Abrogation of Hepatic ATP-Citrate Lyase Protects Against Fatty Liver and Ameliorates Hyperglycemia in Leptin Receptor-Deficient Mice

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Hepatic steatosis is a hallmark of nonalcoholic fatty liver disease (NAFLD) and a key component of obesity-associated metabolic dysfunctions featuring dyslipidemia, insulin resistance, and loss of glycemic control. It has yet to be completely understood how much dysregulated de novo lipogenesis contributes to the pathogenic development of hepatic steatosis and insulin resistance. ATP-citrate lyase (ACL) is a lipogenic enzyme that catalyzes the critical reaction linking cellular glucose catabolism and lipogenesis, converting cytosolic citrate to acetyl-coenzyme A (CoA). Acetyl-CoA is further converted to malonyl-CoA, the essential precursor for fatty acid biosynthesis. We investigated whether dysregulation of hepatic ACL is metabolically connected to hepatic steatosis, insulin resistance, and hyperglycemia. We found that in leptin receptor-deficient db/db mice, the expression of ACL was selectively elevated in the liver but not in the white adipose tissue. Liver-specific ACL abrogation via adenovirus-mediated RNA interference prominently reduced the hepatic contents of both acetyl-CoA and malonyl-CoA, markedly inhibited hepatic de novo lipogenesis, and protected against hepatic steatosis in db/db mice. Surprisingly, liver-specific ACL abrogation markedly inhibited the expression of peroxisome proliferator-activated receptor-gamma and the entire lipogenic program in the liver. Moreover, hepatic ACL deficiency resulted in significantly down-regulated expression of gluconeogenic genes in the liver as well as enhanced insulin sensitivity in the muscle, leading to substantially improved systemic glucose metabolism. Conclusion: These findings establish a crucial role of hepatic ACL in lipid and glucose metabolism; therefore, hepatic ACL may serve as a potential target to treat NAFLD and type 2 diabetes.
from obesity-induced impairments of the coordinate regulation of glucose and lipid homeostasis in the liver, hepatic steatosis occurs as a result of abnormally enhanced de novo lipid synthesis and fat delivery from excessive adipose tissue, as well as decreased fatty acid oxidation and lipid export. Fatty acid biosynthesis occurs in the cytosol through a series of regulated reactions catalyzed by lipogenic enzymes. ATP-citrate lyase (ACL) is a crucial lipogenic enzyme that regulates the flow of glucose carbons to cytosolic acetyl-coenzyme A (CoA) by catalyzing an ATP-consuming reaction to generate acetyl-CoA from citrate, thereby linking cellular glucose catabolism and de novo lipid synthesis. Acetyl-CoA is further converted to malonyl-CoA by acetyl-CoA carboxylase (ACC), the rate-limiting step in de novo fatty acid synthesis. Fatty acids are subsequently synthesized from malonyl-CoA by fatty acid synthase (FAS), long-chain elongase (ELOVL6), and steraryl-CoA desaturase 1 (SCD1); and formation of triglycerides is catalyzed by another series of enzymes, such as glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT). De novo lipogenesis is coordinately controlled in response to nutritional, hormonal, and metabolic stimuli, and is mediated by key transcriptional regulators, including the nuclear hormone receptors peroxisome proliferator-activated receptor (PPAR) γ and liver X receptor (LXR) α, sterol regulatory element-binding protein (SREBP)-1c, as well as carbohydrate-responsive element-binding protein.

Although multiple lines of evidence have indicated that dysregulated lipogenesis considerably contributes to NAFLD, the precise metabolic role of each individual lipogenic enzyme in the progression of hepatic steatosis and insulin resistance has not been fully appreciated until recently. Although ACL is absolutely essential in embryonic development and heterozygous knockout mice with ACL haplo-deficiency in a mixed genetic background exhibit normal lipid metabolism, it remains largely unclear whether ACL plays a critical role in obesity-related derangement of glucose and lipid metabolism, or to what degree dysregulation of hepatic ACL activity is pathogenically connected to NAFLD and loss of glycemic control.

