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REVIEW

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Lipid droplet deposition in the regenerating liver: A promoter, inhibitor, or bystander?

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Abstract

Liver regeneration (LR) is a complex process involving intricate networks of cellular connections, cytokines, and growth factors. During the early stages of LR, hepatocytes accumulate lipids, primarily triacylglycerol, and cholesterol esters, in the lipid droplets. Although it is widely accepted that this phenomenon contributes to LR, the impact of lipid droplet deposition on LR remains a matter of debate. Some studies have suggested that lipid droplet deposition has no effect or may even be detrimental to LR. This review article focuses on transient regeneration-associated steatosis and its relationship with the liver regenerative response.

INTRODUCTION

Transient regeneration-associated steatosis (TRAS) has been observed in rodent models of partial hepatectomy (PH)–induced liver regeneration (LR), and numerous studies have attempted to establish the potential connection between TRAS and LR.[1,2] However, the relationship between TRAS and LR remains a subject of controversy. Following 70% PH, mice with CAV1-/- or hepatocyte-specific knockout of AKT1/2 exhibited coexisting disorders of TRAS formation and inhibition of LR.[3,4] On the other hand, mice with

Abbreviations: ACC, acetyl-CoA carboxylase; ADRP, adipose differentiation-related protein; ALB, albumin; ANXA A6, Annexin A6; ATG5, autophagy related 5; ATG7, autophagy related 7; ATGL, adipose triglyceride lipase; CAV1, Caveolin-1; CE, cholesterol esters; CM, chylomicron; CV, central vein; DDC, 3,5-diethoxycarbonyl-1,4-dihydro-collidine; DNS: de novo synthesis; ET1, endothelin 1; FAS, fatty acid synthase; FABP, fatty acid binding protein; FATP, fatty acid transport protein; FFA, free fatty acid; GC, glucocorticoids; GR, glucocorticoid receptor; HSL, hormone sensitive lipase; LD, lipid droplet; LDLR, LDL receptor; LR, liver regeneration; MTP, microsomal triglyceride transfer protein; NCoR1, nuclear receptor corepressor 1; PARK7, Parkinson protein 7; PCSK9, proprotein convertase subtilisin/kexin type 9; PDK4, pyruvate dehydrogenase kinase 4; PGE2, prostaglandin E2; PH, partial hepatectomy; PPAR, peroxisome proliferator-activated receptor; PV, portal vein; S1P, sphingosine-1-phosphate; TRAS, transient regeneration-associated steatosis; TG, triglyceride.

Yuelei Hu and Ruilin Wang contributed equally to this work.

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hepatocyte Nuclear receptor corepressor 1 (NCoR1) deficiency displayed an accelerated LR process along with enhanced TRAS formation.[5] However, some studies have indicated that the deletion of fatty acid binding protein (FABP) in hepatocytes impairs TRAS formation but does not affect LR.[1] Moreover, in hepatocyte peroxisome proliferator-activated receptor α (PPAR)α-deleted mice, enhanced TRAS during regeneration was accompanied by impaired LR.[6] The disruption of key genes involved in lipid metabolism, transport, and regulation may influence energy metabolism during LR, and the effect of the disturbance of energy metabolism on the progress of LR may play an important role in those contradictory results. The theoretical basis of most studies is the utilization of lipid droplets (LDs) as sources of energy in the regeneration process.[7,8] However, a recent study has suggested that LD formation can inhibit the expression of MIER1 by influencing protein synthesis, thereby promoting the progress of LR.[2] During LR, not only do triglyceride (TG)and cholesterol levels increase in the liver but there is also a transient elevation in the levels of free fatty acids (FFAs) in the peripheral blood.[1,5] However, certain studies have demonstrated a downward trend in the expression levels of genes associated with *de novo* synthesis of FFAs during the early stages of regeneration.[9] In summary, the mechanisms underlying TRAS formation and its effects on LR remain unclear. It is only through an in-depth exploration of these intricate mechanisms that TRAS can be manipulated for the advancement of LR. This review aims to provide valuable insights into the mechanism of TRAS formation and its relationship with LR, thereby facilitating a deeper understanding of this intriguing phenomenon.

LIVER REGENERATION AND GROWTH, HEPATOCYTE PROLIFERATION, AND HYPERTROPHY

Detoxification is a critical function of the liver, whereby it transforms endogenous metabolites, such as ammonia and exogenous drugs, and toxins into less toxic or nontoxic compounds.[10] However, this may result in liver injury. The liver is the "injury-privileged organ" of vertebrates. It has a strong regenerative ability after injury caused by biotic (viruses, parasites, etc.) and abiotic (toxins, drugs, and PH) factors.[11] Long-term chronic injury caused by NAFLD may cause some hepatocytes to proliferate to maintain liver homeostasis. However, in cases of acute injury such as PH, NAFLD may interfere with the process of LR.[12,13] The energy metabolism of hepatocytes changes during LR to meet the rapid proliferation and hypertrophy requirements.[14,15] LR is a complex interactive network involving multiple cellular and molecular interactions,[11] with close relationships between the liver and other organs of the body.[16] The main manifestations of LR are the division and hypertrophy of mature hepatocytes or progenitor cells after the death or loss of hepatocytes, which can restore the original liver weight and function.[7,17] Changes in liver size can also occur under pathological and physiological conditions, such as cachexia, severe wasting, fetal development, individual growth, and pregnancy.[11,18] Although there are many similar biological metabolic processes and signaling molecule transmissions in LR and liver growth, the 2 processes are not the same. In addition, hepatocyte proliferation events, such as the transplantation of an entire liver with a relatively small mass into a recipient with a large body weight, would also result in liver growth. This process can be regarded as an acute loss of parenchyma in the liver of a recipient with a large body weight, which can be categorized as LR despite relatively small transplanted livers without significant injury, as liver resection.[19]

The complexity of LR arises from many factors (Figure 1). First, the source of regenerating hepatocytes was multivariate.[20,21] The reentry of differentiated mature hepatocytes into the cell cycle to divide and proliferate and to promote the recovery of liver weight and function plays an important role in LR after PH.[20] In cases of severe liver injury resulting in significant hepatocyte loss or chronic liver injury inhibiting hepatocyte replication, liver progenitor cells emerge as the primary cellular source of LR.[22,23] Furthermore, apart from liver progenitor cells, biliary epithelial cells have also been identified as one of the sources involved in LR.[24,25] Second, the LR microenvironment is complex. In addition to resident hepatocytes, cholangiocytes, sinusoidal endothelial cells, KC, and HSC within the liver, numerous platelets, neutrophils, and macrophages are involved in regulating LR through extensive and complex intercellular information communication.[11,26–28] Third, changes in nutrients, the vagus nerve, and mechanical forces brought about by blood flow and the extracellular matrix are also important factors affecting LR.[18,29–32] There is a close relationship between the liver and other organs[16]; whether changes in the transcriptional levels of the kidney and lung after PH regulate the LR process need to be further studied.[33] When the liver is damaged and needs to be repaired, "hepatostat"[11] in the body mobilizes multiple regenerative mechanisms to quickly restore the liver's size, weight, and function to guarantee and maintain body homeostasis.

ENERGY METABOLISM OF HEPATOCYTES AND REMODELING IN LR

Hepatocytes do not continuously proliferate in adult vertebrates and play an important role in maintaining body homeostasis. The liver is responsible for the biochemical metabolism of sugars, lipids, proteins, and

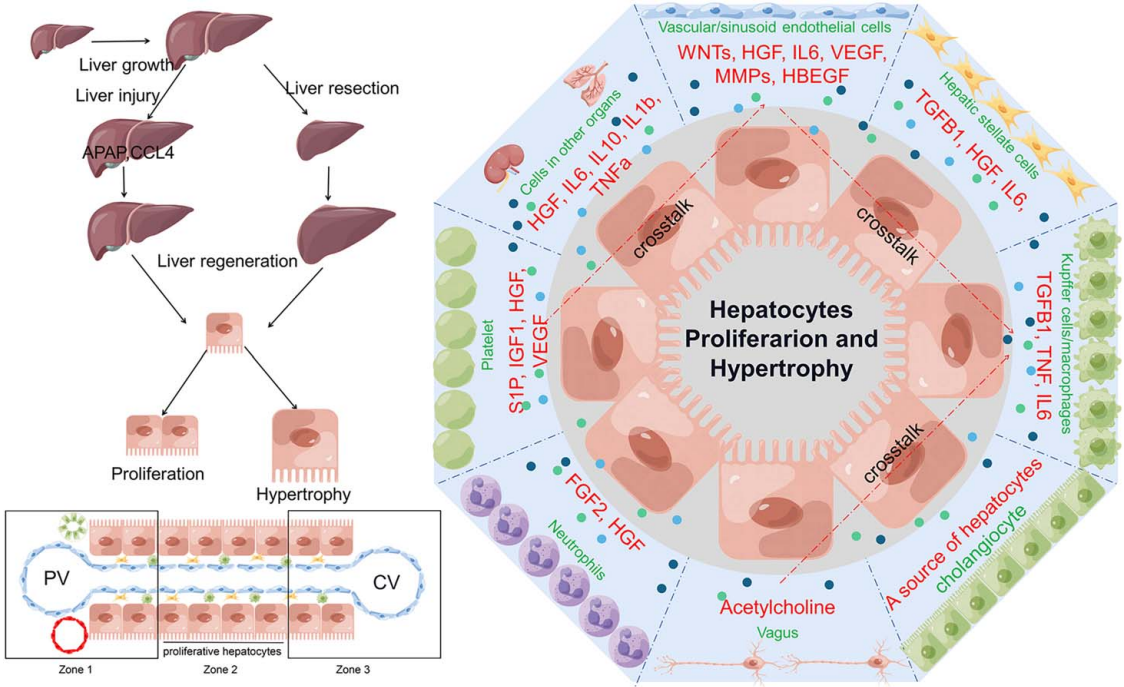

FIGURE 1 A schematic diagram of liver growth, regeneration, and regulation of intercellular crosstalk and secretion in the regenerative microenvironment. Liver growth and regeneration after injury involve the division and hypertrophy of hepatocytes. This process is not only regulated by biliary epithelial cells, sinusoidal endothelial cells, KC, and HSC but also by a large number of platelets, neutrophils, macrophages, and vagus nerves through extensive and complex intercellular communication. Significant transcriptional changes occur in other organs such as the lung and kidneys after PH. Abbreviation: PH, partial hepatectomy.

