Is the slippery slope from steatosis to steatohepatitis paved with triglyceride or cholesterol?

Accumulation of hepatic lipids has been thought to trigger the inflammation, apoptosis, and fibrosis that characterize progression of hepatic steatosis to steatohepatitis and cirrhosis. In this issue of *Cell Metabolism*, Marí et al. (2006) provide evidence for excessive mitochondrial free cholesterol as a cause of the progession of steatosis to more severe liver disease.

Nonalcoholic fatty liver disease (NAFLD) ranges in severity from steatosis to steatohepatitis to the above plus fibrosis leading to cirrhosis. Recent observational studies indicate a prevalence as high as 25% in the United States (Farrell and Larter, 2006; Browning and Horton, 2004), where NAFLD may be a leading cause of cryptogenic cirrhosis. Although most individuals with NAFLD seem to either remain stable or improve over time, little is known about the progression of this disorder to steatohepatitis and cirrhosis.

Much of the increased prevalence of NAFLD is driven by obesity. However, high rates of NAFLD in relatively normal weight people (by Western standards) from the Indian subcontinent and Southeast Asia suggest that, even in the absence of obesity, insulin resistance leads to hepatic fat accumulation. Indeed, patients with total lipodystrophy, who have no adipose tissue, have severe insulin resistance with marked hepatic steatosis. Studies of the molecular basis of NAFLD have largely focused on triglyceride (TG), the major lipid stored in hepatocytes. Although much is known about the regulation of hepatic TG synthesis, secretion, and storage, much less is known about the role of TG and/or its precursors in stimulating the inflammatory changes needed for the progression of steatosis to steatohepatitis. In this issue of *Cell Metabolism*, Marí et al. (2006) move the spotlight to cholesterol, and in particular mitochondrial free cholesterol, as a central molecule in the pathogenesis of steatohepatitis.

Hepatic TG levels are determined by the availability of fatty acids (FA) from the circulation, de novo lipogenesis of FA from glucose, oxidation of FA, and the secretion of TG on very low-density lipoproteins (VLDL) (Figure 1A) (Goldberg and Ginsberg, 2006). Each of these processes may be altered by insulin resistance in ways that predispose to steatosis. Thus, insulin resistance leads to increased lipolysis of adipocyte TG and more FA flux to the liver (Yu and Ginsberg, 2005). Insulin resistance may be associated with reduced lipoprotein lipase-mediated lipolysis of plasma chylomicron or VLDL TG, leading to hepatic uptake of remnant lipoproteins carrying more TG than normal. De novo lipogenesis is increased in insulin resistance; insulin-mediated stimulation of SREBP-1c is a key contributor, although glucose-mediated stimulation of ChREBP can also play a significant role (Browning and Horton, 2004). Aberrant expression of PPARγ2 in insulin resistance livers can also stimulate de novo lipogenesis (Gavrilova et al. 2003). Oxidation of hepatic FA is regulated at several points, but is likely to be limited in the face of adequate hepatic glycogen and increased lipogenesis with elevated levels of malonyl-CoA. Finally, insulin can target apoB for post-translational degradation; the balance between systemic hyperinsulinemia and hepatic insulin resistance will determine how much apoB will be available to carry TG out of the hepatocyte (Figure 1B).