Using adenovirus-mediated RNA interference strategy, we investigated the metabolic effects of targeted suppression of hepatic ACL upon liver steatosis and glucose metabolism in leptin receptor-deficient db/db mice that spontaneously develop fatty livers. We show that ACL, selectively dysregulated in the livers of db/db mice, plays a crucial role in glucose and lipid homeostasis and may serve as a promising therapeutic target for the treatment of NAFLD and diabetes.

**Materials and Methods**

Animal studies, including adenovirus administration and metabolic measurements, were conducted with all experimental procedures approved by the Institutional Animal Care and Use Committee of the Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Detailed materials and methods are described in the Supplementary Material. For statistical analysis, data are presented as the mean ± standard error of the mean (SEM) and differences were analyzed by unpaired two-tailed t test between two groups. For glucose tolerance test, comparison was done with two-way analysis of variance.

**Results**

**ACL Is Selectively Dysregulated in the Livers of db/db Mice**

Leptin is an adipocyte-derived hormone that exerts a broad spectrum of physiological functions in lipid and glucose metabolism, and genetic deletion of its long form receptor results in morbid obesity, fatty liver, and type 2 diabetes. To investigate whether ACL is connected to the derangement of lipid metabolism in the absence of leptin actions, we first assessed the expression levels of ACL in both the white adipose tissue and livers of C57BL/6j db/db mice fed a regular chow diet. In comparison with wild-type (WT) littersmates, quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) and western immunoblotting analyses showed nearly five-fold and 2.7-fold elevations, respectively, in the messenger RNA (mRNA) and protein expression levels of ACL in the livers of db/db mice (Fig. 1A,B). In contrast, comparable ACL expression levels were detected in the white adipose tissue. Consistently, the ACL enzyme activity was also approximately three-fold higher in the livers of db/db mice than in WT control mice (Fig. 1C). In parallel, as compared with WT mice, more than three-
fold and two-fold increases were observed, respectively, in the mRNA and protein expression levels of the lipogenic regulator PPARγ in the livers but not in the white adipose tissue of db/db mice (Supplementary Fig. 1). Thus, ACL expression is dysregulated in a liver-selective manner, which is closely accompanied by the abnormally elevated hepatic expression of PPARγ in db/db mice. These results suggest that loss of lipogenic control occurs in the liver as a result of defective leptin actions, presumably contributing to the development of fatty liver.

**ACL Is Crucial for the Hepatic Production of Acetyl-CoA and Malonyl-CoA as Well as De Novo Lipogenesis in db/db Mice**

The observation that the expression of ACL was prominently elevated in the livers of db/db mice prompted us to hypothesize that hepatic ACL mediates leptin’s crucial metabolic actions in lipid and glucose homeostasis. To investigate the metabolic effects of targeted suppression of hepatic ACL, we took an RNA interference approach using the recombinant adenovirus to deliver the small hairpin (sh) RNA directed against the coding region of ACL (Ad-shACL). C57BL/6J db/db mice were infected with Ad-shACL or Ad-shLacZ adenoviruses via tail vein injection, and sacrificed 15 days after infection. Ad-shACL but not Ad-shLacZ adenoviruses specifically and almost completely abolished ACL expression in the liver (Fig. 2A). In parallel, ACL enzyme activities were also reduced by approximately 95% in the livers of Ad-shACL–infected mice (Fig. 2B). Importantly, evaluation of alanine aminotransferase or aspartate aminotransferase levels in the serum indicated that Ad-shACL did not cause apparent functional damage or inflammation to the livers of db/db mice as compared with Ad-shLacZ (Table 1).

