that hypertriglyceridemia alone did not act as an independent factor for instigating beta cell dysfunction in ApoC3-transgenic mice. Furthermore, we showed that the prevailing hypertriglyceridemia did not exacerbate the effect of hyperglycemia on beta cell function and accelerate the development of diabetes in STZ-treated ApoC3-transgenic mice. These results suggest that hypertriglyceridemia, which is a major therapeutic target for reducing cardiovascular risk, may not be a primary target for preserving functional beta cell mass in obesity and type 2 diabetes. However, we must acknowledge that our studies could not preclude the possibility that hypertriglyceridemia could compound the impact of hyperglycemia and insulin resistance in combination on beta cell function. The resulting combinatory effect, termed "glucolipotoxicity," is known to elicit beta cell oxidative stress and cause beta cell dysfunction in type 2 diabetes (53–56). Therefore, further studies are warranted to address whether hypertriglyceridemia would aggravate the deleterious effect of hyperglycemia and insulin resistance on beta cell mass and function in ApoC3-transgenic mice.

Author Contributions—Y. Z. L., X. C., C. L. J., and S. Q. conceived the idea and coordinated the studies. Y. Z. L. and X. C. conducted the animal studies. T. Z. and S. L. performed real-time quantitative RT-PCR assay and immunocytochemistry. X. C. and J. Y. performed *ex vivo* GSIS studies and low-dose STZ studies in mice. X. X. and G. G. performed anti-insulin and anti-glucagon immunohistochemistry. Y. Z. L., X. C., and H. H. D. analyzed the data and wrote the manuscript. All authors reviewed the results and approved the final version of the manuscript.

References

1. Arsenault, B. J., Boekholdt, S. M., and Kastelein, J. J. (2011) Lipid parameters for measuring risk of cardiovascular disease. *Nat. Rev. Cardiol.* **8,** 197–206
2. Hopkins, P. N., Heiss, G., Ellison, R. C., Province, M. A., Pankow, J. S., Eckfeldt, J. H., and Hunt, S. C. (2003) Coronary artery disease risk in familial combined hyperlipidemia and familial hypertriglyceridemia: a case-control comparison from the National Heart, Lung, and Blood Institute Family Heart Study. *Circulation* **108,** 519–523
3. Stewart, M. W., Laker, M. F., and Alberti, K. G. (1994) The contribution of lipids to coronary heart disease in diabetes mellitus. *J. Intern. Med. Suppl.* **736,** 41–46
4. DeFronzo, R. A. (1997) Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis. *Neth. J. Med.* **50,** 191–197

5. Krauss, R. M. (1998) Atherogenicity of triglyceride-rich lipoproteins. *Am. J. Cardiol.* **81**, 13B-17B
6. Brewer, H. B., Jr. (1999) Hypertriglyceridemia: changes in the plasma lipoproteins associated with an increased risk of cardiovascular disease. *Am. J. Cardiol.* **83**, 3F-12F
7. Bard, J. M., Charles, M. A., Juhan-Vague, I., Vague, P., André, P., Safar, M., Fruchart, J. C., Eschwege, E., and BIGPRO Study Group (2001) Accumulation of triglyceride-rich lipoprotein in subjects with abdominal obesity. *Arterioscler. Thromb. Vasc. Biol.* **21,** 407–414
8. Olivieri, O., Stranieri, C., Bassi, A., Zaia, B., Girelli, D., Pizzolo, F., Trabetti, E., Cheng, S., Grow, M. A., Pignatti, P. F., and Corrocher, R. (2002) ApoC-III gene polymorphisms and risk of coronary artery disease. *J. Lipid Res.* **43,** 1450–1457
9. Lewis, G. F., Carpentier, A., Adeli, K., and Giacca, A. (2002) Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr. Rev.* **23,** 201–229
10. Chan, D. C., Watts, G. F., Barrett, P. H., Mamo, J. C., and Redgrave, T. G. (2002) Markers of triglyceride-rich lipoprotein remnant metabolism in visceral obesity. *Clin. Chem.* **48,** 278–283
