# Glucose-Stimulated Upregulation of GLUT2 Gene Is Mediated by Sterol Response Element–Binding Protein-1c in the Hepatocytes

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**GLUT2 is mainly expressed in the liver, β-cells of the pancreas, and the basolateral membrane of kidney proximal tubules and plays an important role in glucose homeostasis in living organisms. The transcription of the GLUT2 gene is known to be upregulated in the liver during postprandial hyperglycemic states or in type 2 diabetes. However, a molecular mechanism by which glucose activates GLUT2 gene expression is not known. In this study, we report evidence that sterol response element–binding protein (SREBP)-1c plays a key role in glucose-stimulated GLUT2 gene expression. The GLUT2 promoter reporter is activated by SREBP-1c, and the activation is inhibited by a dominant-negative form of SREBP-1c (SREBP-1c DN). Adenoviral expression of SREBP-1c DN suppressed glucose-stimulated GLUT2 mRNA level in primary hepatocytes. An electrophoretic mobility shift assay and mutational analysis of the GLUT2 promoter revealed that SREBP-1c binds to the −84/−76 region of the GLUT2 promoter. Chromatin immunoprecipitation revealed that the binding of SREBP-1c to the −84/−76 region was increased by glucose concentration in a dose-dependent manner. These results indicate that SREBP-1c mediates glucose-stimulated GLUT2 gene expression in hepatocytes. *Diabetes* 54: 1684–1691, 2005**

Adipocyte determination and differentiation–dependent factor 1 (ADD1)/sterol response element binding protein (SREBP)-1c is a *trans*-acting factor that regulates transcription of many genes involved in cholesterol and fatty acid synthesis (1–6). To date, three isoforms of SREBP have been identified. SREBP-1a and -1c are derived from a single gene and use different transcription initiation sites (7). SREBP-2 is transcribed from a separate gene (8) and shows 50% sequence homology to the SREBP-1c isoform. SREBP-2 plays a critical role in the regulation of genes of cholesterol biosynthesis, whereas SREBP-1c regulates lipogenesis genes in liver (9).

The relative contribution of insulin (10–14) or glucose (15–17) to the regulation of specific genes is known to be different. In nonfasted mice, the level of SREBP-1c transcripts in the liver is considerably more abundant than the SREBP-1a transcripts (3). Fasting decreases the amount of SREBP-1c transcripts by 60%, and refeeding increases the level by 3.8-fold (3). In contrast, the fasting/refeeding protocol causes only minor changes in the amount of SREBP-1a transcripts (18). In insulin-resistant animals, such as *ob/ob* mice, where insulin signaling is known to be impaired (19), or in other insulin signaling system knockout animals (20), overexpression of SREBP-1c in the liver was reported (12). These reports suggested that SREBP-1c induction could also occur by glucose.

The facilitative GLUTs are a group of highly related membrane proteins that share significant sequence homology between isoforms (21). GLUT2 is known to transport glucose across the hepatic plasma membrane in a bidirectional manner (22,23). GLUT2 was upregulated by glucose, like pyruvate kinase and fatty acid synthase (24). In diabetic states, GLUT2 in the liver and basolateral membrane of renal proximal tubules is known to be upregulated, indicating that hyperglycemia may play a positive role in its gene regulation (25). And in streptozotocin-induced diabetic rats, where insulin was depleted in vivo (10), SREBP-1c expression was increased in liver. These data lead us to postulate that GLUT2 upregulation in the liver could be due to the activation of the gene by SREBP-1c.

From this background, we have attempted to identify the SREBP-1c response element (SRE) in the promoter region of GLUT2 and demonstrate that glucose increases the binding of SREBP-1c on the GLUT2 promoter, resulting in the activation of GLUT2 transcription.

## RESEARCH DESIGN AND METHODS

All the reagents for cell culture, such as media, fetal bovine serum (FBS), antibiotics, and LipofectAMINE PLUS, were purchased from Life Technologies. [γ-$^{32}$P]ATP, [α-$^{32}$P]dCTP, Rediprime labeling kits, and a rapid hybridization solution were purchased from Amersham Biosciences.

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ChIP, chromatin immunoprecipitation; DMEM, Dulbecco's modified Eagles' medium; EMSA, electrophoretic mobility shift assay; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFP, green fluorescent protein; SRE, SREBP response element; SREBP, sterol response element–binding protein; SREBP-1c DN, dominant-negative form of SREBP-1c.

**Preparation of recombinant SREBP-1 and SREBP-1 antibody.** Recombinant SREBP-1 was prepared as described (5). Briefly, recombinant human SREBP-1 were expressed in *Escherichia coli* BL21(DE3)pLysS. SREBP-1 expression vector pET-SREBP-1a was generated by inserting the cDNA fragments from pCSA10 between the *Eco*RI and *Sal*I sites of pET-21a (Novagen) and used for transformation. The recombinant SREBP-1 was induced for 4 h with 1 mmol/l isopropyl-β-D-thiogalactopyranoside. The recombinant proteins containing $NH_2$-terminal T7 and COOH-terminal polyhistidine ($His_6$) tag were purified to homogeneity by Ni-NTA-agarose (Qiagen) chromatography. The purity and concentration of the recombinant proteins were verified by SDS–polyacrylamide gel electorphoresis followed by Coomassie Brilliant Blue staining.

Polyclonal antibody against the recombinant SREBP-1 was prepared by standard protocol, which was prepared as described previously (26). One milligram of recombinant SREBP-1 protein was suspended in 1 ml of PBS and emulsified with 1 mmol/l of Freund's complete adjuvant (Sigma, St. Louis, MO). The emulsified solution was subcutaneously injected into the back of New Zealand white rabbit. After 3 weeks, the booster injection was administered with the same amount and volume of antigen emulsified with Freund's incomplete adjuvant (Sigma). The booster injections were repeated three times in 3-week intervals. At 10 days after the final booster injection, the blood was collected by the heart puncture under the anesthesia using 50 mg/kg of ketamine hydrochloride and 5 mg/kg of xylazine hydrochloride. The collected blood was incubated in 37°C for 1 h to complete coagulation, and the serum was isolated after centrifugation at 10,000*g* for 10 min.

