Enhanced ethanol catabolism in orphan nuclear receptor SHP-null mice

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Park JE, Lee M, Mifflin R, Lee YK. Enhanced ethanol catabolism in orphan nuclear receptor SHP-null mice. Am J Physiol Gastrointest Liver Physiol 310: G799–G807, 2016. First published March 11, 2016; doi:10.1152/ajpgi.00343.2015.—Deficiency of the orphan nuclear hormone receptor small heterodimer partner (SHP, NR0B2) protects mice from diet-induced hepatic steatosis, in part, by repression of peroxisome proliferator-activated receptor (PPAR)-γ2 (Pparg2) gene expression. Alcoholic fatty liver diseases (AFLD) share many common pathophysiological features with non-AFLD. To study the role of SHP and PPARγ2 in AFLD, we used a strategy of chronic ethanol feeding plus a single binge ethanol feeding to challenge wild-type (WT) and SHP-null (SHP−/−) mice with ethanol. The ethanol feeding induced liver fat accumulation and mRNA expression of hepatic Pparg2 in WT mice, which suggests that a high level of PPARγ2 is a common driving force for fat accumulation induced by ethanol or a high-fat diet. Interestingly, ethanol-fed SHP−/− mice displayed hepatic fat accumulation similar to that of ethanol-fed WT mice, even though their Pparg2 expression level remained lower. Mortality of SHP−/− mice after ethanol binge feeding was significantly reduced and their acetaldehyde dehydrogenase (Aldh2) mRNA level was higher than that of their WT counterparts. After an intoxicating dose of ethanol, SHP−/− mice exhibited faster blood ethanol clearance and earlier wake-up time than WT mice. Higher blood acetate, the end product of ethanol metabolism, and lower acetaldehyde levels were evident in the ethanol-challenged SHP−/− than WT mice. Ethanol-induced inflammatory responses and lipid peroxidation were also lower in SHP−/− mice. The current data show faster ethanol catabolism and extra fat storage through conversion of acetate to acetyl-CoA before its release into the circulation in this ethanol-feeding model in SHP−/− mice.

small heterodimer partner; alcoholic fatty liver disease; acetaldehyde dehydrogenase; inflammation

THE ORPHAN NUCLEAR HORMONE receptor small heterodimer partner (SHP, NR0B2) plays a role in lipid and glucose metabolism (31). Because it lacks a DNA-binding domain, SHP exerts its transcriptional repression activity through protein-protein interaction for these metabolic regulations (22, 23). Previous studies revealed that the loss of SHP protects mice from hepatic lipid accumulation induced by a Western diet (14, 31). The protection is mediated by derepression of peroxisome proliferator-activated receptor (PPAR)-α transactivation, which induces fatty acid oxidation through activation of genes such as carnitine palmitoyltransferase 1A (Cpt1a) and acetyl-CoA oxidase 1 (Acox1) (31), and inhibition of PPARγ2 (Pparg2) gene expression, which results in lower lipogenic and adipogenic programs (14). The Pparg gene gives rise to two mRNAs, Pparg1 and Pparg2, which are generated by alternate transcription start and splicing (7, 45). Even though PPARγ1 is expressed more broadly and abundantly throughout the body, the larger isoform PPARγ2 possesses more potent transcriptional activity and is believed to be a primary isoform responsible for adipogenesis in white adipocytes (33). In normal liver, expression of Pparg, especially Pparg2, is usually maintained at a very low level. However, livers from genetically altered obese subjects or those in whom obesity is induced by a high-fat diet showed significant induction of Pparg gene expression, which accounts for obvious lipid accumulation in these subjects (6, 9, 32). Studies from liver-specific overexpression or knockdown have clearly demonstrated critical roles of PPARγ in the development of hepatic steatosis (9, 26–28, 42, 43). Importantly, PPARγ2 appears to be responsible for hepatic fat accumulation. Our recent study critically tested a novel transcriptional cascade linking SHP to Pparg2 for the diet-induced hepatic fat accumulation. In the cascade, deletion of Shp upregulates expression of a novel repressor, hairy and enhancer of split 6 (Hes6), via derepression of retinoic acid receptor (RAR)-mediated transactivation, which in turn inhibits Pparg2 expression by repression of hepatocyte nuclear factor (HNF) 4α transcriptional activity. In the same scenario, treatment with all-trans retinoic acid (atRA), a ligand for the nuclear hormone receptor RAR alleviates fat accumulation in obese mouse models, at least in part, through the same transcriptional cascade (14).

