Impact of the Liver-Specific Expression of SHIP2 (SH2-Containing Inositol 5’-Phosphatase 2) on Insulin Signaling and Glucose Metabolism in Mice

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We investigated the role of hepatic SH2-containing inositol 5’-phosphatase 2 (SHIP2) in glucose metabolism in mice. Adenoviral vectors encoding wild-type SHIP2 (WT-SHIP2) and a dominant-negative SHIP2 (ΔSHIP2) were injected via the tail vein into db/+m and db/db mice, respectively. Four days later, amounts of hepatic SHIP2 protein were increased by fivefold. Insulin-induced phosphorylation of Akt in liver was impaired in WT-SHIP2–expressing db/+m mice, whereas the reduced phosphorylation was restored in ΔSHIP2–expressing db/db mice. The abundance of mRNA for glucose-6-phosphatase (G6Pase) and PEPCK was increased, that for glucokinase (GK) was unchanged, and that for sterol regulatory element–binding protein 1 (SREBP)-1 was decreased in hepatic WT-SHIP2–overexpressing db/+m mice. The increased expression of mRNA for G6Pase and PEPCK was partly suppressed, that for GK was further enhanced, and that for SREBP1 was unaltered by the expression of ΔSHIP2 in db/db mice. The hepatic expression did not affect insulin signaling in skeletal muscle and fat tissue in both mice. After oral glucose intake, blood glucose levels and plasma insulin concentrations were elevated in WT-SHIP2–expressing db/+m mice, while elevated values were decreased by the expression of ΔSHIP2 in db/db mice. These results indicate that hepatic SHIP2 has an impact in vivo on the glucose metabolism in both physiological and diabetic states possibly by regulating hepatic gene expression. Diabetes 54:1958–1967, 2005

Insulin binding to the insulin receptor in turn phosphorylates insulin receptor substrates (IRSs) at tyrosine residues (1,2). The tyrosine-phosphorylated IRS binds to the p85 regulatory subunit of phosphatidylinositol (PI) 3-kinase, resulting in the activation of the p110 subunit (3,4). PI 3-kinase functions as a lipid kinase to produce PI(3,4,5)P3 from PI(4,5)P2 in vivo (5). PI(3,4,5)P3 is crucial as a lipid second messenger in various metabolic effects of insulin (3,6–8). PI(3,4,5)P3 mediates insulin signals to downstream molecules including Akt (9). Akt is the key signaling molecule in the activation of glucose uptake in the skeletal muscle and fat tissue and in the regulation of mRNA expression for gluconeogenesis, glycolysis, and lipid synthesis in the liver (10). SH2-containing inositol 5’-phosphatase 2 (SHIP2) was identified as a lipid phosphatase that hydrolyzes PI(3,4,5)P3 to PI(3,4)P2 (11,12). Targeted disruption of the SHIP2 gene in mice caused an increase in insulin sensitivity without affecting other biological systems (13). In addition, some polymorphisms of the SHIP2 gene found in British and French populations are associated with metabolic syndromes including type 2 diabetes and hypertension (14). The expression of SHIP2 could be elevated in type 2 diabetic subjects with a 16-bp deletion in the 3’-untranslated regulatory region of the SHIP2 gene (15). Based on these reports, SHIP2 appears to be a physiologically important negative regulator relatively specific to insulin signaling with an impact on the state of insulin resistance (13–17).

We have previously reported that overexpression of SHIP2, via 5’-phosphatase activity, impaired insulin-induced activation of Akt resulting in decreased glucose uptake and glycogen synthesis in 3T3-L1 adipocytes and L6 myocytes (18,19). Although we clarified the role and molecular mechanisms by which SHIP2 regulates insulin signaling in the skeletal muscle and fat tissue (20–22), the impact of SHIP2 in the liver on the metabolism of glucose in vivo is largely unknown. Based on studies with tissue-specific knockout of the insulin receptor in mice, the liver was found to be the most critical target tissue of insulin action in the control of glucose homeostasis (23,24). Insulin regulates hepatic glucose output through suppression of hepatic gluconeogenic gene expression via the IRS-2/PI 3-kinase pathway and augmentation of glycolytic gene expression via the IRS-1/PI 3-kinase pathway (25–30).

