Lipid Metabolism and Liver Inflammation.

II. Fatty liver disease and fatty acid oxidation

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Reddy, Janardan K., and M. Sambasiva Rao. Lipid Metabolism and Liver Inflammation. II. Fatty liver disease and fatty acid oxidation. Am J Physiol Gastrointest Liver Physiol 290: G852–G858, 2006; doi:10.1152/ajpgi.00521.2005.—Fatty liver disease (FLD), whether it is alcoholic FLD (AFLD) or nonalcoholic FLD (NAFLD), encompasses a morphological spectrum consisting of hepatic steatosis (fatty liver) and steatohepatitis. FLD has the inherent propensity to progress toward the development of cirrhosis and hepatocellular carcinoma. It is generally difficult to distinguish AFLD from NAFLD on morphological grounds alone despite the distinctions implied by these etiological designations. The indistinguishable spectrum of histological features of both AFLD and NAFLD suggests a possible convergence of pathogenetic mechanisms at some critical juncture that enables the progression of steatohepatitis toward cirrhosis and liver cancer. From a pathogenetic perspective, FLD may be considered a single disease with multiple etiologies. Excess energy consumption and reduced energy combustion appear to be critical events that culminate in lipid storage in the liver. Energy combustion in the liver is controlled by peroxisome proliferator-activated receptor (PPAR)-α-regulated mitochondrial and peroxisomal fatty acid β-oxidation systems and the microsomal ω-oxidation system. PPAR-α, a receptor for peroxisome proliferators, functions as a sensor for fatty acids (lipid sensor), and ineffective PPAR-α sensing can lead to reduced energy burning resulting in hepatic steatosis and steatohepatitis. Delineation of the pathogenetic aspects of FLD is necessary for developing novel therapeutic strategies for this disease.

steatohepatitis; peroxisome proliferator-activated receptors; nonalcoholic fatty liver disease; obesity

OBESITY and obesity-associated fatty liver disease (FLD) are becoming global health problems in adults as well as children (4, 8, 10). When consumption of energy far exceeds the combustion of calories, the unburned energy is conserved in the form of fat [triacylglycerol (TG)] in adipose tissue, leading to obesity (8). Obesity-associated insulin resistance appears to serve as a pathogenic event responsible for the metabolic syndrome comprising Type 2 diabetes mellitus, dyslipidemia, atherosclerosis, hypertension, and hepatic steatosis progressing to FLD (4, 8, 10). In the past, excess alcohol consumption accounted for the majority of cases of FLD, but in recent years, nonalcoholic causes of FLD have attracted considerable attention (6, 15). FLD is now clinically categorized into two broad entities, alcoholic FLD (AFLD) and nonalcoholic FLD (NAFLD), to bring the emerging nonalcoholic causes, in particular, obesity-associated FLD, into focus and to distinguish this from the long-known alcoholic liver disease (2, 4, 6, 10, 15, 21, 25). Hepatic steatosis is encountered in about 20–35% of the general adult population in the United States with about 10% of these advancing toward NAFLD (10). In contrast, the prevalence of steatosis in obese individuals is about 75%, and nearly 35% or more of these, with no evidence of excess alcohol consumption, develop NAFLD (2, 4, 6, 10). Other causes of NAFLD include parenteral nutrition, gastric bypass surgery, and certain disorders associated with fatty acid metabolism. FLD, whether it is AFLD or NAFLD, encompasses a morphological spectrum consisting of hepatic steatosis (fatty liver) and steatohepatitis that has the inherent propensity to progress toward the development of cirrhosis and hepatocellular carcinoma (2, 4, 6, 8, 10, 15). Remarkably similar pathological features of alcoholic steatohepatitis (ASH) and nonalcoholic steatohepatitis (NASH) make it generally difficult to distinguish AFLD from NAFLD on morphological grounds alone despite the distinctions implied by these etiological designations (2, 6, 8). Furthermore, this indistinguishable spectrum of histological features of both AFLD and NAFLD suggests a possible convergence of pathogenetic mechanisms at some critical juncture that enables the progression of ASH and NASH toward liver cell death including apoptosis, inflammation, hepatocellular regeneration, stellate cell activation, and fibrogenesis, events that culminate in cirrhosis and liver cancer (1–3, 6, 15, 19, 21, 25). Given the possibility of multifactorial interplay, for example, hepatitis C viral infection with AFLD or NAFLD, or consumption of even small amounts of alcohol (<20 g/ alcohol daily) by obese individuals, it may not always be possible to clearly separate FLD into distinct etiological entities. In this review, we consider FLD as a single pathological entity (in its unity) with multiple etiologies that include alcohol consumption, obesity associated-insulin resistance, and many metabolic perturbations. While excess energy consumption is an important factor in FLD, we bring attention to the contributory role of defective energy burning with a focus on fatty acid oxidation in the liver. In essence, excess energy consumption coupled with reduced energy combustion can contribute to an expected sequence of events beginning with hepatic steatosis and ending in cirrhosis and liver cancer.