11. Nieves, D. J., Cnop, M., Retzlaff, B., Walden, C. E., Brunzell, J. D., Knopp, R. H., and Kahn, S. E. (2003) The atherogenic lipoprotein profile associated with obesity and insulin resistance is largely attributable to intra-abdominal fat. *Diabetes* **52,** 172–179
12. Herbert, P. N., Assmann, G., Gotto, A. M. J., and Frederichson, D. S. (1983) Disorders of the lipoprotein and lipid metabolism. In: *The Metabolic Basis of Inherited Diseases* (Stanbury, J. B., Wyngaarden, D., Frederichson, A. S., Goldstein, J. L., and Brown, M. S., eds) 5th Ed. pp. 589–651, McGraw-Hill, New York
13. Jong, M. C., Hofker, M. H., and Havekes, L. M. (1999) Role of apoCs in lipoprotein metabolism: functional differences between apoC1, apoC2 and apoC3. *Arterioscler. Thromb. Vasc. Biol.* **19,** 472–484
14. Wang, C. S., McConathy, W. J., Kloer, H. U., and Alaupovic, P. (1985) Modulation of lipoprotein lipase activity by apolipoproteins: effect of apolipoprotein C-III. *J. Clin. Invest.* **75,** 384–390
15. McConathy, W. J., Gesquiere, J. C., Bass, H., Tartar, A., Fruchart, J. C., and Wang, C. S. (1992) Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein C-III. *J. Lipid Res.* **33,** 995–1003
16. Kinnunen, P. K., and Ehnolm, C. (1976) Effect of serum and C apolipoproteins from very low density lipoproteins on human post-heparin plasma hepatic lipase. *FEBS Lett.* **65,** 354–357
17. Quarfordt, S. H., Michalopoulos, G., and Schirmer, B. (1982) The effect of human C apolipoproteins on the *in vitro* hepatic metabolism of triglyceride emulsions in the rat. *J. Biol. Chem.* **257,** 14642–14647
18. Mann, C. J., Troussard, A. A., Yen, F. T., Hannouche, N., Najib, J., Fruchart, J. C., Lotteau, V., André, P., and Bihain, B. E. (1997) Inhibitory effects of specific apolipoprotein C-III isoforms on the binding of triglyceride-rich lipoproteins to the lipolysis-stimulated receptor. *J. Biol. Chem.* **272,** 31348–31354
19. Qin, W., Sundaram, M., Wang, Y., Zhou, H., Zhong, S., Chang, C. C., Manhas, S., Yao, E. F., Parks, R. J., McFie, P. J., Stone, S. J., Jiang, Z. G., Wang, C., Figeys, D., Jia, W., and Yao, Z. (2011) Missense mutation in APOC3 within the C-terminal lipid binding domain of human ApoC-III results in impaired assembly and secretion of triacylglycerol-rich very low density lipoproteins: evidence that ApoC-III plays a major role in the formation of lipid precursors within the microsomal lumen. *J. Biol. Chem.* **286,** 27769–27780
20. Chan, D. C., Watts, G. F., Nguyen, M. N., and Barrett, P. H. (2006) Apolipoproteins C-III and A-V as predictors of very-low-density lipoprotein triglyceride and apolipoprotein B-100 kinetics. *Arterioscler. Thromb. Vasc. Biol.* **26,** 590–596
21. Taskinen, M. R., Adiels, M., Westerbacka, J., Söderlund, S., Kahri, J., Lundbom, N., Lundbom, J., Hakkarainen, A., Olofsson, S. O., Orho-Melander, M., and Borén, J. (2011) Dual metabolic defects are required to produce hypertriglyceridemia in obese subjects. *Arterioscler. Thromb. Vasc. Biol.* **31,** 2144–2150
22. Cohn, J. S., Patterson, B. W., Uffelman, K. D., Davignon, J., and Steiner, G. (2004) Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. *J. Clin. Endocrinol. Metab.* **89,** 3949–3955
23. Petersen, K. F., Dufour, S., Hariri, A., Nelson-Williams, C., Foo, J. N., Zhang, X. M., Dziura, J., Lifton, R. P., and Shulman, G. I. (2010) Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N. Engl. J. Med.* **362,** 1082–1089
24. Norata, G. D., Tsimikas, S., Pirillo, A., and Catapano, A. L. (2015) Apolipoprotein C-III: from pathophysiology to pharmacology. *Trends Pharmacol. Sci.* **36,** 675–687
