

Adiponectin deficiency impairs liver regeneration through attenuating STAT3 phosphorylation in mice

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Liver regeneration is a very complex and well-orchestrated process associated with signaling cascades involving cytokines, growth factors, and metabolic pathways. Adiponectin is an adipocytokine secreted by mature adipocytes, and its receptors are widely distributed in many tissues, including the liver. Adiponectin has direct actions in the liver with prominent roles to improve hepatic insulin sensitivity, increase fatty acid oxidation, and decrease inflammation. To test the hypothesis that adiponectin is required for normal progress of liver regeneration, 2/3 partial hepatectomy (PH) was performed on wild-type and adiponectin-null mice. Compared to wild-type mice, adiponectin-null mice displayed decreased liver mass regrowth, impeded hepatocyte proliferation, and increased hepatic lipid accumulation. Gene expression analysis revealed that adiponectin regulated the gene transcription related to lipid metabolism. Furthermore, the suppressed hepatocyte proliferation was accompanied with reduced signal transducer and activator of transcription protein 3 (STAT3) activity and enhanced suppressor of cytokine signaling 3 (Socs3) transcription. In conclusion, adiponectin-null mice exhibit impaired liver regeneration and increased hepatic steatosis. Increased expression of Socs3 and subsequently reduced activation of STAT3 in adiponectin-null mice may contribute to the alteration of the liver regeneration capability and hepatic lipid metabolism after PH.

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The liver has a central role in metabolic homeostasis, as it is responsible for the metabolism, synthesis, storage, and redistribution of nutrients, carbohydrates, fats, and vitamins. Paradoxically, it is also the main detoxifying organ of the body, which is frequently challenged by chemical, traumatic, or infectious injuries. Consequently, the liver has evolved a unique ability to regenerate in response to liver mass loss because of injuries.^{1,2} Liver regeneration, which is driven by the replication of existing hepatocytes, is a process of compensatory hyperplasia rather than a differentiation process of stem cells.³

One of the most effective models for studying liver regeneration after hepatocellular loss is partial hepatectomy (PH) in rodents. This technique, which was first described by Higgins and Anderson and performed in rats,⁴ can be modified to be safely and reproducibly performed in mice.⁵

After PH, resection of about 2/3 of liver mass results in quiescent hepatocytes rapidly re-entering the cell cycle. This highly regulated process is primed by different cytokines and growth factors that activate the downstream kinases and transcription factors. As a result, the hepatocytes initiate the transcription of more than 100 early genes, accumulate triglyceride and cholesterol to supply the energy and materials required for restore the liver mass. After one or two rounds of replication of hepatocytes, the original liver mass is restored within 5–7 days. Thus, liver regeneration constitutes a unique model to study signal transduction, lipid metabolism, and cell cycle events in a synchronized manner *in vivo*.^{2,3}

Adipocytokines are soluble mediators mainly produced by adipocytes. They influence energy homeostasis and regulate neuroendocrine function as hormones; in addition, they affect immune functions and inflammatory processes as cyto-

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kines. Adiponectin (also known as APN, Acrp30, and GBP28) and leptin are the two most abundant adipocytokines produced by adipocytes. Adiponectin is decreased with obesity and is involved in many obesity-related metabolic disorders, such as insulin resistance, atherosclerosis, and fatty liver disease.^{6,7} Adiponectin interacts with at least two known cellular receptors (AdipoR1 and AdipoR2), which are both expressed in the liver. Binding of adiponectin to AdipoR1 and/or AdipoR2 stimulates the activation of peroxisome proliferator-activated receptor- α (PPAR α) and AMP-activated protein kinase (AMPK).^{8,9} Disruption of PPAR α -mediated lipid signaling pathway delays the initiation of liver regeneration.¹⁰ Activation of AMPK stimulates β -oxidation to influence the lipid metabolism in hepatocytes.¹¹ The first discovered adipocytokine, leptin, has been proved to be crucial during liver regeneration,^{12,13} but whether adiponectin is required during liver regeneration is still unknown. In addition, many evidences have proved that adiponectin has protective effects on many experimentally induced liver diseases.^{14–16} Taken together, we hypothesized that adiponectin plays a role in liver regeneration. To test this hypothesis, we studied the regeneration process triggered by PH in the liver of adiponectin knockout mice. Our results showed that adiponectin plays an essential role during liver regeneration.

MATERIALS AND METHODS

Animal Studies

All the following studies were approved by the Animal Use and Care Committee of Shanghai Jiao Tong University School of Medicine. Adiponectin knockout and wild-type mice maintained in a 129S1 background were kept on 12-h dark–light cycles and maintained on standard mouse chow and water before and after surgery.¹⁷ PH was performed on 8–12-weeks-old male mice as described earlier.⁵ Briefly, animals 8–12 weeks of age were anesthetized by pentobarbital. The three most anterior liver lobes (right upper, left upper, and left lower lobes), totaling about 68% of the liver, were tied at the origins of the lobes with three knots and then resected. The peritoneum was re-approximated with a running suture and then the skin was closed. Sterile saline (1 ml) was administered subcutaneously on the back after closing the abdomen to replace fluid loss from the surgery. Mice were placed under warming lights for at least 1 h after surgery. All the surgeries were performed between 9:00 am and 12:00 pm by one person. At least four animals in each cohort were killed at each time point analyzed. For determination of total hepatocyte proliferation, BrdU (Sigma, St. Louis, MO, USA) was continuously given in drinking water to the mice after surgery. In another set of experiments, BrdU was injected intraperitoneally 1 h before killing as described earlier.¹⁸ At the time of killing, mice were anesthetized, and the remaining liver lobes were removed and weighed. For protein and RNA analysis, liver lobes were frozen in liquid nitrogen, and stored at -80°C until use. For histological analysis, liver lobes were

fixed in 10% formalin then embedded in paraffin, or soaked in 30% sucrose (in PBS) at 4°C overnight then frozen in OCT Compound (Sakura Finetek, Torrance, CA, USA).