Because ACL catalyzes the conversion of cytosolic citrate to acetyl-CoA that is further converted by ACC to malonyl-CoA, the hepatic contents of both acetyl-CoA and malonyl-CoA were measured via LC-tandem mass spectrometric analysis (LC-MS-MS). Hepatic ACL abrogation resulted in a >50% reduction in the hepatic level of acetyl-CoA (Fig. 2C) and, notably, a >70% decrease in that of malonyl-CoA (Fig. 2D) in db/db mice. To examine the effect of ACL knockdown on lipogenesis, we determined the rate of hepatic fatty acid synthesis by measuring the incorporation of [3H]water into hepatic fatty acids. Ad-shACL–mediated knockdown of hepatic ACL decreased hepatic lipogenesis by approximately 78% (Fig. 2E). Hence, these results demonstrate that hepatic ACL is crucial for the production of both acetyl-CoA and malonyl-CoA as well as de novo lipogenesis in the liver. Moreover, given the regulatory role of malonyl-CoA as the metabolic intermediate between lipogenesis and fatty acid β-oxidation, the reduction in hepatic malonyl-CoA resulting from ACL deficiency may directly elicit significant metabolic alterations in hepatic lipid metabolism.

**Ablation of Hepatic ACL Prevents Hepatic Steatosis and Reduces Circulating Levels of Free Fatty Acids in db/db Mice**

We next tested whether ACL is connected to obesity-induced development of hepatic steatosis in association with defective leptin actions. In C57BL/6J db/db mice that have dramatically enlarged livers, Ad-shACL–mediated hepatic ACL ablation markedly alleviated this hepatomegaly, as shown by a significant decrease in the liver weight (by ~25%) as compared with Ad-shLacZ–infected control mice (Fig. 3A). Histological examination of liver sections revealed that hepatic ACL abrogation markedly reduced liver steatosis in db/db mice, because greatly diminished amounts of lipid droplets were observed in the livers of mice infected by Ad-shACL (Fig. 3B) compared with control mice. Consistently, hepatic ACL ablation dramatically decreased (by ~50%) the TG contents (Fig. 3C) while slightly increasing the cholesterol levels in the liver. Moreover, abrogation of hepatic ACL resulted in a dramatic reduction (by ~50%) in the serum levels of free
Entire Lipogenic Program in the Livers of \textit{db/db} Mice

To investigate the mechanisms whereby hepatic ACL abrogation led to protection against hepatic steatosis, we examined the impact of ACL deficiency upon the gene expression programs of hepatic lipid metabolism, namely the expression of key lipogenic regulators and metabolic enzymes for the biosynthesis of fatty acids and triglycerides, as well as those involved in fatty acid oxidation.

In C57BL/6J \textit{db/db} mice infected with Ad-shACL for 15 days and sacrificed under \textit{ad libitum} feeding conditions, quantitative RT-PCR analysis revealed an approximately 56% reduction in the mRNA expression level of PPAR\(\gamma\) but little changes in that of SREBP1c or LXR\(\alpha\) (Fig. 4A). Interestingly, hepatic ACL abrogation resulted in considerable increases in the expression of liver X receptor \(\beta\) (LXR\(\beta\)) (by \(\approx 86\%\)) and modest elevations in the expression of SREBP2 (by \(\approx 26\%\)) (Fig. 4A). This may explain the marginal elevation in hepatic cholesterol levels observed (Fig. 3C), given the role of LXR\(\beta\) and SREBP2 in cholesterol homeostasis.\(\textsuperscript{30,31}\) In the fasted state, however, as analyzed in C57BLKS/J \textit{db/db} mice infected by Ad-shACL for 23 days during the subsequent study of glucose homeostasis, ACL deficiency led to similarly repressed levels of PPAR\(\gamma\) but significant reductions in both SREBP1c (by \(\approx 56\%\)) and LXR\(\alpha\) (by \(\approx 70\%\)) (Supplementary Fig. 2A). On the other hand, no significant changes were detected in the expression of two other lipogenic regulators, PPAR\(\gamma\) coactivator 1\(\beta\) (PGC-1\(\beta\))\(\textsuperscript{32}\) and carbohydrate-responsive element-binding protein, either under fed or fasted conditions (Fig. 4A; Supplementary Fig. 2A). In parallel with ACL deficiency–induced PPAR\(\gamma\) suppression both in the fed and fasted states, the mRNA expression levels were prominently reduced for FAS (by \(\approx 67\%\) and \(\approx 77\%\), respectively), ELOVL6 (by \(\approx 70\%\) and \(\approx 60\%\), respectively), GPAT (by \(\approx 62\%\) and \(\approx 42\%\), respectively) and DGAT2 (by \(\approx 35\%\) and \(\approx 51\%\), respectively), suggesting a predominant role for PPAR\(\gamma\) in regulation of this subset of lipogenic enzymes (Fig. 4B and Supplementary Fig. 2B). Notably, corresponding to the decreased expression of LXR\(\alpha\) and SREBP1c in the fasted state, more dramatic suppression was observed for SCD1 (by \(\approx 57\%\)) and DGAT1 (by \(\approx 50\%\)) (Supplementary Fig. 2B), indicating the involvement of the LXR\(\alpha\)-SREBP1c pathway\(\textsuperscript{33}\) in control of their expression.

