Altered Glucose Homeostasis in Mice with Liver-specific Deletion of Src Homology Phosphatase 2*

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The Src homology 2 domain-containing protein-tyrosine phosphatase Shp2 has been implicated in a variety of growth factor signaling pathways, but its role in insulin signaling has remained unresolved. In vitro studies suggest that Shp2 is both a negative and positive regulator of insulin signaling, although its physiological function in a number of peripheral insulin-responsive tissues remains unknown. To address the metabolic role of Shp2 in the liver, we generated mice with either chronic or acute hepatic Shp2 deletion using tissue-specific Cre-LoxP and adeno viral Cre approaches, respectively. We then analyzed insulin sensitivity, glucose tolerance, and insulin signaling in liver-specific Shp2-deficient and control mice. Mice with chronic Shp2 deletion exhibited improved insulin sensitivity and increased glucose tolerance compared with controls. Acute Shp2 deletion yielded comparable results, indicating that the observed metabolic effects are directly caused by the lack of Shp2 in the liver. These findings correlated with, and were most likely caused by, direct dephosphorylation of insulin receptor substrate (IRS)1/2 in the liver, accompanied by increased PI3K/Akt signaling. In contrast, insulin-induced ERK activation was dramatically attenuated, yet there was no effect on the putative ERK site on IRS1 (Ser612) or on S6 kinase 1 activity. These studies show that Shp2 is a negative regulator of hepatic insulin action, and its deletion enhances the activation of PI3K/Akt pathway downstream of the insulin receptor.

Type 2 diabetes is a complex, polygenic disease wherein a number of tissues are rendered insulin-resistant, a phenotype associated with obesity and sedentary lifestyle (1). Insulin is secreted from pancreatic β cells into the portal circulation and acts as a major regulator of glucose homeostasis by means of a complex network of signaling events (2). Upon binding to the insulin receptor (IR),7 insulin induces trans-phosphorylation of several tyrosyl residues, leading to the recruitment and phosphorylation of insulin receptor substrates (IRSs) and Grb2-associated binder (Gab) family proteins. These serve as docking sites for Src homology 2 domain-containing signal relay molecules, such as Src homology phosphatase 2 (Shp2) and the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K). PI3K activity is stimulated by its binding to IRS proteins (3), leading to activation of downstream targets such as Akt (4). Akt inhibits tuberous sclerosis complex 2 (TSC2), an inhibitor of mammalian target of rapamycin (mTOR), which phosphorylates p70 ribosomal protein S6 kinase 1 (S6K1) and other targets to regulate protein synthesis (5, 6). Insulin also activates ERK1/2, which phosphorylate various substrates, including p90 ribosomal protein S6 kinase (RSK), to promote gene expression (7). By phosphorylating IRS proteins on specific serine residues, ERK1/2 and S6K1 also are implicated in negative feedback regulation of the IR signaling pathway (8).

Shp2 is a ubiquitously expressed non-transmembrane protein-tyrosine phosphatase that contains two Src homology 2 domains, a tyrosine phosphatase (catalytic) domain and a C-terminal region with phosphorylation sites and a proline-rich domain (9). Shp2 plays an essential role in most receptor tyrosine kinase signaling pathways, where it is required for normal activation of the ERK pathway and its transcriptional targets (10, 11). In response to IR activation, Shp2 binds to IRS1/2, Gab1, as well as the transmembrane protein Shps1/Sirpα (12). There have also been reports that Shp2 can bind to and dephosphorylate the IR and IGF1 receptor (13). Yet, although multiple studies have addressed the potential role of Shp2 in regulating insulin signaling and glucose homeostasis, its overall function