Cell Culture

Human hepatoma, HepG2, cells were cultured in DMEM supplemented with 10% FBS (Invitrogen, Carlsbad, CA, USA). HepG2 cells were serum starved overnight, then treated with or without recombinant human adiponectin (A kind gift from Dr Yan Chen, $10\ \mu\text{g}/\text{ml}$) and leukemia inhibitory factor (LIF) (Chemicon, Temecula, CA, USA, $0.1\ \mu\text{g}/\text{ml}$) for 1 h. Total RNA was isolated using the TRIzol reagent (Invitrogen).

Liver Histology and Immunohistochemical Stainings

Liver morphology was assessed based on 5- μm hematoxylin and eosin-stained paraffin sections. To assess the number of hepatocyte mitoses, 30 high-power microscope fields ($\times 400$) from different liver sections for each sample were counted; counts were expressed as mitotic figures/10 high-power fields. Hepatocyte nuclear staining for BrdU was performed with primary BrdU antibody (sc-56258, Santa-Cruz Biotechnology, Santa Cruz, CA, USA) using the commercial kits (Santa-Cruz Biotechnology). Total BrdU-labeled hepatocytes were determined by counting positively stained hepatocyte nuclei in 10 high-power microscope fields ($\times 400$) per liver. The cell proliferation index was calculated by dividing the number of labeled hepatocyte nuclei by the total number of hepatocyte nuclei counted, and the results were expressed as a percentage. Sections of liver frozen in OCT compound (Sakura Finetek) were stained with Oil Red O (Amresco, Solon, OH, USA) for analysis of hepatic fat accumulation.

Liver Lipid Content

Liver triglyceride and cholesterol were extracted as described earlier.^{19,20} Triglyceride and cholesterol levels were then measured using the Beckman-Coulter Synchron Dx800 chemistry analyzers (Fullerton, CA, USA) according to the manufacturer's instruction.

Quantitative Real-Time Reverse Transcription PCR

Differential gene expression was detected by quantitative real-time reverse transcription PCR. Total RNA was isolated from frozen liver tissue using the TRIzol reagent according to the manufacturer's protocol (Invitrogen). DNase I-treated (Promega, Madison, WI, USA) total liver RNA was reverse transcribed using AMV reverse transcriptase (Takara, Otsu, Japan). cDNA were reverse transcribed, respectively, and then the cDNA of the four mice with the same genotype in the same time point were mixed equally. PCR reaction mixes were assembled using the SYBR Green real-time PCR Master Mix (Toyobo, Osaka, Japan). The reactions were performed on the 7900HT Sequence Detection Quantitative PCR System (Applied Biosystems, Foster City, CA, USA). Cycling

Table 1 Sequences of Primers for Quantitative Real-time RT-PCR

Genes	Forward	Reverse
<i>AdipoR1</i>	AATGGGGCTCCTTCTGGTAAC	GCAGACCTTATACACGAACTCC
<i>AdipoR2</i>	GGAGTGTCGTGGGCTTAGG	GCAGTCCGGTGATATAGAGG
<i>Cd36</i>	TCCTCTGACATTTGCAGGTCTATC	AAAGGCATTGGCTGGAAGAA
<i>Fas</i>	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCAG
<i>Acc1</i>	ATGGGCGGAATGGTCTCTTTC	TGGGGACCTTGTCTTCATCAT
<i>Cpt-1</i>	AAAGATCAATCGGACCCTAGACA	CAGCGAGTAGCGCATAGTCA
<i>PPARα</i>	GGGAAAGACCAGCAACAA	TCAAGGAGGACAGCATCG
<i>m-Socs3</i>	ATGGTCACCCACAGCAAGTTT	TCCAGTAGAATCCGCTCTCCT
<i>m-β-actin</i>	CTGGCCGGGACCTGACAGACTACC	ATCGGAACCGCTCGTTGCCAATAG
<i>h-Socs3</i>	TGCGCCTCAAGACCTTCAG	GAGCTGTCCGGATCAGAAA
<i>h-β-actin</i>	CCCAAGGCCAACCCGCGAGAAGAT	GTCCCGGCCAGCCAGGTCCAG

parameters were 95°C for 3 min and then 40 cycles of 95°C (25 s), 58°C (25 s), and 72°C (25 s) followed by a melting curve analysis. All reactions were performed with four technical replicates and repeated independently for three times. The cycle threshold values were normalized to the expression of the housekeeping gene β -actin. Primer sequences are available in Table 1.

Western Blotting

Frozen liver tissue was homogenized in RIPA buffer containing Protease Inhibitor Cocktail (Sigma) and PMSF with a homogenizer and a sonicator at 4°C. After centrifuging at 10 000 g for 10 min at 4°C, the supernatant protein was quantified by the Bio-Rad DC Protein Assay (Hercules, CA, USA). Liver homogenates (50 μ g) were separated by SDS-PAGE and were transferred to the nitrocellulose membrane as referred by the manufacturer's protocols. Antibodies of STAT3 (BD, Franklin Lakes, NJ, USA), phospho-STAT3 (Tyr705) (Cell Signaling Technology, Danvers, MA, USA), and β -actin (Sigma) were used as probes. HRP-conjugated secondary antibodies were from Kirkegaard & Perry Laboratories (Gaithersburg, MD, USA). Immune complexes were detected using the enhanced chemiluminescence system (Pierce, Rockford, IL, USA).

Statistical Analysis

Quantitative data are expressed as mean \pm s.d. Statistical significance between any two groups was determined by the two-tailed Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS

The Effect of Adiponectin Deficiency on Liver Mass Restoration and Hepatocyte Proliferation

To test the hypothesis that adiponectin is required for liver regeneration *in vivo*, we induced liver regeneration by

performing PH on male wild-type and adiponectin-null mice. Mice were killed and analyzed during the first 96 h after surgery. Before PH, there was no significant difference in the liver/body weight ratio between wild-type and adiponectin-null mice. However, the liver/body weight ratio was persistently lower in adiponectin-null mice from 24 to 96 h after PH (Figure 1a). During liver regeneration after PH, the restore of liver mass is predominantly contributed by proliferation of the hepatocytes. To examine whether adiponectin deficiency causes an impairment of hepatocyte proliferation, wild-type and adiponectin-null mice were continuously labeled with BrdU and killed at 72 and 96 h after PH. Adiponectin-null mice showed significantly decreased proliferation than wild-type mice (Figure 1b). These results demonstrate that liver regeneration is impaired in adiponectin-null mice.