amino acids, and its function is conserved in mammals.[34] The hepatic lobule is the basic structural and functional unit of the liver. As early as 100 years ago, Noël proposed the regionality of hepatic lobules,[35] and Deane first proposed the concept of zonation.[36] The application of advanced techniques has facilitated the identification of markers and metabolic functions of hepatocytes in different compartments of the hepatic lobule, as shown in Figure 2. *PCK1*, *HAL*, *CPS1*, *CDH1*, *IGF1*, *CYP7A1*, *GLS2*, *HSD3B7*, *HMGCS1*, *HSD17B13*, *ASS1*, and *ARG1* were highly expressed in portal vein zone hepatocytes. *GLUL*, *CYP2E1*, *CYP1A2*, *CYP3A4*, *OAT*, *IGFBP1*, *NT5E*, *ADH4*, and *BCHE* were highly expressed in central vein zone hepatocytes. *HAMP*, *HAMP2*, *IGFBP2*, *CYP8B1*, *HINT1*, *COX7C*, *APOC1*, *FABP1*, *MT2A*, *MT1G*, and *NDUFB1* were highly expressed in the hepatocytes in the middle lobule. The marker genes in the central region of the lobule have not been completely determined, and some genes in the central vein and portal vein regions extend into the hepatocytes in the middle lobule, such as *CYP2E1* or *ARG1*.[35,37] The physiological direction of blood flow and the differential expression of proteins in hepatocytes and endothelial cells at different sites determine, to some extent, the distribution of metabolic functions of hepatocytes in the hepatic lobule. Oxygen content is higher around the portal vein than around the central vein, which may indicate that periportal hepatocytes are mainly responsible for high oxygen consumption biological functions, such as protein secretion and FFA oxidative metabolism. The differential distribution of enzymes in hepatocytes further controls the spatial differential distribution of hepatocyte metabolism. Overall, hepatocytes in zone 1, in addition to being responsible for protein secretion and oxidative metabolism of FFA, perform functions such as gluconeogenesis, cholesterol biosynthesis, glutaminolysis, urea synthesis, and FFA uptake. The main functions exerted by the hepatocytes in zone 3 are glycolysis, lipid synthesis, bile acid synthesis, glutamine synthesis, and drug metabolism (high expression of *CYPs*). A detailed description of liver lobule zonation and metabolic heterogeneity is available in 4 review articles by Martini, Ben, Moshes, and Paris J.[35,37–39]

The liver is chronically exposed to damage by multiple biotic and abiotic factors, and LR is important for maintaining liver homeostasis. Two main aspects are involved in the maintenance of LR: hypertrophy and division of hepatocytes.[40] However, massive loss of liver parenchyma, as described earlier, leads mature hepatocytes to reenter the cell cycle and become rapidly proliferating. Rapidly proliferating cells, such as hepatocytes during LR and cancer cells, often have a metabolic state that is distinct from that of quiescent cells.[41,42] Metabolic reprogramming is one of the hallmark features of cancer, such as characteristic changes in glucose, glutamine, and lipid metabolism, and similarly, proliferating hepatocytes have metabolic

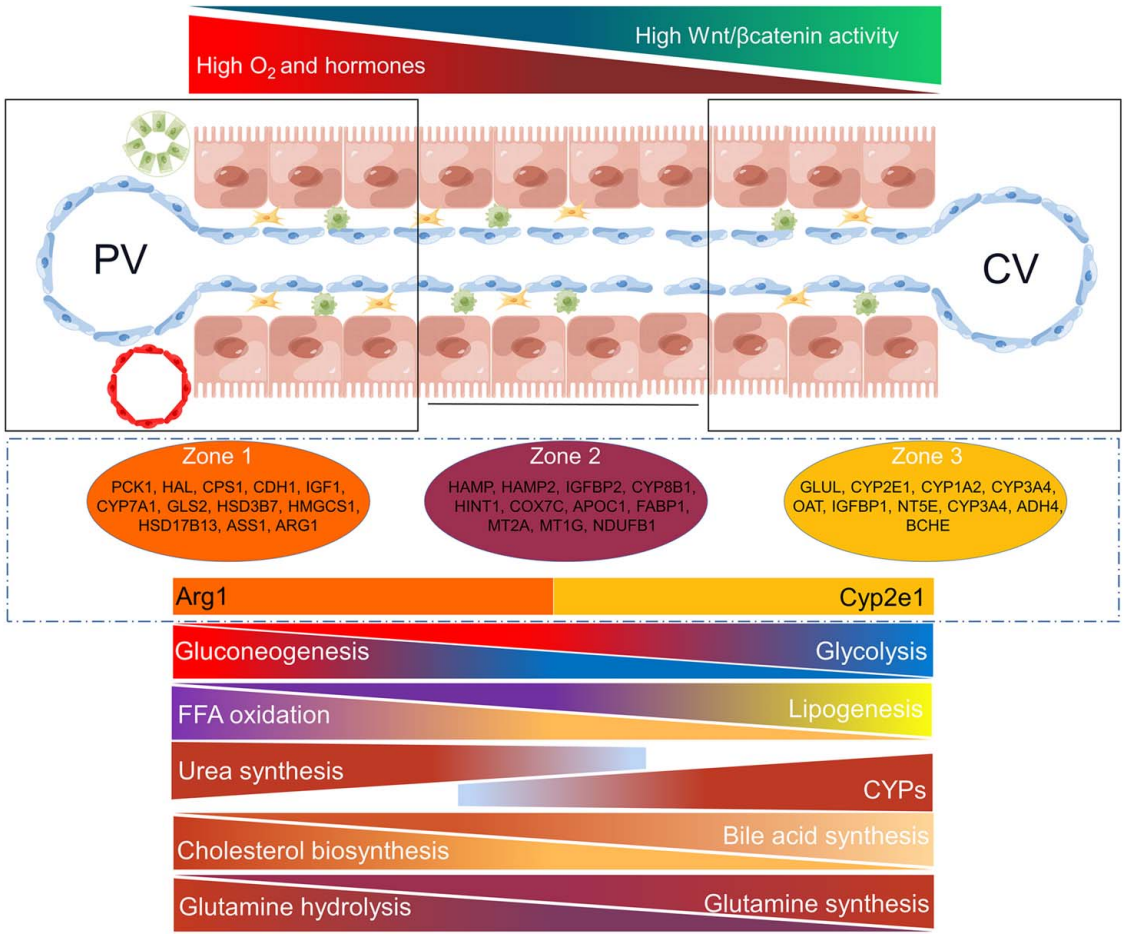

FIGURE 2 Heterogeneity of metabolism, environment, and gene expression in the hepatic lobules. The hepatic lobule has a strict spatial structure, and different regions have different environments and gene expression profiles of hepatocytes and endothelial cells. The differences in the microenvironment and gene expression together construct spatial metabolic heterogeneity at different locations in the hepatic lobule. Abbreviations: CV, central vein; PV, portal vein.

reprogramming.[42–44] The coordinated regulation of cellular metabolic patterning, growth, and division during tissue renewal and regeneration is a prerequisite for tissue recovery after injury, and cellular metabolism sets the stage for tissue homeostasis and regeneration.[14] In terms of LR, some studies have revealed that zone 1 hepatocytes are one of the cellular sources of early regenerating liver,[45,46] and lipid metabolism is the main source of energy. From recent studies, it can be inferred that after massive loss of liver parenchyma, dramatic glucose reduction may enhance lipolysis in the peripheral adipose tissue, resulting in increased plasma FFA content. FFA uptake and *de novo* synthesis in the liver may eventually lead to increased lipid levels in regenerating hepatocytes and serve as an energy source during LR.[3–5,7,47,48] *PTEN*-/- mice developed hepatocyte hypertrophy and mitotic inhibition after PH, which mainly provided energy for liver hypertrophy by enhancing FFA β oxidation.[7] The proliferation of hepatocytes in zone 2 plays an important role in maintaining liver homeostasis, but for zone 1 and zone 3 liver injuries caused by the 3,5-diethoxycarbonyl-1,4-dihydro-collidine (DDC) diet and CCl4 injection, the source of proliferating hepatocytes is distributed in the noninjured area.[49] These findings indicate that hepatocytes with proliferative capacity are distributed in different positions within the hepatic lobules, enabling them to better respond to liver damage caused by different factors and contribute to the maintenance and restoration of liver homeostasis. While some researchers believe that the process of LR after PH is mainly derived from hepatocytes in zone 2, there is also evidence of proliferating hepatocytes in zone 1 and zone 3.[50] The energy sources on which the LR process depends are not always lipids in different genetic backgrounds in mice.[8] The division of hepatocytes in the regenerating liver of *CDK*1-/- mice is inhibited, and the original liver size is restored mainly through hepatocyte hypertrophy. The level of mitochondrial oxidative metabolism is reduced in hypertrophied hepatocytes; however, the metabolic flux of ALT is markedly increased for energy supply through pyruvate oxidative metabolism.[15] Significant metabolic remodeling occurs during LR, which can meet the demand for energy and substance-synthetic substrates required for cell hypertrophy and proliferation to maintain homeostasis of liver metabolism and structural function.