7 The abbreviations used are: IR, insulin receptor; IRS, insulin receptor substrate; Ad, adenovirus; FAS, fatty acid synthase; fl, floxed; G6Pase, glucose-6-phosphatase; Gab, Grb2-associated binder; GH, growth hormone; GTR, glucose tolerance test; HET, heterozygous; IGF1, insulin-like growth factor 1; ITT, insulin tolerance test; LDKO, liver-specific Gab1 knock-out; LSHKO, liver-specific Shp2 knock-out; mTOR, mammalian target of rapamycin; PEPCK, phosphoenolpyruvate carboxykinase; P3K, phosphatidylinositol 3-kinase; PTP, protein-tyrosine phosphatase; RSK, p90 ribosomal protein S6 kinase; S6K1, S6 kinase 1; Shp2, Src homology phosphatase 2; SREBP1c, sterol regulatory element-binding protein 1c; TSC2, tuberous sclerosis complex 2.
in this pathway in different insulin-responsive tissues remains unresolved.

In vitro experiments have been conflicting, suggesting both negative and positive regulatory roles. Studies of 32D myeloid cells expressing an IRS1 mutant lacking Shp2 binding sites demonstrated no effect on insulin-stimulated Ras/ERK activation, but rather, argued that Shp2 negatively regulates (likely by directly dephosphorylating) the PI3K binding sites of IRS1 (14). Similarly, overexpression of Shp2 in 3T3L1 cells negatively modulates insulin signaling and downstream events such as glycogen synthesis by reducing tyrosyl phosphorylation of IRS1 and the concomitant activation of PI3K (15). On the other hand, CHO-IR cells expressing catalytically inactive mutants of Shp2 (C459S) show marked attenuation of insulin-stimulated Ras activation (16), suggesting that Shp2 functions as a positive regulator of insulin action. The reason(s) for these discrepancies is (are) not clear and could be due, at least in part, to the different cell types studied, distinct effects of Shp2 on different pathways downstream of the IR and/or potential off-target effects of dominant negative mutants.

In vivo studies also have not completely resolved the physiological role of Shp2 in insulin signaling and glucose homeostasis. Targeted mutation of Shp2 exon 3 in mice leads to embryonic lethality (17), precluding detailed studies of the effects of global Shp2 deletion. Hemizygous mice are viable but do not manifest any apparent defects in insulin action (18). On the other hand, transgenic mice that express a presumptive dominant negative mutant of Shp2 in skeletal muscle, liver, and adipose tissue exhibit insulin resistance and impaired insulin-stimulated glucone uptake (19). Shp2 deletion in striated and cardiac muscle results in insulin resistance, impaired glucose uptake in muscle cells, and glucose intolerance (20), although these mice also exhibit marked dilated cardiomyopathy (20, 21). Recently, it was reported that Shp2 deletion in the pancreas causes defective glucose-stimulated insulin secretion and impaired glucose tolerance (22). The role of Shp2 in regulating insulin signaling in the liver remains unknown, although the metabolic effects of deleting the Shp2-binding protein Gab1 in the liver have been reported. Liver-specific Gab1 KO mice (LGKO) exhibit enhanced insulin sensitivity, elevated IRS1/2 tyrosyl phosphorylation, enhanced Akt, and suppressed ERK activation (23).

We assessed the effects of chronic or acute deletion of Shp2 in the liver using tissue-specific knock-out and adenoiral-Cre approaches, respectively. We determined the metabolic effects of hepatic Shp2 deletion on insulin signaling and glucose homeostasis and investigated the underlying molecular mechanism.

**EXPERIMENTAL PROCEDURES**

**Mouse Studies**—Shp2-floxed (Shp2fl/fl) mice were generated previously (24). Albumin-Cre mice were obtained from Dr. CR. Kahn (Joslin Diabetes Center). All mice studied were matched males on a mixed 129Sv/J-Kahn (Joslin Diabetes Center). All mice studied were age-matched and were maintained on a 12-h light-dark cycle with free access to water and food. Mice were placed on standard laboratory chow (Purina laboratory chow, #5001) at weaning. Genotyping for the Shp2-floxed allele and for the presence of Cre was performed by PCR, using DNA extracted from tails (25). Ad5-Cre and Ad5-LacZ (control) were purchased from the Gene Transfer Vector Core at the University of Iowa, and their titers were confirmed using cell viability assays, as described previously (26). Where indicated, viruses (3 × 10⁹ plaque-forming units/g of body weight) were injected into the tail veins of 13-week-old male mice. Please note that in all studies, male mice were used. Mouse studies were approved by the Institutional Animal Care and Use Committee at University of California Davis.