Similar to high-fat diet feeding, hepatic fat accumulation has been manifested as a metabolic response to excessive ethanol consumption, which can lead to more serious alcoholic fatty liver diseases (AFLD) such as hepatitis, fibrosis, and cirrhosis. Among many underlying mechanisms suggested for AFLD pathogenesis, increased consumption of NAD+ by two major ethanol-catabolizing enzymes, alcoholic dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH), has been the most prominently discussed among the factors that induce fat accumulation (1, 29). Because these enzymes also participate in retinol oxidation, alcohol consumption also impacts retinol metabolism, thereby decreasing production of atRA, which acts as endogenous ligand for the nuclear hormone receptor RAR (3, 40). Therefore, the current study has assessed the impact of ethanol feeding on hepatic fat accumulation in SHP-null (SHP−/−) mice, in which the upregulated atRA signaling cascade due to SHP deletion inhibits Pparg2 mRNA expression, thereby inducing fat mobilization (14). Contrary to our expectation, we discovered that SHP deletion enhanced ethanol clearance and reduced ethanol-induced liver inflammation and peroxidation but failed to protect the liver from development of steatosis due to increased production of acetate, a precursor for fatty acid synthesis.

MATERIALS AND METHODS

Animals. Male mice were used unless otherwise mentioned. SHP−/− (10th generation against C57BL/6NHzd, Harlan) and wild-type (WT, C57BL/6NHzsd) mice were maintained in a temperature- and moisture-controlled facility with a 12:12-h light-dark cycle. The protocol for the chronic and binge ethanol-feeding experiment is...
described elsewhere (1). Briefly, the ethanol-fed group was provided with a 5% ethanol Lieber-DeCarli diet (Bio-Serve, Flemington, NJ) ad libitum for 10 days and gavaged with a bolus of 31.5% ethanol (5 g/kg body wt) at 7 AM on day 11. For pair-fed controls, age-matched mice were provided with a diet and gavaged with a solution containing isocaloric maltodextrin, instead of ethanol. The mice were euthanized at 4 PM for collection of blood and tissues. All animal care protocols were approved by the Institutional Animal Care and Use Committee of Northeast Ohio Medical University.

RNA isolation and quantitative real-time PCR. Total RNA was isolated from the liver using TRIzol solution (Life Technologies, Grand Island, NY). Synthesis of cDNA from the RNA was carried out using PrimeScript RT master mix (Clontech, Mountain View, CA) or iScript RT supermix (Bio-Rad Laboratories, Hercules, CA). Quantitative PCR (qPCR) was performed on the CFX96 Touch real-time detection system (Bio-Rad Laboratories) using iTaq Universal SYBR supermix (Bio-Rad Laboratories) to measure mRNA levels of the genes. Relative expression was calculated by the cycle threshold (ΔCt) method, with GAPDH used as an internal control. Information about primers used for the qPCR is available upon request, and most of the primer sequences were obtained from PrimerBank (http://pga.mgh.harvard.edu/primerbank).

Western blot analysis. Proteins were extracted from liver samples, and their concentrations were measured using the bicinchoninic acid kit (Thermo Scientific, Arlington Heights, IL). Ten micrograms of protein were loaded onto a 10% SDS-polyacrylamide gel, transferred to a polyvinylidene difluoride membrane, and blocked with 5% nonfat dry milk for 1 h at room temperature. The membrane was probed with rabbit polyclonal or monoclonal antibodies against ADH1 (1:1000 dilution), ALDH2 (1:2000 dilution), and rabbit polyclonal or monoclonal antibodies against ADH1 (1:100 dilution), ALDH2 (1:2000 dilution), and β-actin (1:1000 dilution; Novus Biologicals, Littleton, CO) and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:3000 dilution; Bio-Rad Laboratories). The bound antibodies were detected by enhanced chemiluminescence (Thermo Scientific), and the specific bands were quantified by densitometry analysis (ImageJ) and normalized by corresponding β-actin band intensity.