In the fed state, hepatic ACL deficiency also led to dramatically reduced expression (by \(\approx 68\%\)) of PPAR\(\alpha\), fatty acids (FFA) but did not alter those of TGs and cholesterol (Fig. 3D). These data thus reveal a crucial link between dysregulated ACL and hepatic steatosis in \textit{db/db} mice, suggesting that targeted suppression of hepatic ACL can effectively prevent the occurrence of obesity-associated fatty liver.

\textbf{Deficiency in ACL Results in Suppression of the Entire Lipogenic Program in the Livers of \textit{db/db} Mice}

Fig. 2. Hepatic ACL knockdown reduces the hepatic levels of acetyl-CoA and malonyl-CoA as well as hepatic lipogenesis in \textit{db/db} mice. (A-D) Male C57BL/6J \textit{db/db} mice at 8 weeks of age were infected with control adeno-virus Ad-shLacZ or targeting adeno-virus Ad-shACL via tail-vein injection. At 15 days postinfection, animals were sacrificed in the fed state. (A) Western immunoblot analysis of ACL protein levels from livers and white adipose tissue. Tubulin expression levels were used as the loading control, as the mean \(\pm\) SEM. **\(<\) 0.01 versus Ad-shLacZ-infected control mice. **

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the key regulator of β-oxidation,\textsuperscript{34} in the liver of \textit{db/db} mice (Fig. 4C). In parallel, the PPARγ target genes, long-chain acyl-CoA dehydrogenase and medium-chain acyl-CoA dehydrogenase, which catalyze the initial steps in mitochondrial fatty acid oxidation, displayed significantly decreased expression levels (by \(\approx 31\%\) and \(\approx 36\%\), respectively). Notably, acyl-CoA oxidase (ACOX) 1, another PPARα target gene for peroxisomal β-oxidation of fatty acids, was also markedly suppressed (by \(\approx 72\%\)) as a result of hepatic ACL deficiency (Fig. 4C). On the other hand, few changes were detected in the expression of genes related to fatty acid transport, fatty acid transport protein (FATP) 1, CD36, and carnitine palmitoyltransferase (CPT) 1a. Similar hepatic ACL deficiency-induced alterations were also observed in the expression patterns of these genes in the fasted state (Supplementary Fig. 2C).

To confirm that the lipogenic program was blocked as a result of hepatic ACL abrogation, we analyzed the expression levels by western immunoblotting of PPARγ, FAS, and ACC. In fed C57BL/6J \textit{db/db} mice (Fig. 5A), hepatic ACL abrogation markedly diminished (by \(\approx 90\%\)) the protein expression levels of PPARγ and significantly reduced those of FAS (by \(\approx 69\%\)) and ACC1/2 (by \(\approx 68\%\)) (Fig. 5B). Of note, no alterations in the phosphorylation status of ACC1/2 were detected. Conceivably, the decreased ACC1/2 expression, rather than its phosphorylation inactivation, was likely responsible for the more pronounced reduction in the level of malonyl-CoA than acetyl-CoA resulting from ACL abrogation (Fig. 2C,D).

