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A Unifying Hypothesis Linking Hepatic Adaptations for Ethanol Metabolism to the Proinflammatory and Profibrotic Events of Alcoholic Liver Disease

DR. Zhi Zhong, MD., Ph.D.[1] and **John J. Lemasters, MD., Ph.D.**[1,2]

[1]Department of Drug Discovery & Biomedical Sciences and [2]Department of Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC 29425

Abstract

The pathogenesis of alcoholic liver disease (ALD) remains poorly understood but is likely a multi-hit pathophysiological process. Here, we propose a hypothesis of how early mitochondrial adaptations for alcohol metabolism lead to ALD pathogenesis. Acutely, ethanol feeding causes a near doubling of hepatic ethanol metabolism and oxygen consumption within 2–3 h. This Swift Increase in Alcohol Metabolism (SIAM) is an adaptive response to hasten metabolic elimination of both ethanol and its more toxic metabolite, acetaldehyde (AcAld). In association with SIAM, ethanol causes wide-spread hepatic mitochondrial depolarization (mtDepo), which stimulates oxygen consumption. In parallel, voltage dependent anion channels (VDAC) in the mitochondrial outer membrane close. Together, VDAC closure and respiratory stimulation promote selective and more rapid oxidation of ethanol first to AcAld in the cytosol and then to non-toxic acetate in mitochondria, since membrane-permeant AcAld does not require VDAC to enter mitochondria. VDAC closure also inhibits mitochondrial fatty acid oxidation and ATP release, promoting steatosis and a decrease of cytosolic ATP. After acute ethanol, these changes revert as ethanol is eliminated with little hepatocellular cytolethality. mtDepo also stimulates mitochondrial autophagy (mitophagy). After chronic high ethanol exposure, the capacity to process depolarized mitochondria by mitophagy becomes compromised, leading to intra- and extracellular release of damaged mitochondria, mitophagosomes and/or autolysosomes containing mitochondrial damage-associated molecular pattern (mtDAMP) molecules. mtDAMPs cause inflammasome activation and promote inflammatory and profibrogenic responses, causing hepatitis and fibrosis. We propose that persistence of mitochondrial responses to ethanol metabolism becomes a tipping point, which links initial adaptive ethanol metabolism to maladaptive changes initiating onset and progression of ALD.

Keywords

ethanol metabolism; inflammation; fibrosis; mitochondria; VDAC

Address correspondence to: Dr. Zhi Zhong, Department of Drug Discovery & Biomedical Sciences, Medical University of South Carolina, MSC140, 280 Calhoun Street, Charleston, SC 29425; Phone: 843-792-2163; Zhong@musc.edu.

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

1. Introduction

Alcohol is consumed worldwide, and excessive alcohol consumption leads to alcoholic liver disease (ALD) (Rehm, et al., 2013). In the U.S., approximately two-thirds of adults drink alcohol, and more than 2.5 million people have ALD (Kim, et al., 2002;Chacko and Reinus, 2016). The major pathological features of human ALD are steatosis, hepatitis, and fibrosis/cirrhosis, which often co-exist and eventually progress to end-stage liver disease and/or liver cancer (Ishak, et al., 1991;Bataller and Gao, 2015;Chacko and Reinus, 2016). ALD accounts for about half of global mortality due to cirrhosis (Rehm, et al., 2013;Gao and Bataller, 2011). In severe alcoholic hepatitis (AH), mortality is 30–50% and exceeds 50% in patients with cirrhosis (Fung and Pyrsopoulos, 2017;Beier, et al., 2011). Estimated alcohol-related health-care costs in the United States are more than $26 billion (Kim, et al., 2002). Therefore, ALD remains a major concern for public health and medicine.

ALD is a multistage disease with a pathogenesis generally recognized to be mediated by multiple-hit mechanisms (Tsukamoto, et al., 2009;Szabo and Petrasek, 2017). In recent years, proinflammatory gut microbiome-liver interactions, adipose tissue dysfunction, genetic polyphorphisms, epigenetic changes, perturbation of methionine metabolism, endoplasmic reticulum (ER) stress, and various mitochondrial alterations have all been proposed to contribute to ALD pathogenesis (Garcia-Ruiz, et al., 2013;Williams, et al., 2014;Xu, et al., 2017;Szabo and Petrasek, 2017;Wang, et al., 2016a;Wang, et al., 2016b). Despite extensive study, mechanisms by which ethanol initiates hepatic damage and promotes ALD progression remain incompletely understood.

Many early studies have shown mitochondrial alterations in ALD (Ishak, et al., 1991;Hoek, et al., 2002;Hoek and Pastorino, 2004;Mansouri, et al., 2018). Mitochondrial morphological changes, such as megamitochondria, are an early and constant finding in ALD patients (Matsuhashi, et al., 1998;Hoek, et al., 2002;Ishak, et al., 1991). Moreover, adaptive alcohol metabolism leads to a mitochondrial respiratory burst, an effect that depends on Kupffer cell activation (Thurman, et al., 1982;Yuki and Thurman, 1980b;Rivera, et al., 1998;Forman, et al., 1988;Bradford and Rusyn, 2005). Deletions of mitochondrial DNA (mtDNA) are also common in ALD patients (Larosche, et al., 2010;Fromenty, et al., 1995). Thus, mitochondrial stress and damage are likely important early events in the “multi-hit” pathogenesis of ALD. This article presents a unifying hypothesis of how mitochondrial adaptations that acutely augment alcohol metabolism and detoxification also promote the pathological features of chronic ALD, including steatosis, inflammation and fibrosis.

2. Adaptive alcohol metabolism-related mitochondrial alterations

2a) Hepatic ethanol metabolism

Ethanol undergoes two-step oxidation to acetaldehyde (AcAld) and then to acetate, a process occurring predominantly in the liver (Fig. 1). Alcohol dehydrogenase (ADH) in the cytosol, cytochrome P450 2E1 (CYP2E1) of the microsomal ethanol-oxidizing system (MEOS) in the endoplasmic reticulum (ER), and catalase in peroxisomes catalyze the first oxidation step, which converts ethanol to acetaldehyde (AcAld), a toxic and reactive ethanol

metabolite. ADH is quantitatively the most important first step enzyme, whereas CYP2E1 has a higher Km for ethanol than ADH. Thus, the relative contribution of CYP2E1 to overall ethanol metabolism increases with increasing blood ethanol concentration (Gonzalez, et al., 1991;Lu and Cederbaum, 2008;Leung and Nieto, 2013).