**Animals and treatments.** Male ICR mice (7 weeks of age) were fed a fat-free, high-carbohydrate diet containing 82% (wt/wt) carbohydrates (74% starch and 8% sucrose), 18% (wt/wt) casein, 1% vitamin mix, and 4% (wt/wt) mineral mix and then fasted for 24 h. All the materials for the diet were purchased from Harlem Teklad (Madison, WI).

**Primary hepatocytes preparation from mouse liver.** Hepatocytes were isolated from a male ICR mouse (7 weeks of age) by the collagenase perfusion method (27). Dissociation into individual hepatocytes was performed in Dulbecco's modified Eagles' medium (DMEM) (Invitrogen) containing 10% heat-inactivated FBS, 100 nmol/l insulin, 10 μmol/l dexamethasone, 25 mmol/l glucose, 100 unit/ml penicillin G, and 100 μg/ml streptomycin. For each hepatocyte preparation, cell viability was estimated by the exclusion of trypan blue.

**Isolation of total RNA and Northern blot analysis.** Total cellular RNA was extracted from primary hepatocytes using the TRIzol reagent method (Life Technologies, Rockville, MD) (28) and prepared according to the manufacturer's protocol. Twenty micrograms of each sample was denatured and subjected to electrophoresis in a 0.9% denaturing formaldehyde-agarose gel and transferred to a nylon membrane. The membranes were hybridized with the probe for 3 h at 65°C with Rapid-Hybrid buffer (Amersham Biosciences). After hybridization, the membrane was washed twice with a high-salt washing buffer (0.1% SDS, 2 × SSC [sodium chloride/sodium citrate]) at room temperature for 30 min, followed by a low-salt washing buffer (0.1% SDS, 0.2 × SSC) at 65°C for 15 min. The membrane was exposed to Kodak BioMax film using an intensifying screen at −70°C.

**RNA preparation and RT-PCR.** Total RNA was isolated from primary hepatocytes using TRIzol reagent according to the manufacturer's protocol (Life Technologies). For RT-PCR, the first strand of cDNA was synthesized from 3 μl of total RNA using random hexamers and Superscript II reverse transcriptase (Life Technologies). One microliter of the reverse transcription reaction mixture was amplified with primers specific for GLUT2 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in a total volume of 50 μl. Linearity of the PCR was tested with amplification cycles between 25 and 30. According to the test amplification profile, samples were amplified at 94°C for 30 s, 56°C for 30 s, and 72°C for 28 s. GAPDH was used as an internal control for quality and quantity of RNA. The PCR primers used were as follows: GLUT2 sense, 5′-GGCTAATTTCAGGACTGGTT-3′; GLUT2 antisense, 5′-TTTC TTTGCCCTGACTTCCT-3′; GAPDH sense, 5′-ACCACAGTCCATGCCATCAC-3′; and GAPDH antisense, 5′-TCCACCACCCTGTTGCTGTA-3′.

**Quantitative real-time PCR.** Primers for GLUT2, SREBP-1a, SREBP-1c, SREBP-2, and α-tubulin genes were designed using MacVector software (Accelrys). The PCR mixture was performed in 10 μl (final volume) in glass capillary tubes (Roche Diagnostics). The PCR mixture contained 1 μl of 100 ng cDNA template, 1 μl of a commercial ready-to-use mixture of LightCycler-DNA Fast Start master hybridization probes (Roche Diagnostics), 5 mmol/l $MgCl_2$ (final concentration), and primers and probes at final concentrations of 5 and 1 μmol/l, respectively. Real-time PCR was carried out using a LightCycler instrument (Roche Diagnostics). The conditions for thermal cycling were as follows: initial denaturation for 10 min, followed by 40 amplification cycles at 95°C for 10 s, 58°C for 5 s, and 72°C for 14 s. Fluorescence was measured at 640 nm (F2 channel) at the end of each annealing phase. The amplification was followed by a melting program, which started at 45°C for 15 s and then increased to 95°C by 0.1°C/s, with the fluorescence signal continuously monitored online. Primers and probes were as follows: GLUT2 sense, 5′-GGCTAATTTCAGGACTGGTT-3′; GLUT2 antisense, 5′-TTTCTTTGCCCTG ACTTCCT-3′; SREBP-1a sense, 5′-GGCCGAGATGTGCGAACT-3′; SREBP-1a antisense, 5′-TTGTTGATGAGCTGGAGCATGT-3′; SREBP-1c sense, 5′-GGAG CCATGGATTGCACATT-3′; SREBP-1c antisense, 5′-GGCCCGGGAAGTCACT GT-3′; SREBP-2 sense, 5′-GCGTTCTGGAGACCATGGA-3′; SREBP-2 antisense, 5′-CACAAGTTGCTCTGAAAACAAATCA-3′; α-tubulin sense, 5′-CTCG CATCCACTTCCCTC-3′; and α-tubulin antisense, 5′-ATGCCCTCACCCACGTA C-3′.

**Construction of GLUT2 promoter luciferase plasmids.** The GLUT2 promoter fragments of −1,112/+1 bp were PCR amplified from mouse genomic DNA and subcloned into a pGLM vector and mutated at the E-boxes upstream of the multiple cloning site of the pGL3 basic vector. 5′ serial deletions of mouse GLUT2 promoter reporter were constructed by amplifying the regions of −891/+1, −389/+1, −286/+1, −116/+1, and −57/+1 bp, respectively. SRE mutant construct pmGLUT2d-389m was generated from pmGLUT2d-389 with the mutagenic oligonuleotides (30 mer) using plaque-forming unit polymerase. The sequences of all constructs were confirmed using a T7 sequencing kit (Amersham Biosciences). All transfection plasmids were prepared with the Qiagen Plasmid Midi Kit.