POTENTIAL LINK BETWEEN LIPOLYSIS IN ADIPOSE TISSUE AND LR

When the body's energy supply is adequate, adipocytes store FFA inside the cells as nontoxic TG, which is an important energy depot for the body. In the fasted state, FFAs are released from white adipose tissue lipolysis into the blood, where transport through serum albumin to energy-consuming tissues serves to supply energy and biosynthetic substrates. Both neutral and acidic lipolysis are involved in the lipolysis of peripheral adipose tissue. Acid lipolysis is carried out mainly by lysosomal acid lipase in lysosomes, whereas neutral lipolysis is mediated mainly by adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and monoacylglycerol lipase[51]; 90% of TG breakdown is carried out by ATGL and HSL.[52] In addition, lipolysis also occurs in non-adipose tissues and is mediated by noncanonical pathways that have not yet been fully defined.[51] The regulatory mechanisms of ATGL and HSL are highly complex, with multiple endocrine, autocrine, and paracrine factors, hormones, and neurotransmitters that regulate ATGL and HSL at the transcriptional and posttranscriptional levels to induce lipolysis of adipose tissue. These include catecholamines (adrenaline and noradrenaline), glucocorticoids, thyroid hormones, eicosanoids, atrial natriuretic hormone, growth hormone, IL (IL6 and IL1β), TNFα, and leptin.[51] In contrast, insulin is a well-defined inhibitor of lipolysis.[51] In addition, endothelin 1; sphingosine-1-phosphate, and prostaglandin E2 can participate in the regulation of lipolysis in adipose tissue to a certain extent.[53–58] During LR, hepatocyte proliferation undergoes significant alterations in energy metabolism, and the demand for energy sources shifts accordingly. Cytokine, hormone, and metabolite storms occur during LR. After PH, serum leptin levels increase markedly, accompanied by a marked decrease in blood glucose.[59,60] Many metabolites, such as sphingosine-1-phosphate derived from sphingolipid metabolism and the prostaglandin family of arachidonic acid metabolites (eg, prostaglandin E2), are generated in the regenerative microenvironment.[61,62] Inflammatory factors and cytokines also produce changes as LR occurs and progresses, such as the IL family (IL6 and IL1β), the TNFα family, the TGFβ family, the EGF and FGF families, and others.[11] Changes in mechanical forces are sensed by vascular endothelial cells, with the consequent secretion of a large number of vascular endothelial cell-derived cytokines and regulatory proteins such as endothelin 1.[63] During LR, a large number of cytokines and metabolites are present in the peripheral blood, some of which are the same factors that regulate lipolysis in peripheral adipose tissue. However, whether LR regulates TG hydrolysis in peripheral adipose tissue and whether the secretory cytokine storm has an impact on the adipose tissue lipolysis process remain unclear. Herein, we delineated the cues that may regulate adipocyte lipid breakdown during LR based on literature data, as shown in Figure 3.

METABOLISM OF FFA IN HEPATOCYTES

Hepatocytes are the core sites of lipid metabolism, participating in balancing and buffering lipid metabolism within the body, and therefore, often experience increased LD deposition in pathological hepatocytes. The sources of TG or FFA in hepatocytes mainly include the following: (1) excess dietary TGs are transported to the liver through chylomicrons (CM), (2) increased FFA synthesis from *de novo* lipogenesis, (3) excess FFA influx into hepatocytes from lipolysis within adipose tissue, (4) reduced export of lipids from the liver through VLDL, and (5) reduced oxidation of FFA.[64] Dietary TGs are decomposed into monoacylglycerol and FFA by treatment with bile acid and pancreatic lipase. After being absorbed by intestinal mucosal epithelial cells, TGs are resynthesized, packaged as CM, and enter blood circulation through the lymphatic system.[65–67] CM utilization is facilitated by lipoprotein lipase in the microvasculature of adipose and muscle tissues.[68] CM remnants not utilized by adipose and muscle tissues are transported to the liver, where they are further metabolized.[69] Hydrolysis of TG in peripheral adipose tissue proceeds through a series of lipase-mediated lipolyses, which releases FFA.[51] Multiple proteins have been identified to be involved in the transport of FFA from plasma into hepatocytes, including plasma membrane FABP, CD36, caveolin-1, and very long-chain acyl CoA synthetases (ACSVL/FA transport proteins, also known as FATP/solute carrier family 27A1-6, SLC27A1-6).[64,68,70] In addition to FFA released from peripheral adipose tissue, *de novo* synthesis of FFA in hepatocytes can also lead to an increase in TG content in hepatocytes.[68,69] *De novo* synthesis of FFA is carried out in the cytoplasm, and its synthetic substrate acetyl-CoA is mainly in the mitochondria and cannot freely cross the mitochondrial membrane. Therefore, a citrate-pyruvate transport system in the cell promotes the transport of acetyl-CoA from the mitochondria to the cytoplasm for FFA synthesis. Acetyl-CoA is first converted to malonyl-CoA by ACC, and malonyl-CoA is then converted to palmitate by FAS.[68] FFA oxidative metabolism is used to provide energy, mainly in the mitochondria, which, in turn, reduces lipid levels in the liver. Once the intracellular FFA oxidative metabolism is inhibited, it is obvious that the intracellular FFA utilization is reduced, resulting in fat accumulation, manifested as intracellular LD deposition. In addition to FFA oxidative metabolism, outward transport is the only way hepatocytes reduce intracellular lipids. Because of its hydrophobicity,

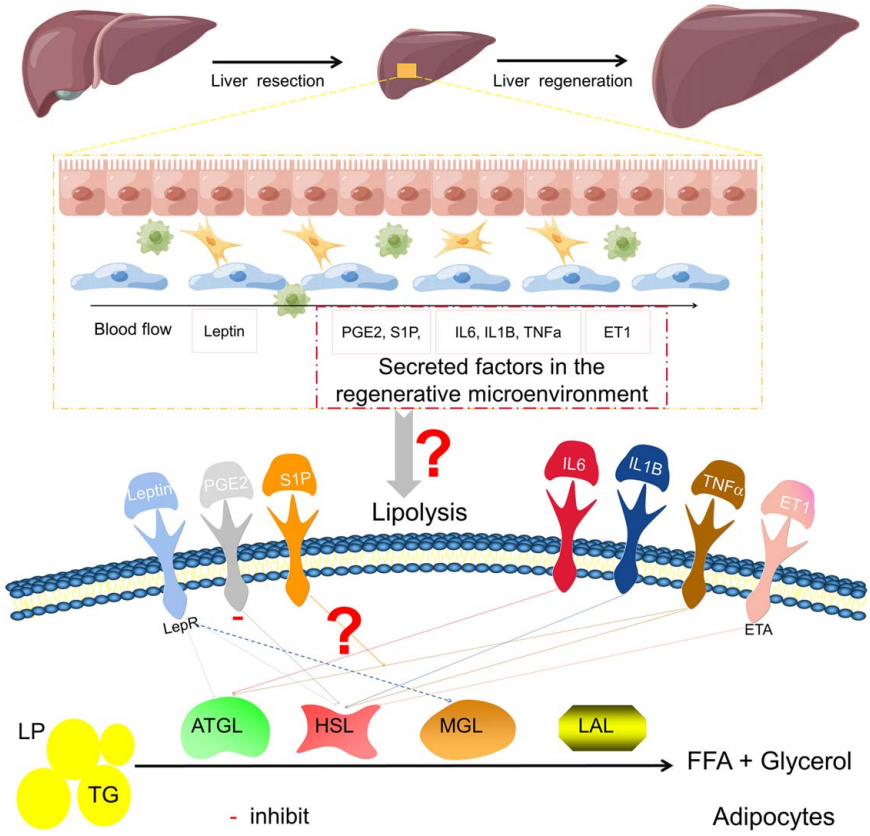

FIGURE 3 The potential link between LR and peripheral adipose tissue lipolysis. During LR, cytokines and metabolite storms are produced, such as interleukin family, TNF family, ET1, S1P, and PGE2, some of which have been shown to affect lipolysis in peripheral adipose tissue. Abbreviations: ET1, endothelin 1; LR, liver regeneration; PGE2, prostaglandin E2; SIP, sphingosine-1-phosphate.

microsomal triglyceride transfer protein (MTP) assists TG in the endoplasmic reticulum and Golgi apparatus with cholesterol, phospholipids, and ApoB100 together packaged into water-soluble VLDL before being outputted from the liver.[64,68,70] A schematic diagram of FFA metabolism in hepatocytes is shown in Figure 4.

LD DEPOSITION IN LR

During the early phase of LR, large amounts of TGs are stored in hepatocyte LD, mainly composed of TG and cholesteryl esters.[48,71,72] This phenomenon has been defined by some scholars as TRAS.[7] Interestingly, some investigators have revealed that there are 4 times increased levels of hepatocyte proliferation during LR after PH, accompanied by 3 times increased levels of LD accumulation.[73] In comparison to standard PH (70%), extended PH (86%) has been associated with higher mortality rates and a lower rate of hepatocyte proliferation. MRI, lipid content analysis, and pathological examination have consistently demonstrated significantly higher lipid content in the liver of the extended PH group compared with the standard PH group at 48 hours after operation. This increased LD deposition is closely associated with mortality in the extended PH group.[74] Metabolism is dynamically altered during LR after PH, and the division of labor among hepatocyte populations balances the proliferation and metabolic requirements of the regenerating liver.[50] The change in gene expression of lipid metabolism can clarify that TRAS is a biological process with strict regulation.[75] With more in-depth studies on LR, the mechanism of TRAS development and its effects on LR are gradually being recognized. However, there is no consensus regarding the relationship between TRAS and LR.[1,48,76,77] According to earlier studies, there are 3 possible combinations of the interaction between TRAS and LR: (1) the formation of LD had little impact on LR; (2) the accumulation of LD promotes LR; and (3) the formation of LD impedes LR. Although studies have shown that the expression levels of genes related to *de novo* synthesis of FFA are inhibited during LR,[9] some researchers still believe that LD deposition may also be partially derived from *de novo* FFA synthesis in hepatocytes.[5] In addition to *de novo* synthesis and transport, VLDL-mediated lipid output and oxidative metabolism of hepatocytes also affect TRAS. The relationship between TRAS and LR is summarized in Supplemental Table S1, http://links.lww.com/HC9/A521. At the same time, we also described the interference of those influencing factors on lipid metabolism in the liver (Figure 5).