Aldehyde dehydrogenase (ALDH) further oxidizes AcAld to acetate. Of 19 ALDH isoforms, ALDH2 in the mitochondrial matrix is most important for AcAld oxidation, and thus AcAld must enter mitochondria to be oxidized to acetate (Lieber, 2005). Together, ADH and ALDH form two moles of NADH for each mole of ethanol oxidized to acetate. In the cytosol, ADH increases the NADH/NAD^+ ratio, and NAD^+ supply becomes rate-limiting for the ADH reaction. Reducing equivalents of NADH formed by ADH enter mitochondria via the malate-aspartate or other shuttle for oxidation by the mitochondrial respiratory chain with regeneration of NAD^+ (Lieber, 2005). Mitochondrial NADH formed after AcAld oxidation by ALDH2 must also be oxidized by the respiratory chain for ethanol oxidation to continue.

2b) Swift increase in alcohol metabolism

The liver is the major organ eliminating ethanol and its toxic metabolic AcAld to protect other organs, especially the central nervous system, after alcohol ingestion. Exposure to ethanol leads to an adaptive increase of ethanol metabolism in both rodent and human livers (Thurman, et al., 1982;Yuki and Thurman, 1980b;Videla and Israel, 1970). Occurring within 2–3 h after ethanol treatment, this phenomenon is named Swift Increase in Alcohol Metabolism (SIAM) and is defined experimentally as a rapid increase in hepatic alcohol metabolism and mitochondrial respiration after a single bolus dose of alcohol (*e.g.*, 5 g/kg), but even small doses (1 g/kg) can produce SIAM (Thurman, et al., 1982;Yuki and Thurman, 1980b;Shimamoto, et al., 2010). SIAM also occurs in human subjects after alcohol consumption of 0.85 g/kg (Thurman, et al., 1989).

Although increased respiration should in theory lead to increased ATP generation, ethanol treatment actually decreases hepatic ATP by 50–60% (El-Assal, et al., 2004;Bailey, et al., 1999;Zhong, et al., 2014). Overall, the respiratory burst in SIAM is an adaptive response to oxidize toxic AcAld more rapidly by increasing NAD^+ supply for ADH- and ALDH-dependent ethanol metabolism. This hepatic hypermetabolism persists during chronic ethanol exposure and was first described in a chronic ethanol feeding model (Videla, et al., 1973;Israel, et al., 1975a;Israel, et al., 1973;Israel, et al., 1975b;Ribiere, et al., 1994;Han, et al., 2012;Han, et al., 2017). The mechanisms of SIAM are not well understood but most likely are multifactorial.

Occurrence of SIAM requires ethanol oxidation to AcAld (Bradford and Rusyn, 2005). Neither MEOS nor hepatic ADH activity is altered when SIAM first develops after acute ethanol (Yuki and Thurman, 1980b). However, ADH deficiency and inhibition, and to a lesser extent, cytochrome P450 inhibition, block SIAM (Yuki and Thurman, 1980a;Wendell and Thurman, 1979;Glassman, et al., 1985). Other studies suggest that catalase also plays a role in SIAM (Bradford, et al., 1999).

2c) Ethanol-induced VDAC closure

The voltage dependent anion channel (VDAC) is a highly conserved 30 kDa mitochondrial outer membrane protein with three isoforms in mice and humans – VDAC1, 2 and 3 (Shoshan-Barmatz and Gincel, 2003;Neumann, et al., 2010). VDAC forms a barrel comprised of a transmembrane alpha helix and 19 transmembrane beta strands enclosing a ~2.5 nm aqueous channel, which in the open state allows passage of non-electrolytes up to 5 kDa in size, although electrostatic profile is also an important determinant of channel permeance (Bayrhuber, et al., 2008;Colombini, 2012). Except for a relatively few membrane-permeant lipophilic compounds, metabolites that enter and leave mitochondria must cross the outer membrane through VDAC (Shoshan-Barmatz and Gincel, 2003;Colombini, 2012). In the open state, anions are somewhat favored over cations. Membrane potential ($\Delta\Psi$) closes VDAC symmetrically with half maximal closure at ±50 mV. VDAC closure effectively blocks movement of most organic anions, including respiratory substrates, acyl-CoA, ATP, ADP and Pi (Shoshan-Barmatz and Gincel, 2003;Vander Heiden, et al., 2000;Lemasters, 2017).

VDAC is generally assumed to be open during mitochondrial metabolism, but more recent data suggest that VDAC can close and inhibit metabolite exchange (Das, et al., 2008;Holmuhamedov and Lemasters, 2009;Lemasters and Holmuhamedov, 2006;Vander Heiden, et al., 2000). Various degrees of VDAC closure modulate substrate supply for respiration, exchange of ADP and Pi for ATP during oxidative phosphorylation (OXPHOS), and other mitochondrial functions. In this way, VDAC acts as a dynamic limiter, or 'governator', of global mitochondrial function (Lemasters and Holmuhamedov, 2006;Lemasters, 2017;Maldonado and Lemasters, 2012).

As noted above, hepatic ATP paradoxically decreases after acute ethanol despite increased mitochondrial respiration and without activation of an identifiable ATPase. Moreover, steatosis occurs, indicative of inhibition of mitochondrial β-oxidation (Zhong, et al., 2014). However, rats fed triglycerides containing short to medium chain fatty acids have less steatosis after ethanol treatment than rats fed triglycerides containing long chain fatty acids, indicating that mitochondria continue to metabolize shorter chain fatty acids after ethanol (Nanji, 2004). To explain these phenomena, closure of VDAC was proposed to occur (Lemasters and Holmuhamedov, 2006). In this way, VDAC becomes rate-limiting for both release of mitochondrial ATP and uptake of fatty acyl-CoA to explain hepatic ATP depletion and steatosis. However, VDAC closure does not block mitochondrial oxidation of short chain fatty acids and AcAld that pass directly through membrane lipid bilayers. Nonetheless, simple permeance of AcAld is insufficient to stimulate mitochondrial respiration over that supported by other respiratory substrates, because the respiration-driven protonmotive force across the inner membrane comprised predominantly of $\Delta\Psi$ inhibits respiration, the well-known phenomenon of respiratory control. Thus, it was further hypothesized that mitochondrial uncoupling and depolarization must also occur to stimulate respiration during SIAM (see below) (Lemasters and Holmuhamedov, 2006).

Subsequent studies showed that outer membranes of hepatocellular mitochondria do indeed become less permeable to adenine nucleotides and low molecular weight dextrans after ethanol and AcAld exposure (Holmuhamedov and Lemasters, 2009). Ureagenesis requires

extensive exchange of different metabolites through VDAC. Both ethanol and AcAld suppress respiration stimulated by ureagenic substrates in cultured rat hepatocytes, and urea formation declines proportionately (Holmuhamedov, et al., 2012). For AcAld, the 50% inhibitory concentration (IC_{50}) is 125 μM. Inhibitors of AcAld-forming ADH, Cyp2E1, and catalase partially restore ureagenic respiration inhibited after ethanol treatment. By contrast, inhibition of ALDH exacerbates suppression of ureagenic respiration by ethanol. Additionally in plasma membrane-permeabilized rat hepatocytes, AcAld suppresses entry of 3-kDa rhodamine-conjugated dextran into the mitochondrial intermembrane space, indicative of VDAC closure (Fig. 2) (Holmuhamedov, et al., 2012). These findings support the conclusion that AcAld underlies ethanol-induced inhibition of ureagenesis through closure of VDAC.