**Transient transfection assay.** Alexander cell lines (American *Type Culture* Collection number CRL-8024), which are human epithelial hepatoma cell lines, were plated at a density of $2 \times 10^5$ cells/35-mm dish. On the following day, 0.5 μg of each construct of the GLUT2 promoter, 0.1 μg of pCMV–β-galactosidase, with or without 0.1 μg of SREBP-1c, and 0.5 μg of dominant-negative form of SREBP-1c (SREBP-1c DN) expression vector were mixed. Transient transfection and luciferase assays were performed as described previously (29). Total protein concentrations of the lysates were determined by the Bradford method (30). Luciferase activities were normalized by the amount of total protein. Each transfection was performed in triplicate and repeated three to five times.

**Electrophoretic mobility shift assay.** The oligonucleotide probes GLUT2-SRE and GLUT2-SREm (GLUT2-SRE mutant) were labeled as described previously (31). Ten picomoles of the single-stranded sense oligonucleotide were labeled with [γ-$^{32}$P]ATP using T4 polynucleotide kinase and annealed with 50 pmol of unlabeled antisense oligonucleotides. For competition assay, unlabeled oligonucleotides (100-fold molar excess) were added to the reaction mixture. The oligonucleotide sequences used in electrophoretic mobility shift assay (EMSA) were as follows: GLUT2-SRE sense, 5′-CCAGGTAGAG TGAGCACTCT-3′; GLUT2-SRE antisense, 5′-AGAGTGCTCACTCTACCTGG-3′; GLUT2-SREm sense, 5′-CCAGGTAGAG***aac***GCACTCT-3′; GLUT2-SREm antisense, 5′-AGAGTGC***gtt***CTCTACCTGG-3′; mLDLR-SRE sense, 5′-TTGAAA ATCACCCCATTGCAGACTCCTCCCCGGC-3′; and mLDLR-SRE antisense, 5′-GCCGGGGAGGAGTCTGCAATGGGGTGATTTTCAA-3′. Mutated bases are represented in bold.

**Preparation of recombinant adenovirus.** Recombinant adenovirus encoding SREBP-1c or SREBP-1c DN was prepared according to Choi et al. (32). Briefly, the respective cDNAs were cloned into pAd-YC2 (5 μg) and transfected into 293 cells with a rescue vector, pJM17 (5 μg), which is necessary for homologous recombination of the recombinant adenovirus. After 12–15 days, recombinants were screened by PCR using upstream primers derived from the cytomegalovirus promoter and downstream primers from the bGHp(A) sequence. The recombinants were amplified in 293 cells and purified and isolated using $CsCl_2$ (Sigma). The recombinant adenoviruses were collected and desalted, and titers were determined by the measurement of plaque counts.

**Treatment of recombinant adenovirus.** Null, adenovirus, or adenovirus containing SREBP-1 or SREBP-1c DN were treated according to the method described by Kim et al. (29). The primary hepatocytes were incubated for 2 h with an adenovirus containing DMEM at a titer of 5 pfu per cell for 2 h at 37°C. Culture medium was then replaced by DMEM supplemented with 10% FBS. The infected cells were harvested for RNA isolation 24 h after viral infection.

**Chromatin immunoprecipitation assay.** The chromatin immunoprecipitation (ChIP) assay protocol was adapted from methods described by Duong et al. (32). The primary hepatocytes were fasted in fasting medium composed of 2 mmol/l glutamine, 1 mmol/l sodium pyruvate, 10 mmol/l lactate, 10 nmol/l dexamethasone, and 2% FBS for 16 h. The medium was then changed to one containing 25 mmol/l glucose and/or 100 nmol/l insulin for 24 h. Cells were washed with ice-cold PBS and cross-linked with 5% formaldehyde in serum-free DMEM for 5 min. The cells were homogenized, pelleted by centrifugation at 2,000 rpm for 4 min at 4°C, and resuspended in SDS lysis buffer (1% SDS, 10 mmol/l EDTA, 50 mmol/l Tris-HCl, pH 8.0). The lysate was sonicated on ice for 3 min at a setting of cycle 0.5, amplitude 30, and sheared to between 100 and 600 bps. To provide a positive control (input) for each condition, one undiluted aliquot was retained for further processing in parallel with all the

