SREBP1 is required for the induction by glucose of pancreatic β-cell genes involved in glucose sensing[S]

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Abstract Previous studies have reported both positive and negative effects of culture of islets at high glucose concentrations on regulated insulin secretion. Here, we have re-examined this question in mouse islets and determined the role of changes in lipid synthesis in the effects of glucose. Glucose-stimulated insulin secretion (GSIS) and gene expression were examined in islets from C57BL/6 mice or littermates deleted for sterol-regulatory element binding protein-1 (SREBP1) after 4 days of culture at high glucose concentrations. Culture of control islets at 30 versus 8 mmol/l glucose led to enhanced secretion at both basal (3 mmol/l) and stimulatory (17 mmol/l) glucose concentrations and to enhanced triacylglycerol accumulation. These changes were associated with increases in the expression of genes involved in glucose sensing (glucose transporter 2, glucokinase, sulfonylurea receptor 1, inwardly rectifying K^+ channel 6.2), differentiation (pancreatic duodenal homeobox 1), and lipogenesis (*Srebp1*, fatty acid synthase, acetyl-coenzyme A carboxylase 1, stearoyl-coenzyme A desaturase 1). When cultured at either 8 or 30 mmol/l glucose, SREBP1-deficient (SREBP1$^{-/-}$) islets displayed reduced GSIS and triacylglycerol content compared with normal islets. Correspondingly, glucose induction of the above genes in control islets was no longer observed in SREBP1$^{-/-}$ mouse islets. We conclude that enhanced lipid synthesis mediated by SREBP1c-dependent genes is required for the adaptive changes in islet gene expression and insulin secretion at high glucose concentrations.—Diraison, F., M. A. Ravier, S. K. Richards, R. M. Smith, H. Shimano, and G. A. Rutter. **SREBP1 is required for the induction by glucose of pancreatic β-cell genes involved in glucose sensing. *J. Lipid Res.* 2008.** 49: **814–822.**

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The effects of chronic hyperglycemia on the function of wild-type β-cells have been investigated in several earlier studies (1, 2). Chronically increased glucose concentrations have been proposed to cause a progressive inhibition of glucose-stimulated insulin secretion (GSIS) in vivo and in in vitro studies on islets from human (3, 4) and rat (5) as well as in insulinoma cells (6). By contrast, other studies have reported that chronic culture at high glucose concentrations can lead to a left shift in the response to glucose of mouse islets (7–9).

Increased glucose concentrations stimulate the expression of several genes likely to affect the differentiated function of β-cells. These include genes involved in regulating glycolytic flux [*Slc2a2*, coding for glucose transporter 2 (10), glucokinase (*Gck*) (11)], lipogenesis [fatty acid synthase (*Fas*) (12), acetyl-coenzyme A carboxylase 1 (*Acc1*) (13), stearoyl-coenzyme A desaturase (*Scd1*) (14), carbohydrate-responsive element binding protein (*Chrebp*) (15, 16)], and electrical activity [*Abcc8* and inwardly rectifying K^+ channel 6.2 (*Kcnj11*), coding for the ATP-sensitive potassium channel subunits and the sulfonylurea receptor 1 (14)]. Underlying these changes, high glucose concentrations increase the levels (17) and nuclear accumulation (18) of pancreatic duodenal homeobox 1 (PDX1). Furthermore, in rat islets (19) and clonal β-cell lines (20, 21), glucose increases the expression of the lipogenic tran-

Abbreviations: *Abcc8*, ATP-binding cassette subfamily C member 8; *Acc1*, acetyl-coenzyme A carboxylase 1; *Chrebp*, carbohydrate-responsive element binding protein; ER, endoplasmic reticulum; *Fas*, fatty acid synthase; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *Gck*, glucokinase; GSIS, glucose-stimulated insulin secretion; *Kcnj11*, inwardly rectifying K^+ channel 6.2; KRBH, Krebs-Ringer bicarbonate buffer; *NKx6.1*, NK6 transcription factor locus 1; *Pdx1*, pancreatic duodenal homeobox 1; *Scd1*, stearoyl-coenzyme A desaturase 1; *Slc2a2*, glucose transporter 2; SREBP, sterol-regulatory element binding protein; TG, triglyceride.

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scription factor sterol-regulatory element binding protein-1c (SREBP1c).

SREBP1c belongs to a family of sterol-regulated factors also including SREBP1a and SREBP2 (22). Whereas SREBP2 is involved in the regulation of genes implicated in sterol synthesis (23), SREBP1c controls the expression of genes involved in triglyceride (TG) synthesis (24). SREBPs are basic helix-loop-helix leucine zipper factors and are synthesized as precursor proteins bound to the endoplasmic reticulum (ER) and nuclear membranes. When required, a SREBP cleavage-activating protein (25) escorts SREBPs from the ER to the Golgi, where SREBPs are sequentially cleaved by Site-1 and Site-2 proteases. The processed, mature SREBPs then enter the nucleus to activate the promoters of specific genes.

Several in vitro studies have shown that overexpression of SREBP1c in β-cells induces the lipogenic genes *Fas* and *Acc1*, leading to an accumulation of TGs and an inhibition of GSIS (20, 26). Recent studies in a model cellular system implicated SREBP1 in β-cell glucolipotoxicity (21), and microarray gene expression profiles of rat islets overexpressing SREBP1 using adenoviruses showed changes in the expression of a number of proapoptotic but also antiapoptotic genes (27).