**Metabolic Measurements**—Glucose was measured in blood collected from the tail using a glucometer (Home Aide Diagnostics). Serum insulin was determined by ELISA using mouse insulin as a standard (Crystal Chemical Company). Glucose and insulin concentrations were used to calculate the homeostasis model assessment of IR, a reliable marker of insulin sensitivity (27), which is defined as: fasting glucose (mg/dl) × fasting insulin (microunits/ml)/405. Free fatty acid and triglyceride concentrations were measured by an enzymatic colorimetric method (Wako). Fed glucose measurements were taken between 7 and 9 a.m. and, where indicated, from mice fasted for 12 h. For insulin tolerance tests (ITTs), mice were fasted for 4 h and injected intraperitoneally with 0.65–0.85 milliunits/g of human insulin (Humulin®, Eli Lilly). Blood glucose values were measured before and at 15, 30, 45, 60, 90, and 120 min after injection. For glucose tolerance tests (GTTs), overnight-fasted mice were injected with 20% g-glucose, and glucose was measured before and 15, 30, 60, and 120 min after injection.

**Biochemical Analyses**—For insulin signaling experiments, 20-week-old male mice were fasted overnight, injected intraperitoneally with insulin (10 milliunits/g of body weight), and killed 5 or 10 min after injection. Tissues were ground in liquid nitrogen and lysed using an radioimmunoprecipitation assay buffer as we described previously (28). Protein concentrations were determined using a bicinchoninic acid assay kit (Pierce Chemical). Proteins (500–1,000 μg) were subjected to immunoprecipitation using IRβ (gift from Dr. C. R. Kahn), IRS1, or IRS2 (Millipore) antibodies. Immune complexes were collected on protein G-Sepharose beads (GE Healthcare) and washed with lysis buffer. Proteins were resolved by SDS-PAGE and transferred to PVDF membranes. Immunoblotting of lysates and immunoprecipitates was performed with antibodies for phosphotyrosine (4G10) (Millipore), IRβ, IRS1/2, pIRS1 (Tyr608), pS6K1 (Thr389), S6K1, pS6 (Ser235/Ser236), pERK, pAkt (Ser473) (Cell Signaling), pIRS1 (Ser612) (Invitrogen), pIRS1 (Ser632) (Upstate Biotechnology), ERK, Shp2, and Akt (Santa Cruz Biotechnology). After incubation with appropriate secondary antibodies, proteins were visualized using enhanced chemiluminescence (ECL; Amersham Biosciences). Pixel intensities of immunoreactive bands were quantified using FluorChem 9900 (Alpha Innotech).

RNA was extracted from livers using TRIzol reagent (Invitrogen). cDNA was generated using a high capacity cDNA Archive kit (Applied Biosystems). Expression of IGF1, phospho-nol-pyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), sterol regulatory element-binding protein 1c (SREBP1c), and fatty acid synthase (FAS) was assessed by quantitative real-time PCR (iCycler, Bio-Rad) with appropriate primers (supplemental Table S1) and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
Altered Glucose Homeostasis in Liver-specific Shp2 Knockouts

A and B, immunoblots of Shp2 expression in peripheral insulin-responsive tissues isolated from Shp2fl/fl (fl/fl, control), Alb-Cre Shp22/−/− (HET), and Alb-Cre Shp22/−/− (LSHKO) mice at 20 weeks of age. Liver lysates were also probed for PTP1B (bottom panel). Note that compared with control mice, hepatic Shp2 protein expression was ∼50 and ∼15% in HET and LSHKO mice, respectively, whereas Shp2 expression in muscle and fat was unaffected. C, immunoblot analysis of Shp2 expression in liver lysates of old (23 months) control and LSHKO mice on chow diet. D, hematoxylin and eosin-stained liver sections from 20-week-old (top panels) and 23-month-old (lower panels) control and LSHKO mice.