FATTY LIVER DISEASE

FLD occurs worldwide in those with excessive alcohol consumption and those who are obese with or without added effects of insulin resistance (4, 6, 8, 10). FLD also occurs in several metabolic and genetic conditions that influence fatty acid metabolism (1, 3, 19, 25). The following represent a brief description of relevant histological features that form the core of FLD. These include hepatic steatosis and steatohepatitis with progression to cirrhosis.

Hepatic steatosis. Both AFLD and NAFLD generally begin as hepatic steatosis, and if the cause persists, this steatosis invariably progresses to steatohepatitis, cirrhosis, and liver cancer (2, 4, 6, 10, 15, 21, 25). Hepatic steatosis represents an excess accumulation of fat (triglycerides) in hepatic parenchymal cells (hepatocytes) of the liver, and it occurs in etiologically diverse conditions. Morphologically, hepatic steatosis
manifests as accumulation of large (macrovesicular) or small (microvesicular) intracytoplasmic fat droplets in liver parenchymal cells. The diagnosis of steatosis is made when lipid content in the liver exceeds 5–10% by weight (1, 2, 6, 15, 19, 25). Hepatic steatosis is mostly macrovesicular in type in the alcoholic, obese, and diabetic states as well as in certain malnutrition states, such as kwashiorkor and acquired immune deficiency syndrome. In macrovesicular variety steatosis, hepatocytes contain a large, single vacuole of fat, which fills the cytoplasm and displaces the nucleus to the periphery, giving rise to a characteristic signet ring appearance (Fig. 1A). Macrovaseicular steatosis may manifest in zone 3 or may present predominantly as panacinar with increasing severity. In microvesicular steatosis, hepatocytes are occupied by numerous small lipid droplets that do not displace the centrally located nucleus to the periphery (Fig. 1B). Genetic or toxin-induced abnormalities in mitochondrial and peroxisomal β-oxidation of fatty acids lead to microvesicular hepatic steatosis, and this type tends to be rapidly progressive and more severe. Some hepatocytes in these livers with microvesicular steatosis may also reveal a macrovesicular fatty change, implying that with the progression of disease some of these small lipid vacuoles may fuse to become a large droplet. In AFLD and longstanding NAFLD, hepatic steatosis is generally macrovesicular and, in some cases, may be intermixed with microvesicular droplets (2, 6, 15, 21, 25).

