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Review

Regulation of fat metabolism in the liver: link to non-alcoholic hepatic steatosis and impact of physical exercise

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Abstract. Hepatic steatosis may develop as a consequence of several dysfunctions. An increased circulating non-esterified fatty acid (NEFA) pool seems to be a major determinant in the pathogenesis of non-alcoholic fatty liver disease. Increased activation of the transcription factor sterol-regulatory-element-binding protein-1c, which promotes fatty acid synthesis, also contributes to hepatic fat accumulation. Increased hepatic fat oxidation with hepatic steatosis may be triggered by increased hepatic fat concentrations through the action of hepatic peroxisomes mediated by peroxisome proliferator-activated receptor α. Finally, inhibition in very low density lipoprotein secretion may also result in hepatic steatosis. This appears to be mainly controlled by the esterification of NEFAs into triacylglycerols by diacyglycerol acyltransferase-1 and -2 and the microsomal transfer protein. Physical exercise would interfere with the development of hepatic steatosis by stimulating lipid oxidation and inhibiting lipid synthesis in liver through the activation of the AMP-activated protein kinase pathway.

Keywords. Hepatic gene transcription, hepatic steatosis, transcription factor; AMPK, hepatic insulin resistance.

Introduction

The consumption of high-fat diets in westernized societies is a major concern. The capacity of the human body to respond to an increased fat intake with increased fat oxidation is limited. High-fat diets, thus, preferentially lead to deposition of dietary fat into adipocytes, but also into muscle and liver. The prevalence of non-alcoholic fatty liver disease (NAFLD), defined as a significant accumulation of fat in the liver (>5–10% of its weight) [1], may be as high as 76% in obese subjects [2]. The natural history of non-alcoholic hepatic steatosis indicates that a certain subset of patients may further develop non-alcoholic steatohepatitis (NASH), cirrhosis, liver failure and/or liver carcinoma [3]. Increasing interest in the causes and consequences of liver lipid infiltration has been recently spurred by the findings of a relationship between the accumulation of triacylglycerols (TAGs) in liver and the development of insulin resistance and insulin signaling defects [4–8]. As a result, fatty liver is now considered as the hepatic component of the metabolic syndrome [9]. Consequently, information relative to cellular and molecular mechanisms involved in the development of hepatic steatosis has clinical importance. The development of a state of non-alcoholic hepatic steatosis may be caused by an increased uptake of lipids by the liver, an increased hepatic synthesis of non-esterified fatty acids (NEFAs), decreased NEFA β-oxidation in the liver and/or decreased synthesis or secretion of very low density lipoproteins (VLDLs). Pertinent components of these metabolic pathways will be reviewed to provide a mechanistic understanding of their potential contribution to liver lipid infiltration (see Fig. 1 for an overview). The hypothetical mechanisms by which liver TAGs interfere with insulin and glucagon action in the liver will also be reviewed. Finally, the molecular impact of physical exercise as a

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therapeutic tool to counteract the development and/or the effects of hepatic steatosis will be discussed.

Hepatic fatty acid uptake

The specific origin of the lipids that accumulate in liver remains nebulous. The potential sources contributing to fatty liver, besides de novo lipogenesis, include peripheral fats stored in adipose tissue that, through activated lipolytic activity, flow to the liver by way of NEFAs and dietary fatty acids which can enter the liver through the uptake of intestinally derived chylomicron remnants [10] and through the spillover of the activity of the lipoprotein lipase and the amount of circulating NEFAs generated by this activity [11]. Recently, Donnelly et al. [12], using stable-isotope methodology in obese NAFLD patients with hypertriglyceridemia and hyperinsulinemia, showed that 60% of TAGs accounted for in the liver arose from the NEFA pool. These data reflect the importance of the circulating NEFA pool in the pathogenesis of NAFLD and especially the overproduction of fatty acids in adipose tissues that flow to the liver. In insulin-resistant states, insulin does not fully suppress the activity of hormone-sensitive lipase (HSL) in adipose tissues, which results in enhanced lipolysis and release of fatty acids [13]. As a consequence, the liver becomes infiltrated with lipids. In contrast, the severe insulin resistance developed by A-ZIP/F-1 fatless mice is thought to be caused by increases in muscle and liver triglyceride content. Upon transplantation of fat tissue into these mice, the TAG content in muscle and liver returns to normal as does insulin signaling and action [4]. These two studies illustrate the paradox that insulin resistance may be a cause as well as a consequence of liver lipid accumulation [14].

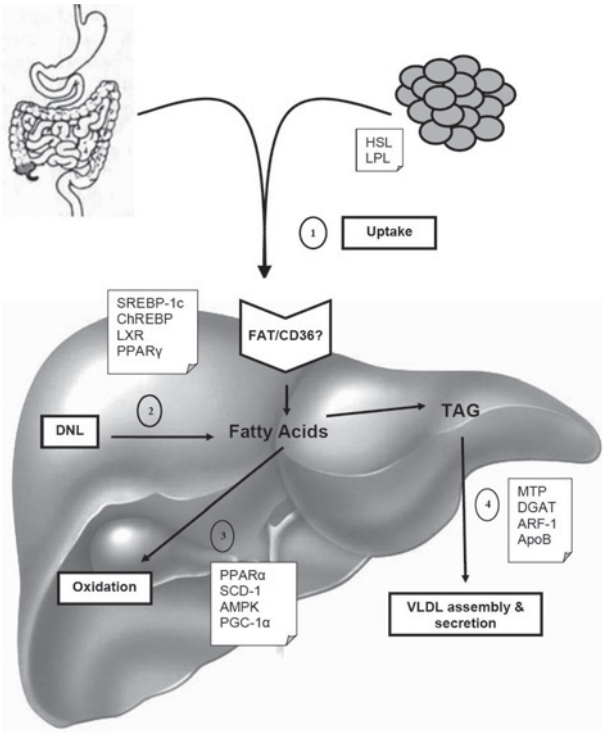

Figure 1. Overview of the four main pathways involved in the development of non-alcoholic hepatic steatosis, and their regulatory factors. Non-alcoholic hepatic steatosis is characterized by (1) an increase in the uptake of lipids by the liver, (2) an increase in hepatic de novo lipogenesis (DNL), and an insufficient elimination of excess liver triacylglycerols (TAGs) by means of (3) hepatic lipid oxidation and (4) very low density lipoprotein (VLDL) assembly and secretion. HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; FAT/CD36, fatty acid translocase/cluster of differentiation 36; SREBP-1c, sterol-regulatory-element-binding protein 1c; ChREBP, carbohydrate-response-element-binding protein; LXR, liver X receptors; PPAR, peroxisomal proliferator-activated receptors; SCD-1, stearoyl-CoA desaturase-1; AMPK, AMP-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1 alpha; MTP, microsomal transfer protein; DGAT, diacyglycerol acyltransferase; ARF-1, ADP-ribosylation factor 1; ApoB, apolipoprotein B.