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Sterol regulatory element–binding protein (SREBP)-1c is the key factor involved in insulin resistance by controlling lipid synthesis via the IRS-1/PI 3-kinase pathway (30). On the basis of these results, it is possible that SHIP2 in the liver functions as a negative regulator of the PI 3-kinase–dependent metabolic action of insulin. Therefore, it would be of particular importance to clarify the impact of hepatic SHIP2 on the regulation of insulin signaling and hepatic gene expression for the control of glucose homeostasis in diabetic model mice as well as in nondiabetic mice.

In the present study, we investigated the impact of liver-specific overexpression of SHIP2 in lean db/+ mice and liver-specific inhibition of SHIP2 in diabetic db/db mice. Adenoviral vectors encoding wild-type SHIP2 (WT-SHIP2) and the dominant-negative mutant of SHIP2 (ΔIP-SHIP2) were injected, via the tail vein, into the db/+ and db/db mice, respectively. We studied the effect of hepatic SHIP2 expression on insulin signaling leading to hepatic gene expression. In addition, we examined whether the liver-specific expression of SHIP2 affects the metabolic actions of insulin in skeletal muscle and fat tissue. Furthermore, glucose metabolism was investigated by conducting glucose tolerance tests and insulin tolerance tests in WT-SHIP2–expressing db/+ mice and ΔIP-SHIP2–expressing db/db mice.

**RESEARCH DESIGN AND METHODS**

Human regular insulin HumalinR was provided by Eli Lilly. A polyclonal anti–IRS-1 antibody and a polyclonal anti-Thr308 phospho-specific Akt antibody were purchased from Cell Signaling Technology (Beverly, MA). A polyclonal anti–IRS-2 antibody and a polyclonal anti-Ser1166 phospho-specific Akt antibody were obtained from Upstate Biotechnology (Lake Placid, NY). A polyclonal anti-Akt antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). A monoclonal anti-phosphotyrosine antibody (PY99) was purchased from Transduction Laboratories (Lexington, KY). The polyclonal anti-SHIP2 antibody has been described previously (12). Enhanced chemiluminescence reagents were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). All other routine reagents were of analytical grade and purchased from Sigma Chemicals (St. Louis, MO) or Wako Pure Chemical Industries (Osaka, Japan).

Male C57BL/KsJ-db/db Jcl (BKS.Cg-Lepdb/+Jcl) mice, their lean heterozygote littermates (db/+ m), and C57BL/6J mice were purchased from Clea Japan (Tokyo, Japan) at 6 weeks of age. Mice were maintained under standard light (12 h light/dark) and temperature conditions. These mice were caged in groups of four and were provided with standard rodent diet and water ad libitum.

**Adenovirus-mediated gene transfer in the liver.** Adenoviral vectors encoding rat WT-SHIP2 and a PI 5'-phosphatase–defective ΔIP-SHIP2 have been described previously (18). Eight-week-old male mice were injected with the adenovirus via the tail vein at a concentration of 5 × 10⁶ pfu (plaque-forming units)/g body wt in a suspension of 200 μl PBS. Experiments were performed 4 days after the injection. All procedures were approved by the Committee of Animal Experiments at Toyama Medical & Pharmaceutical University.