The above observations have suggested that the upregulation of SREBP1 in hyperglycemic states is likely principally to exert a deleterious effect on β-cell function. However, we recently found that SREBP1c inactivation in Zucker diabetic fatty rat islets failed to normalize GSIS in this model of lipotoxicity β-cell dysfunction (28), implying that small increases in SREBP1 level and TG content are not the principal cause of defective secretion.

Culture of mouse islets at high glucose concentrations has been shown to cause hypersecretion of insulin (29, 30), although the mechanisms involved are unclear. Here, we assessed whether SREBP1 induction in response to high glucose concentrations may be important for the enhanced expression of genes, which then mediate the adaptive response to hyperglycemia of mouse islets. We also explored the impact of SREBP1 deletion on the ability of islets from this species to respond to an extended period at high glucose concentrations with enhanced insulin secretion. Specifically, we used islets from wild-type C57BL/6J mice or littermates deleted for SREBP1 by homologous recombination (31).

We show that culture for 96 h at 8 or 30 mmol/l glucose markedly (>50%) impairs GSIS as well as TG content in islets from SREBP1-deficient ($SREBP1^{-/-}$) versus wild-type mice. This difference is associated with the loss, in islets lacking SREBP1, of the induction by high glucose not only of lipogenic genes (*Fas, Acc1, Scd1*) but, unexpectedly, of genes involved in β-cell differentiation, glucose sensing, and electrical activity. Triacsin C, which prevents the synthesis and oxidation of fatty acyl-CoA (32), also blocked the induction of several of the above genes and decreased the TG content and glucose responsiveness of cultured islets. We propose that adequate lipid synthesis is a requirement for the adaptive changes of mouse islets at high glucose concentrations.

MATERIALS AND METHODS

Materials

Collagenase was obtained from Serva (Heidelberg, Germany). Culture medium (DMEM), FCS, and glutamine were obtained from Gibco BRL (Paisley, Renfrewshire, UK). Antibiotics were from Sigma (Poole, Dorset, UK).

Animals and genotyping

$SREBP1^{-/-}$, $SREBP1^{+/-}$, and $SREBP1^{+/+}$ mice were generated as described and bred in the animal facility of the University of Bristol. The mice were fed a normal rodent diet, housed in colony cages, and maintained on a 12 h light/12 h dark cycle. Mice were genotyped by PCR on tail genomic DNA with specific primers. For the triacsin C experiments (see Figs. 3, 5 below), islets were isolated from 3–4 month old C57BL/6J mice from a separate colony (Harlan, Bicester, UK). All animal procedures were carried out in accordance with United Kingdom Home Office welfare guidelines and project license restrictions.

Blood glucose and plasma insulin measurements

Tail blood was assayed for glucose concentration using a Glucometer Accu-chek™ (Roche). Plasma insulin was measured using a rat insulin kit (Chrystal Chem, Inc., Downers Grove, IL).

Isolation and culture of pancreatic islets

Mice (3–4 months old) were euthanized by cervical dislocation, and islets were isolated as described previously (33). Briefly, pancreata were digested with collagenase and hand-picked. The medium used for islet isolation was a bicarbonate-buffered solution (120 mmol/l NaCl, 4.8 mmol/l KCl, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l $MgCl_2$, 24 mmol/l $NaHCO_3$, 10 mmol/l glucose, and 1 mg/ml BSA). It was gassed with O_2/CO_2 (95%/5%) and equilibrated at pH 7.4. For culture in chronically increased glucose concentrations, islets were incubated for 16 h in DMEM containing 10% (v/v) FCS, 11 mmol/l glucose, 2 mmol/l glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin and incubated at 37°C with 95% air and 5% CO_2. Islets were then cultured for 96 h at 8 or 30 mmol/l glucose in the presence or absence of triacsin C (10 μmol/l; Biomol, Exeter, UK) before use. Previous studies have shown that such conditions of chronic high glucose culture had no effect on C57BL/6 mouse islet viability (30) or DNA content (34).

Insulin secretion by static incubation

Cultured islets were incubated for 60 min in a shaking-water bath at 37°C in 1 ml of Krebs-Ringer bicarbonate buffer (KRBH; 130 mmol/l NaCl, 3.6 mmol/l KCl, 1.5 mmol/l $CaCl_2$, 0.5 mmol/l $MgSO_4$, 0.5 mmol/l KH_2PO_4, 2.0 mmol/l $NaHCO_3$, and 10 mmol/l HEPES) supplemented with 11 mmol/l glucose and 0.1% (w/v) BSA. KRBH was equilibrated with O_2/CO_2 (95%/5%), pH 7.4. Batches of three islets were handpicked and incubated for 30 min in 0.5 ml of KRBH as above, containing either 3 or 17 mmol/l glucose. Medium was collected for insulin secretion measurements, and islets were harvested with acidified ethanol to determine cellular insulin content. Insulin was measured by radioimmunoassay (Linco Research, St. Charles, MO).