VDAC closure after ethanol becomes sufficient to be rate-limiting for passage of anionic metabolites across the outer membrane. Such closure need not be complete. Moreover, in the 'closed' state, VDAC becomes a cation selective pore of 1.8 nm in diameter that still conducts small anions like Cl^- (Rostovtseva and Colombini, 1997;Tan and Colombini, 2007). The mechanisms of ethanol-induced VDAC closure remain unknown. In cancer cells, free dimeric α,β-tubulin inhibits VDAC isoforms 1 and 2 but not the minor isoform VDAC3 (Maldonado, et al., 2010;Maldonado, et al., 2013). α-Tubulin is a major target of adduct formation by AcAld, and adduction drastically impairs microtubule polymerization, which might increase free tubulin (Tuma, et al., 1991;Jennett, et al., 1980;Groebner and Tuma, 2015). Kinase cascades may also be involved, since phosphorylation of VDAC by cAMP-dependent protein kinase A (PKA) or glycogen synthase kinase-3β (GSK3β) enhances tubulin closure of VDAC reconstituted into bilayers (Sheldon, et al., 2011), whereas experiments in intact cells indicate that the PKA and GSK3β pathways increase and decrease, respectively, VDAC conductance (Maldonado, et al., 2010;Das, et al., 2008). Phosphorylation by c-Jun N-terminal kinase-3 also decreases VDAC conductance (Gupta and Ghosh, 2015;Gupta, 2017).

2d) Ethanol-induced mitochondrial depolarization in vivo

To examine whether mitochondrial uncoupling occurs to stimulate respiration during SIAM, mitochondrial polarization *in vivo* after ethanol treatment was assessed by intravital confocal/multiphoton microscopy, a technology that allows direct visualization of mitochondrial structure and function in living animals. If the process of OXPHOS remains intact during SIAM, mitochondrial $\Delta\Psi$ should be preserved as respiration increases. Alternatively, if increased mitochondrial respiration is due to uncoupling, then $\Delta\Psi$ should decrease sharply. What was observed is virtually complete mitochondrial depolarization (mtDepo), as assessed by the absence of mitochondrial uptake of $\Delta\Psi$-indicating cationic fluorophores like rhodamine 123 (Zhong, et al., 2014). This mtDepo occurs in an all-or-nothing fashion within individual hepatocytes. The percentage of hepatocytes with depolarized mitochondria increases in a dose- and time-dependent fashion and peaks at 6 to 12 h after single intragastric ethanol feeding (1–6 g/kg) (Fig. 3). At maximum ethanol (~6 g/kg), mtDepo occurs in ~90% of hepatocytes. At 1g/kg ethanol, a dose causing a peak blood alcohol approximating the legal limit for operation of motor vehicles in the U.S., mtDepo

occurs in 10–15% of hepatocytes. mtDepo largely reverses after 24 h as ethanol is metabolically eliminated and is virtually absent after 72 h (Fig. 3) (Zhong, et al., 2014).

High demand for O_2 during SIAM leads to zones of hypoxia, especially in pericentral (centrilobular) regions of liver lobules, which may contribute to liver injury (Ji, et al., 1982b;Ji, et al., 1982a;Ji, et al., 1983;Arteel, et al., 1996). Nonetheless, ethanol-induced mtDepo is not secondary to anoxia, because NAD(P)H autofluorescence in depolarized mitochondria sharply decreases in comparison to adjacent hepatocytes with polarized mitochondria, whereas in anoxia NAD(P)H increases maximally. mtDepo in combination with NAD(P)H oxidation and increased respiration (SIAM) are the hallmarks of uncoupling (Zhong, et al., 2014;Sies, et al., 1974;Nieminen, et al., 1997). In support of an uncoupling mechanism, hepatic ATP decreases ~60% after acute ethanol (6 g/kg)(Zhong, et al., 2014). By increasing mitochondrial respiration, uncoupling is an adaptive response to promote NAD^+ regeneration in support ADH and ALDH2-dependent alcohol metabolism.

Decreased entry into mitochondria of normal NAD^+-linked substrates (pyruvate, acyl-CoA, *etc.*) cannot account for mtDepo and mitochondrial NADH oxidation, since hepatic oxygen consumption does not decrease but instead nearly doubles during SIAM. Rather, oxidation of membrane-permeant AcAld replaces oxidation of other anionic respiratory substrates whose entry into mitochondria is blocked by closure of VDAC after ethanol/AcAld exposure. Several mechanisms for mtDepo are possible, such as futile cycling of H^+, Ca^{2+}, K^+ or other ion, but the specific mechanism remains unknown.

The mitochondrial permeability transition (MPT) is not responsible for ethanol-induced mtDepo *in vivo*, since the MPT blocker CsA did not block ethanol-induced mtDepo, calcein did not re-distribute from cytosol into the depolarized mitochondria, and mtDepo was reversible after acute ethanol (Zhong, et al., 2014). Simple activation of an ATPase to cause futile ATP hydrolysis seems unlikely, since mitochondrial $\Delta\Psi$ falls by a relatively small amount during maximal ADP-stimulated State 3 respiration compared to the virtually complete mtDepo after ethanol (Nicholas and Ferguson, 2013).

Uncoupling proteins (UCP) depolarize mitochondria and stimulate respiration (Diehl and Hoek, 1999;Bouillaud, et al., 2016). In obesity, steatosis and various pathological conditions, liver expresses UCP2 that promotes injury, but UCP2 protein and mRNA are virtually undetectable in normal lean livers (Demori, et al., 2008;Chavin, et al., 1999;Evans, et al., 2008). Thus, UCP2 upregulation seems unlikely to account for mtDepo, since mtDepo begins at a very early time point (1 h) after ethanol and because ethanol-induced mtDepo is not blocked in the livers of UCP2 deficient mice (Zhong et al. 2014 and unpublished).

Interestingly, mtDepo *in vivo* also occurs after chronic ethanol treatment, although the percentage of hepatocytes exhibiting mtDepo is less than after high dose acute ethanol treatment (Rehman, et al., 2015). Thus, mitochondrial uncoupling also occurs after chronic alcohol consumption. In support of such mitochondrial uncoupling in humans, energy expenditure increases whereas metabolic efficiency decreases in both acute and chronic drinkers (Suter, et al., 1994;Levine, et al., 2000;Jhangiani, et al., 1986).

