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

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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 littersmates 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 SREBP1-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.

Supplementary key words islets • sterol-regulatory element binding protein-1c • insulin secretion • pancreatic duodenal homeobox 1 • triacsin C

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 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 (Sle2a2, 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 (Sdl1) (14), carbohydrate-responsive element binding protein (Chrebp) (15, 16)], and electrical activity [Abce8 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-
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 over-expressing SREBP1 using adenoviruses showed changes in the expression of a number of proapoptotic but also anti-apoptotic genes (27).

The above observations have suggested that the up-regulation 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 (20, 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<sup>−/−</sup>) 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. Triassicin 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<sup>−/−</sup>, SREBP1<sup>+/−</sup>, and SREBP1<sup>+/+</sup> 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 triassicin 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-check™ (Roche). Plasma insulin was measured using a rat insulin kit (Chrysalin 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<sub>2</sub>, 1.2 mmol/l MgCl<sub>2</sub>, 24 mmol/l NaHCO<sub>3</sub>, 10 mmol/l glucose, and 1 mg/ml BSA). It was gassed with O<sub>2</sub>/CO<sub>2</sub> (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<sub>2</sub>. Islets were then cultured for 96 h at 8 or 30 mmol/l glucose in the presence or absence of triassicin C (10 μmol/l; Biomol, Exeter, UK) before use.

#### Islets 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<sub>2</sub>, 0.5 mmol/l MgSO<sub>4</sub>, 0.5 mmol/l KH<sub>2</sub>PO<sub>4</sub>, 2.0 mmol/l NaHCO<sub>3</sub>, and 10 mmol/l HEPES) supplemented with 11 mmol/l glucose and 0.1% (w/v) BSA. KRBH was equilibrated with O<sub>2</sub>/CO<sub>2</sub> (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 5 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<sup>®</sup> 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<sup>™</sup> (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 DNA contamination and quantified by RiboGreen assay.
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 (5 mmol/l) and high (17 mmol/l) GSIS (Fig. 1). In contrast to freshly isolated islets, in which
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 8 but not 30 mmol/l glucose (Fig. 2D, G). By contrast, compared with wild-type islets after 4 days of culture at either 8 or 30 mmol/l (Fig. 1A, B).

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 SREBP1$^{−/−}$ 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).

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, Scle2a2, Abec8, 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 Chrepb 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 Srebp-1c, 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), Srebp-1c, 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 < 0.05, ## P < 0.01 for the chronically increased glucose effect.](image)

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 were 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. 1A), when rates of release were compared at 3 or 17 mmol/l glucose in each
Effects of chronically increased glucose concentration; either 8 or 30 mmol/l glucose (Fig. 1), and this change was SREBP1 cose. Thus, a clear impairment of GSIS was apparent in data), consistent with earlier results (26); this difference alleles versus wild-type mice (see supplementary 
slightly in freshly isolated islets from mice deleted for both 
defective insulin secretion. Indeed, GSIS was enhanced 
reflect, at least in part, altered insulin sensitivity rather than 
in glycemia after an intraperitoneal glucose tolerance test 
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type were similar when cultured at 8 or 30 mmol/l glucose 
to 30 mmol/l glucose caused marked decreases in GSIS 
regulation of 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 (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 (48). 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 Srebp1c mRNA induction to the effects of triacsin C (Fig. 4H), compared with the complete abolition of glucose-induced increases in this gene in Slc2a2−/− 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 SREBP1c in the hypersecretion of insulin resulting from chronic exposure of mouse islets to high glucose concentrations in vitro. In addition to the requirement for SREBP1c in the induction of lipogenic genes, SREBP1c 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 (Srebp1c, Gck, Nervf1, Abcc8f). 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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