The interaction of hepatic lipid and glucose metabolism in liver diseases

Lars P. Bechmann, Rebekka A. Hannivoort, Guido Gerken, Gökhan S. Hotamisligil, Michael Trauner, Ali Canbay

1Department of Gastroenterology and Hepatology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany; 2Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; 3Departments of Genetics and Complex Diseases and Nutrition, Harvard School of Public Health, Boston, MA, USA; 4Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Austria

Summary

It is widely known that the liver is a central organ in lipogenesis, gluconeogenesis and cholesterol metabolism. However, over the last decades, a variety of pathological conditions highlighted the importance of metabolic functions within the diseased liver. As observed in Western societies, an increase in the prevalence of obesity and the metabolic syndrome promotes pathophysiological changes that cause non-alcoholic fatty liver disease (NAFLD). NAFLD increases the susceptibility of the liver to acute liver injury and may lead to cirrhosis and hepatocellular cancer. Alterations in insulin response, β-oxidation, lipid storage and transport, autophagy and an imbalance in chemokines and nuclear receptor signaling are held accountable for these changes. Furthermore, recent studies revealed a role for lipid accumulation in inflammation and ER stress in the clinical context of liver regeneration and hepatic carcinogenesis. This review focuses on novel findings related to nuclear receptor signaling – including the vitamin D receptor and the liver receptor homolog 1 – in hepatic lipid and glucose uptake, storage and metabolism in the clinical context of NAFLD, liver regeneration, and cancer.

Keywords: NAFLD; Fatty acid transporters; HCC; Liver regeneration; Nuclear receptors; ER stress.

Introduction

As the main detoxifying organ of the body, the liver also plays a central role in metabolic homeostasis and is a major site for synthesis, metabolism, storage and redistribution of carbohydrates,
proteins and lipids. The rapid increase in obesity worldwide is associated with an increase in the prevalence of non-alcoholic fatty liver disease (NAFLD), making NAFLD the most common liver disease in Western societies [1,2]. In order to understand the pathogenesis of NAFLD, we discuss the basic physiologic mechanisms of hepatic lipid and glucose metabolism. We also aimed at integrating recent clinical and mechanistic data and point out novel links between basic metabolic pathways and the pathophysiologies of NAFLD, liver regeneration, and carcinogenesis.

NAFLD is characterized by lipid accumulation within hepatocytes and may progress to non-alcoholic steatohepatitis (NASH). Lipids derive from circulating fatty acids (FA) upon insulin resistance (IR)-induced dysregulation of peripheral lipolysis. FAs are translocated into the hepatocyte mainly by membrane bound transport proteins [3]. De novo lipogenesis (DNL) further contributes to hepatic steatosis [4]. Hepatocellular accumulation of lipotoxic intermediates such as diacylglycerol (DAG) and ceramides causes hepatic IR [5]. Hepatocytic lipid accumulation predisposes to overproduction of reactive oxygen species (ROS), endoplasmatic reticulum (ER) stress and lipotoxicity [6–8]. Recently, autophagy (especially in the form of macrolipophagy) has been identified to regulate intracellular lipid stores through degradation of lipid droplets and release of FAs into the cytosol as a rapid response to starvation [9–11]. Thus, disrupted autophagy might be essential to the pathogenesis of NASH via lipotoxicity-induced ER stress [12].

The individual steps in hepatic lipid metabolism are orchestrated by a delicate interplay of hormones, nuclear receptors, intracellular signaling pathways and transcription factors. Insulin signaling plays an important role in the regulation of FA metabolism, underscoring the close relation between lipid and glucose metabolism. Insulin affects DNL on multiple levels, including novel mechanisms like vitamin D receptor (VDR) and the bile acid receptor/farnesoid X receptor [FXR] [18–20]. On a transcriptional level, SREBP-1c and carbohydrate-responsive element binding protein (ChREBP), a glucose dependant transcription factor, synergistically induce expression of FAS and ACC [23].

As mentioned above, hepatic FAs either derive from endogenous lipogenesis, are released from lysosomes by autophagy, or derive from the free FA (FFA) plasma pool via active uptake into the hepatocyte. Depending on the metabolic state, FAs are then either processed to TAGs and stored or rapidly metabolized. Indeed, $\beta$-oxidation is the predominant source of energy during the fasting state.

Hepatic lipogenesis includes de novo synthesis of FAs from acetyl-CoA or malonyl-CoA and further processing to TAGs. In mammals, FA synthesis is catalyzed by acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) – an enzyme that is complexly regulated by various nuclear receptors (PPAR, PPAR$\gamma$ and the bile acid receptor/farnesoid X receptor [FXR]) [18–20]. FA elongation requires NADPH as a reducing reagent, which is provided by the pentose phosphate pathway (Fig. 2B). Remarkably, PPAR$\gamma$ itself is activated by a phospholipid synthesized by FAS, indicating a feedback loop [21].

A close link between glucose and lipid metabolism is indicated by the fact that nuclear receptors (NRs) are also important mediators of insulin signaling and since DNL occurs under anaerobic condition. The existence of such a link is further supported by the fact that insulin stimulates FAS expression via the phosphoinositide-3-kinase (PI3K) pathway [22]. On a transcriptional level, SREBP-1c and carbohydrate-responsive element binding protein (ChREBP), a glucose dependant transcription factor, synergistically induce expression of FAS and ACC [23].