TABLE 1

Metabolic parameters of mice with hepatic Shp2 deletion

| Genotype and metabolic parameters | Chow | LSHKO |
|-----------------------------------|------|-------|
| Blood glucose (mg/dl)             |      |       |
| Fed                               | 164.8 ± 3.9 (8) | 152.4 ± 3.7* (8) |
| Fasted                           | 70.6 ± 3.8 (8)  | 59.5 ± 3.3* (8)  |
| Serum insulin (ng/ml)             |      |       |
| Fed                               | 0.05 ± 0.09 (8) | 0.08 ± 0.13 (8)  |
| Fasted                           | 0.19 ± 0.01 (8) | 0.15 ± 0.01* (6) |
| HOMA-IR*                         | 0.73 ± 0.05 (8) | 0.49 ± 0.04** (6) |
| Free fatty acids, fed (mg)        | 0.64 ± 0.03 (9) | 0.61 ± 0.05 (9)  |
| Triglycerides                     |      |       |
| Serum, fasted (mg/dl)             | 63.1 ± 3.5 (10) | 37.9 ± 1.3** (10) |
| Liver, fasted (mg/g liver)        | 77.5 ± 5.4 (9)  | 77.7 ± 8.4 (8)   |

* HOMA-IR, homeostasis model assessment of insulin resistance.

Statistical Analyses—Data are expressed as means ± S.E. Statistical analyses were performed using the JMP program (SAS Institute). Comparisons between groups were made by unpaired two-tailed Student’s t test. ITTs and GTTs were analyzed by repeated-measures analysis of variance (ANOVA). Post hoc analysis was performed using Tukey-Kramer honestly significant difference test.

RESULTS

Generation of Liver-specific Shp2 Knock-out Mice—To clarify the role of Shp2 in regulating glucose homeostasis, we assessed the physiological effects of its deletion in the liver. Mice with liver-specific Shp2 deletion were generated by crossing Shp2fl/fl mice to those expressing Cre recombinase under the control of the albumin promoter (Alb-Cre). The resulting Alb-Cre Shp2fl/fl mice were crossed to Shp22/−/− mice, yielding Alb-Cre Shp22/−/− (hereafter termed liver-specific Shp2 KO; LSHKO), Alb-Cre Shp22+/− (heterozygous; HET), and Shp22+/+ (control) mice. LSHKO mice were born at the expected Mendelian ratio and were morphologically indistinguishable from heterozygous and control littermates. Immunoblot analysis of liver lysates indicated that Shp2 protein expression was decreased by ∼50% in HET and ∼85% in LSHKO mice compared with control mice (Fig. 1, A and B). These findings are consistent with complete deletion of Shp2 in hepatocytes; the residual Shp2 in LSHKO liver lysates likely reflects Shp2 expression in other cell types in the liver, such as biliary canalicular cells, vascular endothelial cells, and macrophages. Shp2 levels were unchanged in other peripheral insulin-responsive tissues (muscle and fat), confirming the specificity of deletion. The expression of another protein-tyrosine phosphatase (PTP) known to regulate hepatic insulin signaling, protein-tyrosine phosphatase 1B (PTP1B) (28, 29), also was unaffected in LSHKO mice (Fig. 1A, bottom panel). The same level of Shp2 deletion was seen in older LSHKO mice; this lack of selection for hepatocytes with undeleted Shp2 suggests that Shp2 is not essential for hepatocyte survival (Fig. 1C). Consistent with this notion, hematoxylin and eosin staining of liver sections from young (20 weeks) or old (23 months) LSHKO mice revealed no overt evidence of hepatotoxicity (Fig. 1D). Therefore, this approach enables efficient and specific deletion of Shp2 in the liver without obvious adverse effects.

