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Alcohol and Lipid Traffic Don't Mix

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Ethanol abuse and chronic ethanol consumption are major causes of liver disease and represent a significant health problem in the United States and worldwide. An early manifestation of alcoholic liver disease (ALD) is the presence of fatty liver (hepatic steatosis) that, with continued insult, can progress to alcoholic hepatitis and cirrhosis¹. A hepatocellular organelle central to the progression of these disease states is the lipid droplet (LD), a sphere of triglycerides encased in a phospholipid monolayer (for reviews see^{2,3}). The excessive formation and accumulation of LDs within the hepatocyte is a defining feature of steatosis. Despite the fact that LDs are central to hepatic steatosis, the mechanisms that support the formation and metabolism of these organelles are only partially understood. As most patients that abuse alcohol display some degree of hepatic steatosis, it seems essential that we define how LD metabolism is altered by both acute or chronic alcohol exposure and how this leads to hepatocellular damage.

As fat accumulation can influence the liver's sensitivity to important triggers such as oxidative and ER stress, as well as the production and accumulation of endotoxins^{4,5}, the aberrant accumulation of lipids in hepatocytes could easily act as the "first hit" in the progression of ALD¹ and might therefore provide a prime target for therapeutic intervention. In this short essay we discuss the concept that LDs in hepatocytes are complex and dynamic organelles that appear to reside in a state of flux between formation and fusion or, conversely, metabolism and vesiculation. This equilibrium is mediated by a variety of different membrane-associated proteins, some of which may be unique to LDs while others appear to be components of the secretory and endocytic membrane trafficking machinery. We propose that these proteins act to vesiculate LDs in a regulated fashion to facilitate lipid metabolism, and that alcohol exposure appears to attenuate this process, leading to the accumulation of LDs that potentiates hepatic steatosis.

LD Dynamics in Hepatocytes

In hepatocytes, there can be an expansive increase in LD number and size by the continued synthesis and accumulation of esterified lipids from lipid-modifying enzymes associated with the surrounding monolayer and/ or the trafficking and subsequent fusion of smaller lipid droplets. In general, the expansion of lipid droplet growth is relatively modest in cultured hepatocytes under normal serum conditions. However, cells exposed to 200 μ M oleic acid alone or in combination with 100 mM ethanol show a dramatic increase in lipid droplet size and number, particularly in the perinuclear regions (Fig. 1). This dramatic

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response occurs in isolated rat hepatocytes exposed to alcohol in culture, hepatocytes isolated from alcohol-pair fed rats, the WIF-B hepatocyte model that maintains polarized characteristics and can metabolize alcohol, as well as VA-13 cells, a stable HepG2 cell line transfected to express alcohol dehydrogenase. Importantly, all of these cell models display a 2-fold to 4-fold increase in LD size in response to alcohol. This may represent either an aberrant increased lipid synthesis and storage or an attenuation in LD vesiculation and breakdown. The fact that LD number and size are maintained in alcohol-treated hepatocytes for prolonged periods of time even when “starved” in serum-free medium suggests that LD vesiculation and lipolysis are indeed compromised upon alcohol exposure (Fig. 1).

Membrane Dynamics Contributing to Lipid Droplet Vesiculation and Lipolysis

The mechanisms by which lipid bilayers are deformed and vesiculated to support the endocytic and secretory pathways in the hepatocyte are well studied (for reviews see^{6,7}). Although LDs are encased in a lipid monolayer, it is reasonable to predict that many components utilized to tubulate the plasma membrane, endosomes, and the Golgi apparatus could also be recruited to LDs to mediate LD breakdown. This vesiculation process could facilitate lipolysis by greatly increasing the surface to volume ratio of LDs and thereby allow greater access of the lipases, lysosomes and autophagosomes required for lipid metabolism. Such a vesiculation machinery appears to be conserved and widely used throughout epithelial cells. It consists of the large GTPase Dynamin that functions as a membrane pincher and acts in concert with the actin cytoskeleton, membrane coat proteins and associated structural adaptors, small GTPases such as Rabs and Arfs that act as molecular switches, and a variety of BAR-domain containing proteins that are capable of bending membranes. It is attractive to postulate that these components could be recruited to the LD surface to deform and break the LD monolayer not unlike what occurs during the endocytosis or secretion. Importantly, this process could be compromised by alcohol, leading to the observed accumulation of LDs.