Taken together, these results demonstrate that hepatic ACL abrogation not only resulted in marked decreases in the production of precursor molecules for lipid synthesis, but also triggered metabolic alterations that blunted the entire lipogenic program in the livers of \textit{db/db} mice, leading to efficient inhibition of hepatic \textit{de novo} lipogenesis. The suppressed expression of hepatic lipogenic genes was likely mediated, at least in part, through normalization of the elevated PPARγ expression levels in obese animals. On the other hand, given the observed decreases in the expression of genes related to fatty acid oxidation, lipid disposal through β-oxidation might play a lesser role in

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<th>Table 1. Physiologic Data and Serum Measurement of \textit{db/db} Mice Infected by Ad-shLacZ and Ad-shACL</th>
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All data are from 8-week-old male C57BL/6J (n = 7 per group) or C57BLKS/J \textit{db/db} mice (n = 6-7 per group) infected for 15 or 23 days by Ad-shLacZ versus Ad-shLacZ, except that the levels of alanine transaminase and aspartate transaminase were determined at 8 (for C57BL/6J mice) or 10 (for C57BLKS/J mice) days postinfection. Data are expressed as the mean ± SEM.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; EF, epididymal fat pad; β-HB, β-hydroxybutyrate; ND, not determined.

*\(P < 0.05\), †\(P < 0.01\) versus control mice infected by Ad-shLacZ.

Fig. 3. Hepatic ACL abrogation protects against liver steatosis in \textit{db/db} mice. Male C57BL/6J \textit{db/db} mice at 8 weeks of age were infected with Ad-shLacZ versus Ad-shACL. Measurements were performed at 15 days postinfection. (A) Liver weight. (B) Representative images of hematoxylin-eosin staining of liver sections (n = 5/group) (magnification \(\times 10\)). The bar indicates 1 mm. (C) TG and cholesterol (CHO) levels in liver extracts. (D) Serum levels of TGs, cholesterol, and FFAs. Data are expressed as the mean ± SEM (n = 7-8/group). *\(P < 0.05\) and **\(P < 0.01\) versus Ad-shLacZ-infected control mice.
the attenuation of hepatic steatosis as a result of hepatic ACL ablation. Consistent with this idea, knockdown of hepatic ACL did not increase serum levels of ketone bodies (Table 1).

**Deficiency in Hepatic ACL Improves Systemic Glucose Metabolism and Insulin Sensitivity in** db/db **Mice**

Because hepatic steatosis and circulating FFA elevations are closely associated with obesity, insulin resistance and hyperglycemia, we next investigated the impact of hepatic ACL ablation upon adiposity, energy balance, and glucose metabolism in db/db mice. C57BL/6J db/db mice infected with Ad-shLacZ for 15 days exhibited slightly reduced body weight and fat mass without changes in food intake, but showed dramatically decreased serum glucose levels in the fasted state (Table 1). However, no appreciable changes in metabolic rate, respiratory exchange ratio, or physical activities were detected (Supplementary Fig. 3). Infection of C57BLKS/J db/db mice, which manifest more severe insulin resistance and hyperglycemia phenotypes,35 by Ad-shACL for up to 23 days led to few changes in body weight and fat content (Table 1) despite similar degrees of knock-down of hepatic ACL expression (data not shown). Therefore, hepatic ACL abrogation exerted little action upon energy homeostasis and adiposity, and the observed slight reduction in the body weight of C57BL/6J db/db mice might similarly reflect the nonspecific effects of the adenoviral infection as described.36 As in C57BL/6J db/db animals, Ad-shACL–mediated hepatic ACL abrogation in C57BLKS/J db/db mice markedly reduced the fasted glucose levels, by ~38%, ~58%, and ~58%, respectively, at 8, 12, and 16 days postinfection compared with control mice infected by Ad-shLacZ (Fig. 6A). Furthermore, glucose tolerance test showed dramatically improved glucose tolerance in Ad-shACL–infected mice at 16 days (Fig. 6B). To exclude the possibility of potential “off-target” effects exerted by Ad-shACL, a second adenovirus (Ad-shACL#) with similar ACL knock-down efficiency was also tested. Consistently, marked amelioration of glucose tolerance and hyperglycemia in db/db mice were observed, along with similarly attenuated liver steatosis and repressed expression profiles of lipogenic genes (data not shown). Thus, these results demonstrate that targeted suppression of
ACL, the lipogenic enzyme that initiates lipid synthesis in the liver, is able to correct hyperglycemia associated with obesity and fatty liver.