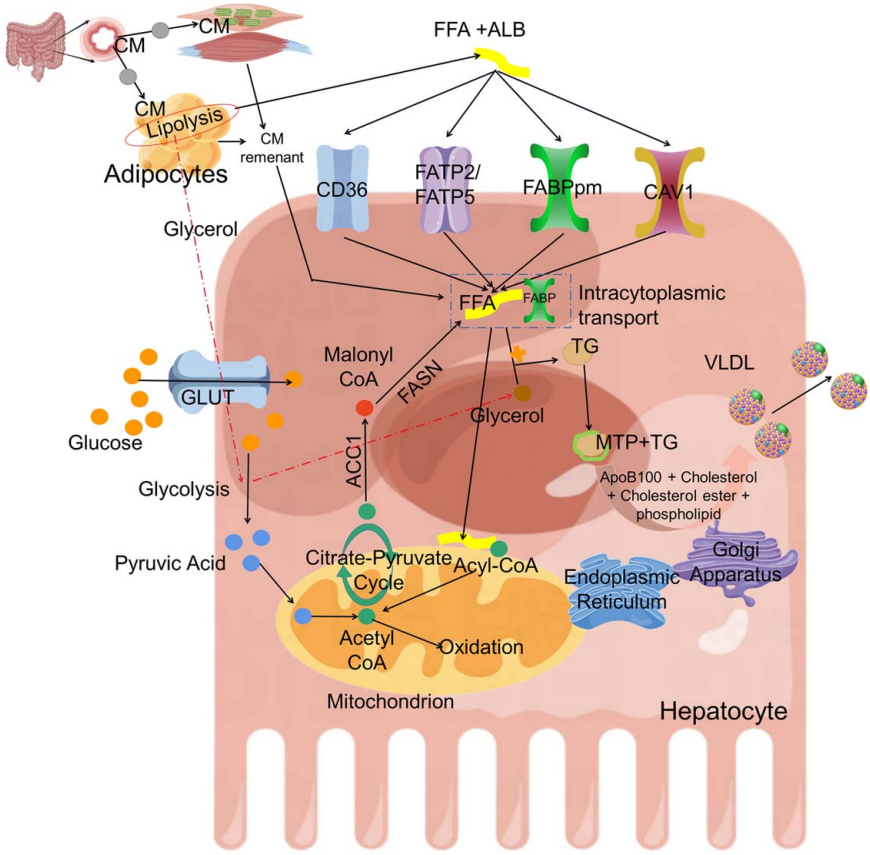

FIGURE 4 Schematic diagram of FFA metabolism in hepatocytes. Hepatocytes are important centers of lipid metabolism. First, fat in the digestive tract is absorbed into the blood in the form of chylomicrons(CM), and part of it is stored and utilized in adipose tissue and muscle tissue. CM remnants enter the liver for further metabolism. In peripheral adipose tissue, TG is hydrolyzed by lipase under the stimulation of various factors. FFAs are released and combined with ALB (albumin) to transport in the blood and then transported into the liver through the FFA transporter on the surface of the liver cell membrane. It can be converted into LD stored in cells and can also be further packaged into VLDL to secrete hepatocytes. FFA provides energy through oxidative metabolism in mitochondria. In addition to the above metabolism, cells can also use acetyl coenzyme A for the *de novo* synthesis of FFA. Abbreviations: ALB, albumin; CM, chylomicrons; FFA, free fatty acids; MTP, microsomal triglyceride transfer protein; TG, triglyceride.

Taken together with the relationship between TRAS and LR in previous literature, we summarize that there may be the following 5 reasons for the above 3 phenotypes: (1) impaired LR coexists with increased LD deposition: metabolic inhibition of FFA leads to increased accumulation of LD, and energy supply impairment of regenerating hepatocytes results in LR inhibition, as typically exemplified by PPAR α effects on LR; (2) reduced LD deposition inhibits LR: severe impairment of hepatic FFA transport induces reduced LD deposition in the liver, leading to insufficient supply for subsequent oxidative metabolism, inhibiting LR, such as *CAV1* deficiency (partly related to the suppression of oxidative metabolism); (3) reduced LD deposition inhibits LR: impaired storage of lipids results in reduced LD that inhibits LR, as knockout of *Plin2* causes increased VLDL formation and facilitates lipid transport out of hepatocytes; (4) LD deposition does not affect LR: some transporters affect lipid transport to a lesser extent, other lipid transporters compensate, and the deposited LD are sufficient to cope with the regenerative energy requirements; (5) LD deposition did not affect LR: there was no difference in the peak of fat accumulation, but the time of fatty accumulation was extended. In LR, hepatocyte hypertrophy substitutes for hepatocyte proliferation to restore liver homeostasis. For example, PTEN deficiency enhances the oxidative metabolism in hepatocytes. The relationship between TRAS and LR is intricate, and genetically engineered mice often harbor numerous associated gene expression changes that influence the relationship between TRAS and LR. Next, we describe the complex relationships in detail.

LD deposition does not affect LR

Annexin A6

Annexin A6 (*ANXA A6*), a member of the annexin family, is involved in a variety of cellular functions, such as membrane transport, cholesterol homeostasis, and signal transduction.[78–81] *ANXA A6* gene knockout has no significant effect on normal growth and development in mice[82] but can avoid weight gain and obesity caused by high-fat feeding.[83] ANXA A6 plays an important role

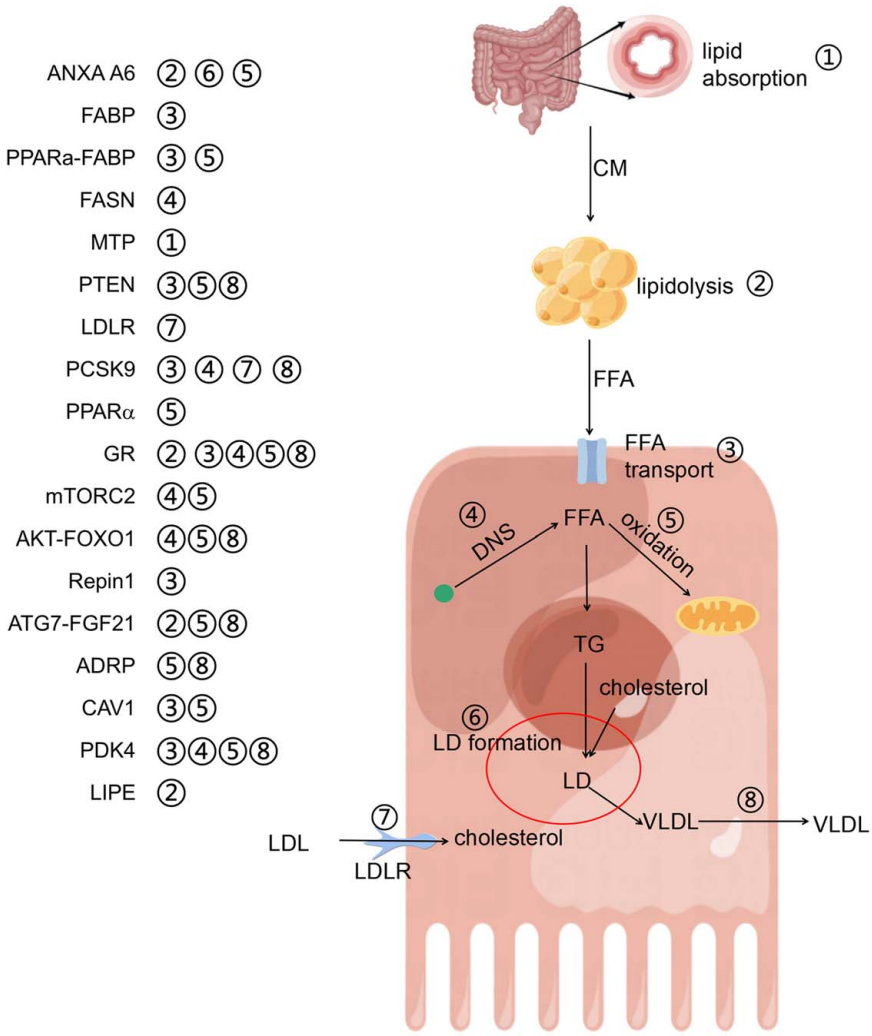

FIGURE 5 A brief summary of the regulatory mechanism of lipid droplet (LD) deposition during liver regeneration as reported in the literature. This figure depicts eight important processes of lipid metabolism: ① lipid absorption in the digestive tract; ② lipid hydrolysis in peripheral adipose tissue; ③ transport of free fatty acids into hepatocytes; ④ *de novo* synthesis of fatty acids; ⑤ oxidative metabolism of fatty acids; ⑥ formation of lipid droplets; ⑦ LDL transport into hepatocytes; and ⑧ VLDL assembly and transport outside hepatocytes. The left-hand side of the figure summarizes important regulatory factors and their potential involvement in lipid metabolism. The red circle represents the formation of LD, while the green dots represent acetyl-CoA. Abbreviations: CM, chylomicron; DNS, *de novo* synthesis; LD, liquid droplet.

in energy metabolism.[14] In *ANXA A6*-/- mice, fatal hypoglycemia occurred after PH, the survival rate was severely reduced, and LD deposition was delayed during LR but did not affect the peak of LD deposition. Although the expression of *ANXA A6* by the adeno-associated virus and glucose supplementation were able to rescue death after PH, the authors did not investigate its effect on LD deposition. Interestingly, despite increased mortality, aggravation of hypoglycemia, and delayed appearance of the peak of LD deposition after PH, reentry into the hepatocyte cell cycle was not inhibited in the early phase of LR.[60] The regulatory mechanism of ANXA A6 on LD deposition during LR is unclear, but previous findings seem to provide some hints. The effect of ANXA A6 on LD metabolism differs among cells. First, primary hepatocytes from *ANXA A6*-/- mice and cell lines in which *ANXA A6* was knocked down exhibited a reduction in LD deposition after OA treatment, possibly through a mechanism involving cytoplasmic phospholipase A2α inhibition[84] because cytoplasmic phospholipase A2α was involved in the formation of LD.[85] Second, loss of *ANXA A6* results in increased LD deposition in adipocytes, along with severely suppressed catecholamine-induced phosphorylation of HSL, impairing lipolysis in adipocytes.[86] These mechanisms may play an important role in inhibiting LD deposition during LR after PH in *ANXA A6*-/- mice. Although there is no study on the effect of downregulation of *ANXA A6* expression on FFA metabolism in hepatocytes, some researchers found that the downregulation of *ANXA A6* expression in triple-negative breast cancer can increase the expression of FABP4 and also promote the uptake and oxidative

metabolism of FFA in mitochondria with intracellular lipid reducing.[87] The decrease in LD deposition caused by *ANXA A6* deficiency may come from many aspects, such as decreased lipolysis of peripheral TG, inhibition of LD formation, and enhanced oxidative metabolism.