As FAs and their metabolites are the major cause for lipotoxicity and promote the formation of ROS, FAs are stored for future use as TAGs, which are relatively inert and consist of three FAs esterified to a glycerol backbone. TAGs are then either stored in lipid droplets within the hepatocyte or processed to VLDL [7]. TAG synthesis is catalyzed by the enzymes mitochondrial glycerol-3-phosphate-acyltransferase (mtGPAT) and diacylglycerol-acyltransferase (DGAT) [24]. TAGs are then packaged into VLDL particles, by conjugation to apoB-100 in a 5:1 TAG/cholesterol ratio.
ratio. These processes are controlled by SREBP-1c, the liver X receptor (LXR), FXR and ChREBP, which again links glucose and lipid metabolism [25].

**Hepatic fatty acid uptake**

Another source for hepatic FAs is FFA recruitment from the plasma pool. FFAs are derived from lipolysis in adipocytes. This occurs usually in the fasting state, where it is promoted by catecholamines, natriuretic peptides and glucagon, while it is usually repressed by insulin [26]. However, the insulin-resistant state (obesity; metabolic syndrome) goes along with increased adipocyte lipolysis, leading to abundant FFAs in the plasma pool independently from the nutritional status [27]. FFAs are then taken up by the hepatocytes in a facilitated fashion rather than by passive processes [28]. FATPs are thus in the focus of NAFLD research in which a variety of FATPs have been identified. While FATP1 is abundant in muscle and adipose tissue and is barely detectable in the liver [29], FATP2 and FATP5 are expressed in hepatocytes and most likely facilitate the major amount of FA uptake in the liver [30]. Other transport proteins include fatty acid–binding protein (FABP), glutamate–oxaloacetate-transaminase 2 (Got2; or mitochondrial aspartate aminotransferase [mAspAT]), a membrane bound protein that mediates the endocytotic uptake of long-chain FAs, and caveolin-1 [31–33]. Fatty acid translocase (CD36/FAT) is a membrane glycoprotein present on platelets, mononuclear phagocytes, adipocytes and hepatocytes with multiple functions, including thrombospondin-1 receptor

<table>
<thead>
<tr>
<th>Nuclear receptor</th>
<th>Natural ligands</th>
<th>Chemical/ligands’ agonists</th>
<th>Function in lipid metabolism</th>
<th>Function in glucose metabolism</th>
<th>Role in NAFLD</th>
<th>Role in liver regeneration</th>
<th>Role in HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα</td>
<td>Fatty acids</td>
<td>Fibrates</td>
<td>Regulates expression of FAS → lipogenesis; CD36/FAT, FATPs → FA-Uptake; Acetyl-CoA-synthetase, CPT-1 → β-oxidation</td>
<td>Regulates PEPCK, GSK3, Glycogen synthase → glycogen metabolism; insulin sensitivity</td>
<td>Fibrate treatment improves IR</td>
<td>Delayed regeneration in PPARα KO mice</td>
<td>PPARα activation is associated with liver carcinogenesis</td>
</tr>
<tr>
<td>PPARy</td>
<td>Prostaglandins</td>
<td>Glitazones</td>
<td>Regulates expression of CD36/FAT → FA-Uptake; SCD-1 → FA-metabolism</td>
<td>Regulates GLUT-4 expression → insulin sensitivity</td>
<td>Activation; PPARy KO mice are protected from diet induced steatosis; Glitazones improve IR and TAG accumulation and increase adiponectin levels</td>
<td>Downregulated; glitazones inhibit hepatocyte proliferation</td>
<td>PPARy KO increases susceptibility to hepatic carcinogens; PPARy activation induces cell cycle arrest in hepatoma cells</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Fatty acids</td>
<td>Glitazones; GW501516</td>
<td>Regulates SREBP-1c → lipogenesis</td>
<td>Induces glycolysis and pentose phosphate pathway shunt</td>
<td>Agonist treatment improves hepatic steatosis in mice</td>
<td>Agonist treatment improves liver regeneration in mice</td>
<td>PPARγ affects COX2 expression in hepatomas</td>
</tr>
<tr>
<td>FXR</td>
<td>Bile acids</td>
<td>Chenodeoxycholic acid (CDCA); GW4064</td>
<td>Regulates SREBP-1c → lipogenesis; Regulates VLDL formation via SHP</td>
<td>Regulates PEPCK, glucose-6-phosphatase → gluconeogenesis</td>
<td>Induced; SHP is upregulated in NAFLD; FXR KO mice develop steatosis</td>
<td>Mediates liver regeneration after partial hepatectomy; impaired regeneratory capacity in FXR KO mice</td>
<td>FXR KO mice develop hepatic tumors; interaction with Wnt/β-catenin signaling</td>
</tr>
<tr>
<td>LXRα</td>
<td>Hydroxysterols</td>
<td>T0901317; GW3965</td>
<td>Regulates SREBP-1c, SCD-1, FAS → lipogenesis; cholesterol metabolism</td>
<td>Regulates insulin receptor expression, GLUT-4 and IRS expression → insulin sensitivity</td>
<td>Induced, LXR promotes hepatic lipogenesis</td>
<td>Reduced activation after partial hepatectomy</td>
<td>Unknown</td>
</tr>
<tr>
<td>VDR</td>
<td>(1,25-Hydroxy- vitamin D3</td>
<td>Calcitriol-derivates</td>
<td>VDR represses PPARα signaling, direct effects unknown</td>
<td>VDR represses PPARα signaling, direct effects unknown</td>
<td>Downregulated; VDR KO mice develop steatosis; vitamin D protects from diet induced steatosis</td>
<td>Vitamin D deficiency leads to impaired hepatic regeneration</td>
<td>VDR polymorphisms are associated with HCC</td>
</tr>
<tr>
<td>LRH1</td>
<td>Phospholipids</td>
<td>Dilauroylphosphatidylcholine (DLPC)</td>
<td>Repression of SHP potential effects on VLDL synthesis; DLPC treatment suppresses SREBP-1c, FAS, ACC-2 and SCD-1 expression in vivo</td>
<td>DLPC treatment reduces hepatic gluconeogenesis and improves insulin response in vivo</td>
<td>DLPC treatment improves hepatic TAG and FA accumulation</td>
<td>Unknown; potential proliferative effects</td>
<td>Unknown; repression of SHP might promote tumor growth</td>
</tr>
</tbody>
</table>

