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Lipids in Liver Disease: Looking Beyond Steatosis

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Abstract

See “Alterations in lipid metabolism mediate inflammation, fibrosis, and proliferation in a mouse model of chronic cholestatic liver injury,” by Moustafa T, Fickert P, Magnes C, et al, on page 140; and “A high-cholesterol diet exacerbates liver fibrosis in mice via accumulation of free cholesterol in hepatic stellate cells,” by Teratani T, Tomita K, Suzuki T, et al, on page 152.

The liver is a key site for energy and lipid metabolism. Accumulation of excess fat in the liver predisposes individuals to the subsequent development of fibrosis, cirrhosis and hepatocellular cancer.¹ Steatosis is also an important comorbidity that amplifies liver injury and disease progression in diseases of other etiologies, as has been demonstrated for toxic, viral, and cholestatic insults. Less is known about the degree to which lipids and lipid metabolism influence liver diseases when steatosis is not present. Clues already exist that lipid accumulation per se is not a key effector of organ injury. Indeed, lipid droplets are highly specialized organelles that allow cells to store and release lipids in a regulated fashion,² and it has been argued that the compartmentalization of lipids in droplets keeps them out of harm's way.^{3–5} Instead, lipid flux into and out of these droplets, with the conversion of inert species into potentially toxic mediators, is emerging as an important mechanism of lipid-mediated liver injury. A second commonly overlooked point is that lipids may affect other cell populations in the liver besides hepatocytes. Hepatic stellate cells (HSCs), previously also called hepatic “lipocytes,” contain large amounts of lipids stored in droplets. It is conceivable that HSCs or other nonparenchymal cell populations may be relevant targets of lipids in liver diseases. A third important consideration is what types of lipids trigger liver injury and disease. Although overall calorie and fat intake is a key determinant for the development of obesity and hepatic steatosis, specific dietary lipids such as cholesterol or trans fats may be more relevant for disease development, including liver injury, inflammation, and fibrosis.^{6,7} In this issue of *GASTROENTEROLOGY*, 2 studies investigate the role of lipids in the development of liver injury and fibrosis.^{8,9} Although these studies investigate different questions, and use distinct models, they challenge our current

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understanding of the role and the targets of lipids in liver disease, and suggest a broader role of lipids in hepatic disease processes than previously thought.

Teratani et al investigate the effects of high dietary cholesterol on hepatic fibrosis induced by bile duct ligation (BDL) and carbon tetrachloride, 2 common models of biliary and toxic liver injury.⁸ The key result of their study lies in the finding that a high-cholesterol diet has no impact on hepatocyte injury as assessed by alanine aminotransferase measurements and TUNEL staining, but that it severely affects HSCs and liver fibrosis. Moreover, the authors excluded a contribution of Kupffer cells, because they still observed fibrosis-promoting effects of high dietary cholesterol in Kupffer cell-depleted mice. Instead, the authors observed increased cholesterol levels in HSCs from mice on a high-cholesterol diet, and an increased up-regulation of profibrogenic genes in response to transforming growth factor (TGF)- β . Although the authors found no effect of high-cholesterol diets on TGF- β receptor expression, they observed an increased expression of the lipopolysaccharide receptor Toll-like receptor 4 (TLR4), a subsequent increase in TGF- β signaling and down-regulation of Bambi, an inhibitory TGF pseudoreceptor that is highly expressed in HSCs and that negatively affects TGF- β signaling in these cells.¹⁰ The authors confirmed these findings in a second genetic model in which intracellular cholesterol levels are increased owing to the absence of the NPC1 protein. Interestingly, in both models cholesterol did not increase TLR4 mRNA levels, leading the authors to suggest that cholesterol prevents TLR4 protein degradation. The novelty of this study lies in the demonstration of cholesterol effects on HSCs and their fibrogenic activation (Figure 1).