The pathogenesis of the fatty change in AFLD and NAFLD appears multifactorial. In AFLD, impairment or inhibition of peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ function and stimulation of sterol regulatory element-binding protein (SREBP)1, the receptor molecules that control the enzymes responsible for the oxidation and synthesis of fatty acids, respectively, appear to contribute to the overall lipid load in the liver (3, 5, 13, 14, 16–19, 20). In addition, chronic alcoholism is known to damage mitochondria, the endoplasmic reticulum, and other cellular structures, further contributing to the inhibition of fatty acid oxidation (6, 17). The relative roles of various factors in the development of hepatic steatosis are difficult to estimate, but the net result may be that progressively steatotic hepatocytes begin to rupture or die. Ruptured or apoptotic hepatocytes release TGs, which, along with the unmetabolized very-long-chain fatty acids in particular, add to the liver injury. Hepatic steatosis in NAFLD is related to insulin resistance, but it is becoming increasingly evident that NASH can occur in the absence of overt insulin resistance (1, 3–5, 8, 10). These observations suggest that hepatic steatosis in NAFLD may begin as a simple overstorage of unmetabolized energy in hepatocytes in those who consume excess energy (3, 8, 10).
fat liver, the precipitating or inciting event may be directly related to rupture or apoptosis of markedly steatotic hepatocytes and the release of TGs and toxic fatty acids. Fatty acid overload in hepatocytes acts as both a substrate and an inducer of microsomal cytochrome P-450 (CYP2E1) and fatty acid oxidation systems that generate reactive oxygen species resulting in oxidative stress (19). Oxidative stress causes the release of several cytokines including TNF-α, transforming growth factor-β, and interleukins by Kupffer cells (7). Reactive oxygen species as well as ethanol activate stellate cells that participate in fibrogenesis. Lipid peroxidation products as well as proteins modified by reactive oxygen species develop immunogenic properties causing an inflammatory response. Furthermore, autoimmune responses toward hepatocyte constituents have also been implicated in liver cell injury. In both ASH and NASH, gut-derived factors such as bacterial lipopolysaccharide and endotoxin are known to activate Kupffer cells, resulting in cytokine generation and a reduction of circulating adiponectin. Although the exact role of adipocytokines (adiponectin and leptin) in NASH is not known, overexpression of both these cytokines by stellate cells has been demonstrated in insulin resistance states. It is generally considered that leptin promotes fibrosis and adiponectin inhibits fibrosis and causes apoptosis of stellate cells.

Hepatic fibrogenesis, hepatocellular regeneration, and progression to cirrhosis. Livers with extensive high-grade steatosis reveal the presence of scattered ballooned hepatocytes that may leak or rupture. Apoptotic ballooned hepatocytes and cells with Mallory hyaline along with many other factors appear to contribute to the onset of steatohepatitis. Liver cell death and inflammatory responses lead to the activation of stellate cells, which play a pivotal role in hepatic fibrosis. The fibrogenic response commences in zone 3 and manifests as perisinusoidal, perivenular (around terminal hepatic veins), and pericellular fibrosis (Fig. 1E). Relentless extension of this fibrogenic response contributes to the formation and expansion of fibrous septa that bridge between terminal hepatic venules and portal triads and also generates portal to portal fibrous tracts. Liver cell injury and smoldering apoptosis serve as mitogenic stimuli for the conditionally dividing liver cells to proliferate. These proliferating and newly regenerated liver cells tend to accumulate less and less fat as their proliferation frequency increases. In end-stage AFLD with cirrhosis, steatosis generally becomes nonexistent due to the overwhelming resistance of proliferated hepatocytes to accumulate fat. The reasons for this refractoriness remain to be elucidated. These expanding colonies of liver cells contribute to the distortion of liver architecture and formation of liver nodules in a milieu of increasing liver fibrosis. This aggressive fibrogenesis as well as sustained hepatocellular proliferation contribute to the development of liver cirrhosis. The progression to cirrhosis may be influenced by the amount of fat and degree of steatohepatitis and by a variety of other sensitizing factors. In AFLD, the transition to cirrhosis with continued alcohol abuse has been well established, but in NAFLD of obesity the process is not well delineated and the magnitude of this entity is not well catalogued, leading to the vague assertion of cryptogenic nature of this resulting cirrhosis (1, 5).