In vivo, ethanol-induced mtDepo depends on AcAld formation (Zhong, et al., 2014). Deficiency of ADH, the major ethanol-metabolizing enzyme, decreases mtDepo after acute ethanol by ~70%. Deficiency of CYP2E1 and pharmacological cytochrome P450 inhibition each decrease mtDepo after acute ethanol by ~20%, indicating that ADH plays a greater role than CYP2E1 in mtDepo *in vivo*. Alda-1, an activator of ALDH2, also decreases mtDepo after acute ethanol, whereas inhibition of ALDH activity with disulfiram increases mtDepo (Zhong, et al., 2014). Alda-1 also decreases mtDepo after chronic ethanol treatment (Rehman, et al., 2015). Thus, increased AcAld levels during ethanol metabolism promote mtDepo after both acute and chronic ethanol exposure. Overall, increased intrahepatic AcAld after ethanol may act as a signal triggering multiple mitochondrial alterations to accelerate alcohol metabolism.

Because small neutral aldehydes like AcAld do not need VDAC or other carrier to cross the outer and inner membranes of mitochondria, VDAC closure and respiratory stimulation by mtDepo together promote rapid and selective oxidation of membrane-permeant AcAld while simultaneously inhibiting oxidation of competing substrates that require VDAC to enter mitochondria. VDAC closure also prevents futile hydrolysis of cytosolic ATP by uncoupled mitochondria, such that tissue ATP levels can be partially maintained by glycolysis (Lemasters and Holmuhamedov, 2006;Lemasters, 2017;Zhong, et al., 2014). Nonetheless, some anion flux across the outer membrane must persist for electron transfer from cytosolic NADH to mitochondrial NAD^+ by the malate-aspartate or α-glycerol phosphate shuttle. This flux may be through incompletely closed VDAC or previously unrecognized exchange pathways in the outer membrane.

Similar mechanisms may also play a role in nonalcoholic steatohepatitis in which oxidative stress is an important pathogenic mechanisms, since lipid peroxidation chain reactions generate small aldehydes like malondialdehyde that close VDAC even more potently than AcAld (Holmuhamedov, et al., 2011;Holmuhamedov, et al., 2012). Although adaptive in promoting faster detoxification of aldehydes, VDAC closure and the associated mitochondrial uncoupling may become maladaptive hits in the multi-hit pathogenesis of ALD and possibly other forms of liver disease, such as non-alcoholic steatohepatitic (NASH) and vinyl chloride-related toxicant-associated steatohepatitis (TASH) (Cave, et al., 2010;Anders, et al., 2016), as further discussed below.

2e) Mitophagy and ethanol-induced mitochondrial remodeling

Mitochondrial autophagy, or mitophagy, is a process leading to lysosomal degradation of mitochondria in response to nutrient deprivation, mitochondrial damage and the need for cytoplasmic remodeling as bioenergetic demands change (Kim, et al., 2007;Lemasters, 2014). Deficient mitophagy is associated with mitochondrial dysfunction and the pathogenesis of many diseases (Jin and Youle, 2012;Redmann, et al., 2014;Zhang, et al., 2018;Chistiakov, et al., 2017), whereas overactive mitophagy can produce mitochondrial depletion and a bioenergetic deficit, as described for cadmium hepatotoxicity (Pi, et al., 2013).

Different signaling pathways initiate mitophagy. In Type 1 mitophagy, Vps34 (Class III phosphoinositide 3-kinase [PI3K]), beclin-1, and other proteins initiate formation of cup-

shaped phagophores that wrap around individual mitochondria and fuse to form mitophagosomes. Type 1 mitophagy often occurs coordinately with mitochondrial fission (Lemasters, 2014). Type 1 mitophagy is typical of nutrient deprivation and cytoplasmic remodeling and is completely blocked by PI3K inhibitors like 3-methyladenine and wortmannin. In Type 1 mitophagy, the outer compartment of mitophagosomes (space between the inner and outer autophagosomal membranes) acidifies, and then mtDepo occurs (Fig. 4A). Subsequently, mitophagosomes fuse with lysosomes (or late endosomes) to form autolysosomes in which hydrolytic digestion of mitochondria occurs within about 15 min (Lemasters, 2014;Eid, et al., 2013). Thus, Type 1 mitophagy removes functional mitochondria to provide metabolic precursors during nutrient deprivation or that are in excess of metabolic needs (Rodriguez-Enriquez, et al., 2009;Lemasters, 2014).

In Type 2 mitophagy, mtDepo initiates autophagic sequestration. After mtDepo, Pink1 accumulates on mitochondria to promote Parkin binding. Parkin is an E3 ligase, and ubiqitination of mitochondrial proteins recruits autophagy receptor proteins like p62/SQSTM-1, followed by association of LC3-containing membranes that appear to fuse to form an autophagosome enveloping the target mitochondrion (Lemasters, 2014). By contrast to Type 1 mitophagy of polarized mitochondria, PI3K inhibitors do not block Type 2 mitophagy of depolarized mitochondria (Fig. 4A). Moreover, cup-shaped phagophores and mitochondrial fission are typically absent in Type 2 mitophagy (Lemasters, 2014;Pickles, et al., 2018). Other autophagy receptors, including BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (Bnip3), Nix, optineurin and double FYVE-containing protein 1 (DFCP1) also associate with depolarized mitochondria to promote LC3 binding and autophagic sequestration (Schweers, et al., 2007;Kubli and Gustafsson, 2012;Wong and Holzbaur, 2014;Wong and Holzbaur, 2015). In a third type of mitophagy, mitochondria-derived vesicles (MDVs) enriched in oxidized mitochondrial proteins bud off from mitochondria and transit to multivesicular bodies. Topologically, internalization of such MDVs by invagination of multivesicular bodies followed by vesicle scission into the lumen is a form of microautophagy. Such Type 3 micromitophagy is also Pink1/parkin-dependent (Soubannier, et al., 2012;McLelland, et al., 2014).

Acute ethanol stimulates mitophagy, and this autophagy decreases acute ethanol-induced hepatotoxicity and steatosis in mice in a parkin-dependent fashion (Ding, et al., 2010;Williams, et al., 2015). Ethanol also causes oxidative mtDNA damage and depletion (Mansouri, et al., 2001;Eid, et al., 2016). The effects of chronic ethanol on autophagy/mitophagy are somewhat controversial. Some studies show enhanced autophagic flux and increased autophagosome numbers, whereas others indicate that chronic ethanol disrupts autophagosomal processing and lysosomal function, leading to autophagosome accumulation (Eid, et al., 2013;Lin, et al., 2013;Kharbanda, et al., 1995;Thomes, et al., 2015). Nuclear translocation of transcription factor EB (TFEB), the master regulator of lysosomal biogenesis, increases after acute ethanol but decreases after chronic ethanol in mice and in patients with alcoholic hepatitis, implying suppressed lysosomal biogenesis and autophagy after chronic ethanol (Thomes, et al., 2015;Chao, et al., 2018). TFEB over-expression decreases chronic ethanol plus binge drinking-induced liver injury (Chao, et al., 2018).

