Hepatic Akt Activation Induces Marked Hypoglycemia, Hepatomegaly, and Hypertriglyceridemia With Sterol Regulatory Element Binding Protein Involvement

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Akt is critical in insulin-induced metabolism of glucose and lipids. To investigate functions induced by hepatic Akt activation, a constitutively active Akt, NH_2-terminally myristoylation signal–attached Akt (myr-Akt), was overexpressed in the liver by injecting its adenovirus into mice. Hepatic myr-Akt overexpression resulted in a markedly hypoglycemic, hypoinsulinemic, and hypertriglyceridemic phenotype with fatty liver and hepatomegaly. To elucidate the sterol regulatory element binding protein (SREBP)-1c contribution to these phenotypic features, myr-Akt adenovirus was injected into SREBP-1 knockout mice. myr-Akt overexpression induced hypoglycemia and hepatomegaly with triglyceride accumulation in SREBP-1 knockout mice to a degree similar to that in normal mice, whereas myr-Akt–induced hypertriglyceridemia in knockout mice was milder than that in normal mice. The myr-Akt–induced changes in glucokinase, phosphofructokinase, glucose-6-phosphatase, and PEPCK expressions were not affected by knocking out SREBP-1, whereas stearoyl-CoA desaturase 1 induction was completely inhibited in knockout mice. Constitutively active SREBP-1–overexpressing mice had fatty livers without hepatomegaly, hypoglycemia, or hypertriglyceridemia. Hepatic acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase 1, and glucose-6-phosphate dehydrogenase expressions were significantly increased by overexpressing SREBP-1, whereas glucokinase, phosphofructokinase, glucose-6-phosphatase, and PEPCK expressions were not or only slightly affected. Thus, SREBP-1 is not absolutely necessary for the hepatic Akt-mediated hypoglycemic effect. In contrast, myr-Akt–induced hypertriglyceridemia and hepatic triglyceride accumulation are mediated by both Akt-induced SREBP-1 expression and a mechanism involving fatty acid synthesis independent of SREBP-1. *Diabetes* 52: 2905–2913, 2003

Phosphatidylinositol 3-kinase activation via insulin receptor substrate proteins and downstream Akt has been shown to play a critical role in insulin-induced metabolic actions (1–3). In fat and muscle, the role of Akt has been widely recognized as mediation of insulin-stimulated glucose uptake (4–6). Indeed, overexpression of constitutively active forms of Akt is sufficient to induce glucose transport in 3T3-L1 adipocytes and L6 muscle cells (7–9), whereas Akt2-deficient mice showed impaired glucose tolerance due to a decrease in insulin-induced glucose uptake in skeletal muscle and increased hepatic glucose production (10).

On the other hand, in the liver, the rate of glucose utilization is determined by rate-limiting metabolic enzymes that catalyze glucose to generate triglyceride and glycogen by pathways known as glycolysis, lipogenesis, and glycogenesis. Moreover, the liver can produce glucose from lactate, pyruvate, and amino acids via gluconeogenesis and also from glycogen, via glycogenolysis, both of which are also rate limited by key metabolic enzymes at each step. Therefore, the rates of hepatic glucose input and output depend on rate-limiting metabolic enzymes, and insulin is the most important regulator of these enzymes (11).

Akt is regarded as a key signal molecule mediating the metabolic actions of insulin in muscle, fat, and the liver (2,12). The first aim of this study was to characterize the phenotype induced by the activation of hepatic Akt. For this purpose, we overexpressed constitutively active Akt by using adenovirus transfer into the mouse liver. This allowed investigation of phenotypic features, including glucose and lipid metabolism. Much remains unclear regarding the pathway from Akt activation to the regulation of the enzymes involved in glucose and lipid metabolism. To date, it has been reported that Akt phosphorylates and activates glycogen synthase kinase (GSK)-3 (13,14). Phos-

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ACC, acetyl-CoA carboxylase; FAO, fatty acid oxidation; FAS, fatty acid synthase; G6Pase, glucose-6-phosphatase; G6PD, glucose-6-phosphate dehydrogenase; GK, glucokinase; GSK-3, glycogen synthase kinase 3; LacZ, *E. coli* β-galactosidase gene; LPL, lipoprotein lipase; myr-Akt, NH_2-terminally myristoylation signal–attached Akt; PFK, phosphofructokinase; PK, L-type pyruvate kinase; PPARα, peroxisome proliferator–activated receptor-α; SCD1, stearoyl-CoA desaturase 1; SREBP, sterol regulatory element binding protein.

TABLE 1
Sequences of PCR primers for cloning mouse cDNA probes

Probe	Primer 1	Primer 2	Position in cDNA
ACC	5′-GACTTCATGAATTTGCTGAT-3′	5′-AAGCTGAAAGCTTTCTGTCT-3′	1640–1809
CPT1	5′-GATCTACAATTCCCCTCTGC-3′	5′-CTGCATCTGCTGCTCCAGCT-3′	915–1114
CPT2	5′-CTAGACACCTCCTGGTCCTA-3′	5′-CAACTTCTGTCTGAGCTCTG-3′	781–980
FAS	5′-GCAGATCCTTTGATGATTCA-3′	5′-TTACACCTTGCTCCTTGCTG-3′	793–942
G6Pase	5′-TGCTGCTCACTTTCCCCACCAG-3′	5′-TCTCCAAAGTCCACAGGAGGT-3′	601–800
G6PD	5′-ACCCTTCATCCTGCGCTGT-3′	5′-CGGATGACCAGCTCATTA-3′	1108–1230
GK	5′-CGACTCTGGGGACCGAAGGCAG-3′	5′-TGATGCGCATCACGTCCTCACT-3′	1133–1304
LCAD	5′-AATGGGAGAAAGCTGGAGAA-3′	5′-TAGGGCATGACAATATCTGA-3′	250–448
LDLR	5′-TCCATCGCAGCTGGGTCTGT-3′	5′-TACACTGTGTCACATTGACGC-3′	752–951
LPL	5′-TGCCACTTCAACCACAGCAG-3′	5′-TCCCACCAGCTTGGTGTAGC-3′	398–595
MCAD	5′-CAGAGAGGAGATTATCCCCG-3′	5′-TAGCAGTTTGCACCCCTGTA-3′	171–370
PEPCK	5′-GACAGCCTGCCCCAGGCAGTGA-3′	5′-CTGGCCACATCTCGAGGGTCAG-3′	196–395
PFK	5′-AGGCTCTCGGCTGAACATCA-3′	5′-TGAAGGCGTCCCTCCTCGCT-3′	826–985
PK	5′-ACCGTGAGCCTCCAGAGGCT-3′	5′-TGTTGGTATAGCCAGAGCCA-3′	1591–1856
PPARα	5′-ATGGTGGACACAGAGAGCC-3′	5′-GAGCTCTCCTCACCGATGGA-3′	439–575
SCAD	5′-ATGGGTGAGCTCGGGCTGCT-3′	5′-ATCCACTGTTGCTTCTGCTG-3′	263–462
SCD1	5′-ATGCCGGCCCACATGCTCCA-3′	5′-TACTCCAGCTTGGGCGGGGG-3′	136–335
VLCAD	5′-GCTTACATGCTGAGTGCCAA-3′	5′-CCTTCAAAGATCCGGAAGAT-3′	1275–1474

CPT, carnitine palmitoyltransferase; LCAD, long-chain acyl-CoA dehydrogenase; LDLR, LDL receptor; MCAD, medium-chain acyl-CoA dehydrogenase; SCAD, short-chain acyl-CoA dehydrogenase; VLCAD, very-long-chain acyl-CoA dehydrogenase.

phorylated GSK-3 then induces the activation of glycogen synthase. Akt is thus involved in one mechanism of insulin-induced activation of glycogen synthase. In addition, recently, Akt was reported (15) to increase the expression of sterol regulatory element binding protein (SREBP)-1, and some previous reports (16–24) have suggested an important role of SREBP-1 not only in lipid metabolism but also in glucose metabolism. Thus, we endeavored to elucidate the contributions of SREBP-1 to hepatic Akt-induced phenotypes by utilizing SREBP knockout mice and hepatic SREBP-1a– and -1c–overexpressing mice. Comparing the mice overexpressing active Akt and SREBP-1 and the SREBP-1 knockout mice overexpressing active Akt revealed the contribution of SREBP-1 to various Akt-induced phenotypes and altered gene expressions. These results contribute to understanding the molecular mechanisms underlying insulin-activating Akt-induced in vivo phenotypes.