**Table 1. Overview of ligands and function of the most abundant nuclear receptors in hepatocytes.**
Fig. 1. Hepatic lipid metabolism in health and disease. (A) Dietary lipids are emulsified in the intestinal tract by bile acids (BAs). Hydrolyzed lipids are absorbed by enterocytes and packed into nascent chylomicrons (NCs). NCs enter the bloodstream via the thoracic duct where they receive important apoproteins (apoE; apoC-II) from HDL. These apoproteins are important for chylomicrons to deliver TAGs and FAs to adipocytes and myocytes via lipoprotein lipase (LPL) degradation. Chylomicron remnants are taken up by hepatocytes via LDL-receptor (LDLR) and LDL receptor-related protein (LRP)-mediated endocytosis. BA synthesis is regulated by LRH1 and FXR, which activate BA export pumps. BA re-uptake by enterocytes stimulates FGF-19 release into the portal blood, which inhibits BA synthesis. (B) Free fatty acids (FFA) derive from lipolysis in adipose tissue and are actively taken up by various FA transporters under the control of insulin (Ins) and nuclear receptor signaling. Under physiologic conditions, the bulk of FAs is oxidized intramitochondrially and provides ATP and acetyl-CoA for the tricarboxylic acid cycle (TCA). Triglycerides (TAGs) derived from de novo lipogenesis are either stored in lipid droplets (LD) or packed into VLDL and exported into the bloodstream. Acetyl-CoA for de novo lipogenesis is provided by the pyruvate dehydrogenase complex (PDC), which catalyzes oxidation of pyruvate, the end product of glycolysis. (C) Under physiologic conditions, β-oxidation of short-, medium- and long-chain FAs (SCFA, MCFA, LCFA) are degraded in mitochondria. Therefore, FAs are activated to acyl-CoA and shuttled across the mitochondrial membrane by carnitine palmitoyltransferase-1 (CPT1). Malonyl-CoA, an intermediate of lipogenesis, inhibits CPT1 and thus FA oxidation in the mitochondria. With FA abundance and in the insulin resistant state, LCFA and very-long-chain FAs (VLCFA) are oxidized in peroxisomes and the ER. This leads to the induction of hepatocyte apoptosis, the invasion and activation of inflammatory cells, as well as fibrogenesis.
activity, which has also been identified to facilitate FA uptake [34,35]. Besides the fact that the regulation of FATP activity is generally complex, the individual contribution of these FATPs to FA uptake has not been entirely clarified yet. Nevertheless, signaling via PPARα again predominantly regulates the transcription of these transport proteins as combined with hormonal regulation via insulin and leptin [30,36].

Macrophagy

Autophagy has recently been implied to play a role in hepatic lipid homeostasis [37]. As a lysosomal pathway, it recycles dispensable cellular constituents into important energy sources during the fasting state [38]. Recent animal studies revealed that autophagy is a key process in hepatic lipolysis and lipid droplet degradation [10,39]. As mentioned above, lysosomes process chylomicron remnants as well as TAGs that accumulate during hepatic lipogenesis. During starvation, macroautophagy leads to the fusion of lysosomes and lipid droplets into autophagosomes, which are then degraded; FAs are thus released and can be catabolized via β-oxidation. Starvation leads to repression of the so-called mammalian target of rapamycin (mTOR), an insulin downstream target that inhibits autophagy. Interestingly, rapamycin treatment also downregulates SREBP-1c in primary hepatocytes, which suggests an effect of rapamycin, independent of mTOR, on the activity of forkhead box protein O (FoxO) [40]. Long-term repression of autophagy is accounted for by insulin action, and Akt mediated de-phosphorylation of FoxO, a transcriptional activator of autophagy related genes (ATGs). Indeed, FoxO simultaneously represses SREBP-1c activation and thus DNL. Thus, insulin receptor activation induces DNL and represses autophagy-mediated lipid droplet degradation, both short- and long-term, via two distinct mechanisms. However, in the insulin-resistant state and in obesity per se, hepatic mTOR is over-activated and calpain, a repressor of