The peak of hepatocyte proliferation appears between 24 and 48 h after PH.^{2,3} We assessed the kinetics of hepatocyte proliferation during liver regeneration in wild-type and adiponectin-null mice by evaluating hepatocyte DNA synthesis (BrdU pulse labeling) and counting mitotic hepatocytes after PH. In wild-type mice, the proliferation index (percentage of BrdU-positive hepatocytes) markedly increased at 36 h post PH, reached a peak at 48 h post PH, then decreased to a low level at 72 h post PH. Nevertheless, in adiponectin-null mice, the proliferation index was persistently lower from 36 to 60 h post PH and a little higher at 72 h post PH (Figure 1c and d). The mitotic figures of hepatocytes were counted and we found that much more mitotic figures were observed at 48 and 60 h post PH in the liver of wild-type mice in comparison with the adiponectin-null mice (Figure 1e and f). HE staining of liver tissue sections also revealed that both of the wild-type and adiponectin-null livers had increased vacuolation after PH. However, the vacuolation level, which may represent the hepatic steatosis (lipid accumulation), in adiponectin-null mice was obviously higher than that in the

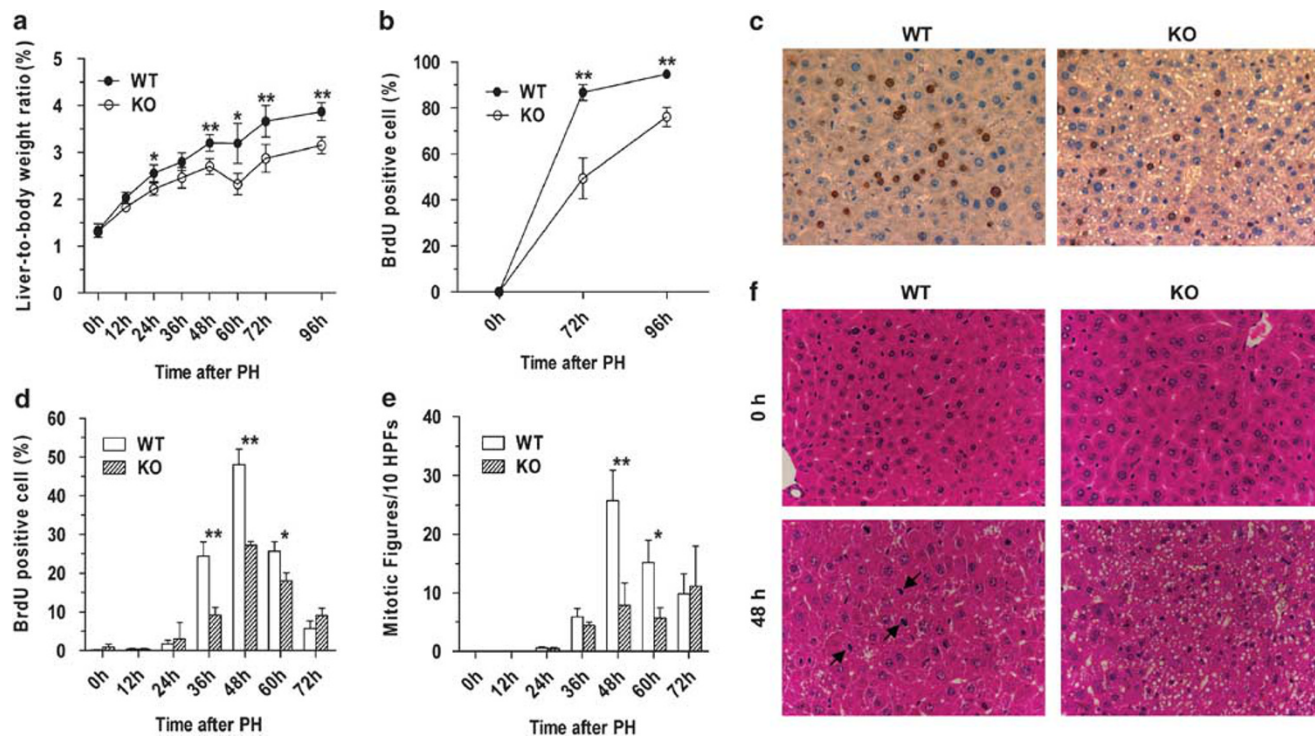


Figure 1 Impaired restoration of liver mass and hepatocyte proliferation in adiponectin-null mice. **(a)** Liver/body weight ratio in wild-type (WT) and adiponectin-null (KO) mice at the indicated time points after PH. **(b)** Cumulative proliferation of hepatocytes after PH. Mice were given BrdU in drinking water until the time of killing. **(c)** 1-h BrdU pulse labeling of hepatocytes in livers from wild-type (WT) and adiponectin-null (KO) mice at 48 h after PH (magnification, $\times 400$). **(d)** Percentage of BrdU-positive hepatocyte nuclei in all nuclei in wild-type (WT) and adiponectin-null (KO) mice at the indicated time points after PH. **(e)** Counts of mitotic figures in wild-type and adiponectin-null mice at the indicated time points after PH. **(f)** Morphologic assessment of livers from wild-type and adiponectin-null mice at 0 and 48 h after PH. The representative photomicrographs of hematoxylin and eosin-stained paraffin sections of the liver are shown (magnification, $\times 400$). The arrows denote the representative mitotic figures. Results are shown as mean \pm s.d. ($n \geq 4$ for each genotype at each time point). * $P < 0.05$, ** $P < 0.01$.

wild-type mice at 48 h post PH. Hepatic steatosis and focal liver injury were more prominent in the adiponectin-null mice (Figure 1f). We also evaluated the apoptosis level in the livers of both wild-type and adiponectin-null mice at 48 h post PH using the TUNEL staining, but no significant difference was found (data not shown). Taken together, these results clearly show that the hepatocyte proliferation of adiponectin-null mice is impeded during the regeneration after PH.