RESEARCH DESIGN AND METHODS

Adenoviruses, animals, and measurements of serum/hepatic components. Full-length mouse Akt cDNA (25) was NH_2-terminally tagged with the sequence corresponding to myristoylation signal peptide as previously described (26). Adenoviruses expressing β-galactosidase (i.e., the *E. coli* β-galactosidase gene [LacZ]) and NH_2-terminally myristoylation signal–attached Akt (myr-Akt) were created as already described (27). Adenoviruses expressing constitutively active human SREBP-1a (amino acids 1–460) and constitutively active SREBP-1c (1–436) were constructed using an Ad Easy kit (Quantum Biotechnology). Male C57BL/6 mice (8–10 weeks of age) or male SREBP-1 knockout mice (28) (15 weeks of age) were injected, via the tail vein, with adenovirus at a dose of 2.5×10^7 plaque-forming units/g body wt. Animals were fasted for 14 h (experiments in Figs. 1 and 2) or 24 h (experiments in Figs. 3–6) before blood sampling and killed. Blood glucose was measured with a portable blood glucose monitor, Glutest-Ace (Sanwa Kagaku Kenkyusho, Nagoya Japan). The plasma insulin level was determined with a radioimmunoassay kit (Pharmacia, Tokyo, Japan). Serum triglyceride and cholesterol were assayed as previously described (29,30). Hepatic total lipid was extracted as described using the Folch method (31), washed with saline and water, dried, and resuspended in Krebs-Ringer buffer containing 3% BSA. Triglyceride content in this resuspension was assayed as previously described (29). Hepatic glycogen content was measured as previously described (32). All animal studies were conducted according to the Japanese guidelines for the care and use of experimental animals.

Preparation of riboprobes for RNase protection assays. Riboprobes of mouse SREBP-1a and SREBP-1c for RNase protection assays were described previously (33). Riboprobes of other enzymes were amplified from mouse embryonic cDNA using the PCR primers described in Table 1. PCR products were subcloned into pCR2.1 (Invitrogen), pBlueScript (Stratagene), or pCMV-Script (Stratagene), and all sequences were certified using a CEQ-2000 sequencer (Beckman).

RNA preparation, Northern blotting, and RNase protection assay. Total hepatic RNA was isolated using Trizol reagent (Isogen; Nippon Gene, Tokyo, Japan). RNA concentrations were estimated based on absorbance at 260 nm. Northern blotting of SREBP-1 was conducted by running 20-μg RNA samples on a 1% agarose gel containing formaldehyde and transferred to a nylon membrane. The 47-mer oligo-DNA probe of SREBP-1 (5′-ATGTAGTCGATG GCCTTGCGCAAGACAGCAGATTTATTCAGCTTTGC-3′), which is the same in humans and mice, was labeled with [γ-^{32}P]ATP using the DNA 5′ End-Labeling System (Promega) and purified with a ProbeQuant G-50 Micro Column (Amersham). The membranes were hybridized with 10^6 cpm/ml of the radiolabeled probe in Ultra-hyb Buffer (Ambion) at 42°C overnight and washed according to the manufacturer's instructions. Blots were exposed using a Molecular Imager GS-525 (Biorad). RNase protection assays were carried out according to the manufacturer's instructions (RPA III kit; Ambion, Austin, TX). RNA samples (10 μg) from each mouse were hybridized with the riboprobes. After treatment with RNase, the intensities of the resultant bands were determined using the Molecular Imager GS-525.

Immunoblotting. For immunoblotting of SREBP proteins, nuclear extracts from mouse livers were prepared as described previously (34). Aliquots of nuclear proteins (20 μg) were subjected to SDS-PAGE. Immunoblot analyses were performed using the ECL Western Blotting Detection System kit (Amersham Pharmacia Biotech). The primary antibodies for SREBPs were as previously described (24,28). For the detection of tissue Akt, each tissue sample was homogenized in 10 volumes of lysis buffer (20 mmol/l Tris, pH 7.5, 150 mmol/l NaCl, 1 mmol/l EDTA, 1% Nonidet P-40, and 1 mmol/l phenylmethylsulfonyl fluoride) and centrifuged. Protein concentrations of the collected supernatant were assayed using a bicinchoninic acid assay kit (Pierce), and samples were adjusted to 3 mg/ml. Forty-five micrograms of each tissue specimen were subjected to SDS-PAGE. The polyclonal antibody for Akt was raised in rabbits using the keyhole limpet hemocyanin–conjugated COOH-terminal 17 amino acids of Akt.

Akt activity assay. Akt activity in the liver was assayed using an Akt Kinase Assay kit (Cell Signaling Technology). Hepatic tissue was homogenized in a 10-fold volume of lysis buffer, and 200 μl of the lysate were incubated with 10 μl of immobilized Akt monoclonal antibody for 2 h. Washing and kinase