**Western blot analysis.** Mice deprived of food for 16 h were injected with human regular insulin (5 units/kg body wt) or saline via the tail vein. After 5 min, the mice were anesthetized and killed to isolate the liver, hindlimb muscle, and epididymal fat. These tissues were homogenized using a polytron at half-maximal speed (15,000 rpm) for 1 min on ice in 500 μl of a homogenization buffer containing 20 mmol/l Tris, 5 mmol/l EDTA, 10 mmol/l Na₃PO₄, 100 mmol/l NaF, 2 mmol/l Na₂VO₃, 1% NP-40, 1 mmol/l phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin (pH 7.5). The tissue lysates were solubilized by continuous stirring for 1 h at 4°C and centrifuged for 30 min at 14,000g. The supernatants (200 μg protein) were immunoprecipitated with indicated antibodies for 2 h at 4°C. The precipitates were washed twice with 5% SDS-PAGE and then suspended in polyvinylidene difluoride membranes using a Bio-Rad Transblot apparatus. The membranes were blocked in a buffer containing 50 mmol/l Tris, 150 mmol/l NaCl, 0.1% Tween 20, and 5% nonfat milk (pH 7.5) for 2 h at 20°C. They were then probed with antibodies for 16 h at 4°C. After the membranes had been washed in a buffer containing 50 mmol/l Tris, 150 mmol/l NaCl, and 0.1% Tween 20 (pH 7.5), blots were incubated with horseradish peroxidase–linked second antibody and then examined by enhanced chemiluminescence (ECL) detection using an ECL reagent according to the manufacturer’s instructions (Amersham). In each experiment, the intensity of the band derived from insulin-induced phosphorylation of IRS-1, IRS-2, and Akt in control db/+ m mice was assigned as a value of 1 arbitrary unit, and the intensity of all treated groups in db/db mice was expressed as a fold value of control.

**Northern blot analysis.** Mice deprived of food for 16 h were anesthetized and killed, and the liver was removed and frozen in liquid N2. Mice were not fasted for the analysis of SREBP1 mRNA. Total RNA was extracted from 50 mg of the liver sample using the QuickPrep total RNA Extraction Kit (Amersham). Total RNA (10 μg) for each sample was separated using a 1% agarose gel and transferred to a Hybond-N+ positively charged nylon membrane (Amersham). Probes for glucokinase (GK) (31), SREBP-1 (32), PEPC-K (33), glucose-6-phosphatase (G6Pase) (34), and GLUT2 (35) mRNAs were kindly provided by T. Noguchi (Nagoya University, Nagoya, Japan), H. Shimano (Tsukuba University, Ibaragi, Japan), D.K. Granner (Vanderbilt University Medical Center, Nashville, TN), H. Nakajima (Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan), and W. Ogawa (Kobe University, Kobe, Japan), respectively. Each probe was chemiluminescently labeled and hybridized, and the mRNA expression was detected using the AlkPhos Direct Labeling and Detection System with CDP-Star (Amersham Biosciences).

**Oral glucose tolerance test and insulin tolerance test.** For the oral glucose tolerance test, mice deprived of food for 16 h were loaded orally with glucose (2 g/kg). Blood samples were collected from the orbital sinus at various time points after the loading. For the insulin tolerance test, mice deprived of food for 8 h were injected intraperitoneally with human regular insulin (0.75 units/kg). Blood samples were collected from the tail vein at various time points. Blood glucose levels were then measured with a Freestyle KISSEI (Kissei, Japan), and blood insulin concentrations were measured with an enzyme-linked immunosorbent assay kit (Moriga, Japan).

**Statistical analysis.** Data are presented as means ± SE. P values were determined using a paired t test or Bonferroni test with ANOVA. P < 0.05 was considered statistically significant.