Improved Glucose Homeostasis in Mice with Liver-specific Shp2 Deletion—Body weights of control and LSHKO mice on chow were comparable (supplemental Fig. S1A), suggesting that any differences in insulin sensitivity and glucose homeostasis in these mice are primary and not due to body weight alterations. Although Shp2 has also been implicated in growth hormone (GH) signaling (30), the head−rump length of mice was comparable between genotypes, suggesting that Shp2 is not required for GH-induced IGF1 production in the liver (supplemental Fig. S1B). Indeed, IGF1 mRNA expression in the liver was comparable between genotypes (supplemental Fig. S1C). We assayed several metabolic parameters in control and LSHKO mice (Table 1). LSHKO mice exhibited significantly lower fed and fasted glucose levels compared with controls, suggesting improved insulin sensitivity. Consistent with this observation, LSHKO mice exhibited lower fasted insulin concentrations compared with controls. In addition, the homeostasis model assessment of IR was significantly lower in LSHKO than control mice. We also assayed several parameters of whole body lipid homeostasis. Fed serum-free fatty acid concentrations were comparable between genotypes, but fasted serum tri-
glycerides were significantly lower in LSHKO mice compared with controls. Finally, fasted liver triglycerides were comparable between genotypes.

To assess insulin sensitivity directly, mice were subjected to ITTs. LSHKO mice exhibited significantly greater reduction in blood glucose following insulin injection compared with controls (Fig. 2, A and B). In addition to their improved insulin sensitivity, LSHKO mice showed increased ability to clear glucose from the peripheral circulation during GTTs (Fig. 2C). There were no significant differences in insulin secretion between LSHKO and control mice during the GTT (Fig. 2D). These differences were amplified in older (17 months) LSHKO and control mice on chow (Fig. 2, E and F). Thus, hepatic Shp2 deletion leads to improved insulin sensitivity and enhanced glucose tolerance.

**Improved Glucose Homeostasis in Mice with Acute Liver-specific Shp2 Deletion**—To determine whether the improved glucose homeostasis is directly caused by deletion of Shp2, we examined the metabolic effects of acute deletion of hepatic Shp2 using a recombinant Ad5 adenovirus expressing Cre recombinase. The high degree of hepatotropism of Ad5 ensures liver-specific targeting (28), and this approach also enables comparison of metabolic parameters of the same cohorts of mice before and after adenovirus injection. Ad-LacZ (control) and Ad-Cre were titrated using cell viability assays as described (28), then injected into the tail veins of 13-week-old male Shp2fl/fl mice. Ad-Cre infection resulted in significant reduction of Shp2 levels in the liver (Fig. 3A), without any alteration in other tissues (data not shown). Prior to viral injection, mice exhibited identical glucose tolerance (Fig. 3B). However, after viral injection, mice with Ad-Cre-mediated Shp2 deletion exhibited enhanced glucose tolerance compared with controls (Fig. 3C).

Insulin regulates glucose and lipid metabolism in the liver in part by modulating gene expression (31, 32). Quantitative real-time PCR was used to determine the effect of hepatic Shp2 deficiency on gene expression. Consistent with improved glucose tolerance, the levels of PEPCK and G6Pase mRNAs were decreased significantly in mice with hepatic Shp2 deficiency, compared with controls (Fig. 3D). Taken together, these findings indicate that deficiency of Shp2 in the liver directly improves glucose homeostasis.