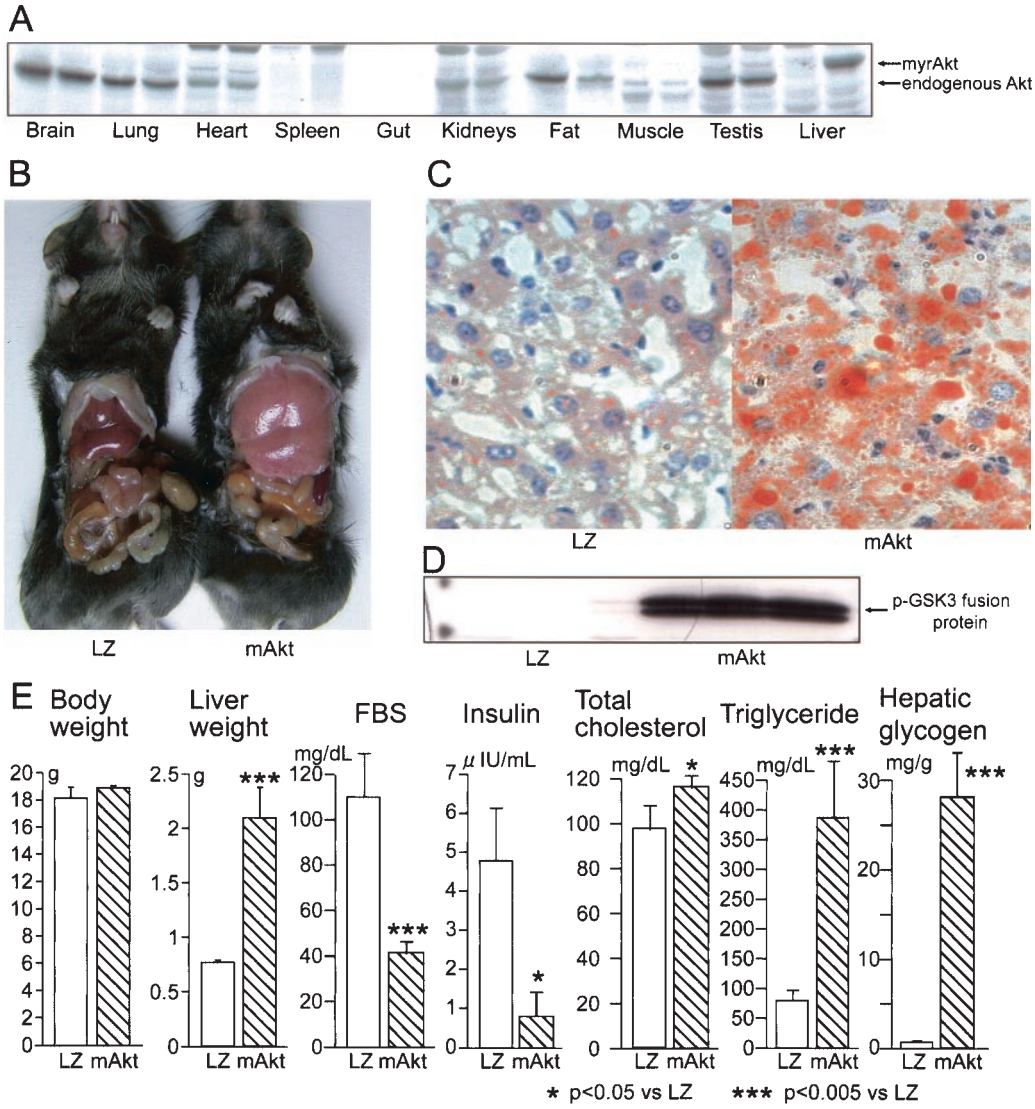

FIG. 1. Phenotypes of mice injected with LacZ or myr-Akt adenovirus. C57BL/6 male mice were injected with 2.5×10^7 plaque-forming units/g body weight of adenovirus-containing β-galactosidase (LZ) or myr-Akt construct via the tail vein. Blood sampling and killing were carried out 4 days after the injections, following 14 h of fasting. *A*: Western blotting of various tissues lysates. Each tissue (45 μg) was electrophoresed and immunoblotted with anti-Akt antibody. Odd numbered lanes represent tissues from LacZ-injected mice, and even numbered lanes represent those from myr-Akt–injected mice. *B*: Abdominal cavity. *C*: Sudan III staining of the liver. *D*: Akt kinase assay of livers. *E*: Body and liver weights, blood glucose, plasma insulin concentration, triglyceride, total cholesterol, and hepatic glycogen content. Results represent means ± SE of three mice. FBS, fasting blood sugar.

reaction with GSK-3 fusion protein were done according to the manufacturer's instructions.

Quantification of the results. Results are expressed as means ± SE, and the significance was assessed using one-way ANOVA and unpaired Student's *t* tests.

RESULTS

Overexpression of constitutively active Akt caused marked hepatomegaly, fatty liver, hypertriglyceridemia, and hypoglycemia. Four days after the intravenous viral injections, the tissue distribution of overexpressed myr-Akt was investigated by immunoblotting with anti-Akt antibody. Results showed that myr-Akt was highly selectively expressed in the liver and undetectable in other tissues (Fig. 1*A*). Endogenous Akt expressions did not differ between LacZ and myr-Akt mice in most tissues, but in fat, endogenous Akt was slightly decreased by the overexpression of myr-Akt. Overexpression of myr-Akt resulted in markedly higher kinase activity than endogenous Akt in control mice, as demonstrated in the experiment using GSK-3 as a substrate for the Akt kinase (Fig. 1*D*).

The livers of constitutively active Akt (myr-Akt)–injected mice were whitish and markedly enlarged as compared with those of control mice (Fig. 1*B*). Liver weight in myr-Akt–injected mice was ~2.5-fold greater than that of the control LacZ-injected mice, without a significant difference in overall body weight (Fig. 1*E*). Sudan III staining showed fine- to medium-sized lipid droplets to have accumulated diffusely in the livers of myr-Akt–injected mice, indicating fatty liver (Fig. 1*C*). Supratesticular fat did not differ significantly in size between control and myr-Akt–injected mice (data not shown).

Serum parameters and hepatic glycogen contents are presented in Fig. 1*E*. Serum glucose levels of myr-Akt–

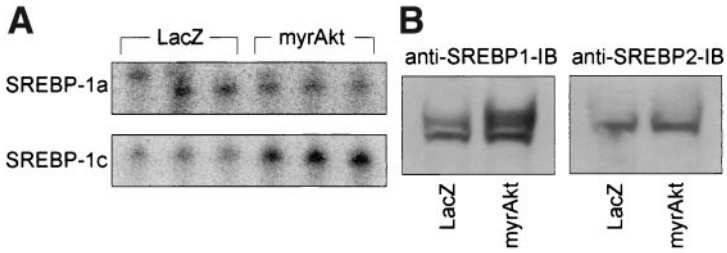

FIG. 2. Detection of SREBP from the liver. *A*: RNase protection assay for detecting all SREBP-1 subtypes. A 10-μg aliquot of liver total RNA from each of three mice was hybridized with riboprobes of SREBP-1a or -1c. *B*: Western blotting of liver nuclear extract. Aliquots of hepatic nuclear extract were subjected to SDS-PAGE and immunoblotted with anti–SREBP-1 or anti–SREBP-2 antibodies.

injected mice were significantly reduced (40 ± 5 and 60 ± 8 mg/dl in the fasted and ad libitum states, respectively), whereas those of the controls were 110 ± 19 and 121 ± 22 mg/dl, respectively. Fasting plasma insulin levels of myr-Akt–injected and control mice were 0.7 ± 0.5 and 4.8 ± 1.4 μIU/ml, respectively. Serum total cholesterol in the fasted state was slightly but significantly higher in myr-Akt–injected mice than in controls. Serum triglyceride in the fasted state was markedly increased, by 4.7-fold, in myr-Akt–injected mice. Hepatic glycogen content was also markedly increased in the myr-Akt–expressing liver.

Induction of SREBP-1c in myr-Akt–overexpressing liver. SREBP-1c has been attracting attention because it is induced by insulin stimulation (20,35,36) and then functions as a transcription factor that induces several rate-limiting lipogenic enzymes in the liver (16,22,24,37,38). Recent reports (15,18) have described insulin-induced SREBP-1c induction as being mediated through the phosphatidylinositol 3-kinase pathway, and it was suggested (15) that Akt is also involved in this induction of SREBP-1c. Thus, we examined the effect of myr-Akt overexpression on SREBP-1c expression in the liver. RNase protection assay revealed an increase in SREBP-1c mRNA with no significant change in the SREBP-1a mRNA level (Fig. 2*A*). Immunoblotting of hepatic nuclear extracts revealed that the nuclear form of SREBP-1 from the myr-Akt–expressing liver to be increased while that of SREBP-2 was not affected (Fig. 2*B*). These results are in good agreement with those of a previous report (15).