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Fig. 2. Regulation of hepatic glucose metabolism. (A) After intestinal absorption, glucose (Glu) reaches the hepatocyte via the portal vein. The insulin-independent glucose transporter 2 (GLUT2) shuttles Glu across the membrane. Abundance of glucose induces conformational changes of the glucokinase regulatory protein (GCKR), which binds to glucokinase (GCK) and keeps it in the nucleus in the fasting state. GCK is then released into the cytosol and phosphorylates Glu to glucose-6-phosphate (Glu-6-P); depending on the nutritional state, it serves as a substrate for glycolysis or glycogen synthesis, respectively. GCK is transcriptionally regulated by insulin and nuclear receptor signaling. (B) Glu-6-P is a central intermediate in the hepatic glucose metabolism. It is degraded during glycolysis, which provides energy in the form of two ATP and two NADH molecules per glucose molecule. The product pyruvate is further decarboxylized to acetyl-CoA, which enters the intramitochondrial tricarboxylic acid cycle (TCA). Alternatively, Glu-6-P is degraded in the pentose-phosphate shunt, which provides NADPH, a cosubstrate for DNL. Acetyl-CoA is an important product of the TCA, linking glucose and lipid metabolism, as it is the substrate for DNL (Fig. 1). Gluconeogenesis and glycogenolysis provide Glu-6-P as a substrate for glucose synthesis in the fasting state. Gluconeogenesis is catalyzed by glycogen phosphorylase, activated by AMP, and repressed by insulin. The key enzyme in gluconeogenesis is PEPCK, which is repressed by insulin signaling via Akt-mediated FoxO phosphorylation and activated by PPARα.
ATGs, is induced [9]. Despite FoxO activation in IR, mTOR and calpain activation account for the repression of hepatic autophagy in obese individuals.

**Fatty acid oxidation**

Oxidation of FAs occurs within mitochondria, peroxisomes and the ER, and facilitates degradation of activated FAs to acetyl-CoA. It is a rapid and effective way of energy allocation since, for example, the oxidation of one molecule of palmitate produces up to 129 ATP equivalents [41]. FAs are activated by acyl-CoA synthetase to acyl-CoA in the cytosol. This process is indispensable for enabling FAs to cross membranes and enter organelles. While short- and medium-chain FAs pass the mitochondrial membrane without activation, activated long-chain fatty acids (LCFAs) are shunted across the membrane via carnitine palmitoyltransferase-1 (CPT1). Malonyl-CoA, an early intermediate of DNL that accumulates upon insulin receptor activation, is an allosteric inhibitor of CPTI [42]. Thus, in the fed state, FA oxidation is inhibited and DNL-promoted, allowing for storage and distribution of lipids [43]. In general, short-, medium- and LCFAs are oxidized within mitochondria (β-oxidation), while toxic, very-long-chain FAs are oxidized within peroxisomes (Fig. 1C). In diabetes or FA overload, cytochrome P450 (a.k.a. CYP4A)-dependent β-oxidation of LCFAs occurs in the ER and induces ROS and lipid peroxidation [44]. During the process of β-oxidation, electrons are indirectly donated to the electron transport chain to drive ATP synthesis. Acetyl-CoA can be further processed via the tricarboxylic acid cycle (TCA) or, in the case of FA abundance, be converted into ketone bodies [45]. PPARα and insulin signaling are again involved in the regulation of FA oxidation and the formation of ketone bodies via transcriptional regulation of mitochondrial HMG-CoA synthase. Interestingly, succinyl-CoA, an intermediate of the TCA, inactivates mitochondrial HMG-CoA synthase by succinyllation, which once more intercalates glucose- and lipid metabolism [46]. For a simplified overview of key metabolic pathways in hepatocellular lipid homeostasis, see Fig. 1B.

**Key Points 2**

- Insulin activates DNL via PI3K-mediated FAS expression
- SREBP-1c and ChREBP transcriptionally activate FAS and ACC as well as VLDL assembly
- Insulin represses peripheral lipolysis and liberation of FFAs from adipocytes
- Insulin represses autophagy via activation of mTOR and repression of FoxO phosphorylation
- Insulin represses FA oxidation

**Hepatic glucose metabolism**

**Hepatocyte glucose uptake**

In the postprandial state, blood glucose is taken up by the hepatocyte via the glucose transporter type 2 (GLUT2) – a membrane-bound transporter with high capacity and low affinity for glucose. In contrast to GLUT4, which is expressed by muscle and adipose tissue, the expression and activity of GLUT2 is independent of insulin signaling. In pancreatic islet cells, GLUT2 is thus also referred to as a “glucose sensor” [47]. Once taken up by the hepatocyte, glucose is phosphorylated to glucose-6-phosphate by liver glucokinase (L-GCK; Fig. 2), the rate limiting enzyme for hepatic glucose utilization [48]. In contrast to other hexokinases, GCK (syn.: hexokinase IV) is not inhibited by its product, which allows for postprandial glycogen storage within the hepatocyte. In the fasting state, L-GCK is inactive and bound to glucokinase regulatory protein (GCKR) within the nucleus. Post-prandial glucose abundance and insulin-action synergistically cause rapid dissociation of L-GCK from GCKR and translocation to the cytoplasm [49]. L-GCK is transcriptionally regulated by SREBP-1c, hepatic nuclear factor-4-alpha (HNF4α), hepatic nuclear factor 6 (HNF6), FoxO1, and upstream stimulatory factor 1 (USF1) (see Fig. 2A). Indeed, mutations in the GCK gene have been associated with IR and the pathogenesis of maturity-onset diabetes of the young (MODY) in several studies [50,51].