It has been shown that increased hepatic TG stimulates increased VLDL TG secretion by targeting apoB away from degradation and toward secretion and/or by increasing the amount of TG on each VLDL (Fisher and Ginsberg, 2002). However, recent studies suggest greater complexity. For example, stimulation of hepatic lipogenesis by an LXR agonist results in increased TG secretion but has no effect on apoB secretion. In vivo overexpression of mtGPAT, an enzyme that synthesizes diglycerides, increases both hepatic TG content and secretion (Linden et al., 2006), but overexpression of either DGAT1 or DGAT2, enzymes that synthesize TG and increase hepatic TG, has had inconsistent effects on TG secretion (Millar et al., 2006). On the other hand, we demonstrated that increased delivery of FA to the liver can increase apoB secretion without increasing TG secretion (Zhang et al., 2004). Together, these results indicate compartmentalization of hepatic TG into pools with tight or loose connections to TG secretion or differential effects of FA and TG on apoB and TG secretion. To further complicate matters, levels of lipid droplet proteins such as ADRP and perilipin, activities of hepatic lipases such as TGH, ATGL, and HSL, and the activity of MTP (which transfers endoplasmic reticulum TG and cholesterol onto apoB) may all confound the relationship between hepatic TG accumulation and the assembly and secretion of VLDL.

How steatosis progresses to steatohepatitis is under intense investigation. Inflammation, together with evidence of apoptosis and necrosis of hepatocytes, differentiates steatohepatitis from steatosis. Most investigators accept a "two-hit" hypothesis; steatosis appears to be the required background abnormality upon which inflammation, cellular dysfunction, and cell death can occur. Increased FA oxidation and ROS formation could lead to a state of oxidative stress, with sequelae that include lipid peroxidation, membrane damage, and mitochondrial dysfunction, the latter leading to more ROS formation. However, the evidence that there is increased FA oxidation in steatotic livers is limited. In their paper, Marí et al. (2006) present evidence supporting an alternative lipid-based mechanism for the progression of steatosis to steatohepatitis (Figure 1C). These authors propose and provide experimental evidence for a specific role for accumulation of free cholesterol in mitochondria leading to mitochondrial glutathione depletion and sensitivity to TNFα and FAS mediated pathways of apoptosis. Using two diets, one that was choline deficient and one with 2% cholesterol plus sodium cholate, they specifically increased either hepatic TG or cholesterol, respectively. They demonstrated that TNFα treatment caused apoptosis, necrosis, and ROS formation only in livers
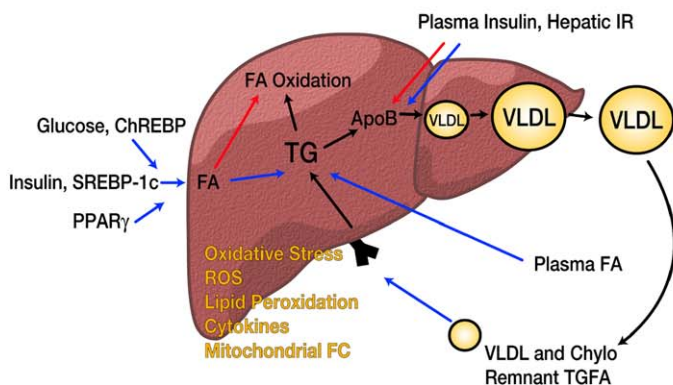
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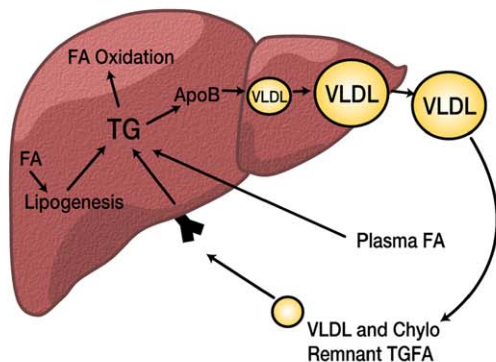
B Dysregulation of Hepatic Lipid Homeostasis Leading to Steatosis
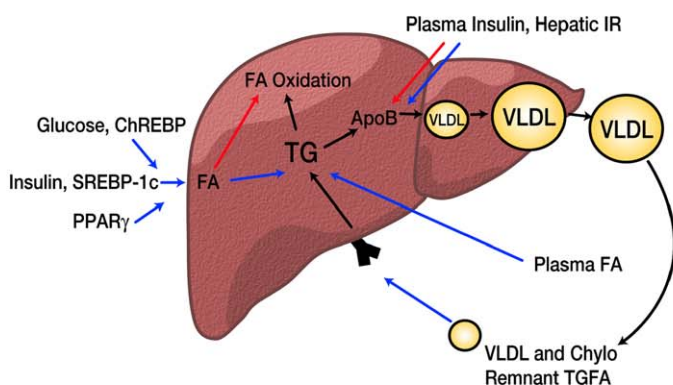
C Dysregulation of Hepatic Lipid Homeostasis Leading to Steatohepatatis