Plasma and tissue chemistry. Blood was drawn from the orbital plexus immediately after completion of the diet regimen. For blood gas analysis, blood samples were analyzed immediately after collection using an STAT hand-held blood analyzer with an EC8+ cassette (Abbott Laboratories, East Windsor, NJ). Plasma was prepared after centrifugation of whole blood at 12,000 × g for 10 min. Hepatic lipids were extracted using the method described by Folch et al. (8). Enzymatic kits were used to measure the levels of free fatty acids (BioVision, Milpitas, CA), triglyceride (Thermo Scientific), cholesterol (Thermo Scientific), ethanol (Sigma, St. Louis, MO), acetate (Sigma), acetaldehyde (Megazyme, Chicago, IL), alanine transaminase (ALT; Thermo Scientific), and aspartate transaminase (AST; Thermo Scientific) according to the manufacturers’ manuals. For hematoxylin-eosin staining, livers were fixed in 10% formalin overnight and processed for paraffin embedding and sectioning (7.5 μm thick).

Lipid peroxidation assay. Hepatic lipid peroxidation induced by alcohol was assessed using the thiobarbituric acid-reactive substances (TBARS) assay (TCA method) kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s protocol. TBARS values were normalized by liver weight.

Blood ethanol clearance test. To test ethanol tolerance, mice were injected with ethanol (3.5 g/kg ip) as previously described (5). Blood samples were obtained from tail veins at the indicated time points for determination of plasma ethanol concentration. A new group of naïve mice was used to measure wake-up time or ethanol-induced loss of righting response (LORR) after the ethanol challenge as mentioned previously (5).

Statistical analysis. Values are means ± SE unless otherwise specified. Student’s t-test was used to compare two different groups. P < 0.05 was considered significantly different.

RESULTS

Induction of hepatic Pparg2 transcript by ethanol diet. Many reports suggest that strong induction of PPARγ2 is a manifestation of fatty liver associated with pathophysiology such as obesity and diabetes (6, 21, 27, 42). A transcriptional cascade containing downstream PPARγ2 is proposed to be an important determinant for hepatic fat accumulation (14). To determine whether the transcriptional cascade also plays a role in AFLD, we challenged C57BL/6 mice with a Lieber-DeCarli ethanol diet and quantified hepatic mRNA levels of the genes in the cascade. Similar to Western diet feeding, ethanol feeding reduced Hes6 mRNA levels and induced Pparg2 and fat-specific protein (Fsp27) gene expression, suggesting that the proposed cascade also plays a role in alcohol-induced hepatic steatosis (Fig. 1).

SHP−/− mice are not protected from the development of alcoholic fatty liver. Deletion of Shp caused downregulation of Pparg2 and Fsp27 expression, which resulted in significant protection from fat accumulation upon Western diet feeding (14). Therefore, we tested whether SHP−/− mice can also be protected from hepatic steatosis induced by alcohol. C57BL/6 WT and SHP−/− mice were subjected to the chronic and binge ethanol-drinking regimen, and their hepatic fat profiles were compared with those of pair-fed controls. Although ethanol feeding significantly increased hepatic triacylglycerol (TG) level in both genotypes (Fig. 2A), there was little difference in TG and total lipid accumulation between WT and SHP−/− mice, which was also supported by hematoxylin-eosin staining (Fig. 2B). As expected, however, hepatic cholesterol accumulation was lower in SHP−/− animals due to upregulation of cytochrome P-450 7A1 (Cyp7a1) expression (data not shown) (31). To test whether the response of the proposed transcriptional cascade controlling hepatic fat mobilization (14) to ethanol in SHP−/− mice was different from the response to a Western diet, we performed a real-time qPCR analysis using hepatic RNA. Interestingly, Pparg2 and Fsp27 expression was lower and Hes6 expression was higher in SHP−/− mice, as observed in a Western diet regimen (Fig. 3A). Similar to the result observed in Fig. 1 and corroborated by a recent observation (41), this 10-day chronic binge-drinking regimen