Because the lipid droplets in HSCs contain about 20% cholesterol or cholesterol ester,¹¹ it seems logical that dietary cholesterol may increase these levels and affect HSC biology. However, the current study leaves open the question of whether other cells in the liver are affected by dietary cholesterol. Hepatocytes are known for their ability to take up large amounts of plasma cholesterol and to eliminate excess cholesterol via excretion into bile in a process known as reverse cholesterol transport.¹² Similarly, macrophages are typically affected by high dietary cholesterol.¹² Importantly, previous studies have demonstrated that a high-cholesterol diet sensitizes mice to tumor necrosis factor- and Fas-mediated steatohepatitis and hepatocellular cell death.⁶ Although Teratani et al show no influence of dietary cholesterol on apoptotic or necrotic injury after BDL or carbon tetrachloride injection, it seems unlikely that dietary cholesterol selectively traffics to HSCs without exerting effects on other hepatic cell populations. One could envision that the predominant target and effects of cholesterol depend on the underlying disease, and that cholesterol may be redirected to HSCs when biliary excretion is blocked or hepatocyte uptake is impaired.

Although the study by Teratani et al shows for the first time how lipids alter responses specifically in HSCs, the implications of these results for liver disease in patients with high cholesterol remain unclear. It is conceivable that dietary cholesterol amplifies the fibrogenic response in HSCs as suggested by Teratani et al, and also affects liver injury and inflammation as “second hit” to hepatocytes in other diseases as suggested by Mari et al.⁶ Importantly, both studies have used a high amount of cholesterol translating to 1000–2000 mg/d, whereas the average human intake is only 300 mg/d, thereby making the extrapolation to the clinical setting difficult. Previous studies using National Health and Nutrition

Examination Survey data have linked increased cholesterol intake to death from liver disease, but did not analyze the underlying liver disease.¹³ Therefore, well-designed trials in specific patient groups, such as hepatitis C and nonalcoholic fatty liver disease, need to assess the impact of cholesterol intake on different liver diseases and fibrosis development. Although many questions about dietary cholesterol and liver disease remain to be answered, one important take home lesson from the study by Teratani et al is that we need to put more focus on the effects of lipids in nonhepatocyte populations, such as the HSCs.

In a second article published in this issue of *GASTROENTEROLOGY*, Moustafa et al⁹ uncovered a unique role for hepatic lipids in a mouse model of cholestatic liver disease caused by genetic deletion of the ATP-binding cassette sub-family B member 4 (*Abcb4*^{-/-}). *Abcb4* encodes a phospholipid flippase that promotes biliary secretion of phospholipid and protects the biliary epithelium from the damaging effects of bile acids. Mice deficient in *Abcb4* develop hepatocellular injury, cholestasis, and liver fibrosis¹⁴; *ABCB4* mutations also occur in humans, and are responsible for the syndrome known as progressive familial intrahepatic cholestasis type 3 (PFIC3).¹⁵

Although *Abcb4*^{-/-} mice develop many of the same biochemical and histologic derangements seen in humans with cholestasis, they also display some unique alterations in lipid metabolism. Specifically, *Abcb4*^{-/-} mice have hypocholesterolemia and low levels of hepatic triglycerides, whereas humans with cholestasis typically have hypercholesterolemia. Despite this discrepancy, there is a hint that the lipid abnormalities in *Abcb4*^{-/-} mice are clinically relevant, because *ABCB4* polymorphisms in humans can lead to a reduction in circulating cholesterol levels.¹⁶ Curious whether the lipid-related alterations in *Abcb4*^{-/-} mice are causally related to liver disease, Moustafa et al compared hepatic gene expression in the knockout mice with that in wild-type controls. They found that genes affecting lipid metabolism were indeed influenced by *Abcb4* deficiency. They also measured hepatic gene expression in *Abcb4*^{-/-} mice before and after treatment with 24-*NorUrsodeoxycholic* acid (*NorUDCA*), a side-chain variant of ursodeoxycholic acid that ameliorates cholestatic liver injury. Again, genes affecting lipid metabolism were among the most markedly influenced by *NorUDCA* treatment.

A closer look at *NorUDCA*-treated *Abcb4*^{-/-} mice showed that the drug reversed the background hypocholesterolemia and hypophospholipidemia and normalized hepatic triglyceride content, thus reversing the abnormalities caused by the gene knockout. These changes coincided with an increase in cholesterol and phospholipid incorporation into lipoproteins and a decrease in the activity of triglyceride hydrolase. The latter finding was particularly intriguing because it suggested liver disease in *Abcb4*^{-/-} mice is due to enhanced triglyceride hydrolysis in the liver.