Figure 1. Hepatic lipid metabolism in steatosis and steatohepatitis

A) Hepatic TG homeostasis is maintained through a balance of the delivery of albumin bound fatty acids (FA) or triglyceride (TG) FA in remnant lipoproteins, de novo synthesis of FA from glucose via lipogenesis, oxidation of FA, and the assembly and secretion of TG and apolipoprotein B (apoB) as very low-density lipoproteins (VLDL).

B) Insulin resistance and obesity can result in steatosis because of increased delivery of FA and TGFA, increased lipogenesis driven by SREBP-1c, ChREPB, and possibly aberrant expression of PPARγ, and decreased fatty acid oxidation secondary to increased lipogenesis and levels of malonyl CoA. Plasma hyper-insulinemia with modest hepatic insulin resistance (IR) could result in increased insulin-mediated degradation of apoB and more steatosis. Blue arrows indicate pathways or processes that will stimulate hepatic TG production and/or secretion; red arrows indicate inhibition of those pathways or processes.

C) The progression of hepatic steatosis to steatohepatitis requires insults in addition to steatosis: these include oxidative stress, production of reactive oxygen species (ROS), lipid peroxidation, the actions of cytokines such as TNFα, and according to the work of Marí et al. (2006), increased mitochondrial free cholesterol (FC) with loss of glutathione and increased sensitivity to TNFα.

with increased cholesterol (with or without increased TG) content. These investigators further showed that the free cholesterol content of mitochondria was increased, at least transiently, on the high-cholesterol/cholate diet and that this was associated with altered membrane fluidity and reduced glutathione content. Additional experiments, in which mitochondrial glutathione was depleted with a small molecule, reiterated the overall effects of increased mitochondrial cholesterol. Finally, treatment with the HMG-CoA reductase inhibitor, atorvastatin, reduced mitochondrial free cholesterol and increased mitochondrial glutathione levels in livers from rats fed the high-cholesterol/cholate diet. Signs of steatohepatitis were ameliorated as well.

So, is altered hepatic cholesterol metabolism, and specifically increased mitochondrial cholesterol concentration, the missing lipid-link between steatosis and steatohepatitis? The data of Marí et al. (2006) are certainly intriguing and suggestive. It would help to know more about the effects of the diet with high cholesterol but without cholate, as these two dietary components have been shown to induce different sets of genes important for hepatic inflammation and fibrosis (Vergness et al., 2003). It would also be helpful if diets lower in cholesterol had been used: a 2% cholesterol diet is equivalent to an intake of more than 2000 mg/day of dietary cholesterol in man, in whom the average dietary cholesterol intake is 300 mg/day. Additionally, the transient nature of the rise in mitochondrial free cholesterol, due to increased acyl-CoA:cholesteryl acyltransferase (ACAT) expression and activity in the high-cholesterol-fed rats, points to the need to determine if mitochondrial free cholesterol levels differed between the cholesterol-fed and the choline-deficient diet-fed mice after 1 week. With those caveats in mind, Marí et al. (2006) have clearly opened the way for more careful examinations of hepatic lipids; it is time to move beyond TG and also investigate the role of hepatic cholesterol in various mouse models of steatosis. If the present data are supported by further studies, new approaches to therapy could follow. Present therapies targeted at increasing insulin sensitivity, increasing FA oxidation, or reducing TG synthesis are all logical based on a large body of data collected over the past two decades. Hepatic cholesterol may become an

adjunctive or alternative target if these new data are confirmed. Of course, prevention of steatosis by proper nutrition and exercise remains our primary goal.