**RESULTS**

Expression of SHIP2 in the liver of mice injected with an adenoviral vector via the tail vein. Systemic adenoviral injection into mice via the tail vein is known to result in the liver-specific expression of the exogenous gene (36). The 5'-phosphatase–defective ΔIP-SHIP2 acts as a dominant-negative mutant possibly by competing with endogenous SHIP2 to bind P[(3,4,5)P₃ (18). By inhibiting the endogenous function of SHIP2, ΔIP-SHIP2 augments insulin signaling mediated by the PI 3-kinase product PI(3,4,5)P₃ (18,19). Hepatic expression of both WT-SHIP2 and ΔIP-SHIP2 was detected 1 day after the adenoviral injection. The abundance was maximal at 4 days, decreased thereafter, and returned to the basal level at 14 days after the injection (data not shown). Therefore, we examined the expression of WT-SHIP2 and ΔIP-SHIP2 in various tissues of mice 4 days after the adenoviral injection. As shown in Fig. 1, we observed fivefold greater amounts of exogenous WT-SHIP2 and ΔIP-SHIP2 than endogenous SHIP2 in the liver. The lower band seen in the liver of mice may be a splicing variant of SHIP2. Alternatively, we cannot rule out the possibility that the band originated from the degradation of SHIP2 preponderantly from the preparation of samples. Except for the liver, expression of either exogenous WT-SHIP2 or ΔIP-SHIP2 was not detectable in various tissues examined. The body weight of the mice was not changed by the liver-specific expression of SHIP2 (data not shown). We analyzed the effect of liver-specific expression of WT-SHIP2 in control db/+ m mice and ΔIP-SHIP2 in diabetic db/db mice on the metabolic action of insulin.
Effect of WT-SHIP2 expression on insulin-induced phosphorylation of IRS and Akt in the liver of db/+m mice. Mice injected with the adenoviral vector encoding WT-SHIP2 or control LacZ were deprived of food for 16 h and then injected intravenously with insulin (5 units/kg) or saline (200 μL) for 5 min. Thereafter, the liver was removed and subjected to immunoblot analysis (Fig. 2). Amounts of IRS-1, IRS-2, and Akt were not altered by expression of WT-SHIP2 in the liver of db/+m mice (Fig. 2A–C). Insulin-induced tyrosine phosphorylation of IRS-1 and IRS-2 was also comparable between WT-SHIP2– and LacZ-injected db/+m mice (Figs. 2A and B). Akt is a crucial mediator of IRS/PI 3-kinase signaling and is known to be activated by phosphorylation at Thr308 and Ser473 residues (10,37,38). Insulin induced phosphorylation of Akt at Thr308 in the liver of LacZ-transfected db/+m mice. The extent of the phosphorylation was decreased by 49.0 ± 13.0% in the liver of WT-SHIP2–overexpressing db/+m mice (Fig. 2C). Similar results were obtained in C57BL/6J mice (data not shown).

Effect of ΔIP-SHIP2 expression on insulin-induced phosphorylation of IRS and Akt in the liver of db/db mice. We next examined the ameliorative effect of the liver-specific expression of ΔIP-SHIP2 on the metabolic signaling of insulin in the liver of diabetic db/db mice (Fig. 3). In db/db mice, the amount of IRS-1 and IRS-2 was decreased by 27.9 ± 4.8 and 73.6 ± 5.2%, respectively, compared with db/+m mice, whereas the amount of Akt

FIG. 1. Expression of SHIP2 in the liver of mice injected with an adenoviral vector via the tail vein. Total homogenates of the indicated tissues obtained from db/+m and db/db mice 4 days after adenoviral injection with WT-SHIP2 (A) and ΔIP-SHIP2 (B), respectively, were subjected to immunoblot analysis with anti-SHIP2 antibody. The amount of SHIP2 in the liver is shown as means ± SE of three separate experiments (C).