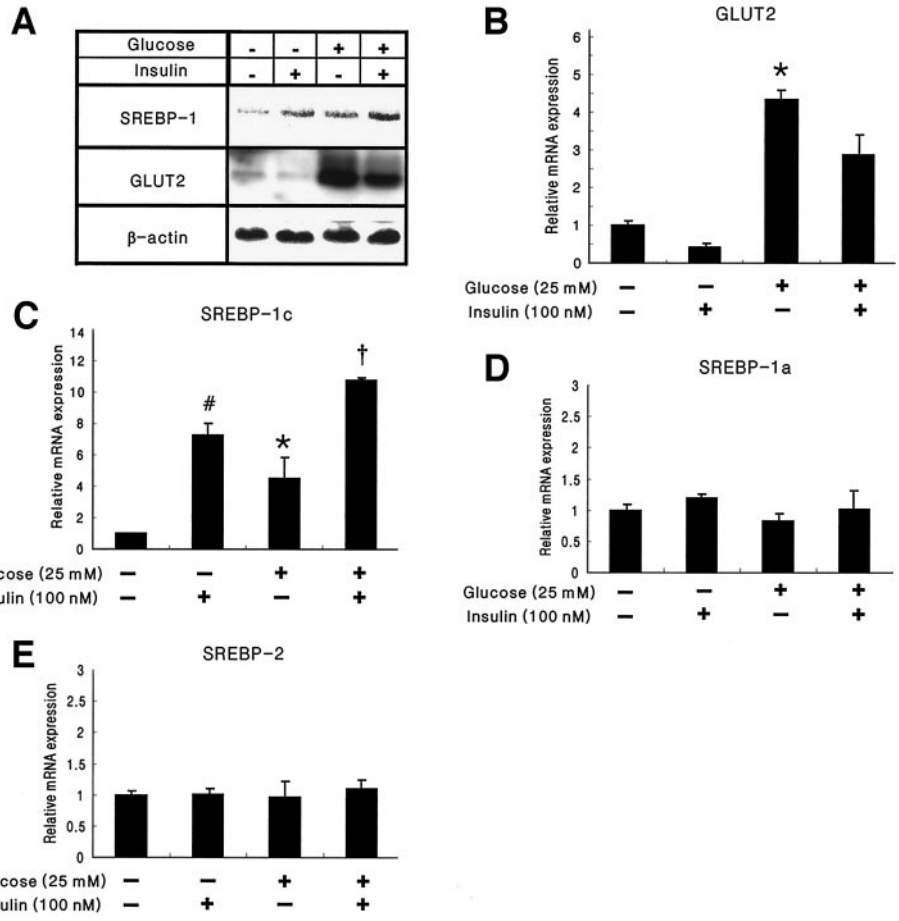


**FIG. 1. Effect of glucose and insulin on the mRNA level of GLUT2 and SREBPs in primary cultured hepatocytes. *A*: Hepatocytes isolated from mice were plated for 5 h and cultured for a further 16 h in the presence or absence of insulin (100 nmol/l) and glucose (25 mmol/l). Total RNA was extracted from each treatment group as described in RESEARCH DESIGN AND METHODS. *B–E*: Quantification of hepatic mRNA for GLUT2, SREBP-1c, SREBP-1a, and SREBP-2. Total RNA (3 μg) used in reverse transcription experiments was subjected to real-time PCR. The data were quantified as described in RESEARCH DESIGN AND METHODS. All mRNA levels were normalized to that of α-tubulin. The results are the means ± SD of three independent experiments with triplicate measurements. *B*: *$P < 0.001$ for the untreated vs. glucose-treated group; *C*: *$P < 0.005$ for the untreated vs. insulin-treated group, #$P < 0.05$ for the untreated vs. glucose-treated group, and †$P < 0.001$ for the untreated vs. glucose/insulin-treated group.**

other samples at the reversal of the cross-linking step. To reduce nonspecific background, each chromatin sample (1 ml) was precleared with 60 μl of protein A/G agarose (Santa Cruz Biotech), supplemented with 200 μg/ml sonicated salmon sperm DNA (Stratagene, La Jolla, CA), and the beads were pelleted. Chromatin complexes in the supernatant were immunoprecipitated overnight at 4°C using either 30 μg of SREBP-1 antibody or without antibody. Immune complexes were collected with 60 μl of protein A/G agarose including 200 μg/ml of salmon sperm DNA (Stratagene). Promoter-specific PCR GLUT2 promoter primers were as follows: sense, 5′-CCATCAATACTCAGCTTCTG-3′; antisense, 5′-TGTGTGTGTGTGGAATTGTC-3′.

**Statistical analysis.** All transfection studies were performed in three to five separate experiments, where triplicate dishes were transfected. The data were represented as a mean ± SD. Statistical significance was determined by independent two-sample *t* tests or paired *t* tests between two groups of datasets.

## RESULTS

**Effect of glucose and insulin on the mRNA level of GLUT2 and SREBPs in primary cultured hepatocytes.** To observe the effects of glucose and/or insulin on the expression of SREBP-1 and GLUT2, we treated primary cultured hepatocytes with high glucose (25 mmol/l), insulin (100 nmol/l), or both. Northern blot and real-time PCR analyses showed that treatment with insulin (100 nmol/l) or glucose (25 mmol/l) increased the SREBP-1 mRNA level (Fig. 1*A* and *B*). However, GLUT2 expression was not affected by insulin alone but was stimulated by addition of high glucose (Fig. 1*B*, *$P < 0.001$ for the untreated vs. glucose-treated group). These data suggest that glucose is a major determinant in GLUT2 gene upregulation. Real-time PCR of the SREBP-1 isoform revealed that insulin or glucose treatment activated SREBP-1c expression (Fig. 1*C*), whereas SREBP-1a and SREBP-2 expressions were not affected by both insulin and glucose (Fig. 1*D* and *E*). Insulin and glucose increased SREBP-1c mRNA level by seven- and fourfold, respectively (Fig. 1*C*, *$P < 0.005$ for the untreated vs. insulin-treated group and #$P < 0.05$ for the untreated vs. glucose-treated group). Insulin and glucose showed additive effect (Fig. 1*C*, †$P < 0.001$ for the untreated vs. glucose/insulin-treated group) in activating SREBP-1c expression.

**Effect of SREBP isoforms on GLUT2 promoter reporter activity in the Alexander cell lines.** As shown in Fig. 2, SREBP-1c activated GLUT2 promoter reporter by 5.2-fold (*$P < 0.001$ for the untransfected vs. SREBP-1c group), whereas SREBP-2 activated the promoter by 2.4-fold (#$P < 0.05$ for the untransfected vs. SREBP-2 group), suggesting that SREBP-1c acts as more potent activator of GLUT2 promoter than SREBP-2 isoform.