FABP, PPARα- FABP, and FAS in hepatocytes and MTP in the small intestine

The complete process of lipid metabolism involves the absorption of lipids in the digestive tract, lipolysis of peripheral adipose tissue, import of FFA by fatty acid transport-related proteins to hepatocytes, export of lipids from hepatocytes through VLDL, and *de novo* synthesis and oxidation in cells.[88] Four knockout models have been used to study the effect of lipid metabolism on TRAS and regeneration, namely, hepatocyte-specific knockout *FABP* mice, small intestine-specific knockout *MTP* mice, hepatocyte *PPARα* and *FABP* double knockout, and hepatocyte-specific knockout *FAS* mice. However, there was no correlation between TRAS and LR.[1] It is important to note, however, that TRAS mainly arises from lipolysis in peripheral adipose tissue and possibly in part from *de novo* synthesis, so TG only partially decreased during LR as a result of small intestine-specific knockout of *MTP*. Serum FFA levels were significantly higher at 6 hours. MTP in the small intestine is very important for CM-mediated lipid absorption. The absence of *MTP* leads to a large accumulation of TG in small intestinal mucosal cells and reduced absorption.[89] There are few studies on MTP and fat metabolism in the small intestine; however, the absence of MTP in adipose tissue significantly promotes ATGL-mediated lipolysis.[90] There is no clear conclusion on whether the deinhibition of ATGL by *MTP* deficiency exists in the small intestine at the same time, but it may also be a possible reason for the increase in serum FFA levels. FABP is a fatty acid transport-related protein in hepatocytes, and hepatocyte-specific knockout of *FABP* resulted in a partial decline in hepatic TG during LR. The overall trend of hepatic TG in both groups of mice was consistent with that in control mice. There are 4 mechanisms of FA transport on the hepatocyte surface: CD36, FABP, FATP, and CAV1.[64] The knockout of *FABP* may only partially affect FA transport and LD formation. In the hepatocyte-specific knockout FAS model, although some researchers have suggested that the *de novo* synthesis of FFA is related to the formation of LD during LR,[5] LD deposition and LR were not significantly affected. The relationship between the *de novo* synthesis of FFA and the formation of LD and the extent of their contribution still needs to be clarified. Finally, in the *PPARα* and *PPARα-FABP* double knockout mouse model, knockout of *FABP* reversed the increased LD deposition resulting from *PPARα* knockout, illustrating that FABP plays a role in the increased TG content during LR. Paradoxically, however, the author did not find a difference in LR, possibly because they did not dynamically analyze changes throughout the LR. Although the above 4 models have found that LR is not related to lipid deposition, the above 4 models may not truly reflect the relationship between TRAS and LR. This may be because other homofunctional proteins or processes may play a compensatory role in the case of interfering with a single factor affecting lipid metabolism.

PTEN

PTEN, a tumor proliferation suppressor with dual-specificity phosphatase activity of lipid phosphatase and protein phosphatase, is involved in the regulation of several signaling pathways and plays an important role in cell growth, cell cycle, and material metabolism.[91] The lipid phosphatase properties of PTEN are mainly able to inactivate phosphatidylinositol triphosphate by converting phosphatidylinositol triphosphate dephosphorylation to phosphatidylinositol bisphosphate, which further inhibits PI3K/AKT and the activation of downstream signaling pathways.[92] PI3K/AKT is a core signaling pathway involved in cell proliferation, cell cycle regulation, and substance metabolism in cancer and LR.[93,94] First, *PTEN*-specific deletion in adipose tissue improved glucose tolerance and increased insulin sensitivity in obese and diabetic mice.[95] Second, the specific knockout of *PTEN* in hepatocytes leads to markedly increased lipid synthesis and lipid import in the liver, resulting in fatty liver disease and even progression to liver cancer.[96–98] Third, the downregulation of *PTEN* expression leads to decreased assembly and secretion of VLDL in hepatocytes.[99] The downregulation of *PTEN* has been found in several patient and animal models of diseases, such as HBV/HCV infection, metabolic syndrome, and hepatoma progression triggered by dietary unsaturated FFA.[100–102] Fat accumulation in the liver occurs early in the LR process, and a decrease in *PTEN* expression in liver tissue occurs during this process.[7] Some evidence indicates that *PTEN* is involved in the process of LR and the regulation of energy metabolism.[7,103] In the LR model of hepatocyte-specific *PTEN* knockout mice, liver weight increased, liver cell area expanded, and hypertrophy was observed, but mitosis decreased. Although the deletion of *PTEN* in hepatocytes resulted in increased LD deposition in nonproliferating hepatocytes, the peak of lipid deposition during LR was not significantly increased but prolonged the time of lipid content elevation. Downregulation of *PTEN* expression induces AKT signaling pathway activation, leading to enhanced lipid oxidation, thereby mediating metabolic adjustments and hepatocyte hypertrophy during LR.[7] However, a recent study found that *PARK7* knockout led to an increase in PTEN expression levels, inhibiting LR and leading to enhanced TRAS, and genomic

depletion or pharmacological inhibition of PTEN restored the delayed LR.[103] According to the 2 studies mentioned above, it can be found that the expression level of *PTEN* affects the deposition of lipids during LR. Loss of *PTEN* expression results in a prolonged period of lipid deposition, whereas increased expression of *PTEN* results in increased lipid deposition. In addition, the knockout of *PTEN* in other cell types also affects LR. The loss of *PTEN* expression in bone marrow-derived cells leads to M2 differentiation, which can promote LR after PH. *In vitro* studies also showed that the conditioned medium of KC with *PTEN* deficiency could promote the proliferation of hepatocytes.[104] In summary, *PTEN* is involved in regulating LR and lipid metabolism during LR, and *PTEN* expression in hepatocytes and other cells has different effects on LR.

LDL receptor (LDLR)

LDLR mainly clears cholesterol esters (CE)in the serum by endocytosis of LDL and other lipoproteins. *LDLR* almost disappeared in the livers of mice overexpressing proprotein convertase subtilisin/kexin type 9 (*PCSK9*), and the whole or hepatocyte-specific knockout of *PCSK9* showed an obvious increase in LDLR expression level.[105] Previous studies have shown that the expression of *PCSK9* (mRNA) increases during LR after PH.[106] Therefore, the expression level of LDLR may decrease during LR. In *LDLR*-/- mice, the LR process was impaired, which was mainly accompanied by a decrease in the expression levels of cytokines such as IL6, TNF, and HGF. LD deposition did not change significantly during LR, but there were significant differences in the lipid profiles. Compared with WT mice, in the basic-state liver of *LDLR*-/- mice, the CE level was higher (increased by 2.5 times), and phosphatidylethanolamine (decreased by 1.7 times) and ceramide (decreased by 2.5 times) were lower. After PH, the CE level in WT mice decreased gradually after 48 hours, but the CE level in *LDLR*-/- mice remained high. In addition to the difference in the changes in CE, the level of sphingolipids in *LDLR*-/- mice liver 3 hours after surgery was significantly higher than that in the control group, and the level of ceramide was significantly higher than that in the control group 2 days later.[107] Mice with *PCSK9*-/- showed increased expression levels of inflammatory factors, such as IL6 and TNFα, which were reversed by small interfering RNA-targeted *LDLR*; thus, there may be an endogenous regulation mechanism of IL6 and TNFα by *LDLR*.[108] Although, in the basal state, loss of *PCSK9* expression levels resulted in increased cytokine expression levels, LR induced by PH was significantly suppressed in *PCSK9*-/- mice.[105] Cholesterol can promote the inflammatory response and proliferation of hepatocytes,[109] but a long-term cholesterol diet resulting in increased intracellular cholesterol content inhibits the hepatic regenerative response after PH.[110] In general, the expression level of LDLR may be reduced during LR; therefore, LDLR may not be important for LR and lipid deposition. The impaired regeneration ability of hepatocytes in *LDLR*-deficient mice may be related to a decrease in cytokine expression and a change in lipid composition. However, the effect of lipid composition on LR remains unclear.

LD deposition is a favorable factor for LR

NCoR1

Studies on interactions between *NCoR1*, nuclear receptors, deacetylases, and transducing beta-like 1 have revealed that they can form a corepressor complex for the downregulation of target genes; therefore, loss of *NCoR1* leads to increased expression levels of its target genes.[111–113] In mice, hepatocyte-specific knockout of *NCoR1* (*NCoR1*$^{hepa-/-}$) results in hepatic steatosis,[111] and downregulation of *NCoR1* expression, leading to elevated expression of metabolism-related genes, may be responsible for hepatic steatosis, such as lipogenesis and lipid oxidation genes.[114] The expression of NCoR1 is suppressed after PH, and this suppression may play an important role in driving the progression of LR. In *NCoR1*$^{hepa-/-}$ mice, *NCoR1* loss accelerated LR but did not alter its outcome. This accelerated LR was accompanied by an increase in the TG content in the liver. Notably, however, basal levels of TG were higher in the livers of NCoR1$^{hepa-/-}$ mice than in the controls, with a similar trend in the LR process in the 2 groups. Orlistat, a FAS inhibitor, reduced and delayed the peak of TG accumulation during LR in both groups while eliminating the difference. Based on the above results, the authors affirmed that *de novo* synthesis of FFA in hepatocytes may be involved in LD deposition during LR.[5] However, the effects of *NCoR1* and orlistat on TG deposition, LR, and lipid synthesis must be assessed objectively. First, the loss expression of *NCoR1* can also promote the enhancement of FFA oxidative metabolism,[115] which provides energy for the early stage of LR and is important for accelerating the progress of LR.[7,45] Second, the main reason why orlistat strongly inhibited the level and rate of TG accumulation during LR may be that orlistat is an inhibitor of FFA synthase and lipases. Orlistat inhibits the absorption of dietary-derived FFA[116] and also reduces lipolysis in adipocytes.[117] Inhibition of lipolysis in peripheral adipose tissue and *de novo* synthesis of FFA may result in decreased TG deposition during LR, and insufficient energy metabolism leads to obvious inhibition of LR in both groups. The effect of *de novo* synthesis of FFA on TRAS and LR rate remains to be further studied, but, in the case of insufficient transport, such as the absence of *CAV1*,[3] the reduction of LD

deposition during regeneration can be reversed by exogenous supplementation of glucose, which, to some extent, suggests that *de novo* synthesis of FFA plays a role in LD deposition and is important evidence that transport and *de novo* synthesis complement each other.