To reveal the contribution of increased SREBP-1 expression to the myr-Akt–induced phenotypes, two models were utilized. In one model, wherein SREBP-1 knockout mice received a myr-Akt–adenoviral injection, we observed the phenotypes that myr-Akt induced independently of SREBP-1 induction. The other, overexpression of constitutively active types of SREBP-1 in the mouse liver, allowed the phenotypes resulting from SREBP-1 induction to be assessed. Figure 3 shows the Northern blotting of liver RNA from six types of mice: *1*) normal mice expressing control LacZ, *2*) normal mice expressing myr-Akt, *3*) SREBP-1 knockout mice expressing control LacZ, *4*) SREBP-1-knockout mice expressing myr-Akt, *5*) normal mice expressing constitutively active SREBP-1a, and *6*) normal mice expressing constitutively active SREBP-1c. Induction of SREBP-1 by myr-Akt overexpression (compare *lane 1* with *lane 2* in Fig. 3) was not observed in SREBP-1 knockout mice (*lanes 3* and *4*). On the other hand, mRNAs of overexpressed nuclear forms of SREBP-1a and -1c, which are known to function as constitutively active SREBP-1s (38), were observed to be comparable to or stronger dense bands (*lanes 5* and *6*) (Fig. 3) than that of myr-Akt–induced SREBP-1 (*lane 2*). Moreover, endogenous SREBP-1 mRNA was induced by the expression of constitutively active SREBP-1 as a result of positive feedback regulation of SREBP-1 (24).

Phenotypes of hepatic myr-Akt–expressing SREBP knockout mice and hepatic constitutively active SREBP-1–expressing mice. The phenotypes of six types of mice are summarized in Fig. 4. Hepatomegaly was similar in myr-Akt–expressing livers, irrespective of the presence or absence of the SREBP-1 gene, whereas SREBP-1 overexpression induced whitish fatty liver with no change in hepatic weight (Fig. 4*A*). Hepatic triglycerides accumulated in response to overexpression of either myr-Akt or SREBP-1, but it should be noted that myr-Akt overexpression induced marked hepatic triglyceride accumulation also in the SREBP knockout mice (Fig. 4*B*).

The hypoglycemic effect induced by hepatic myr-Akt overexpression was also induced in the SREBP knockout mice and was similar to that observed in normal mice (Fig. 4*C*). In contrast, no significant hypoglycemia occurred in association with active SREBP-1 overexpression. Serum triglyceride elevation with myr-Akt overexpression was marked as mentioned before, whereas the increase in serum triglyceride with active SREBP-1 overexpression was minimal (Fig. 4*D*). The serum triglyceride concentration in SREBP-1 knockout mice was very low. Interestingly, however, myr-Akt overexpression had a significant serum triglyceride elevating effect in the SREBP-1 knockout mice.

Regulation of mRNA levels of glycolytic and gluconeogenic rate-limiting enzymes by Akt and

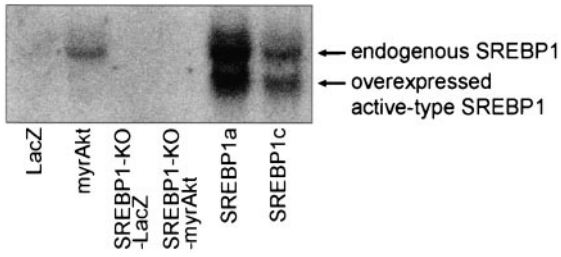

FIG. 3. Northern blotting of liver RNA from six types of mice. Six types of mice were created, and 4 days later, 24-h fasted mice were killed after blood sampling. The six types were *1*) C57BL/6 mice with LacZ adenovirus injection (LacZ), *2*) C57BL/6 mice with myr-Akt adenovirus injection, *3*) SREBP-1 knockout mice with LacZ adenovirus injection (SREBP1-KO-LacZ), *4*) SREBP-1 knockout mice with myr-Akt adenovirus injection (SREBP1-KO-myrAkt), *5*) C57BL/6 mice with constitutively active SREBP-1a adenovirus injection (SREBP1a), and *6*) C57BL/6 mice with constitutively active SREBP-1c adenovirus injection (SREBP1c). Three mice of each type were created in cases *1*, *2*, *5*, and *6*. For *3* and *4*, only two mice each were available, so two hepatic lobes from each mouse were excised, such that four total lobes for each type were manipulated independently to isolate RNA. Aliquots of RNA (20 μg) were electrophoresed, transferred, and blotted with a 5′-labeled oligo-DNA probe detecting SREBP-1 as described in RESEARCH DESIGN AND METHODS. Representative data from each type of mouse are shown. Constitutively active SREBP-1 is a nuclear form whose mRNA is shorter than the endogenous precursor mRNA, which appears below the endogenous SREBP-1.

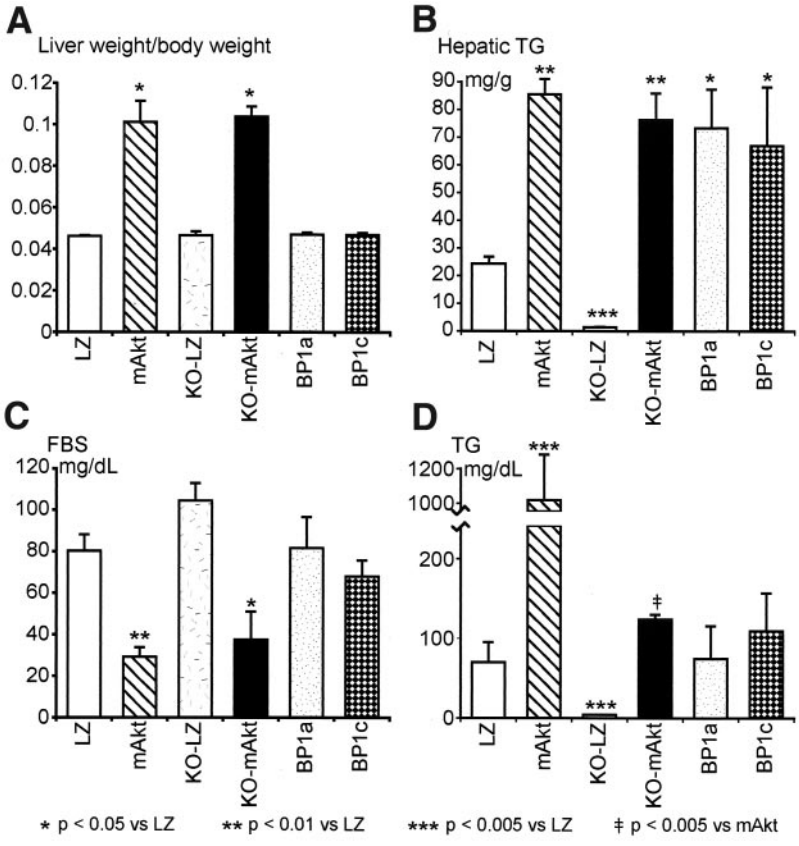

FIG. 4. Physiological and biochemical data of six types of mice. As described in the legend of Fig. 3, all three mice of each type were injected with LacZ (LZ), myr-Akt (mAkt), SREBP-1a (BP1a) or SREBP-1c (BP1c) adenovirus, or both. SREBP-1 knockout mice were injected with LacZ or myr-Akt adenovirus. Four days after injection, mice were fasted for 24 h, blood samples were taken, and then mice were killed. Graphs show the means ± SE of liver weight/body weight ratios (*A*), hepatic triglyceride content (*B*), fasting blood glucose (*C*), and serum triglyceride level (*D*). FBS, fasting blood sugar; KO, knockout; TG, triglyceride.