To further examine the physiological mechanisms by which deficiency in hepatic ACL improves glucose metabolism in \( \text{db/db} \) mice, we first examined by quantitative RT-PCR the expression levels of key gluconeogenic genes in the liver. Under fasted conditions, \( \text{C57BLKS/J db/db} \) mice at 23 days postinfection by Ad-shACL displayed significant reductions in the mRNA levels of PGC-1\( \alpha \) (by \( \approx 50\% \)), the chief gluconeogenic regulator,\(^{37} \) and its two target genes, phosphoenolpyruvate carboxykinase (by \( \approx 50\% \)) and glucose-6-phosphatase (by \( \approx 75\% \)) (Fig. 7A). Therefore, ACL deficiency–induced repression of the gluconeogenic pathway could contribute to the observed normalization of hyperglycemia in \( \text{db/db} \) mice.

To determine whether hepatic ACL cell-autonomously regulates insulin signaling, insulin-stimulated phosphorylation of Akt and glycogen synthase kinase-3\( \beta \) (GSK-3\( \beta \)) was measured in the liver. When compared with Ad-shLacZ–infected control mice, no apparent increases were observed in the phosphorylation of hepatic Akt or GSK-3\( \beta \) in \( \text{C57BLKS/J db/db} \) mice infected with Ad-shACL for either 17 days (Fig. 7B) or 23 days (data not shown). Consistently, in the primary hepatocytes isolated from \( \text{C57BL/6J db/db} \) mice, ACL knockdown prominently reduced the expression of FAS and ACC but did not significantly alter the insulin-stimulated phosphorylation of Akt and GSK-3\( \beta \) (Fig. 7C). Notably, ACL abrogation both in the liver and in primary hepatocytes led to increased basal levels of GSK-3\( \beta \) phosphorylation (Fig. 7B,C). In contrast, hepatic ACL abrogation led to apparent improvement of insulin resistance in the skeletal muscles. Insulin stimulated phosphorylation of both Akt and GSK-3\( \beta \) in soleus muscles to a greater extent in Ad-shACL–infected than in Ad-shLacZ–infected \( \text{db/db} \) mice (Fig. 7D; Supplementary Fig. 4). These data demonstrate that amelioration of hepatic steatosis and a reduction of serum FFA levels as a result of hepatic ACL abrogation can improve muscular insulin sensitivity.

**Discussion**

In the current study, we have established an important role for hepatic ACL, which is dysregulated in an obese and diabetic mouse model with spontaneous progression of liver steatosis, in lipid and glucose metabolism. Our findings illustrate a close pathological link between elevated expression of hepatic ACL and derangement of lipid metabolism in the liver under defective leptin signaling conditions, and further substantiate the contribution of \( \text{de novo} \) lipogenesis to the development of obesity-associated NAFLD and loss of glycemic control.