We also measured the expression of the insulin-regulated lipogenic genes SREBP1c and FAS. Surprisingly, SREBP1c and FAS levels were significantly lower in mice with hepatic Shp2 deficiency compared with controls (Fig. 3E). Although these results are consistent with the decreased serum triglyceride in LSHKO mice, they also contrast their increased insulin sensitivity (see “Discussion”).
Increased Insulin Signaling in LSHKO Mice—To investigate the molecular basis for enhanced insulin sensitivity in LSHKO mice, we injected fasted LSHKO and control male mice with insulin or saline (control) and analyzed insulin signaling in the liver. Overall tyrosyl phosphorylation (Fig. 4A) and basal and insulin-induced tyrosyl phosphorylation of the IR (Fig. 4B) were comparable in livers from control and LSHKO mice. There also was no effect of hepatic Shp2 deficiency on IR phosphorylation at the sites Tyr1162/Tyr1163, which are preferentially dephosphorylated by PTP1B in vitro (33) and in the liver (28). In contrast, basal and insulin-stimulated IRS1 tyrosyl phosphorylation, normalized to IRS1 expression, as well as phosphorylation of Tyr608, a PI3K binding site, was significantly higher in LSHKO mice compared with controls (Fig. 4C). In addition, insulin-induced IRS1 phosphorylation at Ser307, which was recently reported to promote insulin sensitivity in mice (34), was significantly increased in LSHKO mice compared with controls (Fig. 4C). In line with increased IRS1 tyrosyl phosphorylation, there was more p85 subunit of PI3K in IRS1 immunoprecipitates from LSHKO livers compared with controls (Fig. 4D). Basal tyrosyl phosphorylation of IRS2 also was increased in LSHKO mice, and there was a trend toward increased IRS2 tyrosyl phosphorylation following insulin stimulation (Fig. 4E).

Consistent with the increased IRS tyrosyl phosphorylation and PI3K binding, insulin-stimulated Akt phosphorylation on Ser473 was enhanced in LSHKO mice (Fig. 5A). Conversely, but in agreement with the effects of Shp2 deficiency in fibroblasts, insulin-stimulated ERK activation was dramatically impaired in LSHKO mice (Fig. 5B). LGKO mice also show defective ERK activation and enhanced insulin-stimulated Akt activation, which have been attributed to decreased ERK-mediated phosphorylation of IRS1 Ser612 (23). Because Gab1 binding to Shp2 promotes ERK activation in other settings (35–37), we asked whether Ser612 phosphorylation also was decreased in LSHKO mice compared with controls. Surprisingly, however, basal and insulin-induced phosphorylation of Ser612 was nominally increased, although this did not reach statistical significance (Fig. 5C). Defective ERK activation could also impair another major negative feedback pathway in insulin signaling, mediated by S6K (38). Both ERK (39) and RSK (40) phosphorylate and inhibit TSC2, which negatively regulates the S6K activator mTOR (6) (see schematic in Fig. 6). RSK also phosphorylates the mTOR regulator Raptor, helping to promote mTOR activity (41). However, phosphorylation of S6K1 (Thr389) and its downstream target ribosomal protein S6 (Ser235/Ser236) was not inhibited in LSHKO mice (Fig. 5, D and E); if anything, they were slightly increased, indicating that alteration of the S6K1 negative feedback mechanism is not a major contributor to the observed increase in hepatic insulin signaling. Taken together, these data suggest that the increased insulin sensitivity in LSHKO mice results, at least in part, from enhanced activation of the PI3K/Akt pathway downstream of the IR.

**DISCUSSION**

The overall role of Shp2 in regulating glucose homeostasis, as well as its specific functions in the liver, has heretofore remained unresolved. To begin to address these issues, we generated mice with liver-specific Shp2 deletion. Using mice with hepatocyte-specific Cre expression (Alb-Cre), we generated mice with liver-specific Shp2 deletion. Using mice with chronic Shp2 deletion in the liver. We also took advantage of the high degree of hepatotropism of adenovirus 5 Cre to delete liver Shp2 acutely, enabling us to determine whether phenotypes seen in LSHKO mice reflected proximal effects of Shp2 deficiency, as opposed to potential adaptive mechanisms. Acute or chronic deletion of hepatic Shp2 resulted in comparable metabolic phenotypes. Mice lacking Shp2 in the liver exhibit increased hepatic insulin action and enhanced systemic insulin sensitivity, indicating that Shp2 is a negative reg-
ulator of insulin signaling in the liver. Body weights of control and LSHKO mice on chow were comparable, suggesting that alterations in glucose homeostasis are primary. Although multiple studies implicate Shp2 as a key positive regulator of GH signaling (30), the comparable body size and liver IGF1 mRNA expression in LSHKO and control mice argue that Shp2 is not required for GH-induced IGF1 production in the liver.