Glucocorticoid receptor (GR)

Glucocorticoids (GC) promote gluconeogenesis in the liver by facilitating the transport of protein and lipid metabolites in skeletal muscle and liver, respectively. Long-term administration of GC leads to severe metabolic syndrome with enhanced expression of genes involved in glycolipid metabolism after binding to GR in the liver.[118] One study demonstrated that *GR* mRNA expression is elevated twice, 3–6 hours and 24–36 hours, after PH.[119] TG accumulation and regenerative capacity are significantly inhibited following PH in mice with hepatocyte-specific knockout of *GR*.[75] However, exogenous supplementation with GC has been found to inhibit DNA synthesis and perturb LR after PH in rats.[120] These contradictory results seem to indicate that the biological effects mediated by *GR* expression are not only due to the direct effect of GC but also by internal regulation. Many studies have been conducted on the regulation of *GR* activation during lipid metabolism in the basic state. Dexamethasone is a commonly used GC. Resection of the adrenal gland in rats did not change *FABP* expression in the liver, but dexamethasone treatment significantly reduced the expression of *FABP*.[121] Long-term chronic administration of dexamethasone can increase the expression level of *CD36* in the liver of mice, increase the transport of FFA into the liver, and lead to fatty liver.[122] Similarly, dexamethasone treatment increased the expression of *FATP* in the chicken liver.[123] GR activation affects LD metabolism in the liver in 2 ways. The activation of GR by CORT118335 can only induce the partial effect of GC, mainly by promoting the secretion of VLDL in hepatocytes, reducing the deposition of lipids in hepatocytes, and not increasing the transfer of FFA into hepatocytes.[124] GC/GR not only affects the inward and outward transport of FFA in hepatocytes but also increases the synthesis of FFA,[125] lipolysis in adipose tissue,[126] and mitochondrial oxidative phosphorylation.[127] The effect of GR on lipid metabolism in the liver is multilevel, but there are few studies on GR and TRAS, and their relationship needs to be further explored.

Caveolin-1 (CAV1)

In many mammalian cell types, there are abundant submicroscopic plasmalemma pits, which are named caveolae. The functions of caveolae are complex, mainly involving endocytosis, signal transduction, lipid regulation, calcium signal transduction, mechanical sensation, and other processes.[128] Caveolins are the main protein components of caveolae, mainly caveolin-1 (Cav-1), Cav-2, and Cav-3, which are selectively distributed and expressed in tissues,[129–131] and CAV1 and CAV3 are essential for the formation of caveolae.[128] Cav1 is closely related to the physiological and pathological functions of the liver.[132] In all tissues, the expression of CAV1 was the lowest in the liver, but there were obvious differences in different liver cells.[128,133] Three knockout mouse models were used to investigate the relationship between CAV1 and LR expression. The Jackson Laboratory produces ^{jax}*CAV1*-/-, ^{k}*CAV1*-/- mice bred from the kurzchalia laboratory, and $^{BALB/C}$*CAV1*-/- mice produced by mating ^{k}*CAV1*-/- mice with BALB/C mice. The expression of *CAV1* during LR is a process of change. After increasing at the early stage of LR, it becomes phosphorylated at the Y14 site and translocated to the cell membrane, returning to normal levels at approximately postoperative day 5. These results seem to reveal that *CAV1* plays a role in LR. However, another study reported an increased rate of LR in ^{jax}*CAV1*-/- mice, with the liver-to-body weight ratio exceeding that of control mice at 80 hours after PH.[134] However, in both ^{k}*CAV1*-/- and $^{BALB/C}$*CAV1*-/- mice, Manuel et al found that the 3-day survival rate of *CAV1*-/- mice was significantly decreased after 70% PH,[3] accompanied by a significantly poorer level of regenerative liver recovery.[8] In addition to the different effects on the LR process, the effect of *CAV1* on LD deposition during LR was also different in those studies. Using 2 different genetic backgrounds of *CAV1*-/- mice, Manuel et al asserted that the loss of expression of the *CAV1* gene severely inhibits LD deposition during LR. However, a completely different result was reported by Rafael et al. The differing results of the 2 studies may be attributed to several reasons. First, the genetic backgrounds of the mice used by the 2 investigators were different, with the former using C57BL/6J (75% 129 Sv, 20% C57BL/6 J, and 5% SJL mixture) and BALB/C strains and the latter using *CAV1*$^{tm1mls/J}$ strain mice. Second, the sites of gene knockdown in the 2 groups were inconsistent, with the former mainly targeting the exon 3 region and the latter mainly targeting exons 1 and 2. Third, the 3 strains of mice differ in their energy sources, and studies on different genetic backgrounds have found that ^{JAX}*CAV1* mice are more reliant on carbohydrates for energy supply and rely primarily on aerobic glycolysis during LR, not lipid metabolism, unlike the reliance on lipid metabolism exhibited by the other 2 strains of mice.[8,135,136] The effects of CAV1 on LR and lipid deposition may result from multifaceted causes. First, *CAV1* is involved in the mitochondrial FFA oxidative metabolism.[132] Second, it has recently been shown that loss of *CAV1* can lead to enhanced lipid metabolism resulting from the activation of the cAMP signaling

pathway by autocrine PGI2, leading to reduced LD accumulation in cells.[137] CAV1 is an important fatty acid transporter involved in FFA input into hepatocytes.[138] Taken together, the impact of CAV1 on LR has been well-established. The difference in outcomes resulting from different exon knockouts still needs further exploration, and the impact on the occurrence of TRAS may also be a result of their multifaceted interference.

AKT1/2-FoxO1

AKT (protein kinase B) is a serine/threonine protein kinase that contains 3 isoforms, AKT1, AKT2, and AKT3, encoded by 3 separate genes.[139] These 3 AKT isoforms exert various effects and exhibit different biological functions in different disease models. In inflammatory models, *AKT1* null mice exhibit impaired growth, vascular dysfunction, and reduced leukocyte recruitment. Mice deficient in *AKT2* exhibit defects in glucose homeostasis and insulin resistance. *AKT3*-/- mice exhibit impaired brain development.[140–144] The distribution of AKT varies among organs throughout the body, as does its distribution in different cell types in the same organ. Only 2 isoforms, AKT1 and AKT2, are present in the liver, with the expression level of AKT2 being predominant, reaching approximately 85% of the total, and the expression level of AKT1 is relatively high in endothelial and HSC.[139] AKT is an important kinase that mediates LR and plays various roles in different cell types in the LR microenvironment. The activation of AKT in liver parenchymal cells regulates LR after PH.[145] Chronic liver injury resulting from the DDC diet is also accompanied by the activation of AKT, but predominantly AKT1.[146] Activation of AKT is not always favorable for the liver, and p-AKT in endothelial cells enhances the ability of endothelial cells to activate stellate cells, promoting the progression of liver fibrosis. Targeted inhibition of AKT in endothelial cells by nanomaterial-loaded honokiol, which activates ERK1/2 and inhibits AKT, can promote LR in a state of liver fibrosis resulting from bile duct ligation and CCL4 without promoting the progression of liver fibrosis.[147] Adenoviruses expressing AKT can rapidly increase the growth of the liver and, in a short time, can also lead to an obvious increase in liver weight and intracellular stored lipids and glycogen. Inhibition of AKT phosphorylation by blocking PI3K or PDK1 impairs LR.[148–150] These results suggest that the effects of AKT on LR within different cells are 2-sided in different liver disease states. Although AKT1 and AKT2 share their respective functional characteristics, LR is not impaired by specific AKT1 or AKT2 knockout in hepatocytes and is perturbed in AKT1 and AKT2 double knockout mice, resulting in impaired LR. In mice with AKT1/2 hepatocyte-specific knockout, poor LR is accompanied by reduced LD formation.[4] AKT is at the crossroads of exerting biological functions and has complex effects on cell function. There are many regulatory targets upstream and downstream, such as PI3K, GSK3, FOXO, and mTORC1.[151] FoxO1, an important member of the FOXO family, is involved in the regulation of substance metabolism, and the selective regulation of FoxO1 function can regulate hepatocyte glycolipid metabolism.[152,153] Hepatocyte-specific knockout of FoxO1 alleviated the inhibitory effect of AKT1/2 double knockout on LR while restoring the normalization of lipid deposition in the liver.[4] The above results illustrate that the effect of AKT knockout on LD content during LR partially results from the attenuation of FoxO1 phosphorylation with the function of FoxO1 enhancement. However, it is strange that targeted liver FoxO1 gain-of-function mutations can induce diabetes.[154] FoxO1 transcriptionally activates *MTP* and *apolipoprotein CIII* genes, promotes hepatic VLDL assembly, inhibits postsecretory catabolism, enhances LD and VLDL transport outside the liver,[155] and is also able to suppress *de novo* synthesis of FFA in cells.[156] Interestingly, the inhibition of FoxO1 can inhibit the formation of LD in adipocytes, suggesting that FoxO1 has multiple effects on lipid metabolism.[157] Taken together, multiple mechanisms are involved in TRAS caused by reduced inactivation of FoxO1 resulting from AKT knockout.

Replication initiator 1 (Repin1)

Repin1 is widely distributed throughout the body but is relatively highly expressed in the liver and intra-abdominal adipose tissue[158] and is closely associated with obesity, abnormal lipid metabolism, and NAFLD.[159,160] In adipocytes, *Repin1* regulates the expression of genes involved in adipogenesis, LD formation and fusion, and glucose and FFA transport.[161] Hepatocyte-specific *Repin1* deficiency results in dyslipidemia, reduced hepatic lipid deposition, and altered hepatic lipidome structure, preventing adipocyte hypertrophy induced by high-fat feeding.[162] Loss of *Repin1* does not affect *de novo* FFA synthesis but rather affects LD content in hepatocytes. In hepatocytes from Repin1-/- mice, the levels of vehicle-associated membrane protein 4 and synaptosomal-associated protein expression were severely decreased (80% and 40%, respectively), and the expression of CD36 was reduced by half. These results imply that the function of Repin1 is tightly linked to lipid metabolism in the body. Paradoxically, however, a sharp decrease in the mRNA expression level of *Repin1* occurs early after PH (more than 80% reduction at 6h after surgery), but a marked increase in LD accumulation occurs early in LR.[163] This trend indicates that the expression of Repin1 does not seem to have a direct effect on LD deposition during LR. Depletion of hepatocyte Repin1 delays but does not inhibit the outcome of LR and concomitantly inhibits the deposition of LD in the

liver, mainly as indicated by the reduced number of LD and total area of LD.[163] In summary, the relationship between *Repin1* and LD deposition during LR is puzzling, and its effect on TRAS is not a simple surface relationship observed in current studies.