There is no evidence that hepatic uptake of fatty acids is regulated and, as a result, plasma fatty acid concentration is directly related to the influx of fatty acids to the liver [15]. This would be in line with the concept that the liver acts as a buffer for the incoming flux of fatty acids [16, 17]. In a recent paper, we observed that the increase in liver lipid infiltration induced by a high-fat diet was similar in obesity-prone and obesity-resistant rats [17]. This suggests a close relationship between dietary fat and liver lipid content, independent of body weight and fat accretion. The elasticity of the liver in terms of TAG storage capacity allows it to accommodate excess plasma NEFAs [18]. This storage function may neutralize the potential toxicity of fatty acids released into the blood from adipose tissue [19]. Other organs, such as the pancreas, are particularly susceptible to the lipotoxicity of circulating fatty acids [20]. Even though hepatic fatty acid uptake is believed to be concentration dependent, Ravikumar et al. [21] recently suggested that regulation of fatty acid uptake into liver and skeletal muscle may be a potential target in the treatment and prevention of type 2 diabetes. In their study, they developed a method to allow real-time tracking of ingested fatty acids in humans using ^{13}C magnetic resonance spectroscopy, and assessed postprandial fat storage in liver and skeletal muscle in normal and type 2 diabetic subjects. They observed faster incorporation of dietary fatty acid into liver triglyceride in the type 2 diabetic subjects (4 vs 6 h) and almost 50% higher accumulation compared with control subjects without any differences in plasma NEFAs or ^{13}C appearance in the NEFA fraction between the groups. These data suggest the existence of a regulatory mechanism controlling the hepatic fatty acid uptake. Bonen et al. [22] showed that skeletal muscle in obese and type 2 diabetic subjects has increased long-chain fatty acid uptake compared with that in lean humans. The increased uptake was associated

with increased skeletal muscle expression of fatty acid translocase/cluster of differentiation 36 (FAT/CD36). Whether the FAT/CD36 transporters are also involved in hepatic fatty acid uptake remains to be established. In a mouse model of global suppression of the NADPH-cytochrome P450 reductase, these animals developed hepatomegaly and fatty liver along with a specific induction of FAT/CD36 [23]. Lipoatrophic A-ZIP/F-1 mice that develop steatotic livers have tenfold elevated levels of liver FAT/CD36 mRNA [24]. Finally, the use of oleoylethanolamide, an endogenous peroxisome proliferator-activated receptor α (PPARα) agonist, increased the expression in liver of FAT/CD 36 and L-fatty-acid-binding protein (FABP), both involved in the uptake and transfer of free fatty acid in cells [25].

Hepatic de novo lipogenesis

Hepatic steatosis during obesity and insulin resistance occurs as a result of the increased uptake of free fatty acids mainly released from adipose tissue, but also, at least in rodents, through an increase in endogenous hepatic fatty acid biosynthesis [26, 27]. This is a perplexing aspect of an insulin-resistant state, since lipid synthesis in liver is increased rather than decreased, as might be expected if the normal stimulatory effects of insulin on hepatic synthesis were inhibited [28]. During the past few years, it has become increasingly clear that many of the effects of insulin are mediated through downstream effectors of phosphoinositide 3-kinase (PI3K), namely atypical protein kinase C (aPKC) and protein kinase B (PKB or Akt). Lipid synthesis in the liver has been postulated to be controlled largely by the activation of aPKC, which appears to mediate insulin effects on the expression of sterol-regulatory-element-binding protein-1c (SREBP-1c) [29]. Mice in which the aPKC isozyme PKCλ was deleted in the liver exhibited decreased TAG content and reduced expression of the SREBP-1c gene as well as increased insulin sensitivity [29]. SREBP-1c is an important transcription factor that regulates a battery of genes that promote fatty acid and TAG synthesis [i.e. fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 (SCD-1)] [30]. An increased expression of SREBP-1c has been found in different animal models of hepatic steatosis including the corpulent JCR: LA-cp rats [31], the IRS2 –/– mice [32], the ob/ob mice [33] and the lipodystrophic A-Zip/F1 mice [24]. High-fat diets also increase the expression and the activity of SREBP-1c in livers of 129S6/SvEvTac and C57B1/B6 mice and Sprague-Dawley rats [34, 35]. Mice overexpressing SREBP-1a develop severe fatty liver due to overproduction of cholesterol and fatty acids (Fig. 2) [36].

In contrast with muscle, in the livers of insulin receptor substrate (IRS-1) knockout mice, insulin activation of PKB is markedly diminished, but aPKC activation is intact [37]. The same observation was made in the livers of type 2 diabetic, non-obese, Goto-Kakizaki rats [38]. This tissue specificity has been explained by the finding that in mouse liver, aPKC activation is largely dependent on IRS-2, rather than IRS-1, whose function seems to be maintained in these diabetic livers [39]. In contrast to diabetic rodents, the activation of IRS-1 and IRS-2-dependent PI3K, and both PKB and aPKC, appear to be intact in livers of high-fat-fed mice [38, 40]. The maintenance of aPKC activation would provide an explanation for the maintenance of increased activation of SREBP-1c and thus hepatic lipid synthesis [28], as has been observed in insulin-resistant lipodystrophic and ob/ob diabetic mice [33]. This does not imply, however, that the glucose metabolism in the liver is normal. Defects of glucose handling by the liver might be more reflective of fatty-acid- or other substrate-dependent effects on neoglucogenesis and/or glucose storage or release [28].

Several lines of evidence indicate that fatty liver in insulin-resistant states is caused by activation of SREBP-1c, which is elevated by insulin even, as discussed above, in resistant states [26]. Analysis of the SREBP-1c promoter has revealed an insulin-responsive region that maps to a binding site for liver X receptors (LXRs), suggesting a common pathway of action [41, 42]. LXRs are nuclear hormone receptors that, after ligand binding, form active heterodimers with retinoid X receptors (RXRs). They are activated by oxysterols and serve as key sensors of intracellular sterol levels by regulating the expression of genes that control cholesterol absorption, storage, transport, and elimination [43]. Studies have shown that activation of the LXR also increases the expression of SREPB-1c in the liver [42, 44], indicating that these nuclear receptors provide an inter-regulatory control of the cholesterol and fatty acid metabolism. Such an inter-regulatory control is illustrated by the recent findings that a soy protein diet in Zucker obese fa/fa rats lowered serum and hepatic cholesterol and TAG levels compared with rats fed a casein diet for 160 days (Fig. 2) [45]. These reductions in hepatic cholesterol and triglyceride levels were associated with a low expression of LXR-α and its target genes (CYP7A1 and ABCA1), along with a decrease in the expression of SREBP-1c and several of its target genes such as FAS and SCD-1. The authors [45] postulate that the molecular mechanism for the regulation of SREBP-1c gene expression by soy protein is through negative regulation of LXR-α, possibly mediated by hepatic cholesterol concentration, independently of insulin or leptin concentration, which, together with a possible increase in the oxidation of fatty acids, leads to a reduction in hepatic steatosis. Conversely, application of LXR agonists, as potential anti-atherosclerotic agent, was found to be associated with the development of severe hepatic steatosis [46, 47].