Insulin modulates hepatic glucose and lipid metabolism by regulating gene expression. Consistent with their improved glucose tolerance Shp2-deficient mice exhibit decreased PEPCK and G6Pase expression. However, Shp2 deficiency has unexpected effects on lipogeneic gene (SREBP1c, FAS) expression. Insulin stimulates expression of SREBP1c and its downstream target FAS, and accordingly, liver IR KO mice as a consequence of their hepatic insulin resistance, have low serum triglycerides and decreased SREBP1c gene expression (42). Given their enhanced insulin sensitivity, it is surprising that SREBP1c gene expression is also lower in hepatic Shp2-deficient mice compared with controls. Liver-specific PTP1B KO mice also have increased hepatic insulin signaling and decreased levels of SREBP1c (29).

Previous studies suggest that IRS1 is a more important regulator of glucose homeostasis, whereas IRS2 is more closely linked to lipid metabolism (44). Hepatic Shp2 deficiency results in increased tyrosyl phosphorylation of IRS1 and IRS2, although the former may be increased to a greater extent. Regardless, given the increase in both IRS molecules, it is difficult to see how differential effects of Shp2 on IRS phosphorylation can explain the observed improvements in both glucose and lipid homeostasis.
Interestingly, whereas mice with hepatic Shp2 (or PTP1B) deficiency exhibit “selective” insulin sensitivity, patients with type 2 diabetes mellitus show the converse phenotype: selective hepatic insulin resistance, with the insulin effects on glucose homeostasis impeded while its lipogenic actions persist (1, 45). It will be important to explore whether these phenomena are mechanistically related. On the other hand, SREBP1c mRNA levels are controlled not only by insulin, but also by other factors, such as the liver X receptor (46) and saturated fatty acids (47). Conceivably, hepatic Shp2 deficiency might also affect one or more of these pathways.

Transgenic mice that express a presumptive dominant negative mutant of Shp2 to varying levels in liver, skeletal muscle, and adipose tissue exhibit insulin resistance, impaired insulin-stimulated glucose uptake, and decreased IRS1 phosphorylation and PI3K/Akt activation in skeletal muscle and liver (19). By contrast, our studies show clearly that Shp2 is a negative, not a positive regulator of hepatic insulin action and IR signaling. Conceivably, the overexpressed dominant negative mutant interfered with other PTPs and/or other Src homology 2

FIGURE 5. Enhanced Akt and attenuated ERK phosphorylation in LSHKO mice. A and B, total cell lysates were immunoblotted for p-Akt (A) and p-ERK (B) and the corresponding total proteins. C, IRS1 was immunoprecipitated, immunoblotted with phospho-specific antibodies for Ser612, and then reprobed with IRS1 antibodies. D and E, total cell lysates also were immunoblotted for pS6K1 (Thr389) (D) and pS6 (Ser235/Ser236) (E). All blots were scanned and quantified using FluorChem 9900 (A–E, right panels). Statistical analysis was performed using two-tailed Student’s t test (**, p ≤ 0.01; *, p ≤ 0.05).

FIGURE 6. Proposed model for regulation of insulin signaling by hepatic Shp2. Following insulin stimulation, activated IR phosphorylates IRS1 on multiple tyrosyl sites and leads to the recruitment and activation of signal relay molecules including PI3K and Shp2. Shp2 binds to the C-terminal tyrosyl residues of IRS1 and dephosphorylates p85 binding site(s), thus decreasing PI3K binding to IRS1 and attenuating downstream signaling, namely Akt.
Altered Glucose Homeostasis in Liver-specific Shp2 Knockouts

How Shp2 plays diverse roles in individual peripheral insulin-responsive tissues remains unclear. Conceivably, Shp2 has distinct substrates in different tissues. Alternatively, Shp2 may affect the same pathways in different tissues, but the effects of those pathways and/or the feedback regulatory pathways may differ in a tissue-specific manner. The latter explanation is consistent with our finding that Shp2 deficiency differentially affects ERK and Akt activation in the liver. In either case, these studies highlight the need to dissect the tissue-specific roles of PTPs methodically.