**Localization and characterization of SRE in the GLUT2 promoter.** To identify a putative SRE in the mouse GLUT2 promoter, serial deletion constructs of the 5′-flanking region of the GLUT2 promoter were prepared (from −1,112, −890, −389, −283, −166, and −57 to +1, shown in Fig. 3*A*), and their responsiveness to SREBP-1c in Alexander cell lines was observed. The SREBP-1c effect was decreased by deleting the bases down to −57 (pmGT2d-57), indicating that a putative SRE could be located between the −166- and −57-bp regions (Fig. 3*B*, *$P < 0.005$ for the −166- vs. −57-bp deletion construct). A consensus sequence search suggested that a highly conserved SREBP-1 binding site could be present in this

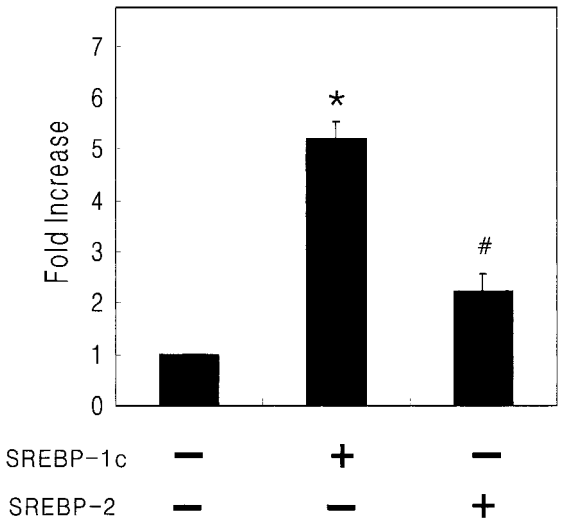


**FIG. 2. Effect of SREBPs on GLUT2 promoter activity in the Alexander cell lines. pmGT2-1112 promoter reporter was cotransfected with SREBP-1c (100 ng) or SREBP-2 (100 ng) to Alexander cell lines, which were plated at a density of $2 \times 10^5$ cells/35-mm dish. Transient transfection, luciferase assays, and measurement of total protein concentration of the lysates were performed as described in RESEARCH DESIGN AND METHODS. Luciferase activities were normalized by the amount of total protein. Each transfection was performed in triplicate and repeated three to five times. *$P < 0.001$ for the untreated vs. SREBP-1–transfected group; #$P < 0.05$ for the untreated vs. SREBP-2–transfected group.**

region. To identify an SRE in this region, EMSA was performed. Recombinant SREBP-1 (5) bound well with the probe in a dose-dependent manner up to 60 ng (Fig. 3*C*). The specificity of the SREBP-1c binding was further confirmed by the SRE consensus sequence from the LDL receptor (LDLR) promoter (33) (Fig. 3*D*, *lane 3*). Mutation introduced at −78/−76 (TGA → aac, SRE mutant in Fig. 3*D*) resulted in a loss of SREBP-1 binding (Fig. 3*D*, *lanes 5* and *6*). The aac mutant lost its ability to compete the binding of SREBP-1 to the GLUT2-SRE consensus sequence (Fig. 3*D*, *lane 4*). Transfection of the putative GLUT2-SRE mutant promoter construct (pmGT2d-389m) lost its responsiveness to SREBP-1c (Fig. 3*E*, ■). These results indicate that the −84/−76 region acts as a possible SRE regulating GLUT2 gene expression.

**Effect of a GLUT2-SRE mutation on the glucose-stimulated GLUT2 promoter reporter activity.** To confirm that the −84/−76 region is a functional SRE in the GLUT2 promoter, the GLUT2 promoter reporters (pmGT2d-389 and pmGT2d-389m) were transfected to Alexander cell lines maintained at concentrations of 5 and 25 mmol/l glucose. The wild-type promoter reporter activity was increased by glucose in a dose-dependent manner (Fig. 4*A*, *$P < 0.05$ for the 0 vs. 25 mmol/l glucose–treated group). The GLUT2-SRE mutant construct (pmGT22d-389m) showed decreased promoter reporter activity when compared with wild-type promoter (pmGT22d-389, #$P < 0.05$ for the wild- versus mutant-type at 25 mmol/l glucose). Again, the activation of the promoter construct by glucose (25 mmol/l) was suppressed by cotransfection of the SREBP-1c DN (Fig. 4*B*, #$P < 0.05$ for the 25 mmol/l vs. 25 mmol/l glucose plus SREBP-1c DN group), indicating that GLUT2 promoter activation by glucose is specifically mediated by SREBP-1c.

**Glucose-stimulation of GLUT2 gene expression.** To observe the effect of glucose on GLUT2 and SREBP-1c mRNA and protein level, primary hepatocytes were maintained at the indicated concentration of glucose. As shown in Fig. 5*A* and *B*, GLUT2 mRNA and protein level increased with glucose concentration in a dose-dependent manner. To confirm that SREBP-1c mediates glucose-stimulated GLUT2 expression, we transduced adenovirus containing SREBP-1c DN into the primary hepatocytes in the presence or absence of glucose. Real-time PCR (Fig. 5*C*, *upper panel*) and RT-PCR (Fig. 5*C*, *lower panel*) revealed that GLUT2 expression was increased by glucose (25 mmol/l, *$P < 0.005$ for the untreated versus glucose-treated group), and the stimulatory effect was suppressed by SREBP-1c DN at the 25-mmol/l glucose concentration (#$P < 0.05$ for the glucose-treated versus glucose plus SREBP-1c DN group). These data indicate that SREBP-1c is responsible for the glucose-stimulated GLUT2 gene expression.

**Glucose increased the binding of SREBP-1 to the GLUT2 promoter in mouse primary cultured hepatocytes.** To confirm the binding of SREBP-1 to the GLUT2 promoter by glucose in primary cultured hepatocytes, a ChIP assay was performed. Chromosomal DNA from primary cultured hepatocytes was cross-linked using formaldehyde, and an antibody specific to SREBP-1 was used to immunoprecipitate the chromatin fragment. A specific region of the GLUT2 promoter (−84/−76) was amplified by PCR. The binding of SREBP-1 to the GLUT2 promoter was increased by glucose or glucose/insulin treatment (Fig. 6*A*). The SREBP-1 binding to the GLUT2 promoter was increased in a dose-dependent manner (Fig. 6*B*). However, insulin did not affect the binding of SREBP-1 to the putative SRE. These results indicate that glucose could be one of the major factors in the binding of SREBP-1 to the GLUT2 promoter, which may help explain the upregulation of GLUT2 gene expression by glucose.