SREBP-1 activations. Glucokinase (GK), phosphofructokinase-1 (PFK), and L-type pyruvate kinase (PK) are the rate-limiting enzymes determining hepatic glycolytic activity, whereas glucose-6-phosphatase (G6Pase) and PEPCK are hepatic gluconeogenic rate-limiting key enzymes. Their mRNA levels were measured by RNase protection assay.

Overexpression of myr-Akt markedly upregulated GK in both normal and SREBP-1 knockout mouse liver (Fig. 5*A*). Active-type SREBP-1s slightly but not significantly increased GK mRNA. Myr-Akt overexpression also increased PFK mRNA regardless of the presence of SREBP-1, whereas overexpression of SREBP had no effect on PFK mRNA expression (Fig. 5*B*). The PK mRNA level also did not differ significantly from overexpression of myr-Akt or SREBP (Fig. 5*C*).

The mRNAs of the two important rate-limiting enzymes regulating gluconeogenesis, G6Pase and PEPCK, were significantly downregulated by overexpression of myr-Akt, which was similar to the observations in SREBP-1 knockout mice. In contrast, overexpression of the active-type SREBP-1 had no significant effect on G6Pase and PEPCK mRNA levels (Fig. 5*D* and *E*).

Regulation of mRNA levels of rate-limiting lipogenic enzymes by Akt and SREBP-1 activations. Figure 6 shows the effects of myr-Akt and SREBP-1 overexpressions on rate-limiting lipogenic enzymes. Myr-Akt overexpression increased fatty acid synthase (FAS) mRNA slightly (Fig. 6*A*) and stearoyl-CoA desaturase 1 (SCD1) markedly (Fig. 6*B*), whereas amounts of acetyl-CoA carboxylase (ACC) and glucose-6-phosphate dehydrogenase (G6PD) mRNAs did not change significantly (Fig. 6*C* and *D*). In contrast, all four enzymes were induced by constitutively active SREBP-1a or -1c overexpression, and the effect of SREBP-1a was stronger than that of SREBP-1c. Myr-Akt–induced SCD1 mRNA induction was completely abolished in SREBP-1 knockout mice. As for ACC, FAS, and G6PD, the regulatory effect of Akt activation appears to be relatively weak compared with that of SREBP-1.

Effects on hepatic fatty acid oxidation and triglyceride utilization in tissues by adenoviral myr-Akt expression. To investigate whether the effects of myr-Akt on triglyceride metabolism are due to altered lipid breakdown, we measured mRNA levels of key hepatic proteins involved in fatty acid oxidation (FAO) (Table 2). In the myr-Akt–expressing liver, peroxisome proliferator–activated receptor (PPAR)α and FAO enzymes tended to decrease as compared with the control liver, but that effect was not significant. In contrast, when myr-Akt was expressed in the SREBP-1 knockout mouse liver, myr-Akt significantly increased PPARα and FAO enzymes.

To investigate whether the discrepancy in serum versus hepatic triglyceride levels is a result of myr-Akt–induced transcriptional changes in the LDL receptor or lipoprotein lipase (LPL), the mRNA levels of the LDL receptor in the liver and of the LDL receptor and LPL in fat and muscle were measured (Table 3). In the liver, myr-Akt upregulated the LDL receptor similarly in normal and SREBP-1 knockout mice. In fat and muscle, neither LPL nor LDL receptor mRNA levels differed significantly among the six types of mice studied.

DISCUSSION

Akt reportedly plays key roles in various cellular functions, including glucose transport, glycogen synthesis,

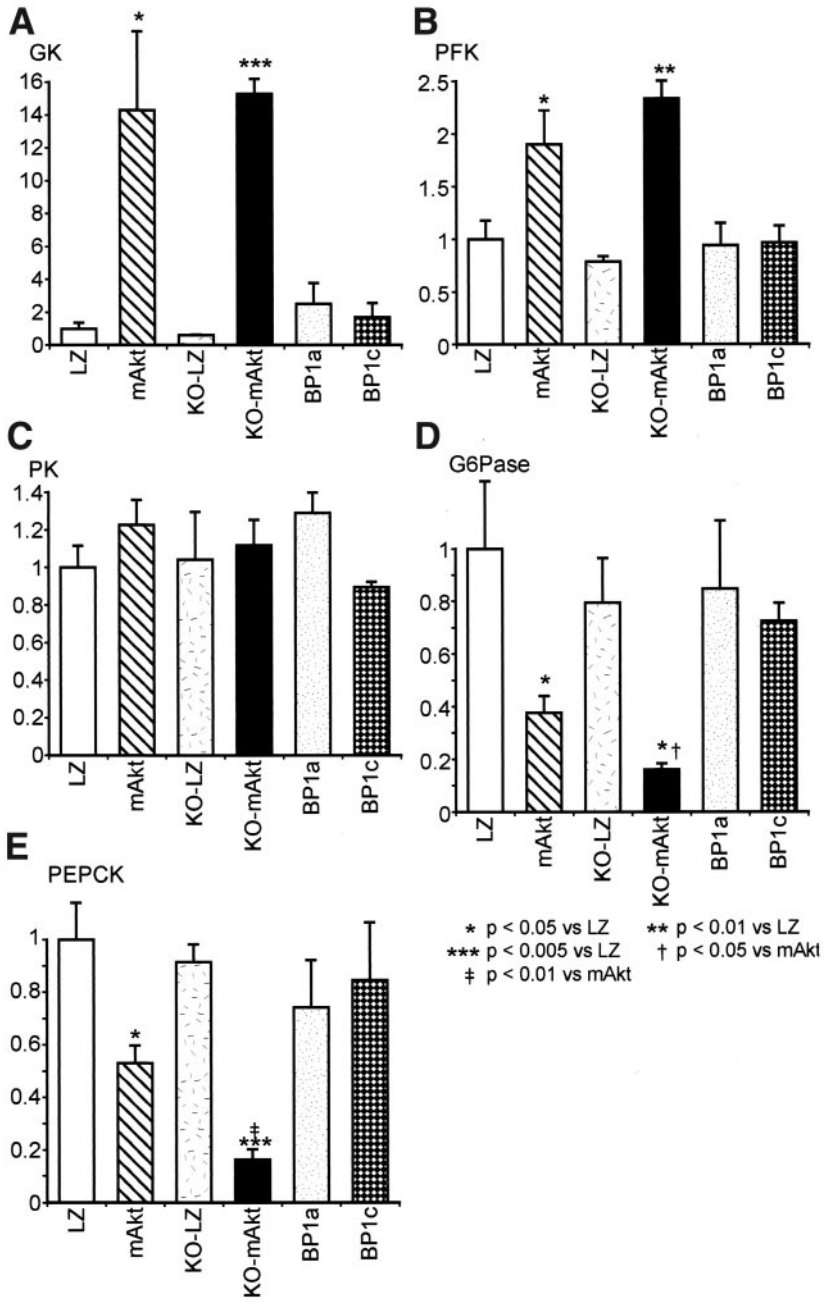

FIG. 5. RNase protection assays of key glycolytic/gluconeogenic enzymes. Three independent samples from three C57BL/6 mice that had been injected with LacZ (LZ), myr-Akt (mAkt), SREBP-1a (BP1a), or SREBP-1c (BP1c) adenovirus or four independent (two lobes from each mouse) samples from two SREBP-1 knockout (KO) mice that had been injected with LacZ or myr-Akt adenovirus were prepared. A 10-μg aliquot of total hepatic RNA was hybridized with riboprobes of each enzyme. The results represent the means ± SE of three or four samples. The scale of the graphs represents the fold increase in the value in LacZ adenovirus–injected C57BL/6 mice.