25. Altomonte, J., Cong, L., Harbaran, S., Richter, A., Xu, J., Meseck, M., and Dong, H. H. (2004) Foxo1 mediates insulin action on apoC-III and triglyceride metabolism. *J. Clin. Invest.* **114,** 1493–1503
26. Waterworth, D. M., Hubacek, J. A., Pitha, J., Kovar, J., Poledne, R., Humphries, S. E., and Talmud, P. J. (2000) Plasma levels of remnant particles are determined in part by variation in the APOC3 gene insulin response element and the APOCI-APOE cluster. *J. Lipid Res.* **41,** 1103–1109
27. Hegele, R. A., Connelly, P. W., Hanley, A. J., Sun, F., Harris, S. B., and Zinman, B. (1997) Common genetic variation in the APOC3 promoter associated with variation in plasma lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* **17,** 2753–2758
28. Chen, M., Breslow, J. L., Li, W., and Leff, T. (1994) Transcriptional regulation of the apoC-III gene by insulin in diabetic mice: correction with changes in plasma triglyceride levels. *J. Lipid Res.* **35,** 1918–1924
29. Ebara, T., Ramakrishnan, R., Steiner, G., and Shachter, N. S. (1997) Chylomicronemia due to apolipoprotein CIII overexpression in apolipoprotein E-null mice: apolipoprotein CIII-induced hypertriglyceridemia is not mediated by effects on apolipoprotein E. *J. Clin. Invest.* **99,** 2672–2681
30. Talmud, P. J., and Humphries, S. E. (1997) Apolipoprotein C-III gene variation and dyslipidemia. *Curr. Opin. Lipidol.* **8,** 154–158
31. Ito, Y. N., Azrolan, N., O'Connell, A., Walsh, A., and Breslow, J. L. (1990) Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. *Science* **249,** 790–793
32. Maeda, N., Li, H., Lee, D., Oliver, P., Quarfordt, S. H., and Osada, J. (1994) Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. *J. Biol. Chem.* **269,** 23610–23616
33. Gerritsen, G., Rensen, P. C., Kypreos, K. E., Zannis, V. I., Havekes, L. M., and Willems van Dijk, K. (2005) ApoC-III deficiency prevents hyperlipidemia induced by apoE overexpression. *J. Lipid Res.* **46,** 1466–1473
34. Jong, M. C., Rensen, P. C., Dahlmans, V. E., van der Boom, H., van Berkel, T. J., and Havekes, L. M. (2001) Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice. *J. Lipid Res.* **42,** 1578–1585
35. Pollin, T. I., Damcott, C. M., Shen, H., Ott, S. H., Shelton, J., Horenstein, R. B., Post, W., McLenithan, J. C., Bielak, L. F., Peyser, P. A., Mitchell, B. D., Miller, M., O'Connell, J. R., and Shuldiner, A. R. (2008) A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. *Science* **322,** 1702–1705
36. Jørgensen, A. B., Frikke-Schmidt, R., Nordestgaard, B. G., and Tybjærg-Hansen, A. (2014) Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N. Engl. J. Med.* **371,** 32–41
37. TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute, Crosby, J., Peloso, G. M., Auer, P. L., Crosslin, D. R., Stitziel, N. O., Lange, L. A., Lu, Y., Tang, Z. Z., Zhang, H., Hindy, G., Masca, N., Stirrups, K., Kanoni, S., Do, R., Jun, G., *et al.* (2014) Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N. Engl. J. Med.* **371,** 22–31
38. Li, W. W., Dammerman, M. M., Smith, J. D., Metzger, S., Breslow, J. L., and Leff, T. (1995) Common genetic variation in the promoter of the human apo CIII gene abolishes regulation by insulin and may contribute to hypertriglyceridemia. *J. Clin. Invest.* **96,** 2601–2605
39. Dallinga-Thie, G. M., Groenendijk, M., Blom, R. N., De Bruin, T. W., and De Kant, E. (2001) Genetic heterogeneity in the apolipoprotein C-III promoter and effects of insulin. *J. Lipid Res.* **42,** 1450–1456
40. Aalto-Setälä, K., Fisher, E. A., Chen, X., Chajek-Shaul, T., Hayek, T., Zechner, R., Walsh, A., Ramakrishnan, R., Ginsberg, H. N., and Breslow, J. L. (1992) Mechanism of hypertriglyceridemia in human apolipoprotein