## DISCUSSION

Recently, it has been suggested that the carbohydrate-responsive element–binding protein (ChREBP) plays a pivotal role in glucose-regulated gene expression (34), whereas SREBP-1c acts as a major mediator of insulin action in the lipogenic genes or in hepatic glucokinase (35,36) and lipogenic gene expression (9,37). However, glucose is required for lipogenic gene induction, where insulin only plays a permissive role (15–17). In insulin-resistant animals in which insulin signaling is impaired, SREBP-1c expression in liver was shown to be increased. For example, upregulation of SREBP-1c was reported in the leptin-deficient obese *ob/ob* mice, lipoatrophic mice overexpressing SREBP-1c in adipose tissue (19), insulin receptor substrate-2 knockout mice (20), and rat primary hepatocytes infected with adenovirus-overexpressing dominant-negative Akt (12). These observations suggest that there may be additional mechanism of SREBP-1c induction other than insulin signaling.

In this study, we demonstrated that glucose induced the gene expression of SREBP-1c and GLUT2 in a dose-dependent manner in the primary hepatocytes. Furthermore, expression of GLUT2 gene is decreased by adenoviral transduction of SREBP-1c DN in the mouse primary cultured hepatocytes, indicating that glucose-stimulated GLUT2 expression is mediated by SREBP-1c. We have