![Fig. 1](http://ajpgi.physiology.org/)
weakly induced Fsp27 mRNA expression in WT mice and blunted Fsp27 mRNA expression in SHP−/− mice, but the difference between the two genotypes was not significant. In addition, mRNA levels of major hepatic fatty acid oxidation genes decreased after ethanol feeding in the WT mice but remained unchanged in the SHP−/− mice, thereby maintaining higher levels in SHP−/− than WT mice (Fig. 3B). Higher expression of these genes is a consequence of derepression by SHP deletion as observed in a Western diet regimen (31) and/or increased fat mobilization due to lower Fsp27 expression (27). Expression of the rate-limiting enzyme in fatty acid synthesis, stearoyl-CoA desaturase 1 (Scd1), was maintained at a lower level in SHP−/− mice independent of diet (Fig. 3C). These results indicate that although lipid metabolism in ethanol-challenged SHP−/− mice is programmed to reduce fat accumulation similar to the Western diet, other pathways drive additional fat accumulation from ethanol in SHP−/− mice. As shown in Table 2, no significant changes in levels of vital gases or ions were manifested in SHP−/− mice, except a significant decrease in blood glucose levels by ethanol feeding was observed in WT and SHP−/− mice. Even though the level of potassium was lower in SHP−/− mice, it was within the normal range (38). To explore ethanol clearance in SHP−/− mice in a condition precluding the effect of gastrointestinal absorption, WT and SHP−/− mice were injected intraperitoneally with an intoxicating dose of ethanol, and LORR (wake-up time) and blood ethanol concentrations were measured as described elsewhere (5). As expected, male and female SHP−/− mice exhibited significantly shorter LORR than WT mice (Fig. 4A), probably because of their greater ethanol clearance rate (Fig. 4B).

**Increased expression of ethanol metabolic genes in SHP−/− liver.** To gain insight into molecular mechanisms underlying the greater ethanol clearance rate in SHP−/− mice, we assessed mRNA expression of ethanol metabolic enzymes from livers of mice challenged with the chronic and binge ethanol-drinking regimen. Transcriptional expression of two major enzymes, ADH1 and ALDH2, was significantly higher in ethanol-fed SHP−/− mice than their WT counterparts (Fig. 5A). Protein levels were also assessed using Western blot analysis. In agreement with the transcriptional levels, protein levels were also significantly higher in ethanol-fed SHP−/− mice than their WT counterparts (Fig. 5B). In the chronic and binge-drinking regimen, however, the plasma level of two metabolites of these enzymes, acetaldehyde and acetate, at the time of tissue col-

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**Fig. 2.** Hepatic fat accumulation in SHP−/− mice challenged with chronic and binge ethanol feeding. Male WT and SHP−/− mice (n = 5–10) were placed on the chronic and binge ethanol-feeding or pair-feeding (control) regimen (see MATERIALS AND METHODS). After the dietary intervention, mice were euthanized for collection of livers. A: triacylglycerol (TG), cholesterol, and total lipid levels after normalization to liver weight. Values are means ± SE. #P < 0.05 vs. pair-fed. *P < 0.05 and ***P < 0.005 vs. WT fed the same diet. B: representative hematoxylin-eosin-stained liver sections. Magnification ×100; scale bars = 100 μm.
lecion was not significantly different between the two genotypes (data not shown). However, the acetate level became significantly higher and the acetaldehyde level became significantly lower in SHP−/− than WT mice 30–60 min after acute ethanol challenge (Fig. 5, C and D), suggesting that SHP deletion increases ethanol catabolism and end-product accumulation. It is likely that the majority of increased acetate in SHP−/− mice is quickly converted to acetyl-CoA and fatty acids, which offset the beneficial effect of SHP deletion on hepatic TG accumulation during the current Lieber-DeCarli diet regimen.