mtDepo triggers Type 2 mitophagy (Narendra, et al., 2008;Hariharan, et al., 2011;Kim, et al., 2008;Lemasters, 2014). Thus, mtDepo after ethanol likely initiates hepatocellular mitophagy. In confirmation, experiments in LC3-GFP transgenic mice show that LC3-GFP mitophagic puncta appear predominantly in hepatocytes with depolarized mitochondria after acute ethanol, implicating strongly that ethanol-induced mtDepo induces mitophagy (Fig. 5). PINK1 also increases after chronic ethanol, indicating PINK1 stabilization on depolarized mitochondria, whereas blunting of mtDepo by Alda-1 decreases PINK1 accumulation (Rehman, et al., 2015). Other studies also show that PINK1 and parkin mediate mitochondrial autophagy after acute and chronic-binge ethanol (Williams, et al., 2015;Eid, et al., 2016). MPT inhibitors like cyclosporin A (CsA) and NIM811 can inhibit hepatocellular autophagy, and deficiency of cyclophilin D, a regulator of MPT pores, impairs autophagy and decreases the sensitivity to the MPT in mitochondria from mice after chronic alcohol treatment, implicating a possible role of the MPT in triggering autophagy (Elmore, et al., 2001;Rodriguez-Enriquez, et al., 2009;King, et al., 2014). VDAC isoforms may serve as the docking sites to recruit parkin onto damaged mitochondria and are the target of parkin-dependent ubiquitination, thus enhancing mitophagy (Geisler, et al., 2010;Sun, et al., 2012). By decreasing mitochondrial release of ATP, VDAC closure after ethanol might also trigger some degree of nutrient deprivation-dependent Type 1 mitophagy.

To maintain mitochondrial homeostasis after mitophagy, loss of mitochondria must be matched by mitochondrial biogenesis. In support of enhanced mitochondrial biogenesis, intragastric alcohol feeding increases PGC-1α, the master regulator of mitochondrial biogenesis, and mitochondrial transcription factor A (TFAM), an activator of mitochondrial DNA transcription and participant in mtDNA replication (Han, et al., 2012;Han, et al., 2017). Nonetheless, alterations of PGC-1α, sirtuins, the deacetylases that regulate PGC-1α activity, and OXPHOS proteins after chronic ethanol treatment are inconsistent between studies (Han, et al., 2012;Han, et al., 2017;Lieber, et al., 2008b;Lieber, et al., 2008a). Taken together, alterations of mitochondrial structure, function, mitophagy and biogenesis indicate that mitochondrial remodeling occurs after chronic alcohol, which may represent repair and regeneration responses or contribute to ALD pathogenesis.

3. Linkage of hepatic adaptations for ethanol metabolism to extrahepatic events

The gut-liver axis plays an important role in both adaptive ethanol metabolism and the pathogenesis of ALD (Szabo and Petrasek, 2017;Scarpellini, et al., 2016;Xu, et al., 2017;Thurman, et al., 1998). Alcohol consumption changes the gut microbiome, causing bacterial over-growth and increasing formation of toxic/proinflammatory products (Yan, et al., 2011;Mutlu, et al., 2012;Engen, et al., 2015). Alcohol also increases intestinal permeability, possibly by altering expression of tight junction-associated proteins (Wang, et al., 2014;Thurman, et al., 1998;Rivera, et al., 1998;Enomoto, et al., 2001). As a result, bacterial components (*e.g.*, endotoxin [lipopolysaccharide] and bacterial CpG-containing DNA) increase in portal blood after acute and chronic ethanol exposure in humans and experimental animals (Bode, et al., 1987;Rivera, et al., 1998;Fukui, et al., 1991;Bala, et al., 2014;Enomoto, et al., 2000a).

The gut-liver axis also modulates SIAM (Bradford, et al., 1993). Gut sterilization and an endotoxin antagonist block SIAM, whereas portal endotoxin infusion mimics the stimulation by ethanol of hepatic oxygen uptake. This latter effect is blocked by the Kupffer cell toxicant $GdCl_3$ (Rivera, et al., 1998;Bradford, et al., 1995). Endotoxin activates Kupffer cells via toll-like receptor 4 (TLR4), which stimulates release of reactive mediators, including proinflammatory and profibrotic cytokines/chemokines (*e.g.*, tumor necrosis factor [TNFα], interleukins, monocyte chemoattractant protein 1 **[**MCP-1]), and reactive oxygen and nitrogen species (ROS and RNS) (Nolan, 2010;Thurman, et al., 1995;Wheeler, et al., 2001a;Enomoto, et al., 2000b;Xu, et al., 2017). Endotoxin in combination with ethanol feeding causes overt liver injury, but endotoxin alone does not or only causes mild steatohepatitis (Bhagwandeen, et al., 1987;Kong, et al., 2017). Moreover, diseases that release endotoxin into the portal blood, such as Crohn's disease, ulcerative colitis and celiac disease, do not manifest the hepatic histopathology of ALD (Rubio-Tapia and Murray, 2008;Rojas-Feria, et al., 2013). Thus, endotoxin is not a sole or sufficient etiological agent in ALD and somehow must act in a synergistic fashion with other ethanol-induced changes.

Kupffer cells also release eicosanoids like prostaglandin E_2 (PGE_2). Kupffer cells isolated from rats receiving ethanol *in vivo* produce PGE_2, and conditioned medium from these cells stimulates hepatocyte oxygen uptake by activating cAMP-dependent pathways (Qu, et al., 1996). PGE_2 increases in blood after acute ethanol (Rivera, et al., 1998;Enomoto, et al., 2000c). Thus, endotoxin activation of Kupffer cells causes PGE_2 production, an effect that is, at least in part, responsible for SIAM. Although SIAM seems linked to mtDepo, how the gut-liver axis and Kupffer cell activation may promote mtDepo remains to be determined.

Hormones are also linked to SIAM. Alcohol increases adrenergic hormone release, and adrenalectomy and adrenergic blockers suppress SIAM (Forman, et al., 1988;Yuki, et al., 1980). Thyroidectomy and hypophysectomy also blunt the hypermetabolic state after ethanol (Israel, et al., 1975a;Bernstein, et al., 1975). Females are more vulnerable to ALD than males, and estrogen enhances sensitivity of Kupffer cells to endotoxin and worsens ethanol-induced liver injury, possibly by increasing expression of CD14 (a co-receptor for LPS) and LPS-binding protein (Ikejima, et al., 1998;Kono, et al., 2002;Enomoto, et al., 2002). By contrast, fasting blunts SIAM, implicating potential roles for increased glucagon or decreased insulin in suppressing SIAM (Thurman and Scholz, 1977). Adipose-derived leptin, a hunger-inhibiting hormone, also increases after ethanol consumption and upregulates Kupffer cell CD14 expression, which could increase sensitivity of Kupffer cells to LPS and therefore possibly SIAM (Imajo, et al., 2012;Roth, et al., 2003).