**Glycolysis and glycogen synthesis**

Glucose-6-phosphate is either further processed in glycolysis or utilized for glycogen synthesis, depending on the systemic metabolic state. Glycolysis, a ten-step process, metabolizes glucose to pyruvate with a net gain of two ATP and two NADH molecules per glucose molecule. Glycolysis is regulated by L-GCK, which provides glucose-6-phosphate, phosphofructokinase, which is inhibited by its product fructose-1,6-bisphosphate, AMP and pyruvate-kinase (PK), the final step in glycolysis. PK is activated by its substrate and inhibited by abundance of ATP. Insulin, epinephrine, and glucagon also regulate PK via the PI3K pathway and ChREBP induces transcription of PK in the presence of glucose [23]. Pyruvate is further decarboxylized to acetyl-CoA and then processed in the TCA or utilized for DNL. The pentose phosphate pathway is an alternative way for degradation of glucose-6-phosphate in hepatocytes, which provides the cell with NADPH, an important antioxidant and co-substrate for DNL and cholesterol synthesis. In hepatocellular carcinoma (HCC), glycolytic activity is dramatically upregulated and associated with increased hexokinase 2 activity and expression of GLUT1, leading to altered glucose utilization, which has therapeutic and diagnostic implications (for the so-called Warburg effect, Fig. 3B and C) [52].

Glycogen synthesis is catalyzed by glycogen synthase (GS) after conversion of glucose-6-phosphate to UDP-glucose [53]. GS is regulated by the allosteric activator glucose-6-phosphate and is inactive in the phosphorylated state. Glycogen synthase kinase 3 (GSK3) phosphorolyses GS and is a downstream target of Akt/PI3K and thus insulin signaling. GSK3 is a multifunctional kinase, involved in cell senescence, apoptosis and lipid metabolism via phosphorylation of SREBP-1c [54]. Other protein kinases that phosphorylate GS are AMP-activated protein kinase (AMPK) and protein kinase A (PKA). Insulin activates glycogen synthesis via repression of PKA. GS synthesizes the glycogen polymer, which is further branched by a branching enzyme.

**Glycogenolysis and gluconeogenesis**

In the fasting state, the liver supplies the body with energy by breaking down glycogen, and following prolonged fasting by gluconeogenesis [55]. Glycogen breakdown is catalyzed by glycogen
Fig. 3. Hepatic lipid and glucose metabolism in HCC. (A) Mediators of NAFLD progression also contribute to carcinogenesis in the liver. In general, obesity and diabetes have been identified as risk factors for cancer development as well as inflammation. Cirrhosis is a precancerous condition and, in fact, most HCCs derive from cirrhotic livers. Important mediators of insulin resistance and lipotoxicity also induce dysplasia and carcinogenesis. This figure gives a brief overview of different cytokines and cell signaling pathways involved in HCC development. (B) Expression of GLUT1 in hepatoma cells leads to increased hepatic glucose utilization. Glucokinase is downregulated, but hexokinase 2 (HK2) is now expressed and phosphorylates glucose with a higher affinity. Aerobic glycolysis leads to a rapid, but rather ineffective energy supply to the proliferating cancer cell. However, this process, referred to as the Warburg effect, facilitates uptake and de novo synthesis of nutrients (nucleotides, amino acids, lipids), available for cell proliferation and tumor growth. (C) Clinically, this effect is utilized in PET diagnostics. An increase in cancer cell glucose uptake, as visualized by FDG PET/CT, acts as an important tool in HCC diagnostics.
phosphorylase (PYGL), which cleaves glucose from the glycogen polymer and produces glucose-1-phosphate, which is converted to glucose-6-phosphate by phosphoglucomutase. A debranching enzyme cleaves the last four glucose monomers. PYGL is regulated through allosteric activation by AMP and via phosphorylation by PKA, which is inhibited by insulin.

**Key Points 3**

- **NRs** are highly conserved ligand-activated transcription factors. Specific ligands of most NRs are known, and are either endogenous (bile acids; fatty acids; hormones; vitamins) or exogenous (drugs; xenobiotic toxins), and induce conformational changes affecting the transcriptional activation of downstream targets [64, 65]. Most NRs heterodimerize with the retinoid-X-receptor.

Table 1 sketches the most abundant NRs in the liver, their ligands, and their roles in hepatic lipid and glucose homeostasis.

- **PPARα** is the most abundant PPAR in the healthy liver. It regulates FA oxidation and uptake as well as gluconeogenesis (PEPCK) and glycogen synthesis (GSK3; GS) (see above). Moreover, PPARα activation has anti-inflammatory effects and (in mice) induces hepatocyte proliferation via induction of microRNA let7c-signaling cascades [66, 67]. In mouse models, but not in humans, activation of PPARα induces hepatic carcinogenesis, which is in part explained by ROS production [68, 69] and induction of microRNA let7c that degrades the c-myc oncogene. Initially an orphan NR, PPARα is known to be the receptor for fibrates in the therapy of hypertriglyceridemia and protects from diet-induced steatosis [74, 75]. PPARα activation increases insulin sensitivity, as enterohepatic hormones that circulate with absorbed dietary lipids and fine-tune their metabolism.

- **PPARδ** is mainly expressed in adipocytes, where it promotes lipid uptake and TAG storage by upregulation of the LDL receptor and CD36/FAT, induces stearoyl-CoA-desaturase-1 (SCD1), increases insulin sensitivity by induction of GLUT4, and decreases TNFα levels and thus systemic IR [73]. In NAFLD, PPARδ is upregulated in liver tissue, and liver-specific PPARδ KO mice are protected from diet-induced steatosis [74, 75]. PPARδ has anti-inflammatory features as it represses NFκB signaling [76]. Recently, activation of PPARδ has been shown to reduce hepatic lipogenesis by suppression of SREPB-1c [76, 77] in addition to increasing the hepatic glucose catabolism as well as muscular oxidation.