(apo) CIII transgenic mice: diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apoE on the particles. *J. Clin. Invest.* **90,** 1889–1900
41. Qu, S., Perdomo, G., Su, D., D'Souza, F. M., Shachter, N. S., and Dong, H. H. (2007) Effects of apoA-V on HDL and VLDL metabolism in APOC3 transgenic mice. *J. Lipid Res.* **48,** 1476–1487
42. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., and Turner, R. C. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28,** 412–419
43. Su, D., Zhang, N., He, J., Qu, S., Slusher, S., Bottino, R., Bertera, S., Bromberg, J., and Dong, H. H. (2007) Angiopoietin-1 production in islets improves islet engraftment and protects islets from cytokine-induced apoptosis. *Diabetes* **56,** 2274–2283
44. Zhang, T., Kim, D. H., Xiao, X., Lee, S., Gong, Z., Muzumdar, R., Calabuig-Navarro, V., Yamauchi, J., Harashima, H., Wang, R., Bottino, R., Alvarez-Perez, J. C., Garcia-Ocaña, A., Gittes, G., and Dong, H. H. (2016) FoxO1 plays an important role in regulating beta-cell compensation for insulin resistance in male mice. *Endocrinology* **157,** 1055–1070
45. Kamagate, A., Qu, S., Perdomo, G., Su, D., Kim, D. H., Slusher, S., Meseck, M., and Dong, H. H. (2008) FoxO1 mediates insulin-dependent regulation of hepatic VLDL production in mice. *J. Clin. Invest.* **118,** 2347–2364
46. Talchai, C., Xuan, S., Lin, H. V., Sussel, L., and Accili, D. (2012) Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. *Cell* **150,** 1223–1234
47. Withers, D. J., Gutierrez, J. S., Towery, H., Burks, D. J., Ren, J. M., Previs, S., Zhang, Y., Bernal, D., Pons, S., Shulman, G. I., Bonner-Weir, S., and White, M. F. (1998) Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* **391,** 900–904
48. Kubota, N., Tobe, K., Terauchi, Y., Eto, K., Yamauchi, T., Suzuki, R., Tsubamoto, Y., Komeda, K., Nakano, R., Miki, H., Satoh, S., Sekihara, H., Sciacchitano, S., Lesniak, M., Aizawa, S., *et al.* (2000) Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory beta-cell hyperplasia. *Diabetes* **49,** 1880–1889
49. Kozlitina, J., Boerwinkle, E., Cohen, J. C., and Hobbs, H. H. (2011) Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. *Hepatology* **53,** 467–474
50. Reaven, G. M., Mondon, C. E., Chen, Y. D., and Breslow, J. L. (1994) Hypertriglyceridemic mice transgenic for the human apolipoprotein C-III gene are neither insulin resistant nor hyperinsulinemic. *J. Lipid Res.* **35,** 820–824
51. Åvall, K., Ali, Y., Leibiger, I. B., Leibiger, B., Moede, T., Paschen, M., Dicker, A., Dare, E., Köhler, M., Ilegems, E., Abdulreda, M. H., Graham, M., Crooke, R. M., Tay, V. S., Refai, E., *et al.* (2015) Apolipoprotein CIII links islet insulin resistance to beta-cell failure in diabetes. *Proc. Natl. Acad. Sci. U.S.A.* **112,** E2611–E2619
52. Størling, J., Juntti-Berggren, L., Olivecrona, G., Prause, M. C., Berggren, P. O., and Mandrup-Poulsen, T. (2011) Apolipoprotein CIII reduces proinflammatory cytokine-induced apoptosis in rat pancreatic islets via the Akt prosurvival pathway. *Endocrinology* **152,** 3040–3048
53. Robertson, R. P. (2004) Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J. Biol. Chem.* **279,** 42351–42354
54. Malhotra, J. D., and Kaufman, R. J. (2007) Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid. Redox Signal.* **9,** 2277–2293
55. Poitout, V., and Robertson, R. P. (2008) Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocr. Rev.* **29,** 351–366
56. Meares, G. P., Fontanilla, D., Broniowska, K. A., Andreone, T., Lancaster, J. R., Jr., and Corbett, J. A. (2013) Differential responses of pancreatic beta-cells to ROS and RNS. *Am. J. Physiol. Endocrinol Metab.* **304,** E614–E622