**Reduced inflammatory response and lipid peroxidation in SHP−/− mice.** Chronic and binge ethanol drinking is reported to induce hepatic inflammation and lipid peroxidation. Greater ethanol clearance in SHP−/− mice may provide protection from these ethanol-induced toxicities. Thus we isolated RNA from the livers of ethanol-fed WT and SHP−/− mice to assess mRNA expression of important inflammatory genes. Some of the major genes involved in inflammatory responses were significantly upregulated in WT mice upon ethanol feeding (Fig. 6A). The upregulation was mostly blunted in SHP−/− animals. In addition, we used a TBARS detection kit to analyze lipid peroxidation in livers of ethanol-fed animals. As shown in Fig. 6B, left, TBARS production was significantly attenuated by ethanol feeding in livers from SHP−/− mice. To assess the molecular mechanism of the decreased peroxidation in SHP−/− liver, mRNA expression of the genes involved in the redox mechanism was quantified (Fig. 6B, right). The antioxidant and oxidant genes, especially a NADPH oxidase gene (17), were significantly upregulated in livers from ethanol-fed WT, but not SHP−/−, animals. Even though serum ALT and AST levels appeared similar between the two genotypes (Fig. 6C), the results suggest that high serum ethanol in WT animals causes oxidative stress due to increased expression of NADPH oxidase, which induces the expression of antioxidant genes; in SHP−/− mice, however, expression of both the oxidant and antioxidant genes remained unchanged, probably because of lower serum ethanol and acetaldehyde concentration. Thus, faster ethanol clearance by SHP deletion may protect the mice from development of steatohepatitis induced by chronic alcohol exposure.

**DISCUSSION**

The orphan nuclear hormone receptor SHP is involved in regulation of many different metabolic processes, such as bile acid synthesis, fatty acid oxidation, and gluconeogenesis (10, 20, 24, 31). Among these, involvement in bile acid synthesis is the best-defined role of SHP. Interestingly, regulation by SHP in other metabolic pathways requires a metabolic challenge to ensure the transcriptional repression activity of SHP. Therefore, phenotypes of these metabolic dysregulations become evident only upon challenge with metabolic stresses. The current study demonstrates that SHP also plays a role in ethanol catabolism in a similar manner. Major genes regulating ethanol metabolism, such as Aldh1l1 and Aldh2, are upregulated in the liver of ethanol-fed SHP−/− mice compared with their WT counterparts. We, along with many others, reported that SHP inhibits transcriptional activities of other nuclear hormone receptors via direct interaction (10, 22–24, 34, 35). Because various nuclear hormone receptors are involved in the regulation of Aldh1 and Aldh2 (4, 19, 37), it can be speculated that

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dead</th>
<th>Mortality, %</th>
</tr>
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<tbody>
<tr>
<td>WT</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>SHP−/−</td>
<td>24</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 1. Mortality of WT and SHP−/− mice on the chronic and binge-drinking regimen

Data are from male mice. WT, wild-type; SHP, small heterodimer partner.
SHP directly regulates these genes through interaction with the nuclear receptors, especially HNF4α and retinoid X receptor, upon ethanol feeding (22). Faster ethanol metabolism in the SHP−/− animals has been manifested by quicker recovery and lower blood ethanol concentration at each time point following acute ethanol challenge. In the chronic and binge ethanol-drinking regimen, the mortality rate of SHP−/− mice was significantly reduced, indicating greater ethanol tolerance in the mutant mice. The high mortality rate of our C57BL/6N (Harlan) WT mice is noteworthy compared with the report of no mortality in an earlier study of C57BL/6N (National Cancer Institute) mice (13). This discrepancy might be due to differ-

Table 2. Gas analysis of blood samples from WT and SHP−/− mice fed ethanol or isocaloric (pair-fed) diet