Autophagy related 7(ATG7)

Autophagy is a protective biological process mediated by lysosomes to degrade intracellular materials and is mainly completed by a cluster of autophagy-related proteins and classified into macroautophagy, microautophagy, and chaperone-mediated autophagy.[164] Autophagy is involved in the regulation of lipid metabolism and is particularly important for energy metabolism in the liver.[164,165] Pharmacological inhibition of autophagy (with 3-methyladenine) and knockdown of *ATG5* expression by RNAi both promoted increased intracellular TG levels, and a conditional knockout of *ATG7* in hepatocytes resulted in high levels of intracellular TG and NAFLD presentation.[165–167] Paradoxically, however, another study suggested that Oil Red O staining of liver tissue at 0 hour after PH with the hepatocyte-specific knockout of *ATG7* showed no significant increase in lipid content.[168] Autophagy is involved in LR, inhibition of autophagy impairs LR, and the promotion of autophagy accelerates this process.[169–172] After 70% portal vein ligation in rats, LR on the nonligated side was accompanied by increased expression levels of LC3B and Beclin1, and positively correlated with the expression levels of CyclinD1.[173] Similarly, enhanced autophagy was observed in LR after PH.[174] The simultaneous presence of enhanced autophagy and increased LD deposition during LR seems to be understandable. Poor diet and reduced blood glucose levels in rodents following PH, similar to the starvation state, induce hepatocyte autophagy and increase FFA delivered from adipose tissue lipolysis to the liver.[165] *ATG7* knockout, similar to *ATG5* knockout, resulted in an enlarged liver in the absence of injury,[168,175] but the inhibition of autophagy resulting from *ATG7* knockdown in hepatocytes attenuated the accumulation of LD and inhibited LR.[168] In wild-type mice, starvation[176] and PH[177] induce an increase in FGF21 expression levels, and FGF21 not only promotes autophagy but also increases FFA metabolism in the liver. Hepatocyte-specific knockout of *ATG7* leads to impaired LR after PH and inhibits LD deposition, which may be due to many reasons. Inhibition of autophagy can inhibit the oxidation of FFA and reduce the transmission of TG by VLDL.[178] Therefore, *ATG7* knockout can also reduce intracellular LD consumption to a certain extent. However, in mice with a hepatocyte-specific knockout of *ATG7*, the expression level of liver FGF21 was significantly increased, which could compensate for the inhibitory effect of autophagy reduction to some extent.[179] In addition to stimulating liver FFA oxidation, FGF21 also reduces FFA flux into the liver by increasing peripheral lipoprotein catabolism and reducing lipolysis of adipocytes.[180] Eventually, in hepatic-specific knockout ATG7 mice, FGF21 may play an important role in impairing LD deposition during LR. In summary, autophagy is involved in the maintenance of organ homeostasis. The effects of autophagy on the liver differ in basal and surgical states, and the relationship between autophagy and the maintenance of liver homeostasis and lipid metabolism is complex.

mTORC2

mTOR is a serine/threonine protein kinase that recruits other proteins to form 2 different complexes: mTOR complex 1 (mTORC1) and complex2 (mTORC2). mTORC2 mainly controls cell survival and migration by phosphorylating various kinases, including AKT, PKC, and SGK1.[181] As one of the important targets of mTORC2, AKT plays an important role in LR and lipid deposition during LR.[4] What role does mTORC2 play in the LR? First, in C57BL/6 mice with hepatocyte-specific knockout *Rictor*, the slight decrease in liver weight and the decrease in liver/body weight ratio in 5–8-week-old mice were mainly due to changes in cell size rather than changes in cell proliferation. The mTOR signaling pathway is activated during LR after PH and is accompanied by AKT activation. Mice with *Rictor* knockout in hepatocytes showed significant inhibition of LR after PH, with increased postoperative mortality and reduced deposition of LD.[145] However, another study found that a hepatocyte-specific *Rictor* knockout resulted in the inhibition of LR and hepatocyte proliferation, accompanied by increased intrahepatic LD deposition. The main reason for this is that the activity of PPARα in the liver is inhibited, and the oxidative metabolism of FFA is severely inhibited.[182] mTORC2 and AKT1 were activated in the DDC diet-induced chronic liver injury model. Since the source of proliferating hepatocytes in LR induced by the DDC diet differs from that following PH, *Rictor* knockdown only reduces the proliferation of oval cells in the liver.[146] LR is clearly inhibited in mice with hepatocyte deficiency of mTORC2. In terms of lipid deposition, it is surprising that researchers have obtained 2 completely opposite conclusions, which may require more objective experimental studies.

Adipose differentiation-related protein (ADRP, Plin2)

LD protein is an important protein component of LD, and in hepatocytes, there are 5 different protein types, Perilipin1(Plin1)–Plin5,[183,184] and each perilipin protein

has distinct roles. Adipose differentiation-related protein is the only widely expressed constitutive LD protein, and its expression level of Plin2 is correlated with the TG content and density of LD.[185,186] The TG content in hepatocytes was significantly reduced in *Plin2*-/- mice; however, hepatic lipogenesis, VLDL secretion, and lipid uptake and utilization were not significantly different from those in WT mice,[187,188] despite uncontrolled VLDL-mediated lipid efflux following PH.[189] *Plin2* deficiency led to lipid reduction, whereas, in Atg7-/- mice, *Plin2* deficiency did not reduce lipid levels. Therefore, the authors conclude that *Plin2* deficiency induces decreased LD deposition caused by autophagy in mouse hepatocytes.[190] In hepatocyte-specific *Plin2* knockout mice, the function of ATGL in hepatocytes is increased, thereby promoting lipolysis.[191] The increased function of autophagy and ATGL may be an important reason for the reduction in lipid deposition in the hepatocytes. The above clues seem to be a reasonable explanation for the decrease in LD deposition during LR. In *Plin2*-/- mice, hepatocyte entry into the cell cycle and LD deposition were impaired after PH.[189] Compared with WT mice, there was no difference in the expression of FFA synthesis-related and FFA transport-related genes in the hepatocytes of *Plin2*-/- mice. The observed LD deposition disorder was mainly due to VLDL-enhanced lipid outward transport, resulting in decreased intracellular lipid storage.[189] The reason for the impaired LR may be that insufficient LD deposition decreases the FFA β oxidation energy supply. On the other hand, β oxidation of regenerated livers in Plin2-/- mice is severely inhibited.[188,189] Plin2 is essential for the storage and oxidative metabolism of FFA, and its effects on TRAS are multiple.

Pyruvate dehydrogenase kinase 4 (PDK4)

PDK4 is a key factor in metabolic regulation. It inactivates the mitochondrial pyruvate dehydrogenase complex by phosphorylating pyruvate dehydrogenase, thus affecting pyruvate conversion metabolism. PDK4 coordinates glucose and fat metabolism and maintains normal blood glucose levels.[192,193] The increased expression of PDK4 is an important marker for the conversion of cellular energy metabolism to FFA oxidation metabolism.[194] The mRNA expression level of PDK4 increases in the early stage of LR, and the protein level of PDK4 increases significantly within 24 hours after operation.[9] To a certain extent, this result also indicates that cell energy metabolism shifts to lipid metabolism during LR, which seems to indicate that it plays an important role in promoting LR. Interestingly, *PDK4*-/- mice showed a significant acceleration of LR after PH and increased LD deposition during LR. There are many reasons for the effect of PDK4 on LD deposition. First, the increase in the expression level of Plin2 is important for the retention of VLDL in hepatocytes and reduces the loss of VLDL caused by insufficient Plin2. Second, the expression levels of CD36 and FABP in *PDK4*-/- mice were increased, which enhanced the transport of FFA into hepatocytes. Third, the gene expression levels of FFA *de novo* synthesis decreased. Fourth, the lack of PDK4 enhances the oxidative metabolism of FFA.[9] Therefore, the mechanism by which PDK4 affects TRAS is complex and requires further investigation.

PCSK9

PCSK9 is a mammalian proprotein convertase with complex biological functions.[106] PCSK9 induces the degradation of LDLR, leading to the accumulation of LDL cholesterol in the blood.[195–198] Therefore, affecting the expression or activity of PCSK9 leads to abnormal serum cholesterol.[199–201] Systemic and hepatocyte-specific knockdown of PCSK9 reduced plasma cholesterol levels by 42% and 27%, respectively.[105] However, a significantly greater proportion of hepatic steatosis and higher visceral fat content were observed in the patient population carrying R46L, a low expression mutation of PCSK9.[202] PCSK9 is highly expressed in both fetal and mature livers, and its levels in the regenerating liver after PH peak at 2–3 days in rodents.[105,106] LR potential is impaired in *PCSK9*-/- mice. Interestingly, in *PCSK9*-/- mice, feeding with high cholesterol markedly reduced necrosis in the liver and seemed to reduce liver damage, but the LR index was not elevated. In contrast, in WT mice, high cholesterol feeding increased the lipid content in the liver but decreased the LR index (4.8 ± 0.7 vs. 3.6 ± 0.5), and the extent of the decrease exceeded that in mice on a chow diet *PCSK9*-/- at the same time (3.7 ± 0.5). It is difficult to understand that, although reduced expression or function of PCSK9 promotes fat accumulation in the liver, a significant reduction in lipid content occurs in the regenerated liver 72 hours after PH in *PCSK9*-/- mice.[105] The decrease in lipid content is accompanied by the inhibition of LR. The effects of PCSK9 on lipid metabolism in hepatocytes are complex. As mentioned earlier, PCSK9 affects cholesterol metabolism by inducing LDLR degradation. Second, because PCSK9 induces CD36 degradation, in *PCSK9* knockdown mice, increased CD36 expression levels promote FFA uptake by hepatocytes, ultimately leading to increased TG content in the liver.[203] Third, PCSK9 promotes VLDL secretion from the liver and increases TG export outside the liver, whereas loss of PCSK9 expression reduces VLDL secretion and promotes TG accumulation within the liver.[204] Fourth, PCSK9 also increases the levels of the lipid-generating enzymes FAS, stearoyl-CoA desaturase, and diacylglycerol acyltransferase 2, thereby

participating in *de novo* FFA synthesis and leading to an increase in FFA.[205] In summary, it is not difficult to determine that the effect of PCSK9 on LR and TRAS is extremely complex, and its specific mechanism requires further exploration.