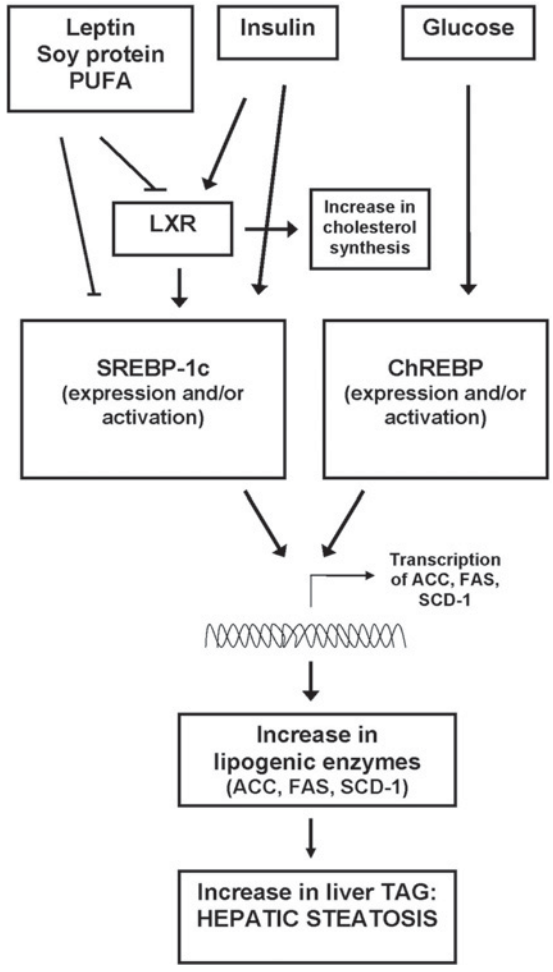

Figure 2. Scheme depicting the putative implication of the transcription factors liver X receptor (LXR), SREBP-1c and carbohydrate-response-element-binding protein (ChREBP) in the development of non-alcoholic hepatic steatosis. PUFA, polyunsaturated fatty acids; ACC, acetyl-CoA carboxylase.

In addition to the transcription factor SREBP-1c, lipogenesis in animals is also regulated by glucose. Glucose activates the carbohydrate-response-element-binding protein (ChREBP), which stimulates gene expression of most enzymes involved in lipogenesis [48]. ChREBP also induces gene expression of key enzymes in glycolysis, thus providing the precursors for lipogenesis [49]. Although beyond the scope of the present review, it is worth mentioning the major effect of other types of sugar, such as sucrose and a fructose-enriched diet, on inducing NAFLD [50]. The underlying mechanisms for the detrimental consequences of dietary fructose in animal models seem to imply increased lipogenesis but also the ability of fructose to induce peroxidation of membrane lipids [51].

Timlin and Parks [52] quantified de novo lipogenesis in healthy human subjects via intravenous infusion of sodium [1-^{13}C] acetate and mass isotopometer distribution analysis. They found that lipogenesis rose from ~5% in the fasted state to ~23% VLDL-TAG fatty acids after meals. Until recently, de novo lipogenesis was believed to be an insignificant pathway in humans consuming a Western, high-fat diet [53]. Schwarz et al. [53], however, reported that with a high-fat, Western diet, hyperinsulinemic obese subjects have significantly higher de novo lipogenesis than normoinsulinemic lean and obese subjects consuming the same diet. In a recent study, cited above, Donnelly et al. [12] found that de novo lipogenesis accounted for ~26% of liver TAG content in hyperinsulinemic subjects with NAFLD. Schwarz et al. [53] suggested that in the presence of hyperinsulinemia, the liver converts excess carbohydrate to fat to control blood glucose and prevent hyperglycemia. Increasing TAG concentrations would, in turn, exacerbate insulin resistance and set up a vicious cycle. On the whole, enhanced de novo lipogenesis appears to be a major abnormality of hepatic fat metabolism in subjects with NAFLD [14].

Hepatic lipid oxidation

An overview of fatty acid oxidation and some of the regulatory molecules involved has been recently presented by Macdonald and Prins [54]. To better understand the importance of lipid oxidation in relation to NAFLD, a brief review of these mechanisms is necessary. The majority of fatty acids are metabolized through β-oxidation occurring principally in the mitochondria [55]. β-Oxidation also occurs in other intracellular sites, particularly in peroxisomes [55]. Peroxisomes are involved in the metabolism of a variety of fatty acids, particularly very long chain fatty acids (VLCFAs) and branched-chain fatty acids [56]. Other types of fatty acid oxidation include α-oxidation and ω-oxidation by members of the cytochrome P450 4A family in the endoplasmic reticulum (microsomes) [56, 57]. The P450 cytochromes are a superfamily of hemoproteins that catalyze the oxidation of endogenous and exogenous compounds, including fatty acids [58]. The dicarboxylic acids formed are further degraded by the classical peroxisomal β-oxidation system [59]. The extramitochondrial fatty acid oxidation systems become more important during periods of increased influx of fatty acids into the liver [60].

Peroxisomes can be stimulated to proliferate by a variety of agents such as organic solvents, herbicides and dehydroepiandrosterone [61]. Such agents are known as peroxisome proliferators as the proliferation they induce is associated with increased expression of genes involved in peroxisomal β-oxidation. PPARs are nuclear receptors activated by peroxisome proliferators [62]. Members of this family include PPARα, PPARγ and PPARδ. PPARα is expressed in tissues involved in fatty acid metabolism, including the liver, and activation of these receptors results in peroxisomal proliferation and increased expres-

sion of enzymes involved in fatty acid oxidation. PPARγ is expressed mainly in adipose tissue and its activation results in facilitation of lipid storage [63, 64]. Thiazolidinediones (TZDs; glitazones) are drugs that improve insulin sensitivity, at least in part, by activation of PPARγ [63]. Normally, PPARγ is expressed at very low intensity in the liver; however, the expression of PPARγ is markedly expressed in animal models with fatty liver [24, 65]. Although previous studies have demonstrated that SREBP-1 can transcriptionally activate PPARγ [66], the precise mechanism mediated by PPARγ that promotes TAG deposition in the liver remains undefined [26]. In a recent publication, Schadinger et al. [67], using the hepatic cell line AML-12 that stably expresses PPARγ2, reported that this phenotype is accompanied by selective upregulation of several adipogenic and lipogenic genes including adipose-differentiation-related protein (ADRP), a protein that coats lipid droplets in hepatocytes, FABP, SREBP-1, FAS and acetyl-CoA carboxylate (ACC). PPARδ is less well characterized but has also been shown to be involved in fatty acid metabolism [63, 64]. PPARs are activated by a number of pharmacological compounds, as well as by fatty acids and fatty-acid-derived molecules. These ligands include the anti-diabetic glitazones, as well as natural ligands like polyunsaturated fatty acids (PUFAs) and fatty acid-derived components of oxidized low-density lipoprotein (LDL) (Fig. 3) [68, 69].