RNA extraction and TaqMan® real-time PCR assay

Total RNA was isolated by cell lysis in TRIzol (Gibco) according to the manufacturer's instructions. RNA samples were treated with DNA-free™ (Ambion, Austin, TX) to remove any genomic DNA contamination and quantified by RiboGreen assay (Molecular Probes). cDNA (100 μl) was synthesized from 1 μg of total

RNA using random hexamer primers and Moloney murine leukemia virus reverse transcriptase (Applied Biosystems, Warrington, UK). Quantitative real-time PCR (TaqMan®) was performed using 25 ng of reverse-transcribed total RNA with 300 nmol/l sense and antisense primers, 100 nmol/l probe (**Table 1**), and 12.5 μl of Master Mix (Qiagen, Crawley, UK) in a total volume of 25 μl in an ABI PRISM 7700 sequence detection system instrument. Probes were labeled with 6-carboxyfluorescein and 6-carboxy-*N,N,N′,N″*-tetramethylrhodamine. Standard curves were constructed by amplifying serial dilutions of untreated mouse islet cDNA (50 ng to 0.64 pg) and plotting cycle threshold values as a function of starting reverse-transcribed RNA, the slope of which was used to calculate the relative expression of the target gene.

TG measurements

Total lipids were extracted from 50 islets using chloroform-methanol (2:1, v/v) (35). Extracted lipids were air-dried, and 10 μl of a detergent (Thesit; Fluka, Gillingham, Dorset, UK) was added to the dry pellet. Samples were air-dried again and resuspended in 30 μl of water (36). TG was measured using a commercial kit (Infinity™ Triglyceride Reagent; Sigma) and a standard curve of triolein (Sigma) treated in parallel with the samples.

Total islet protein assay

Total protein (10 islets) was extracted using radioimmunoprecipitation assay buffer, comprising PBS supplemented with 1.0% (v/v) Nonidet P-40, 0.5% (w/v) sodium deoxycholate, and 0.1% (w/v) SDS. Protein concentration was determined using the BCA kit (Pierce, Rockford, IL).

Statistics

Data are given as means ± SEM, and statistical analysis was performed using one-way ANOVA followed by the Newman-Keuls test.

RESULTS

Metabolic parameters

We observed no significant differences in body weight, blood glucose, or insulin concentrations between SREBP1$^{-/-}$, SREBP1$^{+/-}$, and wild-type mice at 3–4 months of age in either the fasting or the fed state (**Table 2**). Other parameters are discussed below and in the supplementary data.

Effects of culture of wild-type or SREBP1$^{-/-}$ islets at high glucose concentrations on GSIS and TG content

Islets were cultured at glucose concentrations either representing severe hyperglycemia (30 mmol/l) or at a level in the physiological range for fed mice (8 mmol/l) (Table 2); the effects of lower glucose concentrations (e.g., 5.5 mmol/l), which correspond to the starved state (Table 2), were not examined here, because our own data (F. Diraison and G. A. Rutter, unpublished data) and the observations of others (37, 38) indicate that these are associated with increased apoptosis during extended islet culture. Culture of wild-type islets at 30 versus 8 mmol/l glucose concentrations increased basal (3 mmol/l) and high (17 mmol/l) GSIS (**Fig. 1**). In contrast to freshly isolated islets, in which

TABLE 1. Primer and probe sequences used in quantitative real-time RT-PCR analysis (TaqMan®)

Gene	Accession Number	Forward Primer (5′–3′)	Reverse Primer (5′–3′)	Probe (5′–3′)
Sterol-regulatory element binding protein1c	NM_011480	CCACTAGAGGTCGGCATGGT	TCCCTTGAGGACCTTTGTCATT	TGCTTGTCAGCTCACCCTCTGGAA
Cyclophilin	NM_017101	TATCTGCACTGCCAAGACTGA	CCACAATGCTCATGCCTTCTTTCA	CCAAAGACCACATGCTTGCCATCCA
Glyceraldehyde-3-phosphate dehydrogenase	M32599	GTCGTGGATCTGACGTGCC	GATGCCTGCTTCACCACCTT	CCTGGAGAAACCTGCCAAGTATGATGACAT
Fatty acid synthase	NM_007988	CCCTTGATGAAGAGGGATCA	ACTCCACAGGTGGGAACAAG	TCTTTCTCACCAACCTTGGCAAGGT
Acetyl-coenzyme A carboxylase 1	AY451393	TTCTGAATGTGGCTATCAAGACTG	TGCTGGGTGAACTCTCTGAACA	CGATATTGAGGATGACAGGCTTGCAGCT
Carbohydrate-responsive element binding protein	NM_021455	CAACTCAGCACTTCCACAAG	TGGAAACTTTCACCAGGATT	CTGACTGACCCCAGCCTTGT
Stearoyl-coenzyme A desaturase 1	NM_009127	CCTCCGGAAATGAACGAGAG	CAGGACGGATGTCTTCTTCCA	AGGTGAAGACGGTGCCCCTCCAC
Glucose transporter 2	NM_031197	CCCTGGGTACTCTTCACCAA	GCCAAGTAGGATGTGCCAAT	TGGCCCTTGTCACAGGCATTCT
Glucokinase	L38990	TCCCTGTAAGGCACGAAGACAT	ATTGCCACCACATCCATCTCA	CTCTTGATAGCATCTCGGAGAAGTCCCA
Pancreatic duodenal homeobox 1	NM_008814	GAAGAGCCCAACCGCGT	TTGTTTTCCTCGGGTTCCG	CTCCTGCCCACTGGCCTTTCCA
ATP binding cassette transporter c8	L40624	CCCTCTACCAGCACACCAAT	CAGTCAGCATGAGGCAGGTA	CTTTCTCGGCTCTGGATGTCCATCT
Inwardly rectifying K^+ channel 6.2	D50581	TACCACGTCATCGACTCCAA	GTTTCTACCACGCCTTCCAA	ACCACCAGGACCTGGAGATCATTGT
Neurogenin 3	NM_009719	TGCAGCCACATCAAACTCTC	GGTCACCCTGGAAAAGTGA	TGAGTCTGCCCTCATTCAAATCTGC
NKx 6.1	NM_144955	TTCGGAGAATGAGGAGGATGA	ACCGCTCGATTTGTGCTTTT	ACAAACCTCTGGACCCGAACTCTGACG