Our data provide new insights into how Shp2 regulates liver IR signaling (Fig. 6). LSHKO mice have IR tyrosyl phosphorylation similar to that of controls, indicating that the IR is not a substrate of Shp2 in the liver. These findings contrast with previous studies that reported direct association and dephosphorylation of IR and IGF1R by Shp2 (13, 48). Rather, our data strongly suggest that Shp2 regulates hepatic insulin signaling, at least in large part, by modulating IRS1/2 tyrosyl phosphorylation. Basal and insulin-stimulated phosphorylation of IRS1/2 is elevated in LSHKO mice. In addition, there is increased PI3K association with IRS1 in LSHKO mice. Moreover, insulin-induced IRS1 Ser307 phosphorylation is significantly elevated in LSHKO mice compared with controls. The precise role of Ser307 in regulating insulin sensitivity is not fully resolved. Knock-in mice (in which Ser307 is replaced with alanine) reveal that Ser307 is a positive regulatory site in mice and is required to maintain normal insulin signaling, particularly during nutrient stress (34). Although the mechanism underlying Ser307 phosphorylation in LSHKO mice remains to be determined, PI3K inhibition by LY294002 or wortmannin abolishes insulin-evoked IRS1/2 phosphorylation, PI3K association, and Akt activation. Also, although our data argue against a major effect of hepatic Shp2 deficiency on GH action, we do not exclude other, more indirect mechanisms, either ERK-dependent or independent, by which lack of Shp2 in liver results in increased insulin-evoked IRS1/2 phosphorylation, PI3K association, and Akt activation. Rather, our findings are more consistent with direct dephosphorylation of IRS1/2 by Shp2, as previously suggested by Myers et al. (51). Moreover, Zhang et al. (52) and Mattoon et al. (53) reported that Shp2 dephosphorylates the PI3K binding sites on Gab1 in response to EGF stimulation. However, we cannot exclude other, more indirect mechanisms, either ERK-dependent or independent, by which lack of Shp2 in liver results in increased insulin-evoked IRS1/2 phosphorylation, PI3K association, and Akt activation. Rather, our findings are more consistent with direct dephosphorylation of IRS1/2 by Shp2, as previously suggested by Myers et al. (51). Moreover, Zhang et al. (52) and Mattoon et al. (53) reported that Shp2 dephosphorylates the PI3K binding sites on Gab1 in response to EGF stimulation. However, we cannot exclude other, more indirect mechanisms, either ERK-dependent or independent, by which lack of Shp2 in liver results in increased insulin-evoked IRS1/2 phosphorylation, PI3K association, and Akt activation. Rather, our findings are more consistent with direct dephosphorylation of IRS1/2 by Shp2, as previously suggested by Myers et al. (51). Moreover, Zhang et al. (52) and Mattoon et al. (53) reported that Shp2 dephosphorylates the PI3K binding sites on Gab1 in response to EGF stimulation. However, we cannot exclude other, more indirect mechanisms, either ERK-dependent or independent, by which lack of Shp2 in liver results in increased insulin-evoked IRS1/2 phosphorylation, PI3K association, and Akt activation. Rather, our findings are more consistent with direct dephosphorylation of IRS1/2 by Shp2, as previously suggested by Myers et al. (51). Moreover, Zhang et al. (52) and Mattoon et al. (53) reported that Shp2 dephosphorylates the PI3K binding sites on Gab1 in response to EGF stimulation. However, we cannot exclude other, more indirect mechanisms, either ERK-dependent or independent, by which lack of Shp2 in liver results in increased insulin-evoked IRS1/2 phosphorylation, PI3K association, and Akt activation.

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