- **PPARy** co-activator-1α (PGC1α) is a transcriptional co-activator that regulates mitochondrial biology and energy homeostasis [78]. PGC1α is induced by fasting and activates FA oxidation and gluconeogenesis via induction of PPARα, FoxO1 and HNF-4α [79]. Sirtuin 1 (SIRT1) deacetylates PGC1α and thus regulates its activity [80].

- **FXR**, the bile acid receptor and its downstream target, small heterodimer partner (SHP), have recently been identified to play a central role in hepatic lipid metabolism as BA administration lowers blood lipids and the TAGs hepatic content. This effect is partially reversed in Shp KO mice and is mediated by transcriptional repression of the c-myc oncogene. Initially an orphan NR, FXR regulates its activity [80]. FXR activation induces secretion of fibroblast growth factors 15 (mouse) and 19 (human) (FGF15/19), which activates hepatic FA oxidation and insulin response via activation of the FGF receptor 4 [85, 86]. BAs have additional effects on lipid and glucose metabolisms via a G-protein-coupled receptor (TGR5/GBAR), which regulates energy expenditure in brown adipose tissue (and possibly skeletal muscle), and promotes intestinal GLP-1 secretion [87]. Thus, BAs may now be viewed as enterohormones that circulate with absorbed dietary lipids and fine-tune their metabolism.

- **Natural ligands of LXR** are hydroxysterols. LXRα is expressed primarily in hepatocytes, adipose tissue and macrophages, whereas LXRβ is ubiquitously [88]. LXR regulates SREBP-1c, FAS and SCD-1 expression and LXR agonist treatment leads to hyperglycemia secondary to induction of hepatic lipogenesis [89]. LXR increases hepatic glucose uptake by induction of GLUT4 expression and has anti-inflammatory attributes as it modulates the innate immune response [90, 91].

- **LRH1** has recently been implied as a novel NR in BA homeostasis by transcriptional activation of Cyp7A1 [92]. Interestingly, LRH1 activation by dialauroyl phosphatidylcholine improves the hepatic insulin response and hepatic lipid accumulation in high fat diet-fed mice [93]. Accordingly, SREBP-1c, FAS and SCD-1 mRNA levels were downregulated in the treatment group.

- **The VDR** is a novel target in the field of hepatology. Recent studies have shown that vitamin D supplementation protects mice from diet-induced steatosis and VDR KO mice spontaneously develop hepatic steatosis [94, 95]. Polymorphisms in VDR are associated with HCC development in patients with alcoholic cirrhosis, and VDR activation acts antiproliferative effects [96, 97]. In HCV patients, low vitamin D levels are associated with fibrosis progression, and the VDR regulates T-cell activation [97, 98].

Hepatic gluconeogenesis occurs during prolonged fasting episodes and begins intramitochondrially by the induction of pyruvate carboxylase in abundance of acetyl-CoA. Interestingly, inhibition of hepatic CPT-1 and thus mitochondrial fatty acid oxidation significantly represses hepatic gluconeogenesis in mice [56]. Gluconeogenesis is further regulated via allosteric and
Review

transcriptional activation of phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase and glucose-6-phosphatase (G6Pase). Overexpression of PEPCK promotes IR in mice [57]. Insulin action represses PEPCK expression via Akt-mediated FoxO1 phosphorylation. This pathway is repressed by activation of PPARα signaling-mediated induction of the pseudokinase tribbles-homologue 3 (TRB3), and TRB3 expression is associated with IR in patients [58,59]. Other NRs (PGC1α, FXR), glucagon and glucocorticoids also mediate expression of PEPCK and G6Pase [55,60]. FoxO1, a transcriptional activator of PEPCK and G6Pase, is both directly and indirectly activated by PGC1α, HNF4α, CREBP and PPARγ [61]. PEPCK knock-out mice not only show decreased gluconeogenesis, but more importantly a decreased removal of TCA anions, which causes hepatic TAG accumulation and steatosis [62]. In mice, PEPCK overexpression in the striated muscle interestingly leads to longevity and an impressive increase in muscle strength and endurance; this phenotype is partially explained by an optimized mitochondrial oxidation of TAG [63]. These processes again demonstrate the close inter-relation between gluconeogenesis and hepatic lipid metabolism (Fig. 2B).

Hepatic lipid and glucose metabolism in liver injury

Serum FFA levels correlate with hepatocyte apoptosis and FAs were found to activate death receptor-mediated apoptosis [64,65]. On the other hand, high glucose concentrations induce apoptosis in hepatoma cell lines, and the HOMA score is associated with hepatocyte apoptosis in NAFLD patients [66,67]. These observations indicate that hepatocyte apoptosis due to an imbalance in cell metabolism has clinical implications for NAFLD, liver regeneration, as well as fibrogenesis and carcinogenesis. The effects of systemic IR on liver injury via induction of TNFα are discussed in detail below. Intrahepatic fat deposition and obesity decrease hepatic blood flow by direct compression and systemic hypercatecholemia and thus inhibit mitochondrial function and cause formation of ROS, which induces Kupffer cell activation, hypercatecholemia and thus inhibit mitochondrial function and decrease hepatic blood flow by direct compression and systemic lipotoxicity [68]. Interestingly, mitochondrial stress may be partially reversed by treatment with insulin-like-growth factor 1 (IGF-1) and PPARγ agonists [69,70]. Alcohol-fed PPARγ knock-out mice develop a phenotype that mimics alcoholic liver disease in humans, which is linked to ROS accumulation [71]. Overexpression studies identified that the expression of PEPCK links mitochondrial dysfunction with the ER stress response [72].