Probes were labeled with 6-carboxyfluorescein and 6-carboxy-*N,N,N′,N″*-tetramethylrhodamine.

TABLE 2. Serum insulin and glucose levels in SREBP1$^{-/-}$, SREBP1$^{+/-}$, and wild-type mice at 18 h fasted and fed states

Mouse Genotype	n	Weight	Insulin	Glucose
		g	*ng/ml*	*mmol/l*
Fed				
SREBP1$^{+/+}$	9	25.3 ± 0.55	1.27 ± 0.42	8.94 ± 0.46
SREBP1$^{+/-}$	12	24.5 ± 0.54	0.82 ± 0.17	7.86 ± 0.56
SREBP1$^{-/-}$	8	24.7 ± 0.54	1.09 ± 0.42	9.27 ± 0.68
Fasted				
SREBP1$^{+/+}$	10	24.4 ± 1.1	0.40 ± 0.06	4.73 ± 0.28
SREBP1$^{+/-}$	13	22.7 ± 0.8	0.34 ± 0.03	5.46 ± 0.30
SREBP1$^{-/-}$	8	23.2 ± 1.0	0.53 ± 0.09	5.20 ± 0.27

a small increase in the extent of glucose-stimulated (17 vs. 3 mmol/l) insulin secretion was apparent (see supplementary data), SREBP1$^{-/-}$ islets displayed a significantly lower fold change in the acute stimulation of insulin secretion by glucose after culture at either 8 or 30 mmol/l glucose (Fig. 1A, B). A smaller and nonsignificant tendency toward impaired GSIS was also seen in SREBP1$^{+/-}$ islets (Fig. 1A, B).

However, when we compared GSIS between genotypes, we observed that there were no significant differences between the fold stimulation of insulin secretion acutely by 17 versus 3 mmol/l glucose for islets of the same genotype cultured at either 8 or 30 mmol/l (Fig. 1A, B).

Culture for 4 days at 30 mmol/l compared with 8 mmol/l glucose also decreased total insulin content by >85% in each genotype (Fig. 1C), presumably reflecting the sustained stimulation of insulin release under these conditions.

TG accumulation was enhanced significantly by culture of wild-type or SREBP1$^{+/-}$ islets at 30 versus 8 mmol/l glucose (Fig. 1D). Furthermore, with respect to islets from wild-type or SREBP$^{+/-}$ mice, SREBP1$^{-/-}$ mouse islets displayed a substantially (60%) decreased TG content after culture at 8 mmol/l glucose, and no further TG increase was seen in these islets after culture at 30 mmol/l glucose (Fig. 1D).