The Effect of Adiponectin Deficiency on Hepatic Lipid Accumulation and Catabolism During Liver Regeneration

After PH, transient hepatic steatosis occurs during early liver regeneration. Mice exhibit markedly increased hepatic lipid accumulation at 12–24 h after PH. And the hepatic lipid was consumed away 24–48 h after PH, which was proposed to serve as an energy and materials source supporting cell proliferation and tissue re-growth.¹³ However, It is well established that excessive lipid accumulation in liver exhibits profound impairment of liver regeneration after PH.^{12,21–25} Adiponectin functions as a hormone to stimulate lipid catabolism.²⁶ It reduces lipid accumulation by inhibiting hepatic lipogenesis and stimulating fatty acid oxidation.^{14,27} These

reports led us to determine whether adiponectin has effect on hepatocellular lipid accumulation and catabolism during liver regeneration. The timing and magnitude of hepatic steatosis during liver regeneration after PH were examined by Oil Red O staining. Compared to wild-type mice, much more fat accumulated in adiponectin-null livers at 24 h post PH (Figure 2a). Moreover, at 48 h post PH, though the hepatocellular lipid decreased to a low level in wild-type livers, the lipid in adiponectin-null livers was still abundant (Figure 2a). Corresponding biochemical data supported the morphological observation (Figure 2b and c). Increased hepatic triglyceride and cholesterol accumulation were determined in adiponectin-null mice, whereas the levels of triglyceride and cholesterol in serum were not significantly different between wild-type and adiponectin-null mice before PH and after PH (Supplementary Figure 1). These results demonstrate that adiponectin plays an essential role in regulating hepatic lipid homeostasis during liver regeneration.

Expression of Genes Involved in Lipid Metabolism during Liver Regeneration

In an attempt to explore the molecular mechanism underlying adiponectin-mediated hepatic lipid metabolism during

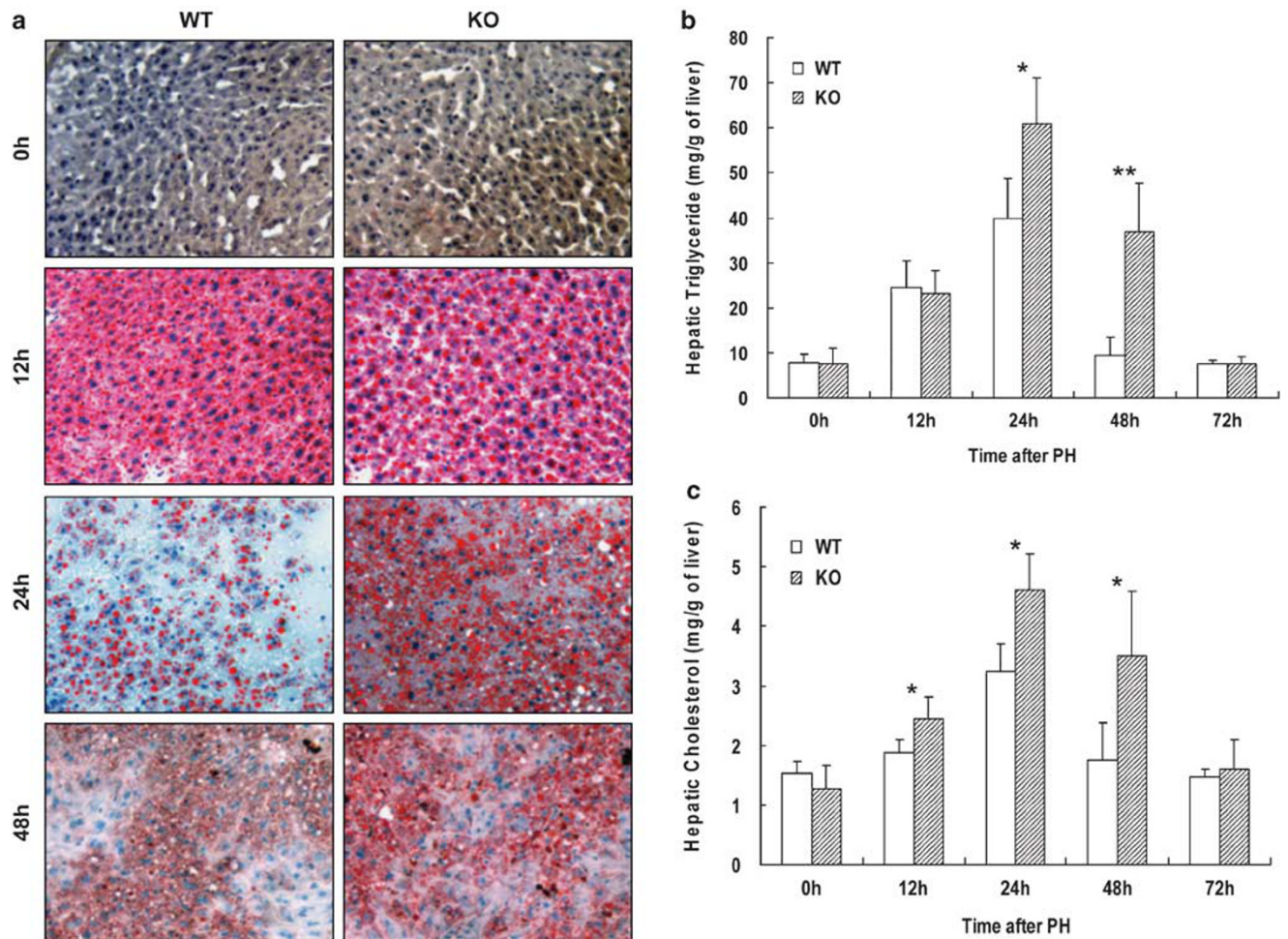


Figure 2 Increased hepatic lipid accumulation in adiponectin-null mice after PH. (a) Oil Red O staining of livers from wild-type (WT) and adiponectin-null (KO) mice at the indicated time points after PH (magnification, $\times 200$). Hepatic triglyceride (b) and cholesterol (c) levels were measured using the livers collected at the indicated time points after PH. Results are shown as mean \pm s.d. ($n \geq 4$ for each genotype at each time point). * $P < 0.05$, ** $P < 0.01$.