4. Adaptive alcohol metabolism and oxidative stress

Ethanol consumption also promotes hepatic ROS and RNS formation (Yin, et al., 2001;Wheeler, et al., 2001b;Hoek and Pastorino, 2004). Ethanol increases CYP2E1, largely by a posttranscriptional mechanism involving stabilization against proteolysis (Gonzalez, et al., 1991;Lu and Cederbaum, 2008). CYP2E1generates superoxide ($O_2^{\bullet-}$), which then forms highly reactive peroxynitrite ($ONOO^-$) by reaction with NO, and hydroxyl radical ($^{\bullet}OH$) by the Fenton reaction (Jaeschke, et al., 2002;Lemasters, 2004). In the presence of ethanol, 1-hydroxyethyl radical is also formed (Thurman, et al., 1998). Therefore, ROS, RNS and other

radical species all increase after ethanol. These radicals attack and damage proteins, lipids and DNA, induce onset of the mitochondrial permeability transition (MPT), cause cell death, and trigger inflammatory processes (*e.g.*, by inflammasome activation) (Jaeschke, et al., 2002;Hughes and O'Neill, 2018). In particular, lipid peroxidation downstream to $^{\bullet}OH$ formation generates toxic aldehydes like malondialdehyde and 4-hydroxynonenal that promote VDAC closure and possibly ethanol-induced mtDepo. Steatosis resulting from VDAC closure and consequent inhibition of beta-oxidation may then increase the vulnerability to lipid peroxidation, creating a vicious cycle.

During mitochondrial metabolism, electrons can escape from 11 identified ubiquinone and flavoquinone-interacting respiratory complexes and dehydrogenases to form $O_2^{\bullet-}$ and H_2O_2 (Young, et al., 2002;Kovacic, et al., 2005;Bunik and Brand, 2018;Brand, 2016). SIAM accelerates NADH oxidation by the respiratory chain, promoting mitochondrial production of ROS, although decreased $\Delta\Psi$ in the absence of other mitochondrial perturbations suppresses ROS formation (Bailey and Cunningham, 2002;Zhong, et al., 2014;Garcia-Ruiz, et al., 2013;Han, et al., 2017;Korshunov, et al., 1997;Starkov and Fiskum, 2003). Ethanol also increases CYP2E1 expression in mitochondria (Anandatheerthavarada, et al., 1997;Bansal, et al., 2010;Robin, et al., 2005).

Cu/Zn superoxide dismutase (SOD1) in the cytosol and Mn SOD2 in the mitochondrial matrix scavenge $O_2^{\bullet-}$. Various reports show increased, decreased, and unchanged Mn-SOD after ethanol treatment (Koch OR, 1994;Nanji, et al., 1995). In the intermembrane space, cytochrome *c* can scavenge $O_2^{\bullet-}$ forming ferrocytochrome *c*. Glutathione-linked anti-oxidant defenses also reside in mitochondria, including GSH peroxidase-1, peroxiredoxin-III, thioredoxin-2 and glutathione reductase. Alcohol consumption selectively depletes mitochondrial GSH, thus increasing mitochondrial oxidative stress (Fernandez-Checa and Kaplowitz, 2005). AcAld aggravates oxidative stress by binding to GSH and promoting GSH leakage (Lieber, 2004). Since GSH, like other metabolites, must pass through VDAC to be taken up into mitochondria, alcohol-induced VDAC closure may also contribute to decreased mitochondrial GSH. Increased consumption of mitochondrial GSH during antioxidant defense causes further depletion.

Oxidative stress also stimulates ER stress. Moreover, ethanol causes ER stress through disturbance of one carbon metabolism, increased homocysteine and activation of acid sphingomyelinase (ASMase) (Ji and Kaplowitz, 2003;Fernandez, et al., 2013). ATP depletion during SIAM may also promote ER stress. ER stress increases expression of StARD1, a mitochondrial cholesterol transporting polypeptide, leading to mitochondrial cholesterol accumulation, which reportedly inhibits mitochondrial GSH transport to exacerbate mitochondrial GSH depletion and promote ROS production, thus forming a vicious cycle (Mari, et al., 2008;Anuka, et al., 2013).

5. Relation of ethanol-induced mitochondrial alterations to pathogenesis of alcoholic liver disease

5a) Adaptive ethanol metabolism and steatosis

Steatosis is a virtually universal feature of ALD except in advanced alcoholic cirrhosis (Magdaleno, et al., 2017). After chronic ethanol, decreased adiponectin, suppressed expression/activation of hepatic PPAR-α and AMPK, disrupted Sirt-1/SREBP-1/lipin-1 signaling, and ER stress inhibit fatty acid oxidation and increase fatty acid esterification (Song, et al., 2008;Ajmo, et al., 2008;Garcia-Villafranca, et al., 2008;Yin, et al., 2012;Correnti, et al., 2014;Ji and Kaplowitz, 2006). Alcohol also activates adipose hormone sensitive lipase (HSL) activity, which increases lipolysis in adipose tissue and mobilizes free fatty acids to other organs, including the liver. In this way, the liver faces an increased fatty acid burden (Zhong, et al., 2012;Dou, et al., 2014;Wood, et al., 1993).

Steatosis develops very quickly after acute ethanol treatment and in parallel with SIAM. Thus, adaptive mitochondria alterations for alcohol metabolism likely play an early role in development of steatosis. Intravital microscopy shows that steatosis occurs principally in hepatocytes with depolarized mitochondria after acute ethanol treatment, strongly implying that steatosis is a response to mtDepo (Zhong, et al., 2014) (Fig. 6). VDAC closure linked to adaptive alcohol metabolism also inhibits entry of fatty acyl-CoA into the matrix space to prevent beta-oxidation, thus promoting steatosis. Similarly, hepatotoxicants that suppress mitochondrial fatty acid entry, inhibit beta-oxidation enzymes, or impair OXPHOS all induce marked hepatic steatosis quite acutely (Fromenty and Pessayre, 1995;Pessayre, et al., 2012;Lemasters, 2013).

Additionally, pericentral hypoxia associated with SIAM upregulates hypoxia-inducible factor-1α (HIF1α) (Ji, et al., 1982a;Arteel, et al., 1996;Zhong, et al., 2014). HIF1α protein induces expression of adipocyte differentiation-related protein (ADRP), which increases triglyceride accumulation and prevents secretion of triglycerides/VLDL in cultured hepatocytes (Nath, et al., 2011;Magnusson, et al., 2006). Hepatocyte specific HIF-1α knockout blocks chronic alcohol-induced ADRP expression in the liver and decreases steatosis (Nath, et al., 2011). Therefore, adaptive mitochondria alterations for alcohol metabolism not only inhibit fatty acid degradation but also prevent triglyceride exportation.