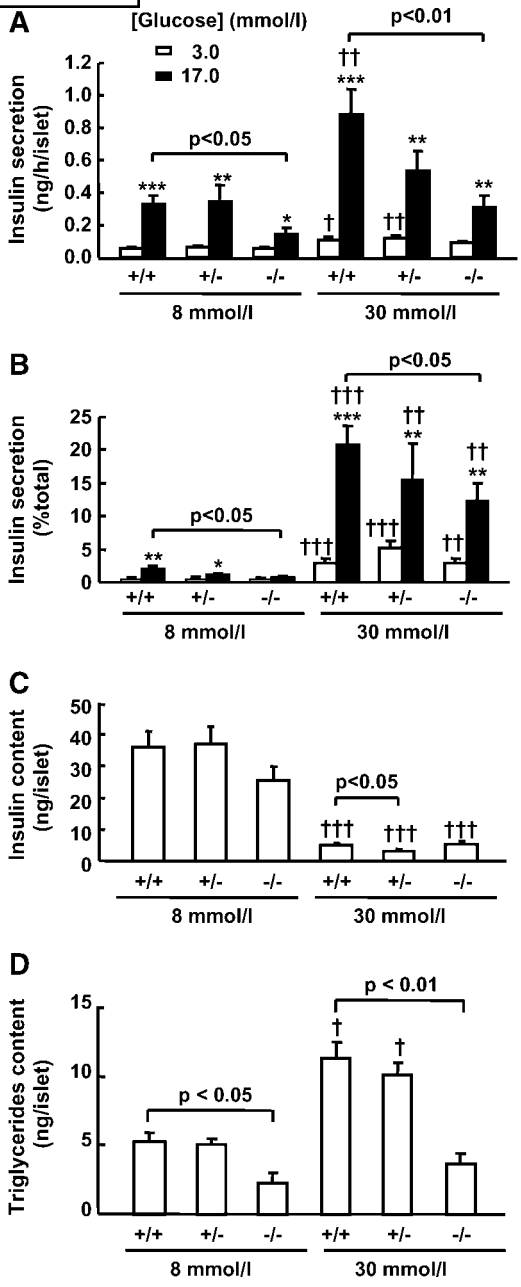

Fig. 1. Effects of glucose and sterol-regulatory element binding protein-1 (SREBP1) deletion on glucose-stimulated insulin secretion (GSIS) and triglyceride (TG) content after chronic exposure of mouse islets to high glucose concentration. After isolation, islets (n = 3/genotype) were cultured for 96 h at 8 or 30 mmol/l glucose before measuring GSIS (five determinations per experiment) and TG content, as described in Materials and Methods. A, B: Insulin release. C: Insulin content. D: TG content. Data are given as means ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for the 17 mmol/l glucose effect; † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ for the effect of chronically increased glucose concentration.

Effects of chronic exposure to high glucose concentrations on gene expression in wild-type or SREBP1$^{-/-}$ islets

To analyze in more detail the mechanisms that may be responsible for the decreases in GSIS in SREBP1$^{-/-}$ versus wild-type islets, we measured the expression of candidate genes using quantitative real-time PCR (TaqMan®) (**Fig. 2**). Deletion of SREBP1 had complex effects on the changes in the lipogenic and other gene expression observed during culture at increased glucose concentrations. Thus, *Fas* and *Gck* gene expression was decreased in SREBP1$^{-/-}$ compared with wild-type islets after 4 days of culture at 8 but not 30 mmol/l glucose (Fig. 2D, G). By contrast, *Acc1*, *Slc2a2*, *Abcc8*, *Kcnj11*, and *Pdx1* mRNA levels were decreased in SREBP1$^{-/-}$ versus wild-type islets at 30 mmol/l glucose but not at 8 mmol/l (Fig. 2E, F, I, J, K). *Scd1* mRNA levels were decreased at 30 and 8 mmol/l (Fig. 2H), whereas SREBP1 deletion had no effect on *Chrebp* or NK6 transcription factor locus 1 (*NKx6.1*) gene expression (Fig. 2C, M). In most cases, the levels of gene expression in heterozygote mice were intermediate between those in wild-type and SREBP1$^{-/-}$ islets at each glucose concentration.

When cultured at 8 mmol/l glucose, neurogenin 3 gene expression was increased significantly in SREBP1$^{+/-}$ and SREBP1$^{-/-}$ mouse islets (Fig. 2L). We observed no changes in the expression of cyclophilin D (Fig. 2A), to