liver regeneration, expression of genes known to be critical in lipogenesis, fatty acid oxidation, and transportation was profiled by relative quantitative real-time reverse transcription PCR. We found that the mRNA levels of carnitine palmitoyltransferases I (Cpt-I), PPAR α , acetyl-CoA-carboxylase 1 (Acc1), fatty acid synthase (Fas), and Cd36 were changed in the absence of adiponectin in comparison with wild-type mice during liver regeneration. The average hepatic mRNA level of each gene in wild-type livers at 0 h was set as 1.0, and the hepatic mRNA level of each gene at 24 h after sham operation was set as a control.

Cpt-I is a rate-limiting enzyme involved in the transport of long-chain fatty acids into mitochondrial matrix.²⁸ It has been well demonstrated that adiponectin can increase the activity of Cpt-I to enhance fatty acid oxidation.¹⁴ Our data showed that, at 0, 12, and 24 h after PH, the hepatic Cpt-I mRNA level was higher in wild-type mice than that in adiponectin-null mice. At 48 h post PH, the hepatic Cpt-I mRNA level in adiponectin-null mice was equal to the level

in wild-type mice. At 72 h post PH, the hepatic Cpt-I mRNA level in adiponectin-null mice was about 1.7-fold higher than that in wild-type mice (Figure 3a). These findings show that, the upregulation of Cpt-I mRNA is delayed in adiponectin-null mice.

PPAR α , which can be activated by adiponectin, is a critical regulator of hepatic lipid oxidation.²⁹ The hepatic PPAR α mRNA level was higher in wild-type mice at 24 and 48 h after PH but a little lower at 72 h after PH as compared to that in adiponectin-null mice (Figure 3b). Obviously, the upregulation of PPAR α mRNA expression, which is similar to that of the Cpt-I mRNA expression, is delayed in adiponectin-null mice.

Fas is another key enzyme involved in hepatic lipogenesis. Expression of hepatic Fas is significantly elevated after PH, and adiponectin can suppress the hepatic mRNA expression of this enzyme.^{14,28} From 12 to 48 h after PH, the hepatic Fas mRNA level was consistently higher in adiponectin-null mice compared to wild-type mice. At 48 h post PH, the level of

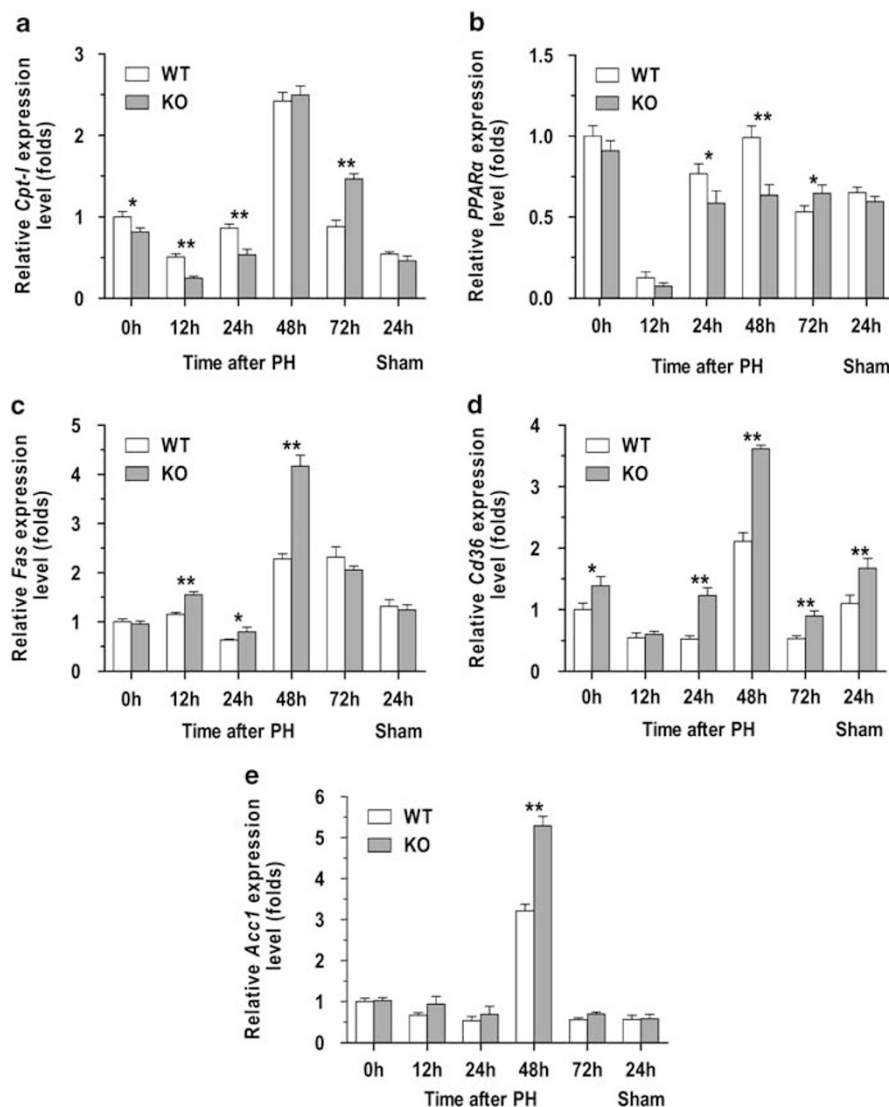


Figure 3 Expression of genes involved in lipid metabolism during liver regeneration. The hepatic mRNA levels of Cpt-1 (a), PPAR α (b), Fas (c), Cd36 (d) and Acc1 (e) of wild-type (WT) and adiponectin-null (KO) mice at the indicated time points after PH or sham operation (Sham) were measured by quantitative real-time reverse transcription PCR. Data are shown as mean of fold changes compared with wild-type mice 0 h after surgery \pm s.d. ($n = 3$). * $P < 0.05$, ** $P < 0.01$.