<table>
<thead>
<tr>
<th></th>
<th>WT (n = 4)</th>
<th>SHP−/− (n = 6)</th>
<th>Ethanol-Fed Mice</th>
</tr>
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<tbody>
<tr>
<td>Na, mmol/l</td>
<td>152 ± 0</td>
<td>152.5 ± 1.1</td>
<td>153.8 ± 3.4</td>
</tr>
<tr>
<td>K, mmol/l</td>
<td>6.75 ± 0.54</td>
<td>6.52 ± 0.35</td>
<td>6.72 ± 0.84</td>
</tr>
<tr>
<td>Cl, mmol/l</td>
<td>118.75 ± 0.5</td>
<td>117 ± 3.0</td>
<td>117.6 ± 1.8</td>
</tr>
<tr>
<td>Total CO₂, mmol/l</td>
<td>22 ± 2.71</td>
<td>24 ± 3.97</td>
<td>26.4 ± 3.05</td>
</tr>
<tr>
<td>BUN, mg/dl</td>
<td>13.75 ± 0.96</td>
<td>17.83 ± 2.90</td>
<td>13.6 ± 4.83</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>184.5 ± 13.1</td>
<td>153 ± 32.3</td>
<td>90.6 ± 13.9***</td>
</tr>
<tr>
<td>Hct, %pcv</td>
<td>48.5 ± 1</td>
<td>47.17 ± 1.72</td>
<td>48.6 ± 1.14</td>
</tr>
<tr>
<td>pH</td>
<td>7.194 ± 0.04</td>
<td>7.279 ± 0.06†</td>
<td>7.255 ± 0.07</td>
</tr>
<tr>
<td>Pco₂, mmHg</td>
<td>51.8 ± 2.80</td>
<td>48.25 ± 4.01</td>
<td>55.54 ± 3.17</td>
</tr>
<tr>
<td>HCO₃, mmol/l</td>
<td>20.1 ± 2.65</td>
<td>22.7 ± 2.88</td>
<td>24.78 ± 2.93</td>
</tr>
<tr>
<td>BEecf, mmol/l</td>
<td>−8.25 ± 3.20</td>
<td>−4.33 ± 3.83</td>
<td>−2.2 ± 3.90</td>
</tr>
<tr>
<td>AnGap, mmol/l</td>
<td>18.67 ± 1.63</td>
<td>16.43 ± 0.59</td>
<td>19.5 ± 3.34</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>16.53 ± 0.35</td>
<td>16.03 ± 0.59</td>
<td>16.54 ± 0.39</td>
</tr>
</tbody>
</table>

Values are means ± SD. Blood was collected from male mice immediately after the chronic and binge ethanol-drinking regimen to obtain gas and ion contents. BUN, blood urea nitrogen; Hct, hematocrit; pcv, packed cell volume; AnGap, anion gap; BEecf, base excess in the extracellular compartment. *P < 0.05 and ***P < 0.001 vs. pair-fed. †P < 0.05 vs. WT fed the same diet.

SHP regulates ethanol-metabolizing genes

Fig. 4. Ethanol clearance in WT and SHP−/− mice challenged with ethanol (3.5 g/kg ip). A: wake-up time after ethanol challenge. *P < 0.05 vs. WT. B: plasma ethanol concentration in ethanol-challenged female (n = 5) and male (n = 8) mice. Values are means ± SE. *P < 0.05; **P < 0.01.
ences in age, substrain (Harlan vs. National Cancer Institute), housing environments, postgavaging care, and other factors.

In our earlier study, we reported that a transcriptional cascade consisting of SHP, HES6, HNF4α, PPARγ2, and FSP27 regulates diet-induced hepatic fat accumulation (14). To investigate the potential role of the transcriptional cascade in the development of alcoholic fatty liver, we launched a chronic and binge-drinking study with SHP−/− mice. Supporting this, a very recent study demonstrated that Fsp27 plays an important role in the development of alcoholic steatohepatitis (41). Interestingly, even though mRNA levels of Pparg2 and Fsp27, critical determinants for fat accumulation in the proposed cascade, were lower in the liver of ethanol-fed SHP−/− mice than their WT counterparts, hepatic fat accumulation was similar between the two genotypes. It also has been known that consumption of NAD⁺, a cofactor required for fatty acid oxidation, by ethanol-metabolizing enzymes contributes to fat accumulation. The data indicate that intrinsic repression activity of SHP controls fatty acid oxidation gene expression by ethanol feeding in the same way as Western diet feeding. We speculate, however, that increased ethanol metabolism depletes NAD⁺, which blocks fatty acid oxidation, and induces acetate.