As mitochondria are the main organelles in energy combustion, the ER is the major site of protein folding and trafficking. Recently, activation of the unfolded protein response (UPR) within the ER has been implied as a key modulator of cellular inflammation and is linked to IR, lipid and glucose metabolism [73]. Three membrane-bound proteins regulate the UPR within the ER; PKR-like eukaryotic initiation factor 2ε kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor-6 (ATF6). The protein kinase PERK affects transcriptional regulation of rRNA, which activates NFxB and ATF4 signaling. NFxB regulates inflammatory signaling (IL-6, TNFα), and ATF4 regulates glucose metabolism [74,75]. SREBP-1c activation occurs during ER stress and thus affects the lipid metabolism – and the ER possibly regulates the number, composition and quality of lipid droplets [76–78]. Furthermore, induction of the mTOR pathway in obese individuals activates SREBP-1c, promotes ER stress and inhibits autophagy, a novel pathway in the biology of hepatic lipid droplets [79,80].

NAFLD, insulin resistance and lipotoxicity

NAFLD represents the most prevalent liver disease in Western societies. It presents with a wide spectrum ranging from simple steatosis or non-alcoholic fatty liver (NAFL) to fully developed NASH with or without fibrosis. NASH can progress to fibrosis with an increased risk to develop end-stage liver disease or hepatocellular carcinoma (HCC) [1]. Hepatic steatosis is defined as an intrahepatic accumulation of TAGs. In parallel, abundant FAs cause lipotoxicity via the induction of ROS release, which causes inflammation, apoptosis, and thus the progression to NASH and fibrogenesis [81]. As described above, most FAs derive from the circulation secondary to increased lipolysis in adipose tissue as well as DNL [3].

Obesity increases the TNFα production in adipocytes, which facilitates adipocyte IR and increases lipolysis rate [82]. Thus, the circulating FFA pool is increased in obese individuals and accounts for the majority of liver lipids in NAFLD [13]. As mentioned, uptake of FFAs into the hepatocyte is facilitated by a variety of FATPs; several studies found an upregulation of these transporters in NAFLD and NASH as well as a correlation with disease severity [65,83,84]. Fifteen percent of the lipid content within the steatotic liver derives from an increased dietary intake of lipids [3]. DNL may account for up to 30% of TAGs in steatotic livers, a mechanism that involves dysregulation in SREBP-1c- and FoxO-mediated hepatic insulin signaling [4,85,86]. Since autophagy-related genes are transcriptionally activated by FoxO and insulin action modulates autophagy, recent studies showed that macroautophagy is dysregulated in the metabolic syndrome [87]. Accordingly, in conditional agα7-knockout mice, Singh et al. observed an increase in hepatic lipid accumulation, and in genetic and dietary mouse models for obesity and hepatic steatosis, autophagy-related genes were downregulated [10,12].

The long-lasting paradigm claiming TAG accumulation to be the “first hit” that predisposes to further liver damage in the pathogenesis of NASH has recently been replaced by a more complex model as emerging evidence points to FAs and their metabolites as the true lipotoxic agents [7]. Interestingly, lipid accumulation and altered composition of phospholipids within ER membranes further promotes ER stress and IR in obese mice [8]. Cytosolic TAGs are thus now considered to be inert and, in fact, lipid droplet accumulation has recently been found to be hepatoprotective [88]. Notably, genetic deletion of DGAT2 (responsible for TAG formation) increases hepatocellular injury in MCD-fed mice despite a reduction in the content of hepatocellular TAGs [89]. However, TAG accumulation and lipid droplet formation accompany and parallel pathophysiologic mechanisms in NASH. FFAs are thus now in the focus of basic research – and FAs as well as acyl-CoA and acetyl-CoA have been identified as potential causes of lipotoxicity [90]. FAs have been found to activate Toll-like receptors and initiate the extrinsic apoptosis cascade [91,92]. FAs also interfere with NR signaling, which might additionally influence the extent of hepatocyte damage and further promote IR and ER stress [93,94]. Accordingly, β-oxidation of LCFA within peroxisomes and ω-oxidation within the ER are upregulated in NASH and contribute to lipotoxicity and ROS formation [95,96]. This might be secondary to inhibition of
mitochondrial β-oxidation due to an accumulation of malonyl-CoA and the inhibition of CPT1 [3,97,98]. In fact, recent studies indicate that activation of mitochondrial FA oxidation protects from steatosis and IR [99,100]. As mentioned above, DAGs and ceramides might as well contribute to hepatocyte damage and IR in NAFLD [7].

These stressors induce a variety of intracellular and paracrine mechanisms that may promote hepatocellular damage. FAs induce the production of TNFα and hepatic TNF receptor expression correlates with the disease severity in NAFLD [101]. TNF receptor activation increases expression of SREBP-1c, which induces hepatic lipogenesis and lipid accumulation [102]. As TNFα-mediated effects are antagonized by adiponectin, adiponectin receptors are actually downregulated in NASH [103]. TNFα activation is further paralleled by death-receptor expression, which facilitates activation of the extrinsic apoptosis cascade. Apoptosis indeed is the predominant form of hepatocellular injury in NASH [64,104]. In fact, apoptotic activity within the diseased liver correlates with disease severity and thus cleaved cyto-keratin-18 fragments in the serum of NAFLD could effectively be utilized as surrogate markers for the progression of NAFLD [105]. As previously mentioned, FA accumulation also leads to induction of ER stress and ROS formation, which again promotes hepatic injury [1,106].