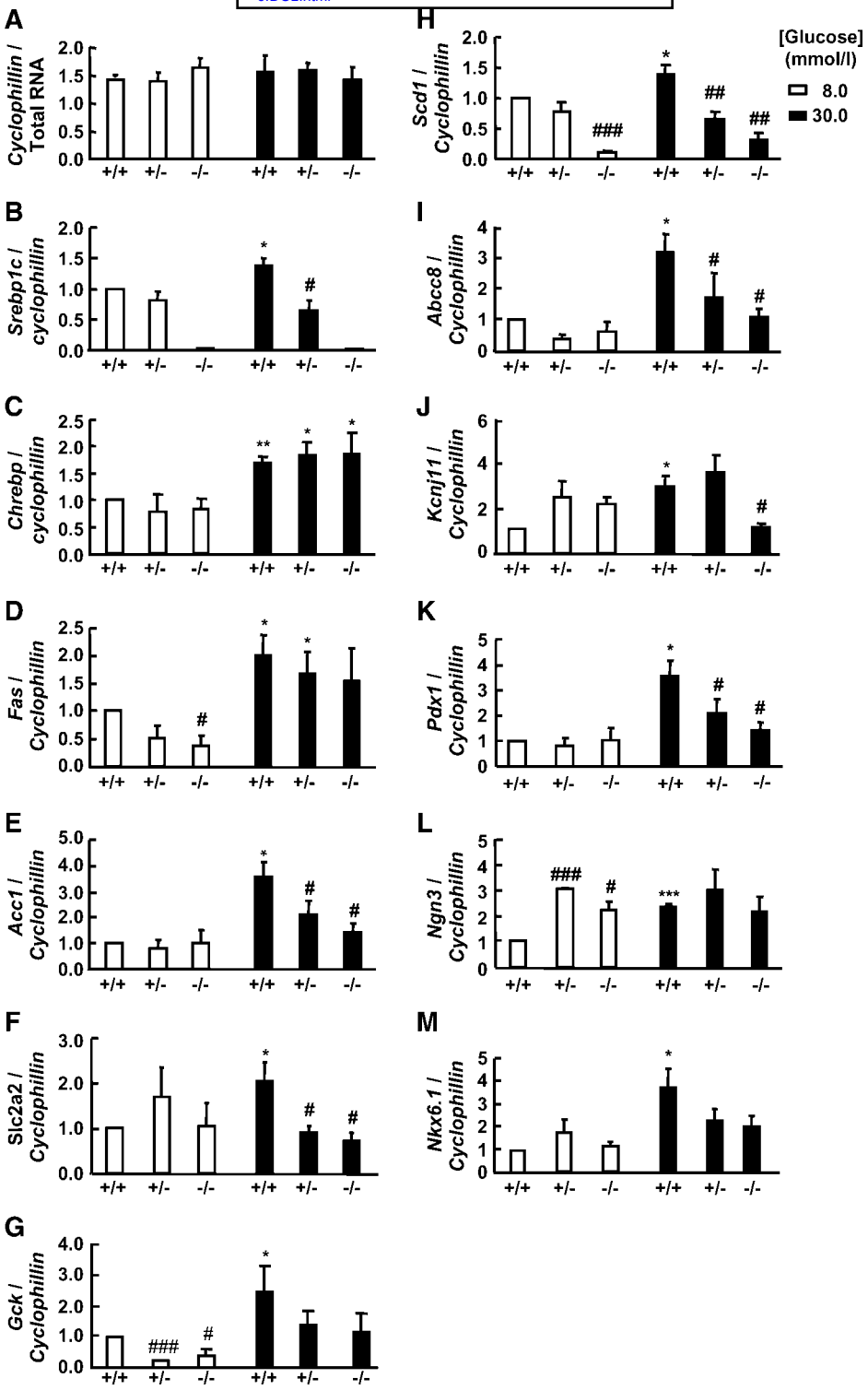

Fig. 2. A–M: Gene expression in islets cultured for 96 h at 8 or 30 mmol/l glucose. After isolation, islets (n = 3/genotype) were cultured for 96 h at 8 or 30 mmol/l glucose before RNA extraction. Data are given as means ± SEM. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ for the genotype effect; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for the effect of chronically increased glucose concentration. *Abcc8*, ATP-binding cassette subfamily C member 8; *Acc1*, acetyl-coenzyme A carboxylase 1; *Chrebp*, carbohydrate-responsive element binding protein; *Fas*, fatty acid synthase; *Gck*, glucokinase; *Slc2a2*, glucose transporter 2; *Kcnj11*, inwardly rectifying K^+ channel 6.2; *Ngn3*, neurogenin 3; *Pdx1*, pancreatic duodenal homeobox 1; *Scd1*, stearoyl-coenzyme A desaturase 1.

which other genes were normalized, or in another "housekeeping" gene, glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) (data not shown), excluding the effects of glucose as being nonspecific.

Effects of triacsin C on GSIS, TG content, and gene expression in islets cultured in chronic high glucose concentrations

To examine the hypothesis that there may be a potential role of acyl-CoA or TG synthesis in the long-term regulation of gene expression and insulin secretion by glucose, we used triacsin C. This pharmacological agent is an inhibitor of long-chain acyl-CoA synthetase and thus of de novo TG synthesis and acyl-CoA oxidation (32).

We first determined whether triacsin C may mimic the effects of SREBP1 deletion on GSIS observed in islets cultured in the same conditions (**Fig. 3**). Islets from wild-type mice were cultured for 96 h at 8 or 30 mmol/l glucose in the presence or absence of 10 μmol/l triacsin C, before measuring GSIS. Addition of triacsin C decreased GSIS by 42.3% and 41.5% when islets were cultured for 96 h at 8 or 30 mmol/l glucose, respectively, but had no effect on basal insulin secretion (Fig. 3).

Under the same conditions, triacsin C had no effect on *Srebp1*, *Chrebp*, *Fas*, *Acc1*, *Scd1*, *Pdx1*, *Gck*, or *Slc2a2* mRNA levels in islets cultured at 8 mmol/l glucose. However, with the exception of *Slc2a2* and *Chrebp* mRNA, which were still induced by 30 mmol/l glucose, upregulation of *Acc1* ($P < 0.05$), *Gck* ($P < 0.05$), *Srebp1*, *Fas*, *Scd1*, and *Pdx1* gene expression was decreased in the presence of triacsin C (**Fig. 4**). Again, we observed no changes in the expression of *cyclophilin D* (Fig. 4) or *Gapdh* (data not shown).

We also measured the effect of this drug on TG content in islets cultured under the same conditions. Triacsin C decreased TG content when islets (vs. nontreated islets) were cultured for 96 h at 8 or 30 mmol/l glucose (**Fig. 5**).

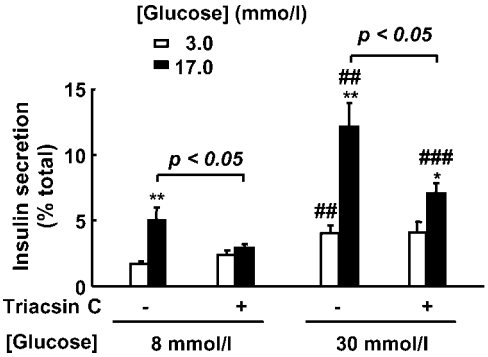

Fig. 3. Effects of triacsin C on glucose-induced insulin secretion in islets exposed to long-term culture at high glucose concentration. After isolation, islets from wild-type mice (n = 3/genotype) were cultured for 96 h at 8 or 30 mmol/l glucose in the presence or absence of triacsin C (10 μmol/l) before measuring GSIS (five determinations per experiment). Data are given as means ± SEM. * $P < 0.05$, ** $P < 0.01$ for the 17 mmol/l glucose effect; ## $P < 0.01$, ### $P < 0.001$ for the chronically increased glucose effect.