DNA synthesis, antiapoptotic activity, and cell proliferation. Thus, the role of Akt is not limited to the metabolic actions of insulin, and intensive studies have focused on Akt in various fields of cell biology, tissue development, and so on. Several reports have described tissue-specific findings in mice transgenic for Akt. The heart (39–41) and pancreas (42,43), in which an active mutant of Akt was overexpressed, showed marked tissue enlargement. To study the role of hepatic Akt, constitutively active Akt was overexpressed selectively in the liver, and the resultant phenotype was investigated. We cannot rule out the possibility that myr-Akt stimulates some signals that endogenous Akt does not; however, because Akt is phosphorylated, translocated to the membrane fraction, and then transmits the signal(s), the overexpression of myr-Akt rather than wild-type Akt is more physiological, although other molecules such as atypical PKC are also likely to transmit signals.

We found that hepatic overexpression of constitutively active Akt led to a phenotype characterized by marked hepatomegaly, hypoglycemia with hypoinsulinemia, and hypertriglyceridemia with fatty liver. Hepatomegaly is likely to result from cell proliferation via Akt's oncogenic activity and the accumulation of lipid and glycogen. It was also demonstrated that constitutively active Akt mimics the effects of insulin in the liver, hypoglycemia, hepatic glycogen accumulation, transcriptional upregulation of GK, and downregulation of G6Pase and PEPCK (11,44). We speculate that, based on the observation that myr-Akt is sufficient for producing a hypoglycemic effect, the agents activating Akt could be novel antidiabetic drugs even if they exert their effects only in the liver. However,

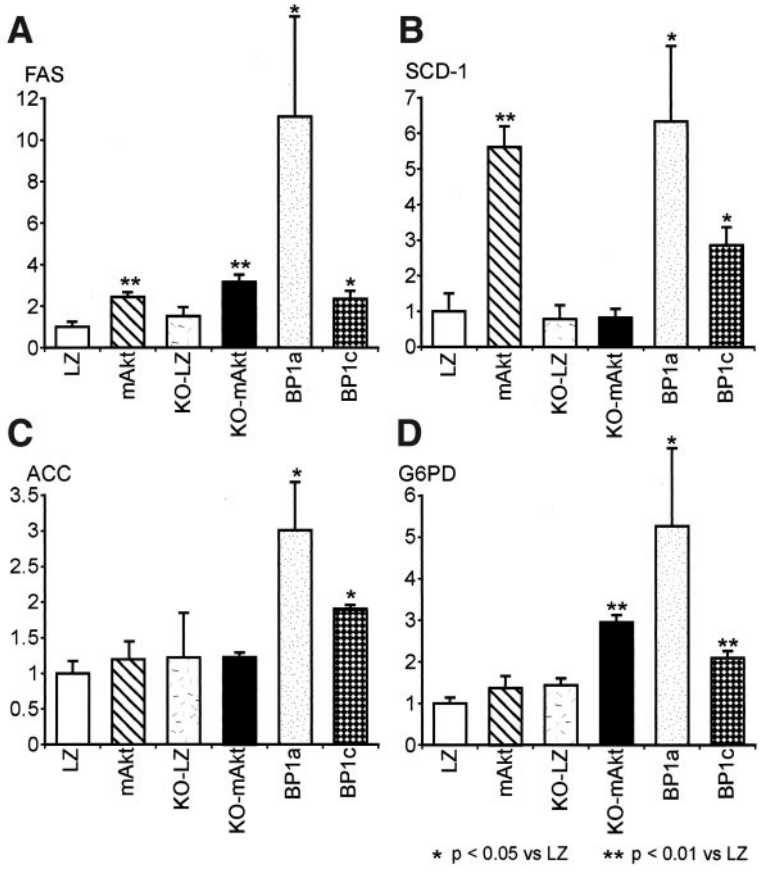

FIG. 6. RNase protection assays of key lipogenic enzymes and G6PD. Three independent samples from three C57BL/6 mice that had been injected with LacZ (LZ), myr-Akt (mAkt), SREBP-1a (BP1a), or SREBP-1c (BP1c) adenovirus or four independent (two lobes from each mouse) samples from two SREBP-1 knockout (KO) mice that had been injected with LacZ or myr-Akt adenovirus were prepared. A 10-μg aliquot of total hepatic RNA was hybridized with riboprobes of each enzyme. The results represent the means ± SE of three or four samples. The scale of the graphs represents the fold increase in the value in LacZ adenovirus–injected C57BL/6 mice.

it is possible that if such a drug was to act continuously or too strongly, undesirable side effects, such as hypertriglyceridemia and hepatomegaly, might develop.

It was recently hypothesized that SREBP-1, the expression of which is increased by Akt activation (15), contributes to the regulation of hepatic glucose-metabolizing enzymes that function to produce hypoglycemic effects. SREBP-1c reportedly enhances transcriptions of GK (19,21) and PK (20) and also inhibits transcription of PEPCK (19,22), suggesting that SREBP-1c is a key factor in hepatic hypoglycemic function (19). However, other reports suggested that expressions of G6Pase and PEPCK are regulated by Forkhead transcription factor (FKHR) (45,46) or GSK-3 (47), which are directly phosphorylated by Akt and inactivated (13,14,48,49).

Our results using myr-Akt expressing SREBP-1 knockout mice clearly demonstrated that SREBP-1 is not necessary for Akt-mediated induction of GK and inhibition of G6Pase and PEPCK transcriptions. As for PK, several reports (50–52) have shown that it is not insulin but rather glucose that regulates its transcription. This is consistent with our observation that myr-Akt overexpression has no effect.

The lipogenic/FAO enzyme and triglyceride results are complicated but interesting. Among the lipogenic enzymes, SCD1 is markedly upregulated by myr-Akt overexpression in normal mice but not in SREBP-1 knockout mice, suggesting that transcriptional regulation of SCD1 by Akt is mediated entirely via the SREBP-1 pathway or that SREBP-1 acts as a strict permissive factor for SCD1

TABLE 2
RNase protection assay of PPARα and fatty acid oxidative enzymes

	LacZ	myr-Akt	Knockout LacZ	Knockout myr-Akt	SREBP-1a	SREBP-1c
PPARα	1.00 ± 0.05	0.80 ± 0.05	0.23 ± 0.04*	0.52 ± 0.03†‡	0.52 ± 0.07†	0.61 ± 0.13§
CPT1	1.00 ± 0.11	0.71 ± 0.07	0.54 ± 0.03‖	0.78 ± 0.22	0.59 ± 0.15	0.71 ± 0.14‖
CPT2	1.00 ± 0.14	0.90 ± 0.03	0.56 ± 0.00§	0.98 ± 0.16¶	0.74 ± 0.10	0.61 ± 0.11§
VLCAD	1.00 ± 0.06	1.08 ± 0.13	0.71 ± 0.05‖	1.08 ± 0.21¶	0.76 ± 0.08	0.75 ± 0.08
LCAD	1.00 ± 0.04	0.81 ± 0.02	0.56 ± 0.11§	0.71 ± 0.12‖	0.79 ± 0.13	0.69 ± 0.13‖
MCAD	1.00 ± 0.06	0.91 ± 0.11	0.48 ± 0.18‖	0.59 ± 0.13	0.45 ± 0.07§	0.48 ± 0.13‖
SCAD	1.00 ± 0.10	1.00 ± 0.05	0.69 ± 0.02†	1.00 ± 0.06‡	0.84 ± 0.07	0.85 ± 0.07

Data are means ± SE of three to four samples. A 10-μg aliquot of total hepatic RNA was hybridized with riboprobes for each enzyme. The scale represents the fold increase in the value in LacZ adenovirus–injected C57BL/6 mice. CPT, carnitine palmitoyltransferase; LCAD, long-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; SCAD, short-chain acyl-CoA dehydrogenase; VLCAD, very-long-chain acyl-CoA dehydrogenase. *$P < 0.001$ vs. LacZ; †$P < 0.01$ vs. LacZ; ‡$P < 0.01$ vs. knockout LacZ; §$P < 0.05$ vs. LacZ; ‖$P < 0.05$ vs. knockout LacZ; ¶$P < 0.1$ vs. LacZ.