In summary, while hepatic TAG accumulation seems to be a benign symptom of hepatic steatosis, FA metabolites contribute to the progression of NAFLD to NASH. IR promotes the recruitment of FFAs from the serum pool as well as intrahepatic FA accumulation, which induces apoptosis and ROS formation. FAs themselves also promote hepatic IR via TNF receptor activation, hence indicating a vicious circle of lipid accumulation and IR as a crucial mechanism in the pathogenesis of NASH (Fig. 1D).

**Acute liver injury and regeneration**

Injured livers produce cytokine signals that trigger adipose tissue to release FAs into the circulation. Indeed, in the acute response, hepatocytes initiate the transcription of lipogenic genes and accumulate TAGs in intracellular lipid droplets. In liver regeneration (e.g., after partial hepatectomy (PHx)), lipid droplet formation is essential to propagate the proliferative response by sufficiently supplying the organ’s energy household. Droplet-stored lipids are also used for synthesizing new lipoproteins, bile acids, and entire membranes [107,108]. As previously mentioned, emerging data supports a close connection between BA and FA metabolism. In this context, FATP-5 has also been suggested to participate in BA metabolism as a bile acid-CoA ligase [109,110]. FATP-5 is expressed preferentially towards the space of Disse and closely follows the hepatic sinusoids [109] where FAs and BAs are absorbed from the enterohepatic circulation. Intriguingly, it has been recently shown that elevated BAs accelerate liver regeneration after PHx by bile acid receptor (FXR)-dependent signals [107,111].

Autophagy in acute liver injury might to a certain degree act hepatoprotectively as it rapidly supplies the hepatocyte with energy. However, hyperactivation of autophagy induces cell death, and the necrosis rate is actually a predictor of liver failure [9,112]. Furthermore, as we previously elucidated key processes in the interplay between adipose tissue and the liver, adiponectin was identified as an important mediator of STAT3 signaling in the regenerating liver [113,114].

**Liver cancer**

Emerging data supports a role for lipid and glucose metabolism in hepatocellular carcinogenesis [115]. First of all, the increase in the prevalence of NAFLD is closely associated with the development of cirrhosis and NASH-related HCC [116]. Accordingly, NASH might account for the majority of HCC in the context of a cryptogenic cirrhosis [117]. Obesity and diabetes have been identified as independent risk factors for developing HCC (Fig. 3A) [118,119]. Several studies could recently dissect the effects of individual confounders of the metabolic syndrome and clearly demonstrate a significant risk for NAFLD patients to develop HCC [120]. Intriguingly, HCC in NAFLD not only arises from cirrhotic tissue; this might imply a special role for lipid metabolism and lipotoxicity in the carcinogenesis within this cohort [121].

To date however, the vast majority of HCC cases arise from patients with cirrhosis secondary to chronic HBV or HCV infection [122]. Wu et al. recently identified various alterations in multiple genes involved in hepatic lipogenesis, FA oxidation, and TAG metabolism in tumors derived from HCV patients compared to HCV-positive control patients [123]. Mechanistically, mediators and signaling cascades involved in the hepatic IR and the regulation of hepatic lipid metabolism are in the focus of HCC research. Several studies identified the insulin receptor downstream targets P3-kinase/Akt and IRS to be activated in HCC when compared to surrounding healthy tissue, which mediates GS43 phosphorylation and mTOR activation [124]. These mediators not only interact with β-catenin signaling, but also with autophagy and glycogen synthesis. In fact, knock-out mice with specific deletions in autophagy-related genes show spontaneous development of hepatocellular dysplasia [125]. As pointed out before, TNFα induces hepatic IR and thus promotes lipid accumulation. TNF and IL-6 signaling is also involved in the activation of the JAK/STAT and ERK pathways; both mediators are crucial for the development of obesity induced-HCC in a rodent model [126]. On the other hand, JAK/STAT signaling is closely linked to, and partially modulated by, the adipocytokines leptin and adiponectin [127].

NRs play a crucial role in hepatic lipid and glucose homeostasis (see also above) and are important mediators of hepatic carcinogenesis. PPARα mediates hepatic FA-oxidation and transport as well as Akt phosphorylation via its downstream target TRβ-3 [15]. PPARα agonists promote carcinogenesis in a rodent model for HCV infection [128]. Interestingly, PPARγ agonists not only interfere with the binding of PPARγ to SREBP-1c, but also with STAT3 in hepatoma cell lines, thus indicating a potential mechanism for its carcinogenic properties [129]. In contrast, PPARγ agonists prevent NAFLD progression and, more interestingly, were in some studies described to act antineoplastically [130,131]. Notably, the majority of mechanistic data in hepatic carcinogenesis derives from hepatoma cell lines and murine models that are only of limited value for the elucidation of hepatic carcinogenesis in humans.

**Conclusions**

Hepatic lipid and glucose metabolism are closely interrelated with inflammatory, proliferative and apoptotic signaling within the liver. In the liver, these catabolic and anabolic pathways can hardly be separated. They share intermediate metabolites and receptor signaling, and go hand in hand in the pathogenesis...
Review

of the most common liver diseases. Intriguingly, the case that these metabolic pathways are also involved in cell proliferation, regeneration and carcinogenesis implies potent future therapeutic approaches for life-threatening diseases. The enhanced understanding of these basic mechanisms is thus imperative as we witness a rising prevalence of obesity and the metabolic syndrome.

Conflict of interest

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Review


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