FIG. 2. Effect of WT-SHIP2 expression on insulin (Ins.)-induced phosphorylation of IRS and Akt in the liver of db/+m mice. WT-SHIP2– and LacZ-injected db/+m mice starved for 16 h were injected with insulin (5 units/kg) via the tail vein. After 5 min, the liver was excised and homogenized. Tissue samples were immunoprecipitated with anti–IRS-1 antibody (A) or anti–IRS-2 antibody (B). The precipitates were subjected to immunoblot analysis with the same antibodies or anti-phosphotyrosine antibody. The tissue samples were subjected to immunoblot analysis with anti-Akt antibody or anti–phospho-Thr308–specific Akt antibody (C). Results are means ± SE of four separate experiments. *P < 0.05 vs. the amount of Akt phosphorylated in LacZ-transfected mice. I.p., intraperitoneal.
was not altered (data not shown). Consistent with the results in Fig. 2, amounts of IRS-1, IRS-2, and Akt in the liver were not changed by the expression of ΔIP-SHIP2 in db/db mice compared with that in LacZ-injected db/db mice (Fig. 3A–C). Insulin-induced phosphorylation of Akt at Thr^{308} was decreased in db/db mice to 19.7 ± 5.0% of that in control db/+ m mice. Interestingly, the extent of phosphorylation was restored to 45.2 ± 4.5% by expression of ΔIP-SHIP2 in the liver of db/db mice (Fig. 3C). In addition, the degree of change in the phosphorylation of Akt well paralleled the alteration of Akt activity in both db/+ m and db/db mice (data not shown). Furthermore, similar results were seen in 5-h–fasted mice in addition to 16-h–fasted mice (data not shown). These results indicate that SHIP2 is involved in the regulation of insulin-induced phosphorylation of Akt in the liver of both nondiabetic and diabetic mice.

Effect of SHIP2 expression on the hepatic gene expression in db/+ m mice and db/db mice. Because the metabolic effect of insulin in the liver is mainly regulated by the hepatic expression of genes involved in glucose metabolism, glycolysis, and fat synthesis (29), we examined the effect of liver-specific SHIP2 expression on the insulin-induced regulation of the hepatic gene expression (Fig. 4A–D). We performed Northern blot analysis of total RNA isolated from the liver of db/+ m mice and db/db mice. The level of G6Pase mRNA and PEPCK mRNA in the liver was increased by 179 ± 78 and 190 ± 57%, respectively, by the liver-specific expression of WT-SHIP2 in db/+ m mice. On the other hand, the abundance of GK mRNA was not altered, and that of SREBP1 mRNA was decreased by 45.0 ± 6.5% by the expression. The levels of G6Pase, PEPCK, GK, and SREBP1 mRNAs in the liver were increased by 872 ± 59, 460 ± 49, 306 ± 26, and 120 ± 16%, respectively, in LacZ-transfected db/db mice compared with control db/+ m mice. The enhanced expression of G6Pase mRNA and PEPCK mRNA was partly reduced by 23.0 ± 8.0 and 36.0 ± 13.0%, respectively, by the liver-specific expression of ΔIP-SHIP2 in db/db mice compared with that of LacZ-injected db/db mice. Conversely, the extent of GK mRNA expression in the liver was further enhanced by 31.0 ± 9.0% in the ΔIP-SHIP2–expressing db/db mice. Elevated expression of SREBP1 mRNA was not changed by the expression. On the other hand, although the expression of Glut2 mRNA in the liver was increased in db/db mice compared with db/+ m mice, neither WT-SHIP2 nor ΔIP-SHIP2 expression affected the level of Glut2 mRNA (Fig. 4E). In addition, the abundance of r18S RNA was equal among the samples to ensure that the same amount of total RNA was used (Fig. 4F).