Decreased mitochondrial fatty acid oxidation has long been considered to be the major mechanism underlying the disturbances in lipid metabolism in liver leading to steatosis [60, 70, 71]. A more recent view is that, in fact, hepatic oxidation is increased with hepatic steatosis but to an insufficient extent to compensate for the important accumulation of lipids [72, 73]. Indeed, hepatic fatty acid mitochondrial and peroxisomal oxidation has been reported to be increased in genetically obese ob/ob mice, a model of NAFLD [74, 75]. The hepatic expression of cytochrome P450 4A genes has also been reported to be greatly increased in ob/ob mice (4a10 and 4a14) and fa/fa Zucker rats (4A2), two models lacking either leptin or a functional leptin receptor [58]. Increased hepatic fat oxidation with hepatic steatosis may be triggered by the increased hepatic fat concentrations, an induction of carnitine palmitoyl transferase-1 (CPT-1) possibly mediated by PPARα, a loss of CPT-1 inhibition by malonyl CoA, and finally by the increased action of hepatic peroxisomes mediated by PPARα [70]. PPARα activation has been reported to increase uncoupling protein-2 (UCP-2) mRNA although it remains unclear whether UCP-2 protein itself is expressed in the liver [76, 77]

It has become clear in recent years that PPARα tightly regulates the expression of genes involved in mitochondrial and extramitochondrial fatty acid oxidation in liver, and any defect in the expression of these genes can affect the degree of hepatic steatosis [78–80]. The generation of mice deficient in PPARα clearly established that this receptor is essential for hepatic peroxisome proliferation. PPARα coordinates transcriptional activation of the peroxisomal fatty acyl-CoA oxidase (AOX), the first enzyme in peroxisomal β-oxidation, cytochrome P450 4A, and other genes, by structurally diverse synthetic peroxisome proliferators [81]. Constitutive levels of mitochondrial fatty acid β-oxidation are lower in these PPARα-deficient mice, but the constitutive or basal oxidation of VLCFAs by the peroxisomal β-oxidation system appears unaffected by PPAR deficiency [82]. On the other hand, disrupting the basal metabolism of VLCFAs by generating mice deficient in AOX resulted in severe microvesicular hepatitis and peroxisome proliferation in liver cells [61, 83]. In these AOX-deficient mice, the hyperactivity of PPARα enhances the severity of steatosis by inducing cytochrome P450 4A activity that generates dicarboxylic acids, and since these are not metabolized in the absence of peroxisomal β-oxidation, they damage mitochondria leading to steatosis. These results suggest that VLCFA- and LCFA-CoA act as PPARα ligands if they remain unmetabolized, thus resulting in the development of microvesicular hepatic steatosis [61]. Accordingly, liver microvesicular steatosis was blunted in mice deficient in both PPARα and AOX [84]. Alternatively, the use of pharmacological and nutritional PPARα agonists has been shown to decrease liver triglyceride content, at least in part by enhancing lipid oxidation [25, 85, 86]. On the whole, PPARα appears to play a central role in fatty acid oxidation in the liver under conditions where the plasma fatty acids are elevated. One of the main natural ligands for the activation of PPARα and hepatic fatty acid oxidation and/or ketogenesis are the fatty acids, both saturated and unsaturated, which thereby control their own fate [87].

Role of leptin and adiponectin

Leptin and adiponectin, the two major fat-derived hormones, have been shown to increase insulin sensitivity and concomitantly reduce hepatic triglyceride content (probably by promoting fatty acid oxidation) in an animal model of insulin resistance or in humans with lipodystrophic diabetes [88 – 90]. Interestingly, there are theoretical considerations that argue against the conventional view that the physiological role of leptin is to prevent its deficiency syndrome, obesity, but rather to confer a metabolic advantage [91]. It has been suggested that the metabolic advantage conferred by the hyperleptinemia of obesity is the prevention of TAG accumulation in non-adipose tissue [19]. In the absence of leptin action, lipogenesis is increased and fatty acid oxidation is reduced accounting for the steatosis that occurs in such circumstances [92]. One pathway by which leptin achieves its anti-lipogenic effect

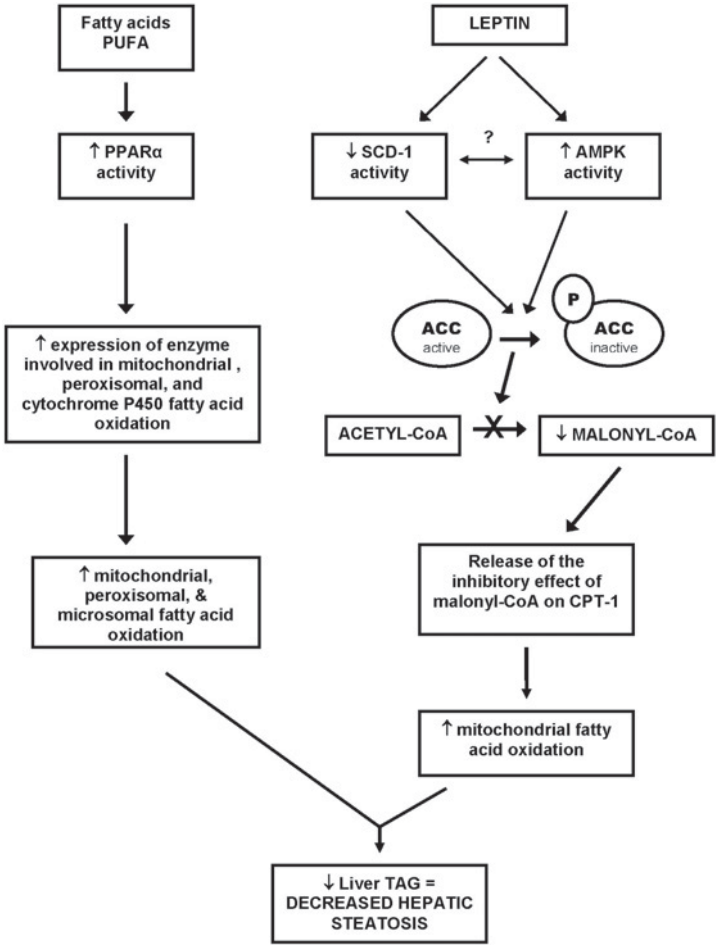

Figure 3. Scheme depicting the role of the transcription factor PPARα and the cytokine leptin in stimulating liver fatty acid oxidation. AMPK, AMP-activated protein kinase; CPT-1, carnitin palmitoyl transferase-1.

in the liver is by lowering expression of SREBP-1c, thus upregulating genes promoting fatty acid β-oxidation and downregulating those involved in lipogenesis [88]. Interpretation of hyperleptinemia in patients with NAFLD could, therefore, be that it reflects a corrective, albeit unsuccessful, response to the presence of hepatic steatosis [93]. This leptin-resistant state might be due to the expression of suppressors of cytokine signaling (SOCS) [94]. Among the eight members of the SOCS family, SOCS-1 and SOCS-3 appear to be relevant to several aspects of hepatic pathobiology [93]. In a recent paper, Ueki et al. [94] reported that a reduction in the hepatic expression of SOCS-1 and SOCS-3 in obese diabetic db/db mice resulted in a marked reduction in the severity of steatosis. The link between SOCS and the expression of SREBP-1c is that SOCS decrease STAT-3 (signal transduction and activator of transcription) phosphorylation, which negatively regulates the SREBP-1c promoter [95]. Liver-specific STAT-3 knockout in db/db mice results in marked increases in SREBP-1c expression and hepatic triglyceride content [96]. In a subsequent study, Ueki et al. [97] demonstrated that the expression of SOCS-1 and SOCS-3 is increased in livers of obese insulin-resistant animals, thus causing a prominent upregulation of SREBP-1c. In summary, in response to proinflammatory cytokines, the expression of SOCS proteins appears to be increased, thereby binding to Jak tyrosine kinase and/or cytokine receptor and inhibiting cytokine signaling [97]. To further elucidate the mechanism whereby leptin reduces hepatic lipid content, Cohen et al. [98] recently used microarrays to identify genes in liver whose expression was modulated by leptin treatment. The gene encoding SCD-1 in liver ranked the highest in this analysis. SCD-1 is a microsomal fatty-acid-modifying enzyme that catalyzes the introduction of the cis double bond between carbons 9 and 10 of saturated fatty acyl-CoA substrates. The preferred substrates for SCD are palmitoyl- and stearoyl-CoA, which are converted to palmitoleoyl- and oleoyl-CoA, respectively. These products are the most abundant fatty acids found in TAGs, cholesterol esters and phospholipids. The cellular roles of monounsaturated fatty acids are diverse; overall, the expression of