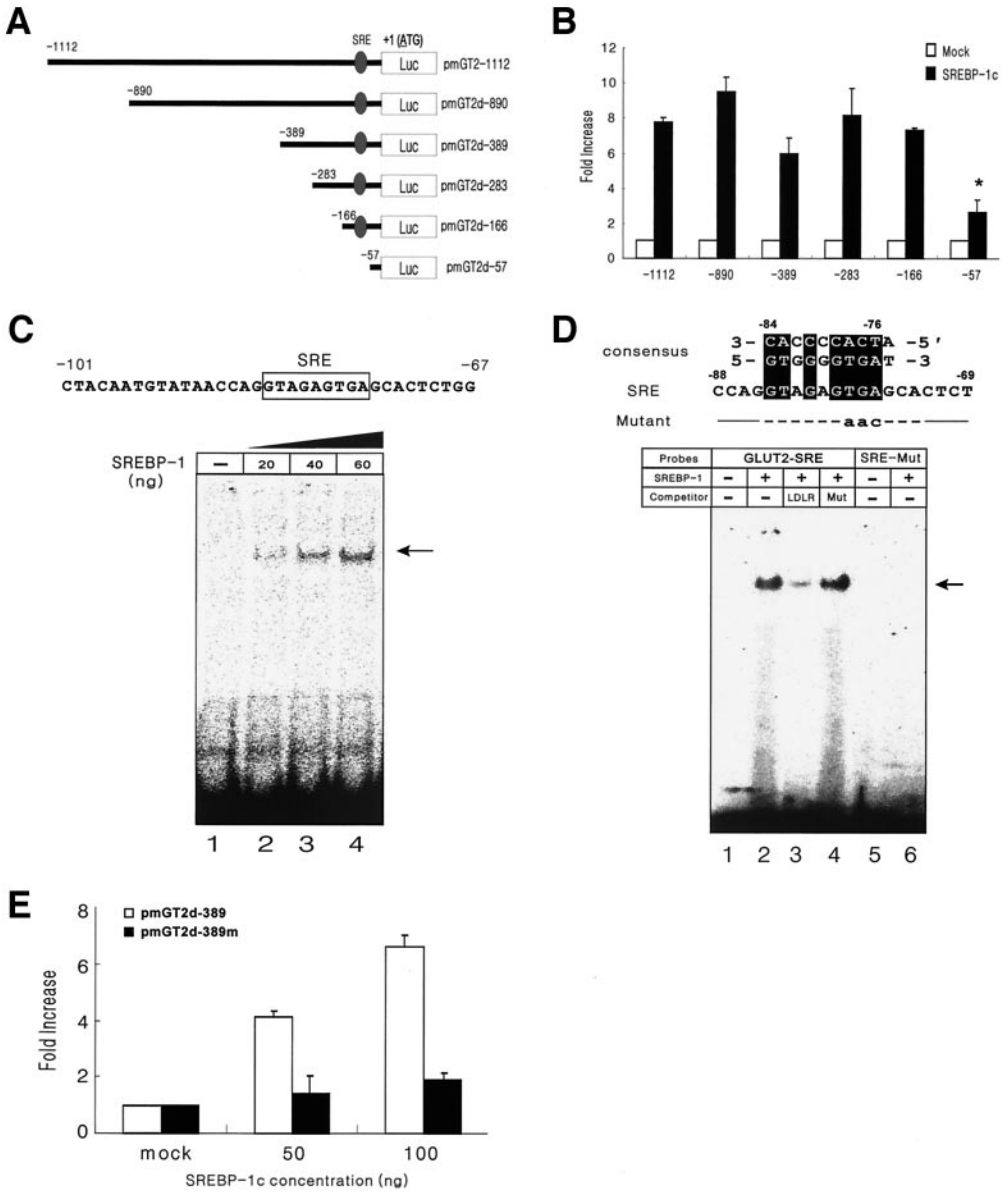


**FIG. 3. Localization and characterizaion of SRE in the GLUT2 promoter. *A*: Schematic diagram of serial deletion constructs of GLUT2 promoter reporter to localization of SRE in the GLUT2 promoter. The indicated numbers represent the number of nucleotides from the ATG codon. *B*: Effect of SREBP-1c on the deletion constructs of GLUT2 promoter. The promoter activities were measured by cotransfecting 100 ng of SREBP-1c expression or empty vectors into Alexander cell lines. The results were normalized by the amount of total protein of lysates, which were determined by the Bradford method (21) and shown as the fold changes of luciferase activities compared with those of the control. Normalized luciferase activities are shown as the means ± SD of three independent experiments in triplicate. *$P < 0.005$ for the −166 vs. −57 deletion construct. *C*: Electrophoretic mobility shift assay of GLUT2-SRE. The assay was performed with recombinant SREBP-1 protein in 4% (wt/vol) nondenaturing polyacrylamide gel. Fifty thousand cpm (0.1 pmol) of $^{32}$P-labeled GLUT2 promoter fragments (−101/−67) containing putative SRE were incubated with 20, 40, and 60 ng of recombinant SREBP-1 protein. *D*: Effect of mutation on the SREBP-1 binding to the putative GLUT2-SRE. Site-directed mutation was introduced into the GLUT2-SRE sequence. SRE mutant was prepared by replacing TGA with aac. Wild-type and mutant probes were labeled with [γ-$^{32}$P]ATP, and EMSA was performed in 4% (wt/vol) nondenaturing polyacrylamide gel. For these experiments, 30 pmol of each double-stranded oligonucleotide and 80 ng of SREBP-1 recombinant protein were used. Consensus sequence of SRE reported in the LDLR promoter and mutant oligonucleotides were used as competitors. The DNA-protein complexes are indicated by an arrow. *E*: Effect of mutation on the GLUT2-SRE on the SREBP-1c–driven promoter activity. A pSV-SREBP-1c expression vector was cotransfected with pmGT2d-389 or pmGT2d-389m into Alexander cell lines. The luciferase activities were represented as fold changes compared with those of the control group. Values are the means ±SD of three independent experiments in triplicate.**

identified a functional SRE in the mouse GLUT2 promoter and demonstrated that SREBP-1c is the *trans*-acting factor mediating glucose-stimulated GLUT2 gene expression.

The question of how insulin-induced SREBP-1c did not activate the GLUT2 gene expression, but glucose-induced SREBP-1c increased GLUT2 mRNA level is not currently understood. As shown in Fig. 6*A*, SREBP-1c induced by insulin did not bind to GLUT2 promoter, whereas SREBP-1c binding to GLUT2 promoter at high glucose concentration was increased. We speculate that this difference in the binding of SREBP-1c to SRE of GLUT2 promoter could be due to *1*) difference in the coregulators recruiting SREBP-1c to SRE by insulin or glucose, or *2*) sequence specificity of GLUT2-SRE for SREBP-1c mediated by insulin or glucose signaling. Mostly, studies on the

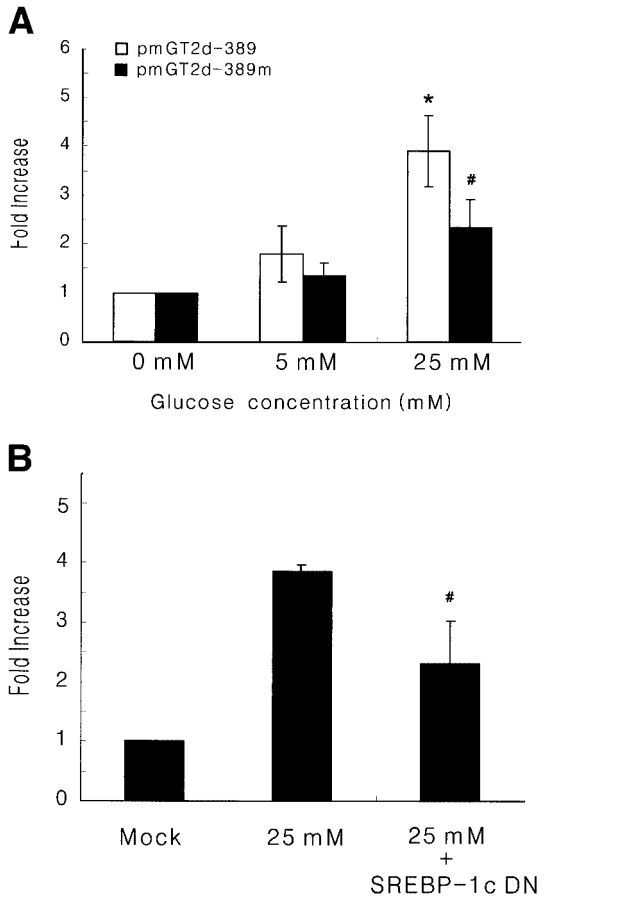


**FIG. 4. Effect of a GLUT2-SRE mutation on the glucose-stimulated GLUT2 promoter reporter activity. *A*: Effect of mutation in the putative SRE on glucose-mediated GLUT2 promoter activity. A pmGT2d-389 (wild-type) or pmGT2d-389m (mutant-type) luciferase reporter vector was transfected into mouse primary hepatocytes with the indicated amount of glucose. **P* < 0.05 for the wild-type 0 vs. 25 mmol/l glucose–treated group; #*P* < 0.05 for the wild-type vs. mutant-type at 25 mmol/l glucose, using paired *t* test. *B*: Effect of SREBP-1c DN on the glucose-stimulated GLUT2 promoter activity. pmGT2d-1112 and SREBP-1c DN were transfected into primary hepatocytes for 16 h, and 25 mmol/l glucose was added to the culture media for 24 h. The luciferase activities were represented as fold changes compared with those of the control group. Values are the means ± SD of three independent experiments in triplicate. #*P* < 0.05 for the 25 mmol/l glucose vs. 25 mmol/l glucose plus SREBP-1c DN, using paired *t* test.**