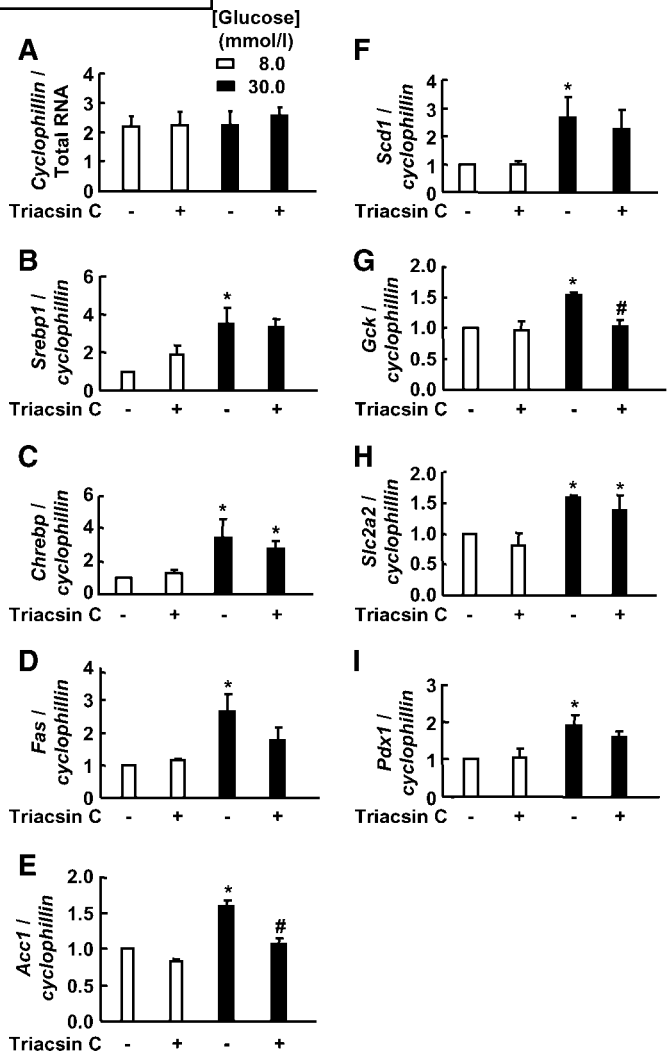

Fig. 4. A–I: Effects of triacsin C on gene expression in islets exposed to long-term culture at high glucose concentration. After isolation, islets from wild-type mice (n = 3/genotype) were cultured for 96 h at 8 or 30 mmol/l glucose in the presence or absence of triacsin C (10 μmol/l) before RNA extraction. Data are given as means ± SEM. * $P < 0.05$ for the chronically increased glucose effect; # $P < 0.05$ for the triacsin C effect.

DISCUSSION

The principal aims of this study were *a*) to reexamine the effects of extended culture at increased glucose concentrations on basal and high GSIS from mouse islets and *b*) to determine whether the induction by glucose of SREBP1 (20), and enhanced fatty acid and/or TG synthesis, might contribute to any of the effects observed.

In agreement with the findings from Khaldi et al. (7) of a "left shift" in the dose response to glucose of mouse islets incubated at high glucose concentrations, we show, first, that both basal and high GSIS are enhanced by culture of C57BL/6 mouse islets at 30 versus 8 mmol/l glucose (Fig. 1). Culture at 8 mmol/l glucose essentially preserved the secretory responses observed in freshly isolated islets (cf. Fig. 1A and supplementary Fig. IA), when rates of release were compared at 3 or 17 mmol/l glucose in each

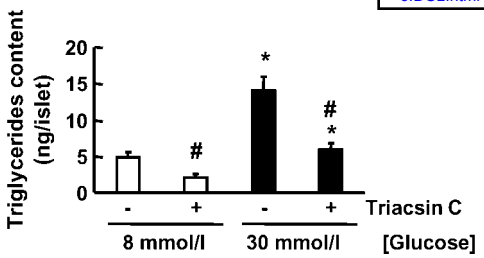

Fig. 5. Effects of triacsin C on TG content in islets exposed to long-term culture at high glucose concentration. After isolation, islets from wild-type mice (n = 3/genotype) were cultured for 96 h at 8 or 30 mmol/l glucose in the presence or absence of triacsin C (10 μmol/l). TG content was measured as described in Materials and Methods. Data are given as means ± SEM. * $P < 0.05$ for the effect of chronically increased glucose concentration; # $P < 0.05$ for the triacsin C effect.

case. Although glucose has been shown to stimulate the growth and proliferation of β-cells, especially in fetal islets (39), it is unlikely that the enhanced secretion of insulin observed in the present study simply reflects an increase in β-cell mass per islet after culture at 30 versus 8 mmol/l.

The present data thus confirm that, in the mouse, culture at mildly or strongly increased glucose concentrations fails to elicit evident "glucotoxic" effects but rather increases basal insulin secretion while preserving glucose-stimulated secretion. In this respect, the response to long-term high glucose treatment of C57BL/6 mouse islets appears to be quite distinct from that of isolated rat islet β-cells, in which even relatively short-term (24 h) exposure to 30 mmol/l glucose caused marked decreases in GSIS (7, 40).