The concept of similarities between the LD and endocytic machineries gains support from several interesting observations generated by a variety of different laboratories. For example, Caveolin-1 (Cav1), is an essential component of caveolae and has been identified in the LD proteome⁸. A functional interaction of Cav-1 with LDs has been reported in hepatocytes and, interestingly, hepatocytes from Cav1^{-/-} mice show reduced LD accumulation after partial hepatectomy⁹. The related family member Cav2 has also been shown to associate with LDs but no functional insights have been provided so far¹⁰. Whether assembled caveolae, or the Cav1 protein, act in the formation or the vesiculation of LDs in the hepatocyte needs to be established.

In addition to caveolae, several small Rab GTPases have been implicated in LD function. Rabs also bind membrane lipids and act as molecular switches to support the targeted transport, docking, and fusion of a donor vesicle to a receptor compartment¹¹. Rab5 supports the fusion of early endosomes¹², while Rab7 performs a similar function between the late endosome and lysosome¹³. Both of these Rabs have been observed to associate with LDs¹⁴, while proteomic studies of LD-associated proteins have identified more than a dozen other Rab proteins. Currently, the contributions these enzymes make to LD dynamics are almost totally undefined.

Most recently, LD metabolism has been linked to the autophagic process in hepatocytes as LD content is delivered to lysosomes and autophagosomes while inhibition of this pathway results in the accumulation of triglycerides¹⁵. The autophagic pathway is well known to

utilize many components of secretory and endocytic machinery¹⁶ and could be a target for ethanol-induced damage.

Alcohol-Induced Perturbations of LD Metabolism

Could ethanol exposure alter the vesiculation, trafficking, and degradation of LDs in hepatocytes? Several studies have identified multiple ethanol-induced impairments of vesicle-based transport in the hepatocyte such as clathrin-mediated endocytosis^{17,18}, including traffic of the hepatocyte-specific asialoglycoprotein receptor (ASGP-R) resulting in altered receptor-ligand binding, internalization, uncoupling of receptor-ligand complexes in endosomes, and degradation by lysosomes¹⁹. Alcohol exposure also results in a marked retention of nascent proteins in the Golgi apparatus of the hepatocyte along with a displacement of Rab2 from this compartment²⁰. These inhibitory effects are likely potentiated by the toxic metabolites of ethanol such as acetaldehyde that has been shown to form inhibitory adducts with cellular proteins and enzymes essential to these pathways, such as the microtubule cytoskeleton^{21,22}. Therefore, it is likely that adduct formation perturbs the enzymatic activity of other GTPases in addition to tubulin, such as the dynamins and Rab proteins, to lead to an impairment of LD degradation. Indeed, it is well documented that alcohol can disrupt lysosomal and proteosomal protein degradation²³, as well as autophagic activity²⁴. In contrast, a recent study has shown that acute ethanol exposure actually stimulates autophagic degradation of both mitochondria and LDs in treated hepatocytes and could provide a protective benefit to the effects of oxidative stress as experimental disruption of this autophagic response leads to apoptosis²⁵. It will be important to define how acute alcohol exposure might lead to these differential responses and whether they are further potentiated by a high lipid environment.

Important Directions for Future Study

In this essay we have provided a very brief overview of how membrane traffic might regulate to LD dynamics in the hepatocyte. Novel concepts that we believe are worthy of experimental pursuit suggest that, first, LDs are dynamic organelles that are reduced in size by vesiculation to facilitate lipolysis. Second, this process is mediated by many of the same membrane trafficking components used at other secretory and endocytic organelles in the hepatocyte. Finally, this vesiculation-supported lipolysis is compromised by both acute and chronic ethanol exposure leading to LD accumulation and steatosis. Superimposed on these predictions is the central issue of how LD dynamics contribute to hepatocellular stress, injury and cirrhosis. The encapsulation of triglycerides into large droplets could be viewed as a protective mechanism by the hepatocyte to remove reactive lipid species that can potentiate oxidative stress, ER stress, and the release of inflammatory cytokines. Thus, LD accumulation per se, either by alcohol or in nonalcoholic steatohepatitis, may not necessarily induce hepatocellular damage directly but rather it may be the subsequent metabolism of these LDs that proves injurious. How the excessive accumulation of LDs in the steatotic liver eventually overwhelms the protective mechanisms of hepatocyte is a seminal question in need of attention.