SCD can influence membrane fluidity, lipid metabolism and adiposity [99]. Oleate is an essential fatty acid for the hepatic synthesis of TAGs and cholesterol esters, both of which are vital for the assembly and secretion of VLDL in the liver [100]. SCD-1 is predominantly located in the endoplasmic reticulum, where it fluctuates in a wide range in response to complex hormonal and dietary factors [101]. The gene is also transcriptionnally regulated by a number of factors including PUFAs and SREBP-1. PUFA repression of SREBP-1 maturation leads to decreased expression of lipogenic genes including SCD-1 [99].

Recently, downregulation of SCD-1 expression and activity was shown to play a major role in leptin-mediated depletion of hepatic lipids [98]. Leptin administration markedly improved the metabolic defects of lipodystrophic mice, indicating that these defects were not due to an absence of TAG storage depots but to the absence of an adipose-derived factor [102]. Interestingly, central leptin repressed SCD-1 mRNA and enzymatic activity in lipodystrophic mice, indicating that the effects of leptin on liver SCD-1 are likely mediated by central action [103]. This is in line with the recent finding that central administration of recombinant leptin reverses diet-induced hepatic insulin resistance mainly by decreasing glycogenolysis [104]. On the whole, SCD-1 appears to be a central metabolic control point in the development of hepatic steatosis. Mice deficient in this enzyme are resistant to both hepatic steatosis and obesity and these effects appear to be due to markedly increased energy expenditure [105]. It was hypothesized that repression of SCD-1 results in an accumulation of saturated fatty acyl- CoAs that potently allosterically inhibit ACC (Fig. 3). The resulting decrease in malonyl-CoA thereby relieves the inhibition on CPT-1, and fatty acids can enter mitochondria and be oxidized. Alternative mechanisms could be that inhibition of SCD-1 increases the levels of a PPARα ligand or could be associated with increased activity of AMP-activated protein kinase (AMPK) [106], or be associated with effects of fatty acids on uncoupling proteins [107]. The interest of the SCD-1 pathway in obesity is illustrated by recent findings reported by Dobrzyn et al. [108] indicating that SCD-1-deficient mice show reduced ceramide synthesis in skeletal muscles and increased phosphorylation of AMPK. Ceramide has been proposed as a likely suspect in lipid-induced damage to tissues caused by intracellular TAG accumulation [109]. Reduced SCD-1 expression by SCD-1-specific anti-sense oligonucleotide inhibitors (ASOs) in mice has also been recently reported to reduce fatty acid synthesis and secretion, and increase fatty acid oxidation in primary mouse hepatocytes [110].

In addition to hyperleptinemia, hypoadiponectinemia has also been reported to be associated with NAFLD [111]. Two adiponectin receptors, AdipoR1 and AdipoR2, have been recently identified [112]. AdipoR1 is expressed abundantly in skeletal muscle, whereas AdipoR2 is predominantly expressed in liver. Bajaj et al. [113] reported that, in type 2 diabetic patients, the plasma adiponectin concentration is negatively correlated with hepatic fat content. Animal models have also indicated that adiponectin confers protective effects against NAFLD [114]. Acute treatment of mice with adiponectin has been shown to decrease insulin resistance and the TAG content of muscle and liver [89].

Very low density lipoprotein synthesis and secretion

Although fat may accumulate substantially in liver, it does not enlarge indefinitely [72]. A new steady state is achieved, whereby the increased hepatic uptake and biosynthesis of fatty acids are compensated for by increased removal of lipids from the liver. Very low density lipoprotein (VLDL) secretion is one pathway which removes fat from the liver. An interesting aspect of VLDL assembly is that the cytosolic TAG pool, newly formed from extracellular NEFAs or de-novo-synthesized endogenous NEFAs, is not directly incorporated into the VLDLs [for a review see ref. 115]. Extracellular NEFAs enter the hepatocyte from the plasma and are esterified into TAGs by diacyglycerol acyltransferase-1 (DGAT-1), also called overt DGAT, and stored in the cytosol. Cytosolic TAGs are thereafter mobilized by the lipolytic action of arylacetamide deacyclase (AADA) and TAG hydrolase (TGH) and reesterified by DGAT-2, also called latent DGAT (Fig. 4). Some TAG products are recycled to the cytosol and some are channeled into a TAG-rich VLDL precursor. The formation of this precursor is enhanced by microsomal transfer protein (MTP) and inhibited by insulin. The working model of VLDL assembly proposes that TAG becomes associated with apolipoprotein B (apoB) in at least two different stages of the assembly process. In the first stage, a small quantity of TAG is transferred to apoB in the endoplasmic reticulum. The formation of this small precursor is catalyzed by the MTP [116]. The second stage, or maturation phase, involves the fusion of the apoB-containing precursor with a larger droplet of TAG. What is known of this less well-characterized process is that it is dependent upon the activity of ADP-ribosylation factor 1 (ARF-1). ARF-1 is a small GTP-binding protein which activates phospholipase D (PLD) to form phosphatidic acid [117]. Overexpression of ARF-1 has been recently shown to increase the assembly of the less buoyant VLDL 2 [Svedberg floatation units (Sf) 20–60] but not of the buoyant (Sf 60–400) VLDL 1 [118]. The importance of the VLDL assembly is also illustrated by the observation that apoB-defective familial hypobetalipoproteinemia is characterized by low levels of LDL cholesterol and fatty liver [119].