Our results demonstrate that targeted suppression of hepatic ACL could effectively prevent liver steatosis in \( \text{db/db} \) mice. The restored lipid homeostasis in the liver most likely arises from the suppression of the entire lipogenic program, which is abnormally enhanced in the livers of \( \text{db/db} \) mice. First, deficiency in hepatic ACL significantly reduced the biosynthesis of malonyl-CoA, the essential precursor for fatty acid and TG synthesis. Second, hepatic ACL abrogation prominently normalized the expression level of PPAR\( \gamma \), which was dramatically elevated in \( \text{db/db} \) animals. This in turn is likely responsible for the observed coordinate repression of a broad set of key enzymes related to fatty acid and TG biosynthesis, including ACC1, FAS, ELOVL6, SCD1, GPAT, and DGAT1/2.\(^{38} \) Consequently, hepatic ACL abrogation markedly inhibited \( \text{de novo} \) fatty acid synthesis in the liver. Consistent with our findings, pharmacological inhibition of ACL results in hypolipidemia in rats.\(^{39} \)
Whereas the resultant decrease in the level of malonyl-CoA, a physiological inhibitor of CPT1,29,40 may lead to increased fatty acid oxidation and hepatic lipid disposal, deficiency in hepatic ACL also led to marked suppression of the hepatic expression of PPARα as well as its target genes long-chain acyl-coenzyme A dehydrogenase, medium-chain acyl-coenzyme A dehydrogenase, and acyl-coenzyme A oxidase 1. Therefore, we suspect that ACL deficiency–elicited repression of lipid synthesis, not increased fatty acid oxidation, most likely played a major role in the prevention of fatty liver in the obese mice. This is in line with the fact that no significant changes in the serum levels of β-hydroxybutyrate were detected in the hepatic ACL-deficient db/db mice (Table 1).

Hepatic steatosis is known to be closely associated with insulin resistance and hyperglycemia.11,41 We found that targeted suppression of hepatic ACL led to marked amelioration of hyperglycemia and improvement of insulin resistance. First, the improved glucose metabolism can be mediated, at least in large part, by the correction of liver steatosis, which may in turn lead to suppression of the gluconeogenic pathway. While the detailed molecular mechanisms responsible for the observed down-regulation of gluconeogenic genes remain to be explored, the decreased expression of PPARα may also contribute to the amelioration of hyperglycemia in hepatic ACL-deficient db/db mice, because PPARα is thought to regulate the gluconeogenic program.42,43 Second, the marked beneficial effects of hepatic ACL abrogation upon glycemic control may arise from the resultant repression of key enzymes in lipid biosynthesis, such as ACC1, SCD1, and ELOVL6. Recently reported studies have revealed that deficiency in these enzymes in the liver can exert profound but distinct impact upon adiposity, hepatic steatosis, gluconeogenesis, and insulin resistance in animal models, presumably via altering the hepatic fatty acid compositions.23,25,26 Third, hepatic ACL deficiency-elicited improvement of insulin signaling in the muscle reflects the
broad metabolic alterations that may involve cross-talks between different tissues, leading to alleviation of insulin resistance at the whole-body level. We speculate that the observed improvement in systemic glucose metabolism in the hepatic ACL-deficient mice, paralleled by the prominent decreases in the levels of hepatic malonyl-CoA and serum FFAs, is metabolically analogous to that reported for the rodent model through adenoviral overexpression of an active form of malonyl-CoA decarboxylase in the liver. When challenged with a high-fat diet, malonyl-CoA decarboxylase–induced hepatic malonyl-CoA reductions in these diet-induced obese rats lead to improved muscular insulin sensitivity, likely through local changes in the muscular fatty acid metabolite compositions in association with decreased plasma FFAs, which are known to induce insulin resistance in muscles. Because db/db mice are known to have elevated expression levels of PPARα and PPARγ, it is of particular note that hepatic ACL abrogation could lead to normalization of the expression of both nuclear receptors through mechanisms that are currently not clear. This may in turn serve to amplify the metabolic effects of ACL suppression, bringing about dramatic changes in the lipogenic pathway and, consequently, hepatic fatty acid compositions.

Our results demonstrate that targeted suppression of ACL in the liver, a critical enzyme that regulates the initial step in lipid biosynthesis and is dysregulated in the obese state, not only leads to protection against liver steatosis, but also to marked improvement in whole-body glucose metabolism. Because impaired leptin signaling (“leptin resistance”) is closely associated with human obesity, fatty liver, and diabetes, these findings provide direct physiological evidence that hepatic ACL represents an attractive therapeutic target for the treatment of both NAFLD and type 2 diabetes.

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References