Cirrhosis to liver cancer: the inevitable progression. End-stage FLD will progress toward the development of hepatocellular carcinoma (Fig. 1F), and, according to various estimates,
hepatocellular carcinoma develops in about 10% of cirrhotic AFLD livers (5, 6, 10, 25). The nature of progression and overall incidence of liver cancer development in cirrhotic NAFLD livers are not well documented because of the emerging nature of this disease. Nonetheless, several reports deal with hepatocellular carcinoma development in cryptogenic cirrhosis related to NASH. Cryptogenic cirrhosis has been estimated to account for 5–30% of end-stage liver disease, and it has been asserted that many of these cases of cirrhosis and the associated hepatocellular carcinoma represent the progression of NAFLD (1, 5). On the basis of these predictions, there is the urgent need for prospective studies to follow the natural history of NAFLD. The absence of steatosis and inflammatory reaction in end-stage NAFLD is the basis for the designation cryptogenic cirrhosis, and, in these cases, it would be important to rule out hepatitis B and C viral infections and alcohol abuse.

**FATTY ACID OXIDATION (ENERGY COMBUSTION)**

The liver is a central player in the whole body energy homeostasis by its ability to metabolize glucose and fatty acids. When energy intake is abundant, mammals preferentially burn carbohydrates to generate ATP and surplus glucose, after replenishing glycogen stores, is converted to fatty acids (lipogenesis) for use in the synthesis and storage of TG in white adipose tissue (20). Although white adipose tissue functions essentially as a limitless reservoir to accumulate TG, the liver is also able to store significant quantities of lipids in conditions associated with prolonged excess energy consumption or impaired fatty acid metabolism manifesting as steatosis. In fasted states, when glucose availability and insulin levels are low, there is a depletion of hepatic glycogen stores and a reduction in fatty acid production. Under these conditions, TGs stored in adipose tissues are hydrolyzed to free fatty acids and mobilized into plasma to reach the liver. In the liver, they undergo oxidation, converted to ketone bodies to be used as fuel by extrahepatic tissues (11, 20).

Sources of increased lipid (TG) content in hepatic steatosis include 1) excess dietary TG associated with overeating that reach the liver as chylomicron particles from the intestine; 2) increased TG synthesis in the liver from fatty acids formed from de novo lipogenesis; 3) excess fatty acid influx into the liver from lipolysis of adipose tissue in obese and insulin-resistant states and subsequent conversion to TG; 4) diminished export of lipids from the liver in very-low-density lipoproteins; and 5) reduced oxidation of fatty acids. High insulin suppresses hepatic glucose production, increases hepatic glucose uptake, and enhances lipogenesis in the liver (3, 8). In essence, perturbations affecting fatty acid influx into the liver, their de novo synthesis, and conversion to TG and/or oxidation to generate ATP contribute to disturbances in hepatic lipid homeostasis (3, 8).

**Lipogenesis and hepatic steatosis.** De novo fatty acid synthesis in the liver is regulated by three known transcription factors: SREBP-1c, carbohydrate response element-binding protein (ChREBP) (11), and PPAR-γ (5, 8, 16). The role of these molecular mediators of lipogenesis in hepatic steatosis has been reviewed recently (for the review, see Ref. 3). Insulin and glucose concentrations regulate fatty acid synthesis in the liver. The activation of genes responsible for lipogenesis in the liver by insulin is transcriptionally mediated by SREBP-1c.

These include fatty acid synthase and stearoyl-CoA desaturase 1. SREBP-1c mRNA and its active nuclear protein form are increased in ob/ob mouse livers, attesting to the notion that SREBP-1c overexpression can lead to hepatic steatosis (3). As expected, overexpression of SREBP-1c in transgenic mouse livers results in steatosis due to increased lipogenesis (11). Disruption of the ChREBP gene in the mouse results in reductions in mRNA levels of all lipogenic genes in the liver (3). The transcription factor PPAR-γ is expressed at very low levels in the liver, and overexpression of this transcription factor in the liver leads to hepatic adiposis (adipogenic hepatic steatosis) with the expression of several adipogenic genes in the liver (16, 24). In an animal model with insulin resistance and hepatic steatosis, increases in hepatic PPAR-γ levels were also noted (16). These studies point to the importance of PPAR-γ in hepatic steatosis and the induction of adipogenic genes in the liver (16, 24).