hepatic Fas transcripts was about 2-fold higher in adiponectin-null mice than that in wild-type mice (Figure 3c). These data demonstrate that adiponectin can suppress the Fas expression to regulate the hepatic lipogenesis during liver regeneration after PH.

Cd36 is a protein responsible for transportation of fatty acids into tissues,³⁰ and adiponectin treatment can markedly downregulate the hepatic expression of Cd36.¹⁴ Both before PH and at 24 h after sham operation, adiponectin-null livers expressed higher Cd36 mRNA level than wild-type livers. This observation suggests that adiponectin might control the basal PPAR α mRNA level. At 24, 48, and 72 h post PH, adiponectin-null livers consistently expressed at about 2-fold higher Cd36 mRNA than wild-type livers (Figure 3d). These data suggest that PH-induced Cd36 gene expression might be modulated by adiponectin.

Acc1 is a lipogenic enzyme known to be suppressed by adiponectin.^{9,14} Marked induction of Acc1 gene expression was seen in both wild-type and adiponectin-null livers at 48 h post PH. However, the level of hepatic Acc1 transcript was 1.6-fold higher in adiponectin-null mice than that in wild-type mice at this time point (Figure 3e). These data indicate that adiponectin is involved in the regulation of Acc1 gene expression during liver regeneration.

The Adiponectin Protein Levels and mRNA Expression of Adipors during Liver Regeneration

To investigate the function status of adiponectin during the process of liver regeneration, we detected the adiponectin protein level and the expression pattern of adiponectin receptors (AdipoRs). As expected, there were high levels of adiponectin protein in livers of wild-type mice but no

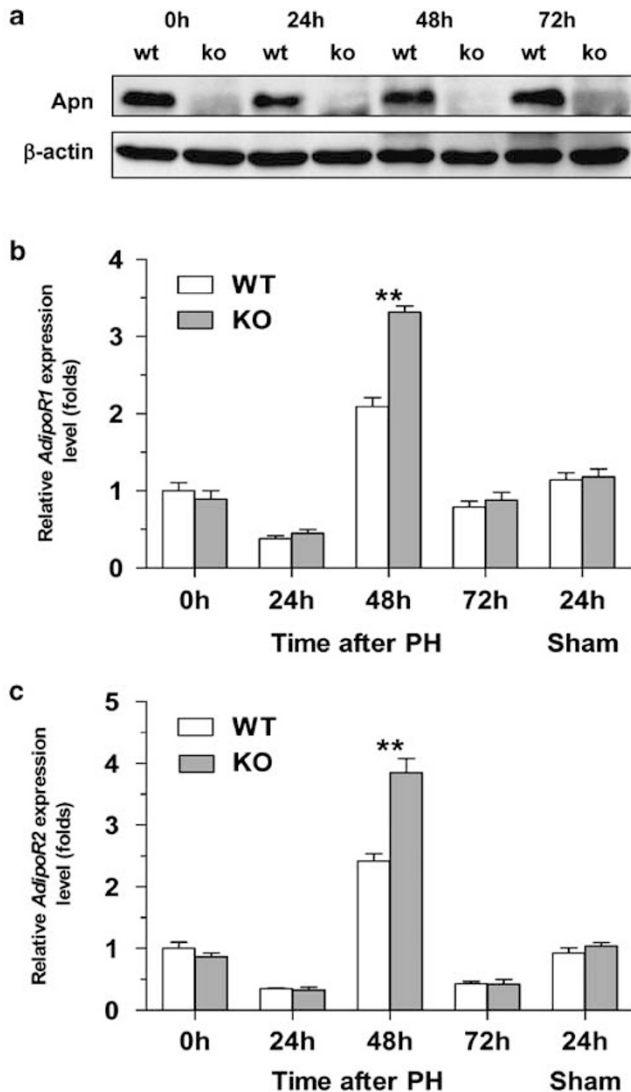


Figure 4 The hepatic protein levels of adiponectin and mRNA levels of AdipoRs during liver regeneration. (a) Immunoblotting of adiponectin in liver homogenates corresponding to wild-type (wt) and adiponectin-null (ko) mice after PH. Immunoblots are representative of three independent experiments. The hepatic mRNA levels of two adiponectin receptors AdipoR1 (b) and AdipoR2 (c) in wild-type (WT) and adiponectin-null (KO) mice at the indicated time points after PH were measured by quantitative real-time reverse transcription PCR. Data are shown as mean of fold changes compared with wild-type mice 0 h after surgery \pm s.d. ($n = 3$). ** $P < 0.01$.

adiponectin protein was detected in livers of adiponectin-null mice at any point in the regeneration process (Figure 4a). Two receptors for adiponectin (AdipoR1 and AdipoR2) have been identified. AdipoR1 is expressed widely in mice, whereas AdipoR2 is expressed mainly in the liver.^{8,9} Interestingly, the hepatic mRNA levels of AdipoR1 and AdipoR2 both increased after PH and reached a peak at 48 h post PH. Moreover, in the absence of adiponectin, the mRNA levels of AdipoR1 and AdipoR2 were higher than those of wild-type mice at 48 h post PH (Figure 4b).