5b) Ethanol-induced mitochondrial alterations and cell death

After high acute ethanol, cell death in liver is actually quite small at about 2%, which makes this phenomenon difficult to study (Zhong, et al., 2014). In chronic ALD, loss of hepatocellular mass is progressive and eventually leads to liver failure (Wang, et al., 2016a;Magdaleno, et al., 2017). Apoptosis and necrosis both occur (Ishak, et al., 1991;Gao and Bataller, 2011;Tsukamoto, et al., 1985). Acute and chronic alcohol treatment causes oxidative modifications of mtDNA, which may trigger apoptosis (Hoek, et al., 2002). AcAld also causes mtDNA damage, and mtDNA deletions occur in >60% of patients with alcoholic steatosis (Fromenty, et al., 1995). Progressive loss of hepatocytes signifies a failure of hepatocellular regeneration, which is otherwise extraordinary in liver, an effect that may be due to AcAld-mediated G2/M cell cycle arrest (Diehl, 2005;Scheer, et al., 2016).

Compromised bioenergetics in hepatocytes with mtDepo may also contribute to failure of regeneration.

Oxidative stress also triggers onset of the MPT (Nieminen, et al., 1995;Hoek, et al., 2002;Malhi, et al., 2006). In cultured hepatocytes, ethanol causes formation of ROS, the MPT and apoptosis (Adachi and Ishii, 2002). However, the MPT is not responsible for ethanol-induced mtDepo *in vivo*, as discussed above (Section 2d) (Zhong, et al., 2014). Nonetheless, mitochondria isolated from rats chronically treated with ethanol show increased susceptibility to MPT onset, which may increase vulnerability to other co-existing risk factors and lead to exacerbation/progression of ALD (King, et al., 2014). High cholesterol intake, which further increases ER stress and mitochondrial ROS production, causes more necrosis and apoptosis after chronic alcohol feeding in mice (Krishnasamy, et al., 2016).

VDAC closes relatively early in the evolution of apoptosis with the consequence that mitochondria no longer release ATP to the cytosol or take up ADP, Pi and respiratory substrates (Shoshan-Barmatz and Golan, 2012;Vander Heiden, et al., 2000). Ethanol-induced oxidative stress promotes Bax translocation to mitochondria, forming a complex with VDAC (Adachi, et al., 2004). VDAC manifests higher permeability to Ca^{2+} in the closed state, favoring Ca^{2+} flux into mitochondria, which is a signal for cell death (Tan and Colombini, 2007). Nonetheless, the specific relationship of mitochondrial metabolic adaptations for ethanol metabolism (VDAC closure and mtDepo) to hepatocellular killing remains to be determined.

5c) Relation of ethanol-induced mitochondrial damage/dysfunction to inflammation and fibrosis: role of inflammasomes and mitochondrial damage-associated molecular pattern molecules

Inflammasome activation.—Inflammasomes are large inflammatory signaling platforms localized to both mitochondria and ER, which are composed of NOD-like receptor proteins (NLRP), caspase-1, and apoptosis-associated speck-like CARD-domain-containing (ASC) protein (Schroder, et al., 2010;Zhou, et al., 2011;Gurung, et al., 2015). In response to ‘danger signals’, NLRP activates caspase-1, also called interleukin-1 (IL-1) converting enzyme (ICE), which proteolytically cleaves precursors of proinflammatory cytokines like IL-1β, IL-18 and the pyroptosis inducer gasdermin D to yield active mature peptides (Gurung, et al., 2015;Yu and Finlay, 2008). AH patients have high IL-1 in blood and increased hepatic NLRP3, caspase-1 and activated gasdermin D (McClain, et al., 1986;Peng, et al., 2014;Tilg, et al., 2016;Khanova, et al., 2018). NLRP3 and caspase-1 deficiency and IL-1β receptor blockade decrease alcohol-induced liver inflammation and damage (Petrasek, et al., 2015;Petrasek, et al., 2012;Tilg, et al., 2016). These findings support a role of inflammasome activation in AH.

Danger signals from mitochondria, such as ROS, mtDNA and cardiolipin externalization to the outer membrane, promote NLRP3 inflammasome formation and sterile inflammation (Zhou, et al., 2011;Gurung, et al., 2015;Iyer, et al., 2013;Shimada, et al., 2012). Ethanol exposure induces acetylation of α-tubulin, which then could promote association of ASC on mitochondria with NLRP3 on ER, leading to NLRP3 activation (Shepard and Tuma,

2009;Misawa, et al., 2013). In human subjects, ethanol consumption increases serum uric acid and ATP, two strong inflammasome stimulators (Petrasek, et al., 2015;Stiburkova, et al., 2014;Lieber, et al., 1962). ATP activates inflammasomes by binding to purinergic receptor P2X7 (P2X7R). P2X7R deficiency decreases ethanol-induced inflammasome activation and steatohepatitis (Iracheta-Vellve, et al., 2015). Uric acid depletion by uricase overexpression also decreases ethanol-induced inflammasome activation (Iracheta-Vellve, et al., 2015). Our recent findings show that chronic ethanol treatment increases NLRP3 inflammasome activation in mice and that blockade of mtDepo by Alda-1 attenuates NLRP3 activation and liver inflammation (Rehman, et al., 2015). Thus, adaptive ethanol metabolism may be upstream of inflammasome activation after ethanol.

NLRP3 activation not only mediates inflammation but also stimulates fibrosis. Mice with constitutively activated NLRP3 exhibit increased HSC activation with collagen deposition, whereas mice lacking NLRP3 and ASC protein show decreased CCl_4- and TAA-induced liver fibrosis (Wree, et al., 2014;Watanabe, et al., 2009). IL-1β directly induces fibrogenic responses in HSCs, and deficiency and inhibition of IL-1 receptor decrease steatohepatitis and fibrosis in a NASH model (Miura, et al., 2010;Wree, et al., 2014).