TABLE 3
RNase protection assay of LDL receptor and LPL

	LacZ	myr-Akt	Knockout LacZ	Knockout myr-Akt	SREBP-1a	SREBP-1c
Liver LDL receptor	1.00 ± 0.15	1.81 ± 0.20*	0.47 ± 0.07†	2.30 ± 0.30‡	2.15 ± 0.50†	0.75 ± 0.05
Muscle LDL receptor	1.00 ± 0.10	0.91 ± 0.21	1.25 ± 0.22	1.09 ± 0.22	1.12 ± 0.06	1.10 ± 0.28
Fat LDL receptor	1.00 ± 0.16	1.10 ± 0.08	1.04 ± 0.22	1.06 ± 0.32	1.01 ± 0.07	1.10 ± 0.20
Muscle LPL	1.00 ± 0.12	1.07 ± 0.03	1.14 ± 0.21	0.93 ± 0.26	1.01 ± 0.05	1.01 ± 0.15
Fat LPL	1.00 ± 0.45	0.88 ± 0.21	1.35 ± 0.33	0.94 ± 0.16	1.24 ± 0.34	1.26 ± 0.16

Data are means ± SE of three to four samples. A 10-μg aliquot of total hepatic RNA was hybridized with riboprobes for each enzyme. The scale represents the fold increase in the value in LacZ adenovirus–injected C57BL/6 mice. *$P < 0.05$ vs. LacZ; †$P < 0.01$ vs. LacZ; ‡$P < 0.01$ vs. knockout LacZ.

gene expression. Alternatively, it is also possible that basal levels of SREBP induce the expressions of some key genes or transcription factors that mediate the effect of Akt. In contrast, FAS and G6PD were upregulated by myr-Akt in both normal and SREBP-1 knockout mice to a similar degree, indicating that Akt increases their expressions via a pathway independent of SREBP-1. Indeed, even in SREBP knockout mice, hepatic triglyceride content was significantly increased by myr-Akt overexpression. The regulations of PPARα and FAO enzymes are complex, but the observation that myr-Akt does not decrease the mRNAs of FAO enzymes in SREBP-1 knockout mice suggests that hepatic triglyceride accumulation is not due to a decrease in FAO. Taken together, these findings strongly suggest that an SREBP-1–independent lipogenic pathway exists downstream from Akt.

Another interesting finding is the effect on serum triglycerides. Although SREBP-1 overexpressions resulted in lipid accumulation in the liver to a degree similar to that obtained with myr-Akt, only myr-Akt produced hypertriglyceridemia. In this study, myr-Akt and SREBP-1a showed comparable upregulatory effects on the hepatic LDL receptor. Furthermore, fat and muscle displayed no significant changes in the mRNA expression levels of the LDL receptor or LPL when myr-Akt adenovirus was injected. In myr-Akt–expressing mice, both hepatic and serum triglyceride levels increased, although in SREBP-1a–expressing mice, only the hepatic triglyceride level increased. These results cannot be explained by modified LDL receptor or LPL gene expressions in the liver, fat, or muscle. Thus, we speculate that triglyceride secretion from the liver is increased in the myr-Akt–expressing mice, but further study is needed to clarify this issue.

Our observations can be summarized as follows. *1*) Hepatic overexpression of Akt led to a phenotype characterized by marked hepatomegaly, hypoglycemia with hypoinsulinemia, and hypertriglyceridemia with fatty liver. *2*) The hypoglycemic effect induced by Akt is associated with upregulation of glycogen synthesis, downregulations of PEPCK and G6Pase, and upregulations of GK, PFK, and FAS. Furthermore, SREBP-1 is not necessary for the Akt-mediated hypoglycemic effect. *3*) Lipogenic enzymes are partially regulated by SREBP-1, but an SREBP-1–independent lipogenic pathway involving transcriptional regulations of FAS and G6PD may exist in the liver downstream from Akt activation.

REFERENCES

1. Saltiel AR, Kahn CR: Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414:799–806, 2001
2. Whiteman EL, Cho H, Birnbaum MJ: Role of Akt/protein kinase B in metabolism. *Trends Endocrinol Metab* 13:444–451, 2002
3. Miyake K, Ogawa W, Matsumoto M, Nakamura T, Sakaue H, Kasuga M: Hyperinsulinemia, glucose intolerance, and dyslipidemia induced by acute inhibition of phosphoinositide 3-kinase signaling in the liver. *J Clin Invest* 110:1483–1491, 2002
4. Czech MP, Corvera S: Signaling mechanisms that regulate glucose transport. *J Biol Chem* 274:1865–1868, 1999
5. Pessin JE, Saltiel AR: Signaling pathways in insulin action: molecular targets of insulin resistance. *J Clin Invest* 106:165–169, 2000
6. Calera MR, Martinez C, Liu H, Jack AK, Birnbaum MJ, Pilch PF: Insulin increases the association of Akt-2 with Glut4-containing vesicles. *J Biol Chem* 273:7201–7204, 1998
7. Kohn AD, Summers SA, Birnbaum MJ, Roth RA: Expression of a constitutively active Akt Ser/Thr kinase in 3T3–L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. *J Biol Chem* 271:31372–31378, 1996
8. Tanti JF, Grillo S, Gremeaux T, Coffer PJ, Van Obberghen E, Le Marchand-Brustel Y: Potential role of protein kinase B in glucose transporter 4 translocation in adipocytes. *Endocrinology* 138:2005–2010, 1997
9. Hajduch E, Alessi DR, Hemmings BA, Hundal HS: Constitutive activation of protein kinase B α by membrane targeting promotes glucose and system A amino acid transport, protein synthesis, and inactivation of glycogen synthase kinase 3 in L6 muscle cells. *Diabetes* 47:1006–1013, 1998
10. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ: Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* 292:1728–1731, 2001
11. O'Brien RM, Granner DK: Regulation of gene expression by insulin. *Physiol Rev* 76:1109–1161, 1996
12. Chen D, Fucini RV, Olson AL, Hemmings BA, Pessin JE: Osmotic shock inhibits insulin signaling by maintaining Akt/protein kinase B in an inactive dephosphorylated state. *Mol Cell Biol* 19:4684–4694, 1999
13. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA: Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378:785–789, 1995
14. van Weeren PC, de Bruyn KM, de Vries-Smits AM, van Lint J, Burgering BM: Essential role for protein kinase B (PKB) in insulin-induced glycogen synthase kinase 3 inactivation: characterization of dominant-negative mutant of PKB. *J Biol Chem* 273:13150–13156, 1998
15. Fleischmann M, Iynedjian PB: Regulation of sterol regulatory-element binding protein 1 gene expression in liver: role of insulin and protein kinase B/cAkt. *Biochem J* 349:13–17, 2000
16. Horton JD, Bashmakov Y, Shimomura I, Shimano H: Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci U S A* 95:5987–5992, 1998
17. Osborne TF: Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. *J Biol Chem* 275:32379–32382, 2000
18. Azzout-Marniche D, Becard D, Guichard C, Foretz M, Ferre P, Foufelle F: Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes. *Biochem J* 350:389–393, 2000
19. Becard D, Hainault I, Azzout-Marniche D, Bertry-Coussot L, Ferre P, Foufelle F: Adenovirus-mediated overexpression of sterol regulatory element binding protein-1c mimics insulin effects on hepatic gene expression and glucose homeostasis in diabetic mice. *Diabetes* 50:2425–2430, 2001
20. Foretz M, Pacot C, Dugail I, Lemarchand P, Guichard C, Le Liepvre X, Berthelier-Lubrano C, Spiegelman B, Kim JB, Ferre P, Foufelle F: ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Mol Cell Biol* 19:3760–3768, 1999