Reduced Hepatic STAT3 Activity in Adiponectin-Null Mice after PH

Cytokine signaling pathways are activated after PH. The interleukin-6 (IL-6)-induced JAK–STAT3 pathway is a key cytokine signaling during liver regeneration. Binding of IL-6 to its receptor (IL-6R), which is associated with two subunits of gp130, stimulates the tyrosine-kinase activity of the Janus-associated kinase (JAK). Activated JAK then phosphorylates the associated gp130 and STAT3 on a tyrosine residue. STAT3 then homodimerizes and translocates to the nucleus, where it induces transcription of a number of target genes to promote the liver regeneration.^{2,3} Suppressor of cytokine signaling 3 (Socs3) interacts with JAK and blocks JAK–STAT3 pathway to suppress the process of liver regeneration.^{31,32} To evaluate whether absence of adiponectin affects STAT3-mediated signaling during liver regeneration, hepatic functional status of STAT3 was analyzed by western blotting. From 24 to 72 h after PH, the phosphorylation of STAT3 was obviously reduced in adiponectin-null mice (Figure 5a). Meanwhile, at 24 and 48 h post PH, the Socs3 mRNA levels in the adiponectin-null mice were about 2-fold higher than those in the wild-type mice (Figure 5b). To determine whether Socs3 mRNA levels can be regulated by adiponectin, human hepatoma, HepG2, cells were treated with human recombinant adiponectin (APN). After APN treatment, the Socs3 mRNA level was reduced by 32.5% in HepG2 cells. The gene expression of Socs3 was acutely induced by LIF treatment,³³ and this induction was reduced by 24.7% on the addition of APN (Figure 5c). These findings suggest that adiponectin can repress the Socs3 gene transcription. For this reason, the reduced STAT3 activation in adiponectin-null mice after PH is partially resulted from the increased Socs3 level.

DISCUSSION

In this study, we have investigated the role of adiponectin in liver regeneration after PH. Our results show that adiponectin is required for normal procession of liver regeneration.

In clinical situations, obese patients with fatty livers tend to have poor outcomes after resection or liver transplantation.^{21–23} In experimental situations, both genetic-based models and high-fat-diet-induced model proved that pre-existing steatosis caused significant impairment of liver regeneration after PH.^{12,24,25} In addition, the augmented hepatic steatosis after PH coexisted with the defective liver regeneration after PH in mice.^{10,34} This phenomenon was observed in adiponectin-null mice as well. These evidences suggest that excessive fat in hepatocytes may harm the normal process of liver regeneration. In the normal progress of liver regeneration after PH, hepatic lipid homeostasis is complex and exactly regulated.¹⁵ In the absence of adiponectin, hepatocytes accumulated much more lipid, and the lipid could not be consumed away efficiently. Meanwhile, mRNA levels of related lipid metabolism genes consistently changed. In the early phase after PH, mRNA levels of Cpt-I and PPAR α had increased higher in wild-type mice in

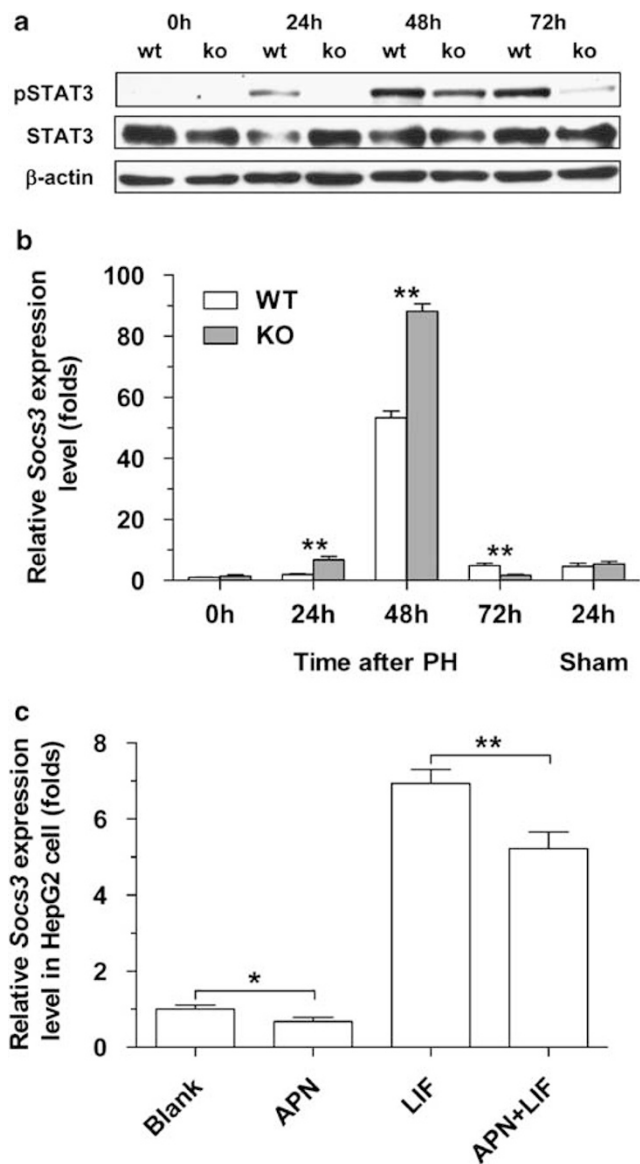


Figure 5 Decreased STAT3 phosphorylation and increased Socs3 mRNA levels after PH in adiponectin-null mice. (a) Total and phosphorylated STAT3 were assessed by western blot in the livers of wild-type (WT) and adiponectin-null (KO) mice at the indicated time points after PH. β -actin is included as a loading control. (b) The hepatic mRNA levels of Socs3 of wild-type (WT) and adiponectin-null (KO) mice at the indicated time points after PH were measured by quantitative real-time reverse transcription PCR. Data are shown as mean of fold changes compared with wild-type mice 0 h after surgery \pm s.d. ($n = 3$). (c) Serum-starved HepG2 cells were treated with or without recombinant human adiponectin (APN) and LIF for 1 h. Relative levels of Socs3 mRNA were determined by quantitative real-time reverse transcription PCR. Results are shown as average of three independent experiments \pm s.d. * $P < 0.05$, ** $P < 0.01$.

comparison with adiponectin-null mice. These adiponectin-dependent changes of gene expression might lead to enhanced lipid β -oxidation. Though the mRNA levels of Cpt-I and PPAR α increased to normal levels and even higher levels later, the lipid β -oxidation might be late for the requirement of energy in the adiponectin-null mice. Moreover, wild-type

mice have reduced mRNA levels of Fas, Cd36, and Acc1. These adiponectin-dependent changes of gene expression might suppress the lipid biosynthesis and uptake to modulate the hepatic lipid homeostasis during the liver regeneration. Taken as a whole, these results suggest that adiponectin-null mice could not catabolize the accumulated hepatocellular lipid efficiently to fuel the fire for the normal progress of liver regeneration.