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Selected reading

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An ARC light on lipid metabolism

The SREBP pathway plays a central role in the regulation of lipid metabolism. In a recent letter, Yang et al. present a comprehensive series of experiments, spanning a wide range of disciplines, that identify ARC105 as a component of the ARC complex that interacts directly with SREBP and is necessary for SREBP function (Yang et al., 2006).

As part of the effort to understand the mechanistic basis for the cellular control of lipid metabolism, much work has been focused on dissecting the sterol regulatory element binding protein (SREBP) pathway. In a recent paper, Yang et al. (2006), demonstrate that a subunit of the ARC complex, ARC105, interacts directly with SREBPs to enable transcription from target promoters (Figure 1).

SREBPs are membrane bound transcription factors that play a central role in regulating lipid production in all metazoans studied. This work has revealed the intricate machinery responsible for regulating the release of SREBPs from the membrane in response to cellular need for lipids. This machinery includes two proteases, an escort factor and retention factors, and is localized to intracellular membranes (Brown and Goldstein, 1999; McPherson and Gauthier, 2004). Each of these components is necessary to ensure regulated release of SREBPs from the membrane and, thus, access to the nucleus. Nuclear access is not the end of the story, however.

Other work has focused on additional proteins needed to form the final transcriptionally active complex, once SREBPs reach the nucleus. These cofactors include CBP (the cAMP response element binding protein [CREB] binding protein), a related protein, p300 (Oliner et al., 1996), Sp1, Sp3, (Athanikar et al., 1997), NFY (Ericsson et al., 1996), and the large, multicomponent activator-recruited cofactor (ARC) complex (or the metazoan Mediator complex) (Naar et al., 1999).

Delineating events at SREBP target promoters more fully, Yang et al. (2006) focused on a single subunit of the large ARC complex. They report that interaction between ARC105 and SREBPs is selective; they detected no interaction between ARC105 and other transcription factors, such as the cellular myeloblast transforming factor (c-Myb) or CREB. Similarly, SREBPs did not bind to other ARC subunits tested. Thus interaction between ARC105 and SREBP is not simply a general phenomenon of the transcriptional machinery.

This selectivity is perhaps surprising. ARC105 was first identified as an essential component of the complex required for TGFβ signaling via Smad2/3-Smad4 binding (Kato et al., 2002). In the present study, the authors show that the SREBP-interacting domain of ARC105 (a domain that does not bind c-Myb or CREB) is structurally similar to the KIX domain of CBP (a domain that does bind c-Myb and CREB). Changing just two residues in the third α helix of ARC105 to the corresponding residues in CBP ($Ile_{64} \rightarrow Tyr$; $Asp_{68} \rightarrow Lys$) substantially improved the ability of ARC105 to interact with both c-Myb and CREB.

Interaction between ARC105 and SREBPs is functionally significant; when the authors used an siRNA strategy to reduce the abundance of ARC105 transcript in cultured cells, the transcription of SREBP-responsive genes was greatly reduced while transcription of several other, non-SREBP-dependent genes remained unaffected. This indicated that the ARC complex could still function with other transcription factors even when levels of ARC105 were artificially low. This is consistent with the selectivity observed in the binding studies. Yang et al. (2006) then used chromatin immunoprecipitation assays to demonstrate joint occupancy of target promoters by SREBP and ARC105.

Experiments conducted in the nematode, *C. elegans*, whose genome harbors orthologs of both SREBP (*SBP-1*) and ARC105 (*MDT-15*), confirmed the physiological relevance of the interaction between ARC105 and SREBP. Disruption of the expression of either gene by RNAi resulted in highly similar phenotypes, including growth defects, infertility, shortened lifespan, and reduced fat storage. A clue to the direct cause of the defects