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Abbreviations

ALD alcoholic liver disease

Cav	caveolin
LD	lipid droplets

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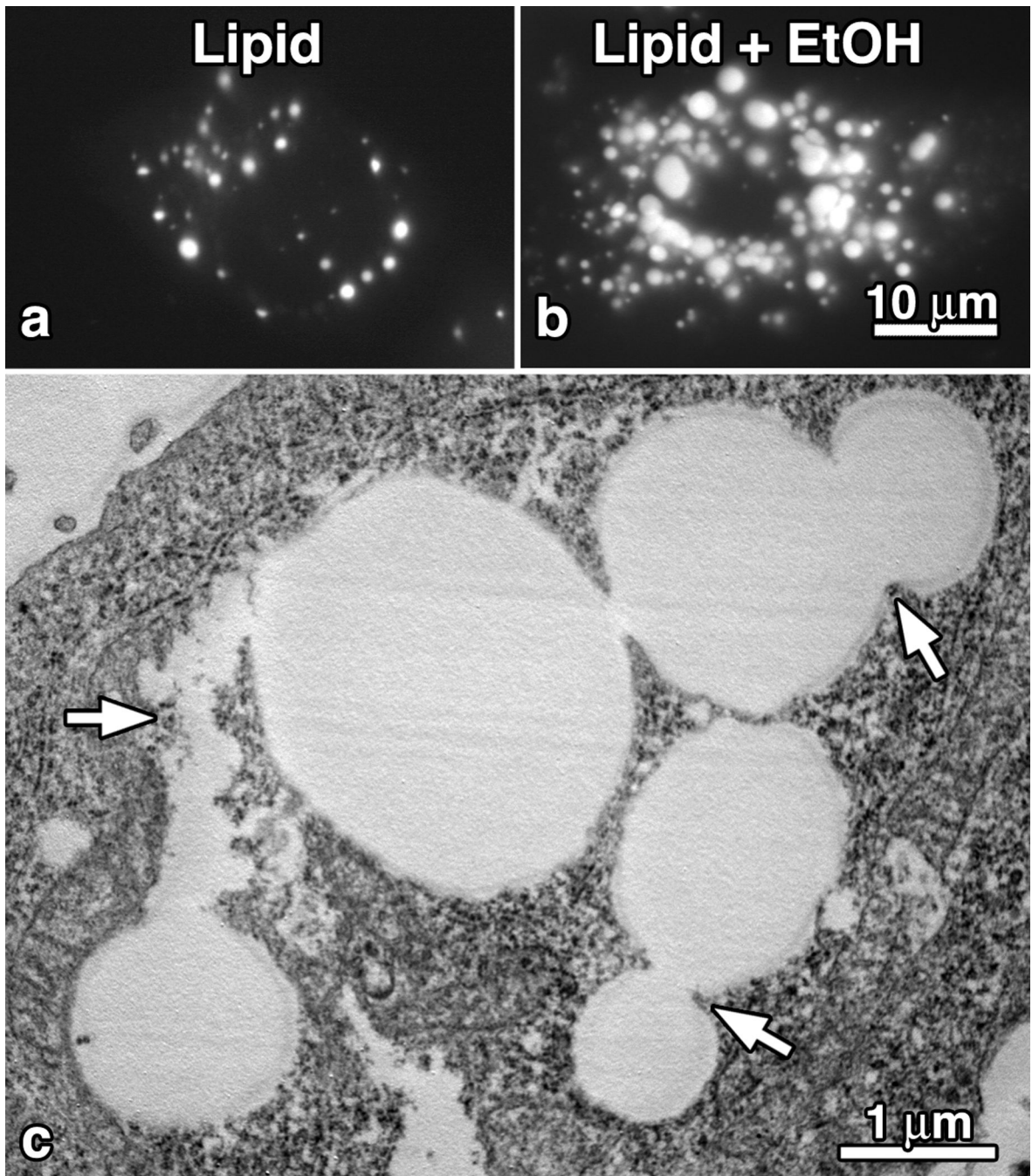


Fig. 1. Lipid Droplets are Dynamic Organelles that Maintain a Fission and Fusion Equilibrium. (a,b) Fluorescence microscopy of VA-13 cells that were incubated in 200 μ M oleic acid (OA) for 15 hours in the absence (A) or presence (B) of 100 mM ethanol and stained with Oil Red O. Note the marked increase in LD size and number induced by the ethanol exposure. (C) Electron microscopy of ethanol treated, OA fed, VA-13 cells showing large LDs undergoing multiple fission and/or fusion events (arrows).