**Fatty acid oxidation and hepatic steatosis.** Disturbances in fatty acid oxidation also account for excess lipid storage in the liver (Fig. 2). Fatty acid oxidation is roughly proportional to the plasma concentration of free fatty acids released from adipose tissue. Fatty acid mobilization is stimulated by glucagon and other hormones and inhibited by insulin. Oxidation of fatty acids occurs in three subcellular organelles (Fig. 2), with β-oxidation confined to mitochondria and peroxisomes and CYP4A-catalyzed α-oxidation occurring in the endoplasmic reticulum (19, 20). Some of the key enzymes of these three fatty acid oxidation systems in liver are regulated by PPAR-α (19, 20).

Mitochondrial β-oxidation is primarily involved in the oxidation of short-chain (<C8), medium-chain (C8–C12), and long-chain (C12–C20) fatty acids, and this process provides energy to cellular processes (20). Mitochondrial β-oxidation results in shortening of fatty acids progressively into acetyl-CoA subunits, which either condense into ketone bodies that serve as oxidizable energy substrates for extrahepatic tissues, especially during starvation (20), or enter into the tricarboxylic acid cycle for further oxidation to water and carbon dioxide (20). Mitochondrial β-oxidation is regulated by carnitine palmitoyltransferase (CPT)1, the carnitine concentration, and malonyl-CoA, which inhibits CPT1 (20). Fatty acids, fatty acyl-CoAs, and several structurally different synthetic compounds known as peroxisome proliferators, which activate PPAR-α, regulate CPT1 levels in the liver (20).

The first step in mitochondrial β-oxidation is the α-β-dehydrogenation of the acyl-CoA ester by a family of four chain length-specific straight-chain acyl-CoA dehydrogenases (20). These include very-long-chain, long-chain, medium-chain, and short-chain enzymes. Medium-chain acyl-CoA dehydrogenase deficiency is the most common inherited disorder of mitochondrial fatty acid oxidation in humans. Mice with disrupted medium-chain and very-long-chain acyl-CoA dehydrogenase deficiency manifest defects in fatty acid oxidation, and these animals develop micro- and macrovascular hepatic steatitis (22). The second, third, and fourth steps in the mitochondrial β-oxidation pathway are performed by 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-keto-acyl-CoA thiolase. All three of these enzyme activities are encompassed in a single mitochondrial trifunctional protein (MTP). This is a heterotrimeric protein that consists of four α-subunits and four β-subunits and catalyzes long-chain fatty
acids and fatty acid metabolism. MTP defects in humans are recessively inherited, and children with defects of any of the three enzymatic activities exhibit mostly microvesicular hepatic steatosis (20). Homozygous null mice for the α-subunit of MTP (MTP-α−/− mice) develop hepatic steatosis immediately after birth and die 6–36 h after birth (20). Aging mice heterozygously mutant for MTP (MTP-α+/− mice) also develop hepatic steatosis and become insulin resistant (22). In addition to genetic disorders affecting mitochondrial fatty acid function, several drugs and toxins, including alcohol, severely inhibit mitochondrial β-oxidation enzymes, leading to hepatic steatosis (20).

Peroxisomal β-oxidation is streamlined exclusively toward the metabolism of less abundant and relatively more toxic and biologically active very-long-chain fatty acids (containing 20 or more carbon atoms), 2-methyl-branched fatty acids, dicarboxylic acids, prostanoids, and C27 bile acid intermediates (20). Very-long-chain fatty acids (>C20) are not processed by the mitochondrial β-oxidation system, and they require peroxisomal β-oxidation to shorten the chain length for further completion of oxidation in mitochondria. Long-chain dicarboxylic acids generated by the microsomal ω-oxidation of fatty acids are metabolized by the peroxisomal β-oxidation system (19, 20). Dicarboxylic acids are generally more toxic than very-long-chain fatty acids and are known to inhibit the mitochondrial fatty acid oxidation system (Fig. 2). An effective peroxisomal β-oxidation system is needed to minimize the deleterious effects of dicarboxylic and other toxic fatty acids to prevent hepatic steatosis.