Although SREBP1$^{-/-}$ mice displayed a slight increase in glycemia after an intraperitoneal glucose tolerance test (see supplementary data), it seems possible that this may reflect, at least in part, altered insulin sensitivity rather than defective insulin secretion. Indeed, GSIS was enhanced slightly in freshly isolated islets from mice deleted for both *Srebp1* alleles versus wild-type mice (see supplementary data), consistent with earlier results (26); this difference was reversed upon culture at either 8 or 30 mmol/l glucose. Thus, a clear impairment of GSIS was apparent in SREBP1$^{-/-}$ (but not SREBP1$^{+/-}$) islets after culture at either 8 or 30 mmol/l glucose (Fig. 1), and this change was associated with decreased islet TG content in SREBP1$^{-/-}$ islets (Fig. 1D). These results also show that even if the fold stimulation of insulin secretion for islets of the same genotype were similar when cultured at 8 or 30 mmol/l glucose (Fig. 1A, B), SREBP1$^{-/-}$ islets tolerated less well than wild-type islets prolonged culture at either glucose concentration.

These results thus challenge the physiological relevance of previous findings from ourselves (19) and others (21, 26) that showed that forced overexpression of SREBP1 in β-cells or islets increased lipogenic gene expression, leading to an accumulation of TGs and an inhibition of GSIS as well as an ER stress response and enhanced cell death (41). Nevertheless, it might be argued that more substantial increases in SREBP1 expression, for example in the combined presence of increased glucose and free fatty acid levels (i.e., "glucolipotoxic" conditions), might contribute to β-cell dysfunction. Arguing against this view, expression in ZDF islets (42) of a dominant-negative form of SREBP1c (28) failed to significantly reverse defective GSIS while substantially decreasing islet TG content and reversing the expression of lipogenic genes.

We also show here that SREBP1 is required for the induction by 30 versus 8 mmol/l glucose, as expected, of lipogenic (*Fas*, *Acc1*, *Scd1*) genes (Fig. 2), given the previously described role of this factor in the control of lipogenic genes (20, 43), and consequently for enhanced TG accumulation (Fig. 1D). More surprising was the apparent action of SREBP1 knockout to block the induction by 30 versus 8 mmol/l on several other genes involved in glucose sensing (*Slc2a2*, *Gck*, *Abcc8*, *Kcnj11*) (Fig. 2).

It was also observed here that *Pdx1* gene expression was increased in normal mouse islets cultured at high glucose concentrations (Fig. 2) and that this effect was inhibited in islets from mice deleted for SREBP1. PDX1 is a key transcription factor involved in pancreatic development (44) and in the maintenance of the β-cell phenotype (45), serving to control insulin (46), *Slc2a2* (47), and *Nkx6.1* (48) gene expression. Liver X receptor (α and β) is a member of a nuclear receptor superfamily of ligand-activated transcription factors. In a recent study (49), downregulation of SREBP1 expression in INS-1 cells by RNA interference blocked the liver X receptor-induced expression of *Pdx1*, compatible with the view that SREBP1 is involved in the regulation of *Pdx1*. Decreases in *Pdx1* levels in SREBP1$^{-/-}$ after culture at 30 mmol/l glucose islets may subsequently underlie the loss of glucose-stimulated expression of *Slc2a2* and *Nkx6.1*. By contrast, *Pdx1* gene expression was higher in freshly isolated islets from SREBP1$^{-/-}$ versus SREBP1$^{+/+}$ mice (see supplementary data), a finding consistent with a negative role for SREBP1c in the control of basal *Pdx1* gene expression. Perhaps reconciling these observations, a recent study proposed that the effects of SREBP1 on β-cell function depend of the level and the duration of its activation (49).

Interestingly, SREBP1 deletion had no effect on *Chrebp* mRNA levels, confirming previous in vitro data in an insulinoma cell line (41) and showing that there were no compensatory increases in the expression of the latter transcription factor in SREBP1$^{-/-}$ islets.

In a complementary approach, we also used triacsin C here to study the potential role of acyl-CoA or TG synthesis in the long-term regulation of gene expression and insulin secretion by high glucose. Blockage of acyl-CoA synthesis using triacsin C affects both fatty acid oxidation and the synthesis of TGs (32). Importantly, using the same culture conditions (4 days at 8 or 30 mmol/l glucose), we could mimic the effects of SREBP1 deletion on both GSIS (Fig. 3) and TG content (Fig. 5). Interestingly, the resistance of *Slc2a2* mRNA induction to the effects of triacsin C (Fig. 4H), compared with the complete abolition of glucose-induced increases in this gene in SREBP1$^{-/-}$ islets (Fig. 2F), may reflect a direct binding of SREBP1c to the *Slc2a2* promoter, as reported in primary rat hepatocytes (50). Nevertheless,

it seems reasonable to conclude that the augmentation of basal and high GSIS by culture at increased glucose concentrations (Figs. 1, 3) may reflect an enhanced fatty acyl-CoA synthesis, resulting in the upregulation of several genes involved in glucose metabolism or sensing.

In summary, the present results demonstrate a requirement for SREBP1 in the hypersecretion of insulin resulting from chronic exposure of mouse islets to high glucose concentrations in vitro. In addition to the requirement for SREBP1 in the induction of lipogenic genes, SREBP1 is also shown, unexpectedly, to be necessary for the upregulation of genes directly involved in the expression of β-cell-enriched genes (*Pdx1*) and in genes whose products are central to glucose sensing (*Slc2a2*, *Gck*, *Kcnj11*, *Abcc81*). Therefore, induction of SREBP1c and enhanced lipid synthesis may play a key role in the adaptive insulin hypersecretion observed in some models of hyperglycemia.

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