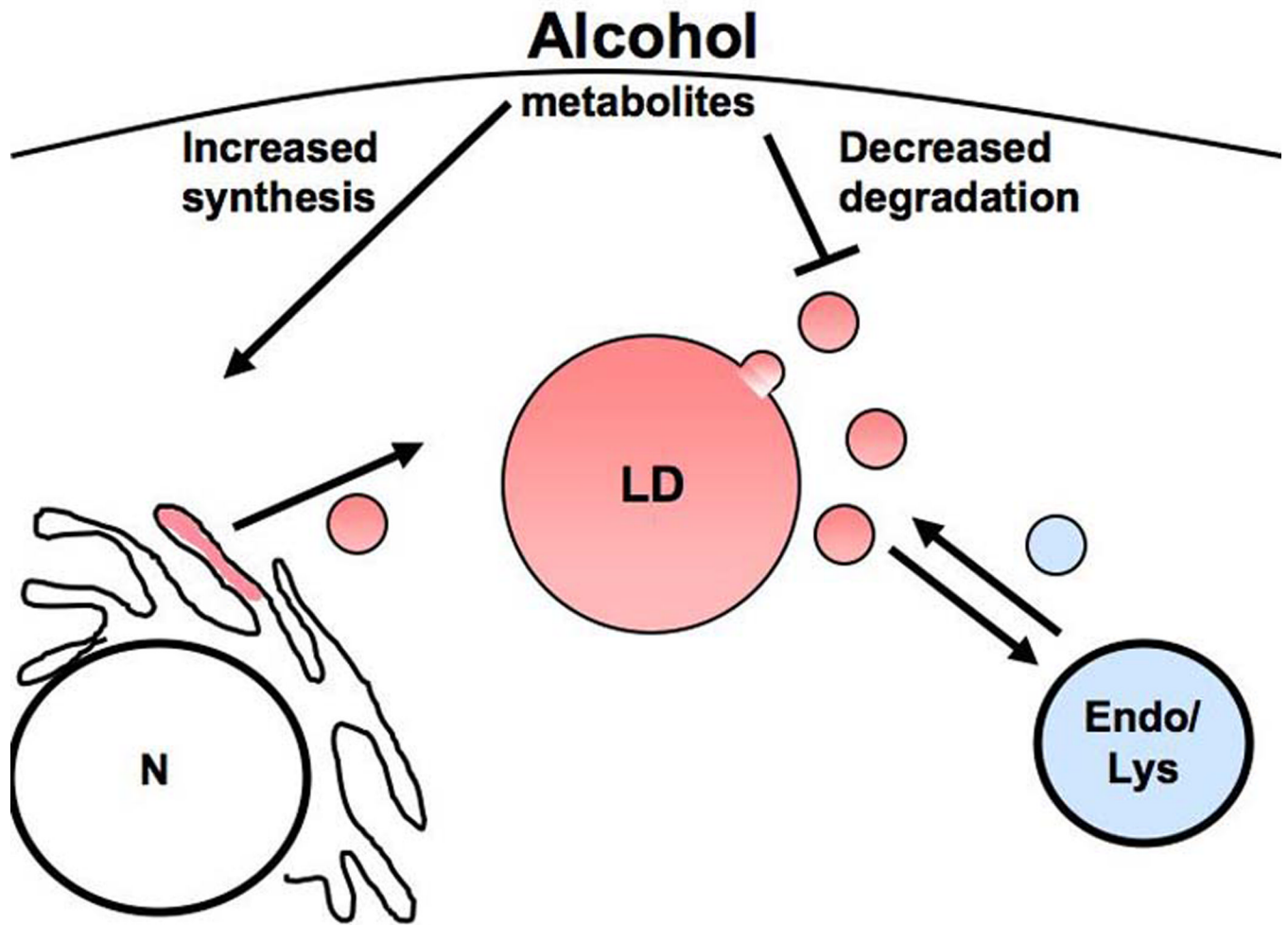


Fig. 2.
The number and size of LDs in the hepatocyte is maintained by a vesicle trafficking-based equilibrium of synthesis and lipolysis. Illustration depicting how ETOH could contribute to steatosis via increased LD formation from the endoplasmic reticulum and/or decreased metabolism, vesiculation, and interaction with degradative compartments such as the endosome/lysosome and autophagosome (not shown).