The reduced STAT3 activity and increased Socs3 mRNA level could also contribute to the augmented hepatic steatosis in the adiponectin-null mice. The liver-specific STAT3 deficiency mice show reduced hepatocyte DNA synthesis after PH were complicated with obesity and fatty liver.^{35,36} In addition, inhibition of the expression of Socs3 in liver ameliorates hepatic steatosis, and Socs3 enhances the hepatic steatosis by attenuating STAT3 activity.³⁷ Earlier study has shown that IL-6 treatment alleviates hepatic steatosis through enhancing STAT3 activation as well as upregulating PPAR α expression and activity in mice.³⁸ Recently, it has been reported that fructose-fed rats show impairment of hepatic STAT-3 activation as well as reduction in PPAR α mRNA expression and activity.³⁹ In our study, adiponectin-null mice also showed reduced STAT3 activity and PPAR α mRNA expression during the early phase of liver regeneration. These results suggest that STAT3 and PPAR α may take part to the regulation of the hepatic lipid accumulation during liver regeneration. However, the detail mechanisms on the cross talk between these two pathways remain to be demonstrated.

Recent studies suggest that adiponectin can activate STAT3 in the rat hypothalamus and mouse cardiac fibroblasts.^{40,41} Our results provide *in vivo* evidence that adiponectin can stimulate the STAT3 activation in mouse livers after PH, which is accompanied by reduced Socs3 transcription. Moreover, our results provide *in vitro* evidence that adiponectin can repress Socs3 transcription. However, we found no significant difference in serum levels and hepatic expression levels of IL-6 between wild-type and adiponectin-null mice (data not shown), which indicate that the decreased STAT3 phosphorylation during liver regeneration represents a direct effect of the adiponectin-dependent Socs3 induction. This is, to our knowledge, the first report to demonstrate that adiponectin-mediated repression of Socs3 gene expression can facilitate STAT3 activation. The direct involvement of the adiponectin-AdipoRs signaling in the induction of Socs3 expression and reduction in STAT3 phosphorylation still requires further investigations.

This study also provides evidence that the transcription of AdipoRs is enhanced after PH. Moreover, the mRNA levels of AdipoRs compensatorily increased in adiponectin-null mice at 48 h post PH. These results suggest that the downstream signaling cascades of AdipoRs triggered by adiponectin are required in the normal progression of liver regeneration. However, one of the principal downstream signal transduction proteins, AMPK phosphorylation, did not significantly change during liver regeneration in both wild-type and adiponectin-null mice (data not shown).

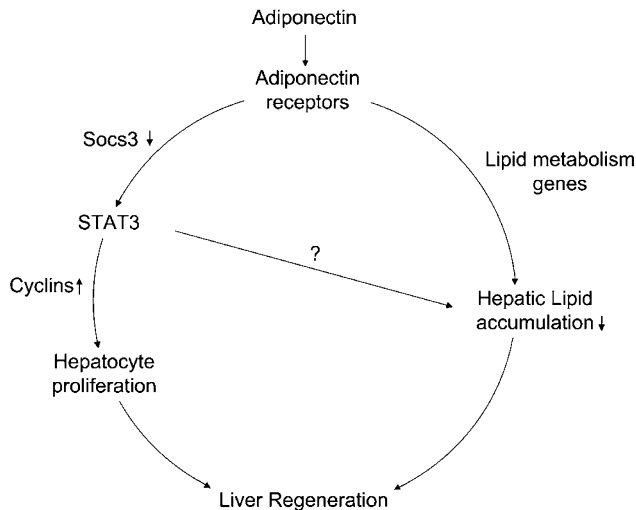


Figure 6 Schematic representation of the potential mechanisms underlies the actions of adiponectin during liver regeneration.

When we were preparing our manuscript, Hisao Ezaki and colleagues published their work about the effects of adiponectin on liver regeneration after PH using different mice strain.⁴² Our study was generally in agreement with their report on the role of adiponectin during liver regeneration after PH. By PH in C57BL/6 mice, they found that delayed liver regeneration was due to reduced expression of cyclins and impairment of fatty acid oxidation. Our investigation on the 129S1 mice indicated that adiponectin-null mice showed impaired liver regeneration and abnormal fatty acid metabolism. Moreover, our results described the reduced phosphorylation of STAT3 and increased transcription of Socs3 in adiponectin-null mice after PH. It has been proved that activation of STAT3 after PH can promote hepatocyte proliferation through enhancing expression of cyclins.³⁶ These evidences collectively showed the potential mechanisms underlie the actions of adiponectin during liver regeneration (Figure 6).

In summary, adiponectin-null mice showed impaired liver regeneration and altered hepatic lipid accumulation after PH. Increased Socs3 and subsequently blocked STAT3 signaling in adiponectin-null mice may contribute to the alteration of the liver regeneration capability and hepatic lipid metabolism after PH. Taken together, these results suggest that adiponectin and AdipoRs could be the novel targets to improve liver regeneration in patients with acute or chronic liver injury.

Supplementary Information accompanies the paper on the *Laboratory Investigation* website (<http://www.laboratoryinvestigation.org>)

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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