LIPE in adipose tissues

LIPE, also known as hormone sensitive lipase (*HSL*), encodes an enzyme responsible for TG decomposition and increased production of FFA.[52] As previously mentioned, we have analyzed and summarized the regulatory mechanism of lipolysis in adipose tissue and its connection to LR. During the early stages of LR, the weights of epididymal and inguinal white adipose tissues were significantly decreased, accompanied by increased phosphorylation of HSL in adipose tissues.[2] These findings not only indicate the influence of HSL in peripheral adipose tissue during LR but also suggest the involvement of peripheral adipose tissue lipolysis in the redistribution of lipids induced by LR. Adipose tissue-specific knockout of LIPE significantly reduced LD deposition during LR and decreased FFA in peripheral blood.[2] The liver weight-to-body weight ratio and Ki67 expression demonstrated that the knockout of HSL in adipose tissue inhibited LR.[2] Most previous studies have focused on the energy supply aspect of TRAS's effect on LR.[7,8] However, through an in-depth exploration of the impact of LD on LR, Chen et al discovered that TRAS formation inhibits MIER1 translation by enhancing EIF2S1 phosphorylation, thereby initiating LR.[2] Notably, a recent study indicated that embryonic stem cells have higher LD content and a lower rate of lipid hydrolysis, with enhanced LD catabolism occurring during differentiation.[206] This suggests a potential role for LD in maintaining the stemness of embryonic stem cells. Does TRAS formation affect the reentry of hepatocytes into the cell cycle during LR? Interestingly, *in vitro* treatment of hepatocytes with palmitic acid increased EIF2S1 phosphorylation and decreased cellular protein synthesis.[2] The enhanced phosphorylation of EIF2S1 caused by TRAS coincides with extensive protein translation during the early stages of regeneration. Inhibition of EIF2S1 phosphorylation in colon cancer cells reversed the inhibitory effect of 3,3'-diindolylmethane on cyclin D1 translation.[207] Studies have shown increased expression of CyclinD1 protein during the early stages of LR.[208] The inhibition of MIER1 translation by TRAS may partially contribute to the initiation of LR, but the process of hepatocyte reentry into the cell cycle for regeneration initiation is complex, and the mechanism of TRAS's effect on LR requires further exploration.

LD deposition inhibits LR

PPARα

PPARs are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily, and FFA is one of their common ligands. They regulate key aspects of energy metabolism within cells. PPARs play pivotal roles in the regulation of hepatic lipid metabolism.[209] In mammals, there are 3 isoforms of PPAR, alpha (α), beta/delta(β/δ), and gamma (γ), which are differentially expressed in various tissues, with PPARα being the predominant isoform in the liver.[209] In rodents, sustained activation of PPARα by endogenous or exogenous compounds leads to hepatocyte swelling, inhibition of apoptosis, and promotion of hepatocyte proliferation, resulting in hepatomegaly, which can eventually progress to liver tumors. However, in humans, it does not cause hepatocarcinogenesis, mainly because of differences in the structure and function of PPARα between mouse and human.[210,211] In the C57BL/6 strain genetic background, increased expression of *PPAR*α accompanies the process of LR after PH[6] and is involved in regulating LR.[212,213] However, global deletion of *PPAR*α in mice in the SV/129 genetic background did not affect the LR process after PH.[214] In addition, in the C57BL/6 mice, Elizabet et al. also showed that *PPAR*α depletion had no significant effect on LR. However, only a one-time point BrdU-positive result was given in the article, and the LR process was not analyzed comprehensively.[1] Several studies have shown that both global and liver-specific knockdown of *PPAR*α expression in the C57BL/6 mice can suppress PH-induced LR.[6,215,216] The reason for the inconsistent effect of PPARα on LR across studies may lie in the fact that the effects of hepatocyte and nonhepatocyte knockout on LR are different. A recent study showed that, in bone marrow cell-specific *PPAR*α knockout mice, knocking down *PPAR*α in bone marrow cells led to the differentiation of macrophages into M1 type, immersed in the liver during LR, and secreted a large number of important cytokines, such as TNFα and IL6,[217] thereby accelerating LR after PH. Both whole-body and hepatocyte-specific PPARα knockdown showed increased LD deposition during LR.[6,216] PPARα is a key factor in controlling lipid β oxidation, and activators of PPARα can mitigate the degree of fatty liver.[218] Knockdown of *PPAR*α leads to severe inhibition of FFA β oxidation and increased lipid accumulation in hepatocytes and is an important trigger for impaired LR and increased lipid deposition during LR. PPARα in different tissues exerts different effects on LR, and the effects of PPARα in hepatocytes on LR and TRAS may largely depend on the function of FFA oxidative metabolism.

PERSPECTIVE: HOW TO GAIN A CORRECT INSIGHT INTO THE RELATIONSHIP BETWEEN LR AND LD IN FATTY LIVER AND TRAS?

To better probe LD deposition during LR, we must clarify that the relationship between LD deposition and LR is not that between fatty liver and LR. Hepatic steatosis, a pathological concept characterized by massive intracellular accumulation of neutral fat, is an important pathological change that occurs during the progression of several diseases, such as viral infection, alcohol consumption, metabolic syndrome, obesity, and diabetes.[219–221] LDs are ubiquitous "organelles" that store neutral lipids in various cells, and the metabolic imbalance of LD is an important reason for their massive accumulation in cells, leading cells to steatosis.[222] The LD membrane originates from the phospholipid monolayer structure in the endoplasmic reticulum, and the proteins on the surface of the LD are essential for coordinating the continued accumulation and decomposition of LD. LDs in hepatocytes are mainly composed of triacylglycerol, cholesteryl esters, or retinyl ester.[223]

Mitochondrial dysfunction has been found in patients and animal models of NAFLD, and endoplasmic reticulum stress further promotes hepatocyte injury and accelerates NAFLD progression.[224–226] Hepatocyte injury activates cells in the regenerative microenvironment, such as stellate cells, which release inflammatory and growth factors[227,228] that together orchestrate and promote hepatocyte proliferation to maintain liver structural, metabolic, and functional homeostasis. However, the regenerative response in steatotic livers after injury remains controversial in the presence of exogenous injuries, such as PH.[1,229,230] In recent years, there has been a gradual consensus that severe steatosis plays a detrimental role in regeneration and function after PH and liver transplantation.[231,232] Severely steatotic livers (>60% macrovesicular steatosis) are routinely excluded from transplant donor livers because they often cause severe postoperative complications, such as primary nonfunction.[232] More than 20% of patients who are prepared for PH have varying degrees of steatosis, which is also a significant risk factor for increased postoperative mortality.[231] In humans, the above evidence indicates that steatosis has significant adverse effects on LR and liver function. However, the effect of hepatocyte steatosis on LR has not yielded consistent results in rodent models of NAFLD. In murine NAFLD models caused by ob/ob and db/db knockouts, significant LR failure was not rescued by leptin supplementation after PH. The relationship between hepatic steatosis and LR may not be accurately revealed by ob/ob and db/db knockout–induced NAFLD models.[233,234] In another model of NAFLD induced by the methionine and choline defi diet (4 wks), steatosis did not affect LR after PH,[235] and in rats (5 wks of feeding), steatosis was able to inhibit LR resulting from portal vein ligation.[13] Svenja et al found that a high-fat diet for 6 weeks enhanced LR induced by 70% PH.[236] However, Gao et al did not find any interference with LR in rats fed a high-fat diet for 6 weeks.[237] Another study indicated that LR after associating liver partition and portal vein ligation for staged hepatectomy was inhibited in a rat model of high-fat diet-induced NAFLD rat model.[12] More interestingly, after PH in mice fed a high-fat diet for 9–10 weeks, LR was accelerated at 2 d and inhibited at 8 d, respectively.[238] The reasons for the different results of the above studies may be complex, including animal strains, fatty liver animal model production methods, liver injury methods, and observation time. Here, we briefly summarize the information on the relationship between NAFLD and LR after PH, as shown in Supplemental Table S2. http://links.lww.com/HC9/A521.

Only by gaining the correct insight into the relationship between LR and LD in fatty liver and TRAS can LD be better used to coordinate LR. Therefore, further improvements are still needed in animal models and methods: (1) avoid changing the metabolic background of the animal liver; (2) does not affect lipid content in the basal state of the liver; (3) advanced techniques should be applied to further analyze the composition of the deposited LD; and (4) relationship between LD deposition and zonation of regeneration might be a new focus. A clear understanding of the relationship between TRAS and LR may provide a safer and more scientific direction and recognition for promoting LR. From the literature, we can find that there is a regulable space for the effect of lipid metabolism on LR. Therefore, targeting the mechanisms of TRAS formation and depletion holds promise for developing safe approaches to harness lipid metabolism to promote LR; however, some issues still need to be addressed. (1) What factors promote adipose tissue lipolysis during LR? (2) Transport of FFA depends on 4 transporters, the malfunction of which one can result in impaired LR? (3) How to maintain the storage of LD and reduce fat output in the liver? (4) How can the LR process be regulated by promoting oxidative metabolism? Ultimately, multifaceted synergistic control may be a safe and effective method to promote LR.

AUTHOR CONTRIBUTIONS

Yuelei Hu and Ruilin Wang: Data curation, formal analysis, and writing and original draft; Yuelei Hu and Juan Liu: Conceptualization; Jiahong Dong and Yunfang Wang: Conceptualization, supervision, validation, and writing and review editing.

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CONFLICTS OF INTEREST

The authors have no conflicts to report.

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