Liver-specific expression of SHIP2 did not affect insulin-induced phosphorylation of IRS and Akt in the skeletal muscle and fat tissue of db/+ m and db/db mice. We further investigated whether the liver-specific expression of SHIP2 affects insulin signaling in skeletal muscle and fat tissue. The level of IRS-1 or Akt did not differ in the skeletal muscle of WT-SHIP2– and LacZ-expressing db/+ m mice, and that of ΔIP-SHIP2– and LacZ-expressing db/db mice. In addition, no apparent difference was found in the amount of basal and insulin-induced phosphorylation of IRS-1 and Akt differed in the fat tissue of WT-SHIP2– and LacZ-expressing db/+ m
Blood glucose and plasma insulin concentrations during oral glucose tolerance and insulin tolerance tests in SHIP2-expressing db/+m and db/db mice. Because the liver-specific expression of SHIP2 affected hepatic insulin signaling leading to the altered expression of genes implicated in glucose homeostasis, we examined the effect of liver-specific SHIP2 expression on glucose and insulin tolerance in db/+m and db/db mice. The blood glucose concentration at 30 min after oral glucose intake was higher in WT-SHIP2–expressing db/+m mice than LacZ-expressing db/+m mice (Fig. 7A). Plasma insulin concentrations remained higher at 0, 15, and 30 min after the glucose loading in WT-SHIP2–expressing db/+m mice than in LacZ-expressing db/+m mice (Fig. 7B). The kinetics of blood glucose concentrations after the insulin injection was comparable between WT-SHIP2– and LacZ-expressing db/+m mice (Fig. 8A). On the other hand, the basal glucose concentration was significantly reduced and the plasma insulin concentration tended to be decreased in ΔIP-SHIP2–expressing db/db mice compared with LacZ-expressing db/db mice. In addition, blood glucose levels (Fig. 7C) and plasma insulin concentrations (Fig. 7D) were significantly lower in ΔIP-SHIP2–expressing db/db mice than in LacZ-injected db/db mice at 15, 30, and 60 min after oral glucose intake. Furthermore, blood glucose levels were lower in ΔIP-SHIP2–expressing db/db mice than in LacZ-expressing db/db mice before and after the insulin injection (Fig. 8B).

DISCUSSION

A previous study (13) with knockout mice demonstrated that SHIP2 plays an important role in the negative regulation of the metabolic action of insulin in vivo. SHIP2 also appears to be implicated in type 2 diabetes with insulin resistance (14,15). Although the functional impact of SHIP2 on insulin signaling has been studied in cultured fat and skeletal muscle cells in vitro (16–22), the impact in vivo of hepatic SHIP2 on glucose metabolism in nondiabetic and diabetic states is unknown. Thus, it would be interesting to clarify how a hepatic excess of SHIP2 promotes insulin resistance and how the inhibition of hepatic SHIP2 ameliorates glucose metabolism in a state of insulin resistance in mice. We have shown that the systemic infusion of adenoviral vectors encoding WT-SHIP2 and a dominant-negative mutant of SHIP2 (ΔIP-SHIP2) in mice resulted in liver-specific expression of the protein.

We utilized db/+m mice as an animal of lean nondiabetic control. The liver-specific overexpression of WT-SHIP2 impaired insulin-induced phosphorylation of Akt without affecting tyrosine phosphorylation of IRS-1 and IRS-2 in the liver. Approximately a 50% decrease in the phosphorylation of Akt was observed even after the overexpression of WT-SHIP2 to a level more than fivefold that of endogenous SHIP2, as shown in Fig. 2C. As a cause of partial inhibition of the phosphorylation, we assume that SHIP2 product PI(3,4)P2 has some signaling ability to transmit the signal for the phosphorylation of Akt, al-
though less ability than PI(3,4,5)P3 (18,19). The overexpression of SHIP2 also resulted in hyperinsulinemia and potentiated the increase in blood glucose levels after oral glucose intake. These results indicate that the excess of SHIP2 in the liver can cause systemic insulin resistance through inhibition of hepatic Akt activation in vivo. Our results are consistent with a previous study (35) showing the effect of the liver-specific expression of a dominant-negative mutant of PI 3-kinase (ΔP85), indicating that the hepatic PI 3-kinase pathway plays an essential role in the metabolic action of insulin. Thus, either inactivation of PI 3-kinase or potentiated hydrolysis of the PI 3-kinase product in the liver appears to result in an exacerbation of glucose metabolism in vivo.