Fig. 5. Enhanced ethanol catabolism in SHP−/− mice. A: expression of major alcohol metabolic enzymes (alcoholic dehydrogenase 1 (Adh1) and acetaldehyde dehydrogenase 2 (Aldh2)) in WT and SHP−/− mice on a chronic and binge-drinking regimen was assessed using real-time qPCR. B: protein levels were assessed using Western blotting, and densitometric intensities were normalized by β-actin level. Values are means ± SE. C: plasma acetaldehyde levels of WT and SHP−/− mice at 0–60 min after acute ethanol challenge (3.5 g/kg ip). D: plasma acetate levels at 30 min after acute ethanol challenge in WT and SHP−/− mice. *P < 0.05 and **P < 0.001 vs. WT. #P < 0.05 and ##P < 0.001 vs. pair-fed.
production, which provides acetyl-CoA for fatty acid synthesis, in the liver of SHP\(^{-/-}\) mice possibly by cytosolic acetyl-CoA synthetase (Acss2) (25, 36). Because of higher acetate production, we expected lower pH and higher levels of HCO\(_3^-\) and base excess in the extracellular compartment (BEecf) in the blood of ethanol-fed SHP\(^{-/-}\) animals. However, results from gas analysis showed no significant difference in these values between the two genotypes, indicating that activation of the acetate to acetyl-CoA is too rapid to change those blood parameters. This assumption is supported by the observation that the higher blood acetate level in the SHP\(^{-/-}\) mice was only evident within 30 min after ethanol challenge. Therefore, these ethanol-altered lipid-mobilizing programs result in additional fat accumulation, which offsets the intrinsic effects of increased fatty acid oxidation and mobilization by SHP deletion.

Increased ethanol clearance in SHP\(^{-/-}\) mice was additionally evidenced by lower inflammatory response and lower lipid peroxidation after chronic and binge drinking than in WT mice (2, 13, 17). These protected outcomes by SHP deletion may be a direct consequence of a lower acetaldehyde level, which is strongly supported by observations from Aldh2\(^{-/-}\) mouse studies (18, 30). Ethanol is also metabolized by cytochrome P-450 2E1 (Cyp2E1), which may be a critical component of oxidative stress (2). Because the role of Cyp2E1 in ethanol-induced oxidative stress and liver injury is controversial (16) and its expression in SHP\(^{-/-}\) mice was not significantly different from that in their WT counterparts for either diet (data not shown), involvement of Cyp2E1 in the current model has not been studied further. However, we cannot completely exclude the direct involvement of SHP in inflammatory signals, because associations between SHP and inflammation, even though contradictory, have been reported (12, 15, 39, 44). Interestingly, reduced inflammatory response and lipid peroxidation did not result in lower serum levels of ALT and AST, biomarkers for hepatotoxicity, in SHP\(^{-/-}\) mice. Either 10-day
ethanol feeding was not enough to induce significant liver damage or the assessment conducted with only surviving mice was not enough to create a significant difference. Nonetheless, our current study identifies a unique role of SHP in ethanol metabolism and provides therapeutic and preventative insights into the development of AFLD.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
J.E.P., M.L., and Y.K.L. developed the concept and designed the research; J.E.P., M.L., and Y.K.L. performed the experiments; J.E.P., M.L., and Y.K.L. analyzed the data; J.E.P., M.L., and Y.K.L. interpreted the results of the experiments; J.E.P. and Y.K.L. prepared the figures; J.E.P. drafted the manuscript; J.E.P., M.L., R.M., and Y.K.L. edited and revised the manuscript; J.E.P., M.L., R.M., and Y.K.L. approved the final version of the manuscript.

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