The peroxisomal β-oxidation system consists of four steps with each metabolic conversion carried out by at least two different enzymes (19, 20). The classical peroxisome proliferator-inducible β-oxidation pathway utilizes straight-chain saturated fatty acyl-CoAs as substrates, whereas the second noninducible group acts on 2-methyl-branched fatty acyl-CoAs (54). Some of the enzymes of these two systems are highly inducible by natural and synthetic ligands of PPAR-α (11, 20). Straight-chain acyl-CoA oxidase is responsible for the initial oxidation of very-long-chain fatty acyl-CoAs to their corresponding trans-2-enoyl-CoAs in the PPAR-α-regulated system, whereas branched-chain acyl-CoA oxidase metabolizes branched-chain fatty acyl-CoAs in the noninducible system. The second and third reactions in the classical β-oxidation system, hydration and dehydrogenation of enoyl-CoA esters to 3-ketoacyl-CoA, are catalyzed by a single enzyme, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase [β-bi/multifunctional enzyme (β-PBE/MFP1)]. Ketocarboxyl-CoAs generated by β-PBE/MFP1 are converted to acetyl-CoA and an acyl-CoA that is two carbon atoms shorter than the original molecule. The shortened acyl-CoA reenters the β-oxidation cycle, and this process repeats for about five cycles, resulting in the removal of about 10 carbon atoms. The appropriately chain-shortened acyl-CoAs are then transported to mitochondria for the completion of oxidation (20). The second and third steps in the noninducible β-oxidation pathway are performed by β-3-hydroxyacyl-CoA dehydrogenase (β-3-hydroxyacyl-CoA dehydrogenase [β-bi/multifunctional enzyme 2]), and the resulting 3-ketoacyl-CoAs are cleaved by the third enzyme of this system, known as sterol carrier protein X, which possesses thiolase activity (20).

Disruption of the straight-chain acyl-CoA oxidase gene in the mouse leads to the development of severe microvesicular hepatic steatosis (9). These mice exhibit high levels of very-long-chain fatty acids (>C22) in serum, growth retardation, and hepatomegaly with steatohepatitis (9). Hepatocyte death and regeneration, lipogranulomas, and hepatocellular carcinomas develop in these mice, and some of these features appear to mimic the spectrum of obesity-associated liver changes.

Fatty acids are also oxidized by the microsomal ω-oxidation system by CYP4A enzymes capable of hydroxylating saturated and unsaturated fatty acids. The first step in microsomal fatty acid oxidation is ω-hydroxylation in the endoplasmic reticulum, and the resulting ω-hydroxy fatty acid is then dehydrogenated to a dicarboxylic acid in the cytosol. Dicarboxylic acids are converted to dicarboxylyl-CoAs for oxidation by the classical β-oxidation pathway. Although ω-oxidation is a minor pathway of fatty acid metabolism, significant amounts of
dicarboxylic acids can be formed under conditions of fatty acid overload in the liver, for example, in obesity and diabetes and in situations where the mitochondrial oxidation system is inadequate to metabolize fatty acids. In addition to serving as substrates for the peroxisomal β-oxidation system, dicarboxylic acids also function as PPAR-α ligands to induce all three fatty acid oxidation systems including the CYP4A gene family in the liver (20).