We next studied db/db mice as an animal model of type 2 diabetes with insulin resistance. Db/db mice are obese and show hyperglycemia and hyperinsulinemia (39,40). The amount of protein and the extent of insulin-induced phosphorylation of IRS-1 and IRS-2 were mildly and markedly reduced, respectively, in the liver of db/db mice compared with that of db/+ m mice. In addition, insulin-induced phosphorylation of Akt was markedly suppressed in the liver of db/db mice. Interestingly, the liver-specific expression of ΔIP-SHIP2 effectively restored the decreased phosphorylation of Akt without affecting the tyrosine phosphorylation of IRS-1 antibody. The precipitates were subjected to immunoblot analysis with anti–IRS-1 antibody or anti–phosphotyrosine antibody (A and C). The tissue samples were immunoblotted with anti-Akt antibody or anti–phospho-Thr308–specific Akt antibody (B and D). Results are means ± SE of four separate experiments. *P < 0.05 vs. the amount of Akt phosphorylated in LacZ-transfected mice. i.p., intraperitoneal.

![FIG. 5. Expression of SHIP2 did not alter insulin (Ins.)-induced phosphorylation of IRS and Akt in the skeletal muscle of db/+ m and db/db mice. WT-SHIP2– or LacZ-injected db/+ m mice (A and B), and ΔIP-SHIP2– or LacZ-injected db/db mice (C and D) starved for 16 h were injected with insulin (5 units/kg) via the tail vein. After 5 min, the skeletal muscle of the mice was excised and homogenized. Tissue samples were immunoprecipitated with anti–IRS-1 antibody. The precipitates were subjected to immunoblot analysis with anti–IRS-1 antibody or anti–phosphotyrosine antibody (A and C). The tissue samples were immunoblotted with anti-Akt antibody or anti–phospho-Thr308–specific Akt antibody (B and D). Results are means ± SE of four separate experiments. *P < 0.05 vs. the amount of Akt phosphorylated in LacZ-transfected mice. i.p., intraperitoneal.](image-url)
are consistent with reports on the 3′-lipid phosphatase PTEN. The PI 3-kinase product PI(3,4,5)P3 can also be hydrolyzed by PTEN (42). Thus, inhibition of PTEN was found to enhance insulin signaling in the liver in studies using tissue-specific deletion and antisense approaches (43,44).

Tissue-specific knockout of the insulin receptor revealed the liver to be the most crucial organ in terms of glucose metabolism in vivo (23,24). Insulin regulates glucose metabolism in the liver by regulating hepatic gene expression (23,24). The conversion of glucose to glucose 6-phosphate catalyzed by GK is the initial step in the utilization of glucose (45). Conversely, the reaction catalyzed by G6Pase is the final step of the production of glucose, and PEPCK is a rate-controlling enzyme of gluconeogenesis in the liver (29). In addition, the production of triglycerides is mediated by lipogenic enzymes mainly regulated in a SREBP1c-dependent manner in the liver, and the enhanced expression of SREBP1c is associated with insulin resistance by causing fatty liver (46). Insulin is known to rapidly inhibit hepatic gluconeogenic gene expression and suppress hepatic glucose output via an IRS-2–mediated PI 3-kinase–dependent pathway mainly involved in Akt signaling (25–28). On the other hand, insulin increases the expression of GK mRNA and SREBP1c mRNA via the IRS-1–PI 3-kinase pathway (30). Based on these findings, inappropriate increases in the mRNA expression of G6Pase, PEPCK, and SREBP1c and/or a reduction in the mRNA expression of GK can lead to systemic insulin resistance (29,45–48). Along these lines, transgenic mice with hepatic overexpression of G6Pase or PEPCK exhibited enhanced hepatic glucose output leading to hyperinsulinemia and hyperglycemia (49,50).