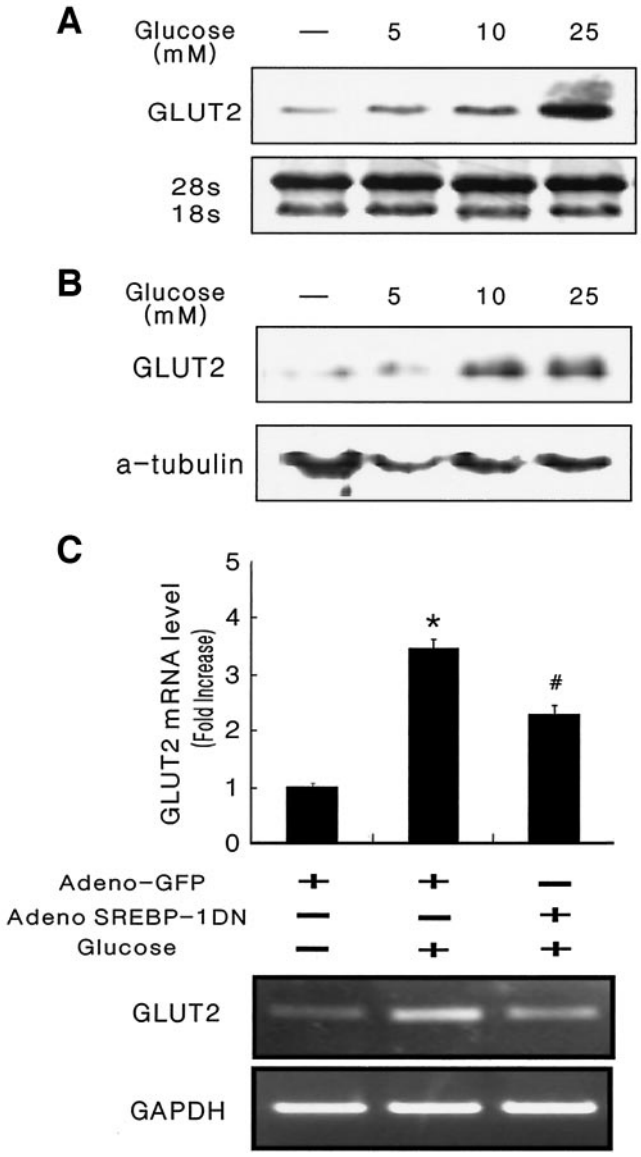


**FIG. 5. Glucose stimulation of GLUT2 gene expression. *A* and *B*: Primary hepatocytes, which were fasted for 24 h, were maintained in media containing the indicated concentration of glucose for 16 h. GLUT2 mRNA and protein levels were quantified by Northern (*A*) and Western (*B*) blot analyses. The blots are representative of two different experiments. *C*: Effect of SREBP-1c DN on glucose-stimulated GLUT2 expression. Adenovirus containing SREBP-1c or null adenovirus (adeno-GFP) was transduced into primary hepatocytes at a titer of 5 pfu per cell for 2 h at 37°C. Cells were incubated in the presence (25 mmol/l) or absence of glucose for 24 h, and RNA was harvested from the cells. The data were quantified as described in RESEARCH DESIGN AND METHODS. The mRNA level was normalized to that of GAPDH. The results are the means ± SD of three independent experiments with triplicate measurements. **P* < 0.005 for Adeno-GFP vs. glucose; #*P* < 0.05 for the glucose vs. glucose plus SREBP-1c DN.**

phenotypic changes in SREBP-1c knockout mice have been focused on the alteration in the lipid metabolism. On a normal diet, mRNAs encoding enzymes of fatty acid and triglyceride synthesis, including fatty acid synthase and acetyl-CoA carboxylase, are shown to be reduced in the liver of SREBP-1c–deficient mice (3). However, a compensatory increase in hepatic SREBP-2 mRNA, accompanied by increased mRNA level for cholesterol biosynthetic enzymes, was observed (38). Thus, these mice showed an increase in hepatic cholesterol content. The SREBP-1–deficient mice showed low level of plasma cholesterol and triglycerides (3). At present, phenotypic changes in SREBP-1c knockout mice with regard to carbohydrate metabolism is not well studied. We assumed that in the insulin-deficient or -resistant state, where SREBP-1c transcription is low and gluconeogenesis is active in liver, the glucose-induced SREBP-1c may play a role in transporting glucose out of hepatocytes into blood, contributing to hyperglycemia. Our studies may provide insight into the understanding of a role of GLUT2 on deranged carbohydrate metabolism in type 2 diabetes.


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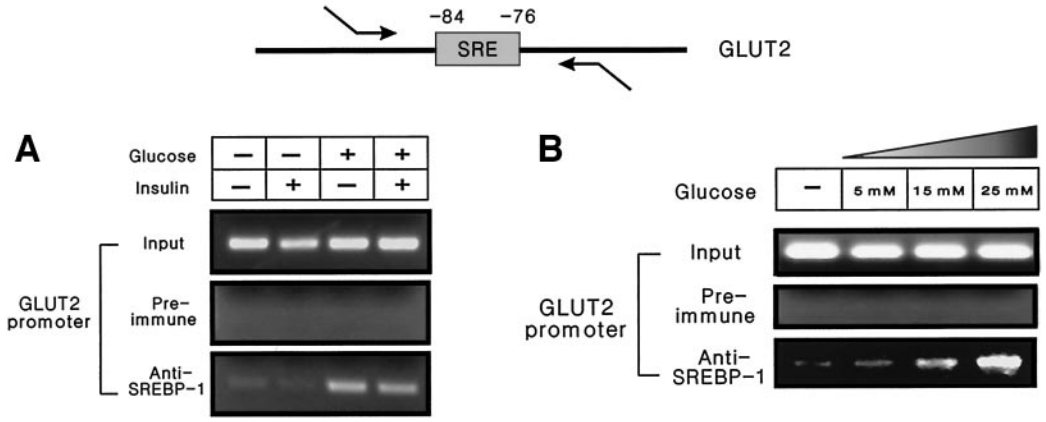


**FIG. 6. ChIP assay. *A*: Effect of glucose or insulin on the SREBP-1 binding to putative GLUT2-SRE. Chromatin was precipitated using a SREBP-1 antibody from primary hepatocytes, and the GLUT2 promoter region was amplified by PCR. The amount of chromosomal DNA used in immunoprecipitation between groups was normalized by input chromatin (100th of chromosomal DNA used for immunoprecipitation). *B*: Effect of glucose concentrations on the SREBP-1 binding to putative GLUT2-SRE. Glucose concentrations are shown on the top. Detailed PCR conditions and methods of sample treatment are described in RESEARCH DESIGN AND METHODS.**



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