The relative activities of the two types of DGAT may have a significant impact on the level of triglyceridemia

as well as on the development of steatosis [120]. The two forms of DGAT are the products of different genes whose expression is differentially regulated [121, 122]. DGAT-1-null mice are viable and can still synthesize TAGs [123]. DGAT-2 is expressed ubiquitously, with high expression levels in the liver and white adipose tissues [121]. In contrast to DGAT-1-null mice, DGAT-2-null mice are lipopenic and die soon after birth from important reductions in substrates for energy metabolism [124]. The roles of DGAT-1 and DGAT-2 in VLDL secretion have been recently examined in mice overexpressing DGAT-1 and DGAT-2 in liver [120]. This study reported that DGAT-1 is the DGAT that is located in the lumen of the endoplasmic reticulum and promotes VLDL secretion, while DGAT-2 has potent overt DGAT activity and plays a major role in cytosolic lipid accumulation. DGAT-1-overexpressing mice showed increased VLDL secretion and an increase in gonadal fat mass. This was not observed in DGAT-2-overexpressing mice. DGAT-1 and DGAT-2-overexpressing mice showed 1.9- and 3.1-fold increases in liver TAG concentration, respectively. The authors conclude that DGAT-1 has a role in VLDL secretion, which may promote obesity, while DGAT-2 has a role in steatosis [120]. This latter conclusion supports the recent findings that a reduction in the expression of DGAT-2 in liver and adipose tissue with an anti-sense oligonucleotide caused a marked reduction in hepatic TAG content and improved blood lipid levels in high-fat-diet-induced obese and ob/ob mice [125]. These effects were related to decreased hepatic lipid synthesis.

MTP also exerts a central regulatory role in VLDL assembly and secretion. This is illustrated by the observation that the inhibition of MTP activity in mice results in an incomplete lipidation of apoB particles and hepatic lipoprotein secretion, causing steatosis [126]. In the latter study, the steatogenic effect of drugs inhibing β-oxidation was exacerbated by MTP inhibition. If on the one hand, inhibition in VLDL secretion results in steatosis,

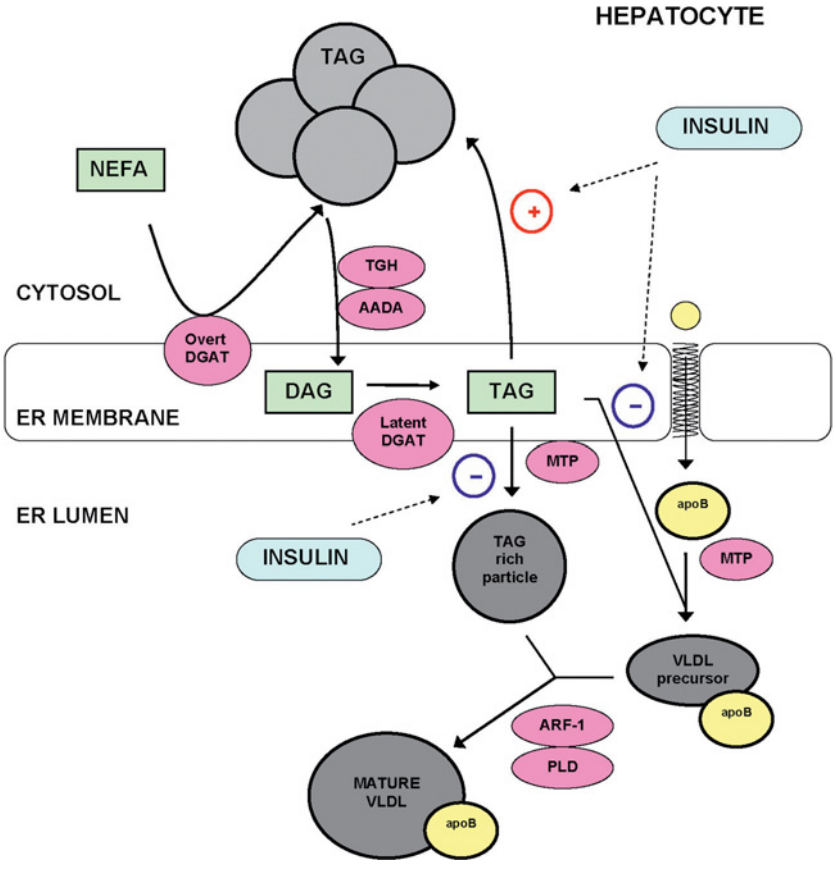

Figure 4. Role of the cytosolic and microsomal pools of liver TAGs in the assembly of VLDL in the endoplasmic reticulum (ER). The cytosolic pool of TAGs is regulated by overt DGAT and serves as TAG storage in the hepatocyte. Cytosolic TAGs are not directly utilized for the assembly of VLDL but are hydrolyzed by TGH and/or AADA and then reesterified in the ER by the latent DGAT. Microsomal TAGs can be recycled back into the cytosol, this phenomenon being enhanced by insulin. Microsomal TAGs can also be incorporated in a TAG-rich particle or a VLDL precursor both of which will serve for the formation of mature VLDL in the ER. The microsomal enzyme microsomal transfer protein (MTP), is an important point of regulation in the formation of VLDL as its action is required in the formation of the microsomal TAG-rich particle but also in the incorporation of a molecule of apolipoprotein B (apoB) into the VLDL precursor. ADP-ribosylation factor 1 (ARF-1) and phospholipase D (PLD) seem to be required for the formation of the mature VLDL particle. DAG, diacylglycerol;

on the other, VLDL overproduction by the liver is also a concern since it is the major source of LDLs in plasma, which are associated with the development of atherosclerosis and cardiovascular disease. It is not surprising, therefore, that several drugs inhibiting MTP activity have been used to decrease plasma TAG and LDL-C concentrations, in most cases with an accumulation of lipids in the liver [127–129]. These data, however, do not give clear evidence that hepatic steatosis *in vivo* indeed results from a decrease in VLDL production. There are instances where the flux of fatty acids is increased, causing steatosis, without any evidence for increase in liver VLDL production [130]. Similarly, the inhibition of glucose-6-phosphatase results in an increase in de novo lipogenesis and the development of hepatic steatosis without affecting VLDL production [131]. On the other hand, the activation of the LXR has been reported to increase lipogenesis and hepatic VLDL production [46]. A recent paper by Grefhorst et al. [132] reported that pharmacological blockage of β-oxidation resulted in severe microvesicular hepatic steatosis, without affecting VLDL-triglyceride secretion. Overall, the demonstration of the implication of VLDL synthesis and production in the development of hepatic steatosis is complicated by the fact that the relationship between availability of fatty acids, hepatic steatosis and VLDL production is not straightforward [130].

As mentioned above, insulin suppresses the secretion of VLDL particles by the liver [133]. This effect may be achieved by interfering with the maturation phase of VLDL assembly via PI3K-mediated events [134]. However, insulin does not inhibit the overall lipolytic mobilization of hepatocellular cytosolic TAG [18]. Instead of being transferred into TAG-rich VLDL, the newly mobilized TAG would be returned back to the cytosolic pool [115]. An increase in plasma insulin levels possibly results in an increase in recycling from the microsomal to the cytoplasmic TAG, thus contributing to steatosis. On the other hand, the development of insulin insensitivity at the hepatic level would contribute to an increase in VLDL secretion. Accordingly, insulin resistance and diabetes have been shown to result in increased VLDL-TAG and VLDL-apoB secretion in the Israeli desert gerbil, a sand rat that appears to be an ideal natural model of the disease in humans [135].