Our results show that the hepatic expression of G6Pase and PEPCK mRNAs was increased by the hepatic overex-
pression of SHIP2 in db/+m mice. The apparent increase in blood glucose levels after the oral intake of glucose in the WT-SHIP2–expressing db/+m mice may be due to an impaired regulation of gluconeogenic gene expression leading to a reduced ability of the liver to dispose of glucose. The abundance of SREBP1 mRNA was reduced and that of GK mRNA was unaltered in the liver of WT-SHIP2–expressing db/+m mice. Based on the data, although the hepatic overexpression of WT-SHIP2 appears to regulate lipid synthesis through SREBP1c, the effect may have less of an impact on glucose metabolism than that caused by the altered levels of G6Pase and PEPCK mRNAs in the liver. On the other hand, the abundance of G6Pase, PEPCK, GK, and SREBP1c mRNAs was significantly increased in the liver of diabetic db/db mice. The inappropriately enhanced expression of G6Pase and PEPCK mRNAs was partially ameliorated, that of SREBP1 mRNA was unaltered, and that of GK mRNA was further increased in the liver of ΔIP-SHIP2–expressing db/db mice. Because these alterations of G6Pase, PEPCK, and GK mRNA expression are able to improve hepatic glucose disposal, the effect of hepatic ΔIP-SHIP2 expression on the improvement of hyperglycemia and hyperinsulinemia is considered to be mainly attributable to these changes in db/db mice. Notably, since hepatic glucose production is controlled by the expression of G6Pase and PEPCK in the liver, the improvement of glucose metabolism appears to be mainly caused by the inhibition of hepatic SHIP2 function leading to the appropriate regulation of hepatic glucose production. Fatty liver is possibly a result of the enhanced expression of SREBP1c mRNA in db/db mice. Since SREBP1 mRNA is already highly expressed in db/db mice, the enhanced PI 3-kinase–dependent signaling caused by the expression of ΔIP-SHIP2 may not have the effect of altering the expression of SREBP1 mRNA. Consistent with this interpretation, the extent of fatty liver was apparently unchanged by the expression of hepatic ΔIP-SHIP2 in db/db mice based on the histological analysis (data not shown).

After the oral intake of glucose, blood glucose levels and
serum insulin concentrations were elevated by the liver-specific expression of WT-SHIP2 in db/+m mice, but the increases were ameliorated by the liver-specific expression of ΔIP-SHIP2 in db/db mice. In contrast, blood glucose levels after intraperitoneal insulin injection were comparable between control LacZ- and WT-SHIP2–expressing db/+m mice. Fasting blood glucose levels were decreased by the hepatic expression of ΔIP-SHIP2 in db/db mice. The degree of the decrease in blood glucose levels appeared similar between LacZ- and ΔIP-SHIP2–expressing db/db mice at all time points after the intraperitoneal insulin injection. Because glucose levels after the intraperitoneal injection of insulin are mainly attributable to the effect of insulin on skeletal muscle and fat tissue (35), our results indicate that the effect of liver-specific expression of SHIP2 may be limited to the hepatic actions of insulin without affecting actions in the skeletal muscle and fat tissue. In this regard, insulin-induced tyrosine phosphorylation of IRS-1 and IRS-2 and phosphorylation of Akt at Thr308 and Ser473 were not affected in the skeletal muscle but not in the liver, to some extent (35). These possible differences may arise from the relatively minor alterations of blood glucose levels and insulin concentrations caused by the expression of WT-SHIP2 compared with those caused by the expression of ΔIP55. Inhibition of hepatic PI 3-kinase for 3 days affected insulin signaling in fat tissue, but not in skeletal muscle, to some extent (35). These possible differences may arise from the relatively minor alterations of blood glucose levels and insulin concentrations caused by the expression of WT-SHIP2 compared with those caused by the expression of ΔIP55. In this context, we cannot rule out the possibility that the skeletal muscle and fat tissue are involved, at least in part, in the alteration of glucose homeostasis caused by the liver-specific expression of SHIP2 in mice. More studies will be necessary to clarify this issue.

In summary, our results indicate that (1) the liver-specific expression of SHIP2 regulates insulin-induced phosphorylation of Akt in the liver, but not in the skeletal muscle and fat tissue, (2) elevation of SHIP2 expression in the liver has an impact on the deterioration of glucose metabolism in vivo, and (3) the inhibition of SHIP2 function in the liver is an effective approach for the amelioration of hyperglycemia with insulin resistance.

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