21. Foretz M, Guichard C, Ferre P, Foufelle F: Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc Natl Acad Sci U S A* 96:12737–12742, 1999
22. Chakravarty K, Leahy P, Becard D, Hakimi P, Foretz M, Ferre P, Foufelle F, Hanson RW: Sterol regulatory element-binding protein-1c mimics the negative effect of insulin on phosphoenolpyruvate carboxykinase (GTP) gene transcription. *J Biol Chem* 276:34816–34823, 2001
23. Shimomura I, Shimano H, Korn BS, Bashmakov Y, Horton JD: Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. *J Biol Chem* 273:35299–35306, 1998
24. Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, Goldstein JL: Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J Clin Invest* 98:1575–1584, 1996
25. Yamada T, Katagiri H, Asano T, Inukai K, Tsuru M, Kodama T, Kikuchi M, Oka Y: 3-phosphoinositide-dependent protein kinase 1, an Akt1 kinase, is involved in dephosphorylation of Thr-308 of Akt1 in Chinese hamster ovary cells. *J Biol Chem* 276:5339–5345, 2001
26. Ono H, Katagiri H, Funaki M, Anai M, Inukai K, Fukushima Y, Sakoda H, Ogihara T, Onishi Y, Fujishiro M, Kikuchi M, Oka Y, Asano T: Regulation of phosphoinositide metabolism, Akt phosphorylation, and glucose transport by PTEN (phosphatase and tensin homolog deleted on chromosome 10) in 3T3–L1 adipocytes. *Mol Endocrinol* 15:1411–1422, 2001
27. Sakoda H, Ogihara T, Anai M, Funaki M, Inukai K, Katagiri H, Fukushima Y, Onishi Y, Ono H, Fujishiro M, Kikuchi M, Oka Y, Asano T: Dexamethasone-induced insulin resistance in 3T3–L1 adipocytes is due to inhibition of glucose transport rather than insulin signal transduction. *Diabetes* 49: 1700–1708, 2000
28. Shimano H, Shimomura I, Hammer RE, Herz J, Goldstein JL, Brown MS, Horton JD: Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. *J Clin Invest* 100:2115–2124, 1997
29. Bucolo G, David H: Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* 19:476–482, 1973
30. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC: Enzymatic determination of total serum cholesterol. *Clin Chem* 20:470–475, 1974
31. Folch J, Lees M, Stanley GHS: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509, 1957
32. Bergmeyer HU, Bergmeyer J, Grassl M: *Methods of Enzymatic Analysis*. 3rd ed. Hoboken, NJ, Wiley, 1984, p. 11–18
33. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS: Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J Clin Invest* 99:838–845, 1997
34. Sheng Z, Otani H, Brown MS, Goldstein JL: Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. *Proc Natl Acad Sci U S A* 92:935–938, 1995
35. Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL: Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc Natl Acad Sci U S A* 96:13656–13661, 1999
36. Kim JB, Sarraf P, Wright M, Yao KM, Mueller E, Solanes G, Lowell BB, Spiegelman BM: Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J Clin Invest* 101:1–9, 1998
37. Kim JB, Spiegelman BM: ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. *Genes Dev* 10:1096–1107, 1996
38. Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, Goldstein JL: Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J Clin Invest* 99:846–854, 1997
39. Condorelli G, Drusco A, Stassi G, Bellacosa A, Roncarati R, Iaccarino G, Russo MA, Gu Y, Dalton N, Chung C, Latronico MV, Napoli C, Sadoshima J, Croce CM, Ross J Jr: Akt induces enhanced myocardial contractility and cell size in vivo in transgenic mice. *Proc Natl Acad Sci U S A* 99:12333–12338, 2002
40. Matsui T, Li L, Wu JC, Cook SA, Nagoshi T, Picard MH, Liao R, Rosenzweig A: Phenotypic spectrum caused by transgenic overexpression of activated Akt in the heart. *J Biol Chem* 277:22896–22901, 2002
41. Shioi T, McMullen JR, Kang PM, Douglas PS, Obata T, Franke TF, Cantley LC, Izumo S: Akt/protein kinase B promotes organ growth in transgenic mice. *Mol Cell Biol* 22:2799–2809, 2002
42. Tuttle RL, Gill NS, Pugh W, Lee JP, Koeberlein B, Furth EE, Polonsky KS, Naji A, Birnbaum MJ: Regulation of pancreatic beta-cell growth and survival by the serine/threonine protein kinase Akt1/PKBalpha. *Nat Med* 7:1133–1137, 2001
43. Bernal-Mizrachi E, Wen W, Stahlhut S, Welling CM, Permutt MA: Islet beta cell expression of constitutively active Akt1/PKB alpha induces striking hypertrophy, hyperplasia, and hyperinsulinemia. *J Clin Invest* 108:1631–1638, 2001
44. O'Brien RM, Granner DK: Regulation of gene expression by insulin. *Biochem J* 278:609–619, 1991
45. Nakae J, Kitamura T, Silver DL, Accili D: The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. *J Clin Invest* 108:1359–1367, 2001
46. Schmoll D, Walker KS, Alessi DR, Grempler R, Burchell A, Guo S, Walther R, Unterman TG: Regulation of glucose-6-phosphatase gene expression by protein kinase Balpha and the forkhead transcription factor FKHR: evidence for insulin response unit-dependent and -independent effects of insulin on promoter activity. *J Biol Chem* 275:36324–36333, 2000
47. Lochhead PA, Coghlan M, Rice SQ, Sutherland C: Inhibition of GSK-3 selectively reduces glucose-6-phosphatase and phosphatase and phosphoenolpyruvate carboxykinase gene expression. *Diabetes* 50:937–946, 2001
48. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME: Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96:857–868, 1999
49. Kops GJ, de Ruiter ND, De Vries-Smits AM, Powell DR, Bos JL, Burgering BM: Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature* 398:630–634, 1999
50. Doiron B, Cuif MH, Kahn A, Diaz-Guerra MJ: Respective roles of glucose, fructose, and insulin in the regulation of the liver-specific pyruvate kinase gene promoter. *J Biol Chem* 269:10213–10216, 1994
51. Lefrancois-Martinez AM, Diaz-Guerra MJ, Vallet V, Kahn A, Antoine B: Glucose-dependent regulation of the L-pyruvate kinase gene in a hepatoma cell line is independent of insulin and cyclic AMP. *Faseb J* 8:89–96, 1994
52. Decaux JF, Antoine B, Kahn A: Regulation of the expression of the L-type pyruvate kinase gene in adult rat hepatocytes in primary culture. *J Biol Chem* 264:11584–11590, 1989