Hepatic insulin resistance

Insulin resistance in humans is not always accompanied by obesity, since insulin resistance is seen in patients lacking subcutaneous fat. However, both obese and lipodystrophic patients have an increase in the amount of fat stored in the liver [for a recent review see ref. 136]. As a consequence, hepatic steatosis is generally associated with hepatic insulin resistance and type 2 diabetes [73, 137]. As mentioned above, however, it is not clear if hepatic steatosis in humans is a cause or a consequence of liver insulin resistance [9]. With the use of a high-fat diet of short duration (3 days vs 3 weeks) in a rat model, Kraegen et al. [138] observed that muscle insulin resistance develops after liver insulin resistance. Using the same dietary approach, Iglesias et al. [139] thereafter specified that this liver insulin resistance was associated with an increased liver fat content. Conversely, the administration of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), an activator of lipid oxidation through the AMPK pathway, resulted in a reduction in liver TAG and malonyl-CoA content and an enhancement of liver and muscle insulin action in insulin-resistant high-fat-fed rats [139]. An association between a reduction in fatty liver and an enhancement in insulin action is also observed with the use of metformin and PPAR agonists [85, 140]. On the whole, there is evidence that liver fat accumulation indeed results in hepatic insulin resistance.

Although there is a good association between hepatic fat accumulation and hepatic insulin resistance, it is still not clear if hepatic insulin resistance is due to systemic factors (i.e. circulating lipids) or to elevated intrahepatic lipids. In a recent study conducted on isolated steatotic livers from high-fat-fed rats, insulin-induced suppression of epinephrine-induced hepatic glucose output was sustained in these rats, indicating that TAG accumulation into the liver was not sufficient to impair hepatic insulin action [141]. The authors concluded that hepatic insulin resistance observed *in vivo* may at least partially result from circulating factors. On the other hand, Samuel et al. [7] used the 3-day high-fat diet protocol, mentioned above [138], to develop hepatic fat accumulation in rats without the confounding effects of peripheral fat accumulation. Hepatic insulin resistance was associated with a threefold increase in liver TAG and total fatty acyl content. The development of hepatic insulin resistance associated with hepatic fat accumulation was attributed to impaired insulin-stimulated IRS-1 and IRS-2 tyrosine phosphorylation. These changes were associated with activation of PKC-ε and JNK1 (Fig. 5). Furthermore, insulin activation of AKT2 and inactivation of GSK3 was impaired in the fat-fed animals. As a consequence, glycogen synthase activity was reduced and gluconeogenesis increased, although the transcription of key gluconeogenic enzymes was not altered by the high-fat feeding. Recently, Neschen et al. [142] used a different approach to study the link between hepatic steatosis and hepatic insulin resistance. They examined whether or not a mouse model, deficient in the mitochondrial isoform of acyl-CoA:glycerol-sn-3-phosphate acyltransferae 1 (mtGPAT 1), would be protected against fat-induced hepatic steatosis and hepatic insulin resistance. GPAT

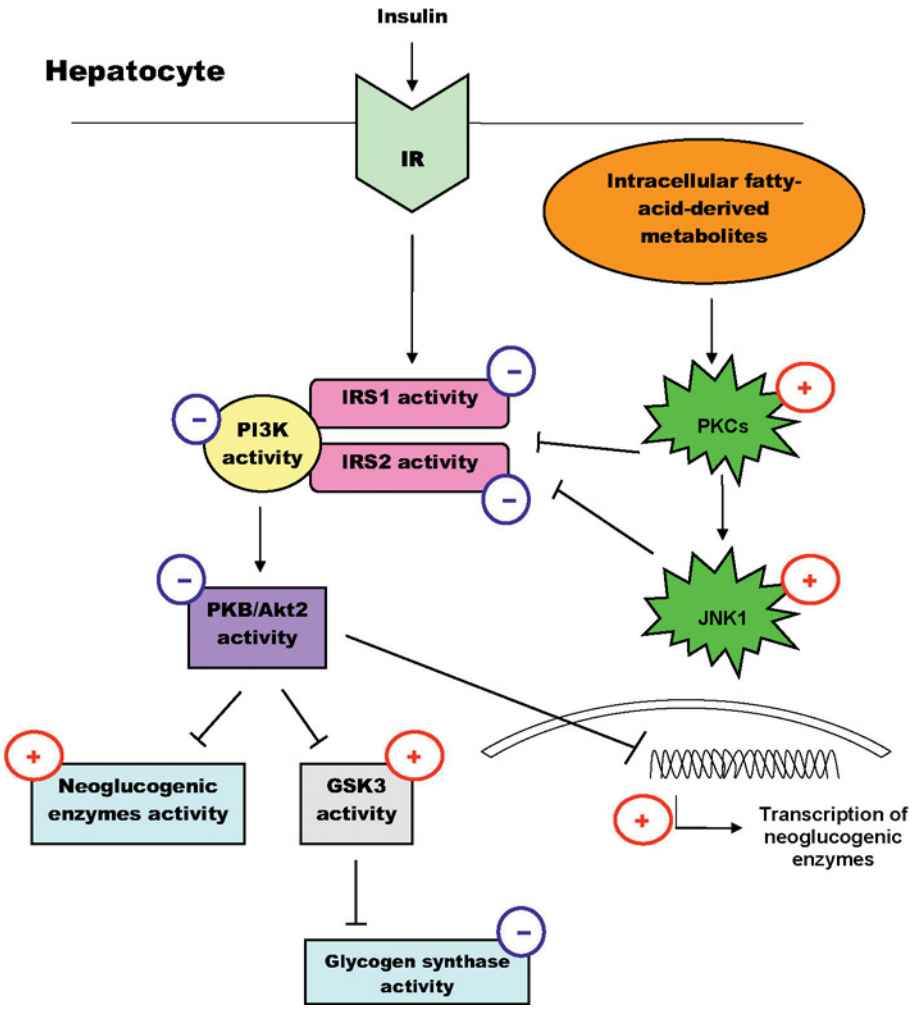

Figure 5. Schematic representation of putative mechanisms that link intrahepatic lipid accumulation and hepatic insulin resistance. Accumulation of fatty-acid-derived metabolites would increase the activity of certain PKC isoforms which increase JNK1 activity. An increase in the activity of PKCs and/or JNK1 would impair insulin signaling and, in turn, decrease insulin-stimulated glycogen synthase activity and insulin-stimulated inhibition of neoglucogenic enzyme activity and transcription. IR, insulin receptor; JNK1, c-Jun N-terminal kinase 1; GSK3, glycogen synthase.

has been hypothesized to be the rate-limiting step in the glycerol 3-phosphate pathway involved in hepatic TAG synthesis. The glycerol 3-phosphate pathway provides lipid metabolites, such as acyl-CoA, lypophosphatidic acid, diacylglycerol and TAG, which have all been suggested to play a role in the development of insulin resistance [7, 86, 143]. Mice deficient in mtGPAT displayed markedly lower TAG and diacylglycerol concentrations and were protected from hepatic insulin resistance. This protection was attributed to a lower diaglycerol-mediated PKCε activation. Surprisingly, however, mtGPAT-deficient mice were protected from high-fat-diet-induced hepatic insulin resistance despite a large increase in hepatic acyl-CoA content, providing evidence against a role of acyl-CoA species in mediating this metabolic process.