**PPAR-α in hepatic steatosis.** The PPAR subfamily of nuclear receptors consists of three members: PPAR-α, PPAR-γ, and PPAR-β/δ, all of which play a role in lipid homeostasis. PPAR-α and PPAR-β/δ facilitate energy combustion, whereas PPAR-γ contributes to energy storage by enhancing adipogenesis (8, 19, 20). PPAR-α functions as a lipid sensor in the liver and recognizes and responds to the influx of fatty acids by stimulating the transcription of PPAR-α-regulated genes (8, 19, 20). These include genes encoding for the mitochondrial and peroxisomal β-oxidation systems and microsomal ω-oxidation system (Fig. 2). Efficient PPAR-α sensing in the liver is crucial for it to recognize and respond to fatty acid influx as in the case of starvation or fasting, where fatty acid influx robustly induces the activities of all three fatty acid oxidation systems to prevent hepatic steatosis. Overnight or prolonged fasting leads to severe hepatic steatosis when PPAR-α sensing is inefficient, as seen in PPAR-α−/− mice (11, 20). PPAR-α−/− mice fail to upregulate fatty acid oxidation systems in the liver and cannot oxidize the influxed fatty acids and thus develop severe hepatic steatosis. PPAR-α−/− mice also develop severe steatohepatitis when maintained on a diet deficient in methionine and choline (13, 14, 18). Also of importance is that administration of PPAR-α agonists to rats not only prevents the development of methionine- and choline-deficient diet-induced steatohepatitis but also reverses hepatic fibrosis (13, 14, 18). Upregulation of fatty acid oxidation systems and the ensuing burning of energy, reduction in the toxicity of fatty acids, and anti-inflammatory effects of PPAR-α activation play a role in the modulation of hepatic steatosis (11, 13, 19, 20).

Recently, the role of PPAR-α in alcohol-induced liver damage (ASH/AFLD) has been examined using PPAR-α−/− mice. PPAR-α−/− mice fed ethanol developed marked hepatomegaly, steatohepatitis, liver cell death and proliferation, and portal fibrosis (17). PPAR-α−/− mice exposed to ethanol also had elevated levels of acetaldehyde and TNF-α, reduced levels of antioxidants enzymes, and activation of the p65 subunit of NF-κB (17). Alcohol-fed PPAR-α−/− mice had higher levels of hepatic microsomal CYP2E1, which contributes to oxidative stress. The induction of CYP2E1 and increases in proinflammatory TNF-α cytokines play a significant role in the pathogenesis of steatohepatitis in these alcohol-fed PPAR-α-deficient mice. The liver lesions induced by alcohol in these PPAR-α−/− mice also reflect a reduced ability to catabolize very-long-chain fatty acids and their metabolites due to an inability to upregulate the fatty acid oxidation systems in the liver in the absence of PPAR-α (17, 19). Transcriptional activation of PPAR-α target genes in the liver is a complex endeavor, which depends not only upon the liganded receptor and its heterodimerization partner but also on an array of multisubunit cofactor complexes (19, 20). Given this complexity, susceptibility to FLD, whether it is AFLD or NAFLD, may be influenced by the levels of PPAR-α, the content of essential transcription coactivators, and the nature of target gene promoters (20). In summary, PPAR-α plays a prominent role in the pathogenesis of FLD and that of PPAR-α ligands in the reduction of FLD by increasing energy utilization (11, 20).

**Perspective**

Hepatic steatosis in its bland form is an excessive accumulation of TG in hepatocytes and, as discussed above, is influenced by lipogenesis and fatty acid oxidation. On the other hand, the death of liver cells and regeneration of surviving hepatocytes, development of steatohepatitis, activation of stellate cells, and progressive and incessant deposition of hepatic fibrosis leading to cirrhosis are all attributed to the “second hit,” or multiple hits. This complex progression of simple steatosis to FLD is related to metabolic abnormalities associated with massive steatosis, insulin resistance, and reductions in fatty acid oxidation (1, 4, 8, 10). Steatohepatitis is attributed to TNF-α, free fatty acid toxicity, toxicity caused by dicarboxylic acids, a decrease in mitochondrial and peroxisomal β-oxidation, the generation of reactive oxygen species, lipid peroxidation, and many more events that lead to liver cell apoptosis and inflammation (7). Hepatocyte death promotes the accelerated proliferation of surviving hepatocytes (9, 12, 23). The inflammatory reaction and reactive oxygen species are known to activate hepatic stellate cells for the progressive deposition of collagen. Elucidation of the mechanisms responsible for hepatic steatosis and steatohepatitis and the associated liver cancer using genetically altered animal models is important for developing strategies for the prevention and treatment of FLD.

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**REFERENCES**