Fatty acids released from triglycerides are important inducers of peroxisome proliferator activated receptor (PPAR)- α . Moustafa et al postulated that PPAR- α was contributing to the excess triglyceride lipolysis in *Abcb4*^{-/-} mice, based on evidence that several enzymes involved in triglyceride catabolism are inducible by PPAR- α .¹⁷ They crossed *Abcb4*^{-/-} mice with *PPAR- α* ^{-/-} mice, which resulted in remarkable protection against cholestasis and liver injury. The double-knockout mice had higher serum lipid levels and higher hepatic

triglyceride levels than *Abcb4*^{-/-} mice along with lower hepatic expression of lipolytic genes. Conversely, when *Abcb4*^{-/-} mice were treated with the PPAR- α agonist fenofibrate, the cholestatic liver injury worsened.

Having shown in 2 experimental scenarios that an improvement in cholestatic liver injury was linked with reduced triglyceride hydrolysis, the authors then tested a third means to shift hepatic equilibrium away from triglyceride hydrolysis, namely, high-fat feeding. Just as with *NorUDCA* treatment and PPAR- α deficiency, high-fat feeding ameliorated cholestasis, hepatic inflammation, and fibrosis in *Abcb4*^{-/-} mice, and the improvement coincided with significant decreases in hepatic phospholipase and triglyceride lipase activities (Figure 2).

These results are striking because they implicate hepatic lipid homeostasis as an important determinant of outcome in a liver disease not characterized by hepatic steatosis. They are also unique in that they demonstrate cholestatic liver disease, at least in *Abcb4*^{-/-} mice, is improved by increasing, rather than decreasing, hepatic triglyceride levels. Does this mean there is some optimal level of hepatic triglycerides that can lead to disease if the supply moves excessively up or down? Probably not! Instead, liver disease may be a function of the metabolic activity of hepatic lipid. Previous studies have shown that excess fatty acids in the liver are injurious if they are prevented from being incorporated into triglycerides.³ The work of Moustafa et al illustrates the converse point— that liver injury can also occur if lipid droplets are unable to hold on to their triglyceride. Taken together, these findings incriminate fatty acids as hepatotoxic compounds and reinforce the notion that triglyceride synthesis in hepatocytes is a cytoprotective mechanism.⁴

Although Moustafa et al effectively reversed hepatocellular injury in *Abcb4*^{-/-} mice with several treatments that reduce triglyceride hydrolysis, it is uncertain why these manipulations also improved cholestasis. There was no evidence that PPAR- α deficiency or high-fat feeding improved bile flow, although this does occur with *NorUDCA*. The beneficial effect of PPAR- α deficiency on liver injury in *Abcb4*^{-/-} mice is also curious in view of other reports that experimental cholestasis owing to BDL, as well as experimental fatty liver disease, are improved by PPAR- α agonists.^{18,19} These results are difficult to reconcile, but are in keeping with the complex and sometimes paradoxical effects of PPAR- α manipulation in other organs.^{5,20} Until the findings can be put into proper perspective, it is premature to consider PPAR- α suppression as a treatment for cholestasis in humans.

Finally, although *Abcb4*^{-/-} mice are used widely as a model of cholestatic liver disease in humans, it is uncertain whether the lipid-related abnormalities in these mice are generalizable to humans—even humans with PFIC3. An important next step will be to determine whether PFIC3 patients display the same reduction of hepatic triglycerides and enhanced triglyceride hydrolysis as *Abcb4*^{-/-} mice. If so, it may lead to a new approach to this disease, and will certainly encourage further exploration of lipid-related abnormalities in other chronic cholestatic disorders.

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Figure 1.

Increased HSC activation by dietary cholesterol. Increased dietary cholesterol up-regulates TLR4 protein levels in HSCs, which in turn down-regulates the inhibitory TGF- β pseudoreceptor Bambi. The resulting increase in TGF- β signaling promotes increased HSC activation and liver fibrosis.

Figure 2.

Hydrolysis of triglyceride in hepatic lipid droplets yields toxic fatty acids, which can activate an injurious positive feedback loop by activating PPAR- α and stimulating triglyceride lipase(s). NorUDCA, PPAR- α inhibition, and high-fat feeding can each interrupt the cycle by preventing further hydrolysis and shifting equilibrium in the hepatocyte toward triglyceride synthesis and storage.