Glucagon hepatic resistance

Along with hepatic insulin resistance, there is some evidence for an association between liver lipid infiltration and liver glucagon resistance. A state of hepatic glucagon resistance has been observed in association with several physiopathologies, including liver cirrhosis, diabetes and malnutrition [144–146]. This has been mainly attributed to a loss of sensitivity of the glucagon receptor by glucagon itself (homologous desensitization). There is also *in vivo* and *in vitro* evidence for an alteration in glucagon receptor mRNA by glucose, cAMP and oxygen [147–149]. Interestingly, several of the physiopathological conditions associated with a state of hepatic glucagon resistance are also associated with an increase in liver lipid infiltration. To further explore this association, we recently reported a reduction in hepatic glucose production in response to glucagon infusion in high-fat-diet-induced

hepatic steatosis in rats [150]. In a subsequent study, we showed that high-fat-diet-induced hepatic steatosis was associated with a reduction in hepatic glucagon receptor density and in Gsα protein content [151]. A negative association was found in that study between hepatic TAG content and glucagon receptor density. The mechanism by which hepatic lipids would reduce hepatic glucagon receptor density might be similar to the known activation of G-protein-coupled receptor kinases by phospholipids [152, 153]. G-protein-coupled receptor kinases mediate phosphorylation of G-protein-coupled receptors and initiate homologous receptor desensitization [154, 155].

Impact of physical exercise

The recognized impact of physical exercise on several aspects of liver metabolism has recently brought several investigators to recommend it as a therapeutic tool for patients with NAFLD [156, 157]. In support of this view, physical exercise conducted concurrently with a high-fat-diet-induced obesity stimulus for a duration of 8 weeks in rats completely prevented the development of macrovesicular hepatic steatosis [158]. This effect was mainly attributed to a reduction in circulating NEFAs, most likely as a result of an increased peripheral oxidation. Furthermore, regular physical exercise introduced mid-way through a 16-week high-fat diet regimen largely reduced fat accumulation and attenuated the deterioration of blood lipid profile [159]. There is little information on the impact of physical exercise on liver lipid infiltration in humans. A recent study, however, reported that 6 months of supervised exercise in a group of moderately obese type 2 diabetic patients resulted in a reduction in VLDL apoB pool size, which may be due to a decrease in VLDL apoB secretion [160]. Together, these data support the positive impact of physical exercise in the overall process of obesity-induced NAFLD and underline the reversibility of liver lipid infiltration and associated metabolic disturbances.

Even though the evidence for the impact of physical exercise in the prevention and attenuation of hepatic steatosis is well supported, there is a paucity of information on the possibility that physical exercise acts directly on intrahepatic mechanisms that would affect fat regulation. There is some evidence that exercise training may downregulate hepatic lipogenic enzymes [161]. We do not know, however, if physical exercise has a direct impact on the molecular processes regulating hepatic lipogenesis and/or lipid oxidation. There is an indication, however, that physical exercise may stimulate lipid oxidation and inhibit lipid synthesis in liver through the activation of the AMPK pathway [162, 163]. Dobrzyn et al. [106] in a recent report mentioned that they have data (not shown) indicating that SCD-1 activity in mouse liver is dramatically decreased after exhausting exercise. These authors postulate that this SCD-1 deficiency could be related to AMPK activation during exercise. AMPK is a fuel-sensing enzyme present in most mammalian tissues [164]. It is activated by stimuli that lead to increases in the AMP:ATP ratio [165]. These stimuli include pathological stresses such as oxidative damage, osmotic shock, hypoxia and glucose deprivation, as well as physiological stimuli such as physical exercise and hormones including leptin and adiponectin [166]. AMPK is activated during exercise and its activation is still present in the postexercise state in muscle, liver and adipose tissue [167]. In the liver, AMPK activation is documented to inhibit lipid synthesis through inactivation of ACC [168], activation of malonyl-CoA decarboxylase [163], an enzyme that degrades malonyl CoA, a decrease in mtGPAT activity [142, 163] and a suppression in the gene expression of the lipogenic enzymes ACC1 and FAS [169], possibly through the reduction in the hepatic expression of SREBP-1 [170]. ACC1 is the predominant carboxylase found in the liver and is involved in lipogenesis, whereas ACC2 is mostly found in cardiac and skeletal muscle where it has been found to regulate β-oxidation [171]. Activation of AMPK stimulates hepatic lipid oxidation mainly through a reduction in malonyl-CoA, an allosteric inhibitor of CPT1, the enzyme that controls the transfer of cytosolic LCFA-CoA into mitochondria [163, 167]. In a recent study [unpublished data], we observed that a high-fat-diet-induced hepatic insulin resistance was accompanied by a reduction in the protein content of activated AMPK. Both of these effects were completely restored by an acute bout of exercise. Altogether, it is legitimate to speculate that a regular program of physical exercise would be an asset to reduce hepatic steatosis and hepatic insulin resistance. The precise mechanism by which these effects would take place, however, still remains to be explored.

As mentioned in a previous section, hepatic steatosis has also been associated with the development of hepatic glucagon resistance attributed to a decrease in hepatic glucagon receptor number [151]. In contrast, physical exercise has been associated with increased glucagon action in the liver. Drouin et al. [172, 173] reported that under glucagon stimulation, trained individuals and animals depicted higher hepatic glucagon production than sedentary counterparts. Similarly, Légaré et al. [174] as well as Podolin et al. [175] demonstrated that endurance training increases plasma membrane hepatic glucagon receptor density. There is also evidence that an acute period of exercise may enhance hepatic glucose production in response to glucagon [176]. In a recent study [unpublished data], we observed that a high-fat-diet-induced hepatic steatosis in the rat was associated with a decrease in total glucagon receptor number accompanied by an increase in endosomal and lysosomal internalisation of glucagon receptor content.

Conclusions

There is no doubt that the liver plays a central role in the regulation of systemic lipid homeostasis. These regulatory processes are controlled to a significant extent at the level of gene transcription by DNA-binding transcription factors. A promising novel research avenue in the area of fat metabolism regulation in the liver is the powerful transcriptional activity of transcriptional coactivator proteins, such as the PGC-1 family [for a review see ref. 177]. PGC-1α is induced in situations where fatty acids become the main energetic substrate (birth, fasting and exercise) [178]. In liver, PGC-1α coinduces the transcription of CPT-1 in cooperation with the transcription factor HNF-4α [179]. Mice lacking PGC-1α develop hepatic steatosis upon fasting [180]. In response to pancreatic glucagon and adrenal cortisol, the cAMP-responsive transcription factor CREB activates gluconeogenesis and fatty acid oxidation in the liver by stimulating expression of PGC-1 [181]. Mice deficient in CREB activity have a pronounced hepatic TAG content and stimulated expression of lipogenic genes on a high-fat diet [182]. PGC-1α is also induced in liver through the relief of the inhibition by insulin on the PGC-1α promoter via the transcription factor FOXO1 [183]. The fact that during prolonged exercise glucagon secretion is increased while insulin secretion is reduced constitutes an interesting basis to explore the effects of exercise training on this mode of regulation in the liver. It is revealing that the increase in mitochondrial biogenesis in skeletal muscle with physical exercise is associated with an increase in PGC-1α mRNA [184], most likely through AMPK activation [185]. It is thus plausible that physical exercise, similarly to dietary PUFAs [186], regulates hepatic gene expression through key transcription factors.

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