Mitochondrial damage-associated molecular pattern molecules.—Mitochondrial stress and damage lead to the release into the cytosol and/or extracellular space of mitochondrial damage-associated molecular pattern molecules (mtDAMPs), including mtDNA, ATP, formyl peptides, cardiolipin, cytochrome *c,* succinate, and mitochondrial transcription factor A (TFAM), which activate immune responses (Arnoult, et al., 2011;Nakahira, et al., 2015;Raoof, et al., 2010). We propose that mtDepo associated with adaptive ethanol metabolism triggers mitophagy. As autophagic processing of depolarized mitochondria and mitophagosomes becomes compromised during chronic ethanol exposure, mtDAMPs leak to the cytosol and are released extracellularly by exosome formation or fusion of depolarized mitochondria, mitophagosomes or autolysosomes with the plasma membrane (Fig. 4). In immune cells, insufficiency or discoordination of autophagy leads to release of autophagosomal/autolysosomal contents as inflammatory mediators (Bhattacharya, et al., 2014;Lapaquette, et al., 2015;Fesus, et al., 2011). If after ethanol a markedly increased autophagic burden overwhelms the lysosomal processing capacity, or if normal processing of autophagosomes is suppressed, then release of mtDAMPs is likely to occur. Indeed, chronic ethanol disrupts autophagosomal processing and lysosomal function (Kharbanda, et al., 1995;Thomes, et al., 2015;Chao, et al., 2018). Moreover, mtDAMPs increase after ethanol (Rehman, et al., 2015;Petrasek, et al., 2015;Rolla, et al., 2001). For example, after chronic ethanol treatment in mice, we found that mtDNA increases in serum, an event associated with mtDepo (Rehman, et al., 2015). mtDNA stimulates TLR9, which in turn activates p38-MAPK in neutrophils (Zhang, et al., 2010b;West, et al., 2011;Zhang, et al., 2010a). mtDAMPs upregulate MyD88 and NF-κB, increase cytokines TNFα, IL-1, IL-6 and IL-10, and induce cell death (Hu, et al., 2015;Miura, et al., 2010). IgG recognizing oxidized cardiolipin also increases in ALD patients (Rolla, et al., 2001). Overall, mitochondrial stress/dysfunction associated with ethanol exposure appears to exacerbate inflammation through mtDAMP release and inflammasome activation.

mtDAMPs may also stimulate fibrosis. In a zebrafish alcoholic fatty liver model, the mtDAMP succinate is released, and succinate activates HSC through G-protein coupled receptor-91 (GPR91) (Jang, et al., 2012;Li, et al., 2015). Another mtDAMP, mtDNA, binds to TLR9. TLR9 is expressed in HSC, and activation of TLR9 directly causes HSC activation and fibrosis (Gabele, et al., 2008;Aoyama, et al., 2010). Nonetheless, information regarding the profile and time course of mtDAMP release in ALD, the relation of mtDAMPs to ALD severity, and which mtDAMPs are most critical for ALD pathogenesis is currently very limited.

In addition to mtDAMP release and inflammasome activation, some other events related to mitochondrial alterations due to adaptive alcohol metabolism may also contribute to fibrosis. Oxidative stress and mitochondrial dysfunction stimulate expression of osteopontin (OPN), a multifunctional protein that can induce activation, migration and collagen production by HSCs (Wen, et al., 2016;Riew, et al., 2017;Zhang, et al., 2017). Expression of OPN and its receptors increases in animals and cells treated with ethanol and in patients with ALD (Seth, et al., 2006;Morales-Ibanez, et al., 2013;Apte, et al., 2005;Seth, et al., 2014). Oxidative stress also promotes formation of profibrogenic TGFβ (Barnard, et al., 1990;Yue and Mulder, 2001). HIF-1α, which is upregulated after ethanol consumption due to hypoxia, induces expression of profibrogenic genes (Higgins, et al., 2008). Together, persistence of mitochondrial responses to ethanol links adaptive ethanol metabolism to maladaptive pro-inflammatory and pro-fibrotic changes initiating onset and progression of ALD.

6. Conclusions and future directions

After ethanol consumption, a respiratory burst occurs due to mtDepo in the liver. Such mitochondrial uncoupling after ethanol treatment occurs coordinately with VDAC closure (Fig. 7). These alterations promote more rapid and selective oxidation of toxic AcAld by inhibiting oxidation of competing respiratory substrates, including fatty acyl-CoA, and by more rapidly regenerating NAD^+ needed for ethanol oxidation to acetate. VDAC closure also limits futile mitochondrial ATP hydrolysis that would otherwise occur after uncoupling. Depolarization in turn activates mitophagy that may have the function of removing mitochondria damaged by ethanol-dependent oxidative stress, an apparent protective mechanism. Mitochondrial remodeling and biogenesis subsequently occur to restore mitochondrial homeostasis.

After chronic alcohol, an excessive mitophagic burden leads to dysfunctional autophagic processing and release of mtDAMPs intracellularly and extracellularly to activate inflammasomes and other receptors that mediate inflammatory/profibrotic responses. We propose that such mtDAMP release is a primary pro-inflammatory/pro-fibrotic event in ALD (Fig. 7). mtDAMPs then synergize with a variety of other pro-inflammatory/profibrotic events, especially in relation to endotoxin uptake from the gut. Although adaptive alcohol metabolism is a response to remove ethanol and its toxic metabolites more rapidly, the same adaptation occurring chronically appears to promote mitochondrial and autophagic dysfunction. Such dysfunction may then act as a tipping point from adaptation to maladaptation, leading to other pathogenic consequences in ALD including: 1) steatosis due to inhibited beta-oxidation, 2) mitochondrial glutathione depletion, 3) oxidative stress, 4) ER

stress, and 5) cell death. Perhaps most importantly, mtDAMP release enhances other inflammatory and profibrogenic responses to ethanol, thus exacerbating hepatitis and leading to fibrosis (Fig. 7). In this way, adaptive mitochondrial alterations for alcohol metabolism constitute an initial "hit" in the multi-hit pathogenesis of ALD that converges and synergizes with other ethanol-dependent events in the gut, endocrine system and adipose tissue to promote development and progression of ALD.

Future studies are needed to elucidate further the mechanisms and pathogenic effects of adaptive alcohol metabolism/mitochondrial alterations in ALD, including: 1) Determining how ethanol causes mtDepo. For example, do Kupffer cells release mediators like PGE_2 to cause heterogeneous mtDepo in nearby hepatocytes? 2) Identifying the uncoupling circuit in mitochondria that causes ethanol-induced mtDepo. Multiple depolarizing mitochondrial ion channels and transporters, such as the mitochondrial ATP-sensitive potassium channel ($mitoK_{ATP}$), adenine nucleotide transporter (ANT), Ca^{2+} transporters, and the F_O portion of ATP synthase might underlie mtDepo. 3) Elucidating the mechanism of ethanol-induced VDAC closure. How do phosphorylation, acetylation and acetaldehyde adduct formation modify VDAC conductances after ethanol? 4) Better characterizing the relation of mtDepo, mitophagy and mtDAMP release, particularly the mechanisms by which chronic ethanol impairs lysosomal processing and leads to mtDAMP release, 5) Identifying which specific mtDAMPs are most important in promoting ALD and how these mtDAMP act on effector cells of inflammation and fibrosis. 6) Clarifying the role of mitochondrial biogenesis and remodeling in repair and regeneration processes in ALD. Such new information potential will lead to useful new biomarkers to monitoring ALD development/severity (e.g., mtDAMPs) and novel therapeutic strategies (*e.g.,* mtDepo blockade, enhancing mitophagosome processing, antagonists of mtDAMPs).

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REFERENCES

Adachi M, Higuchi H, Miura S, Azuma T, Inokuchi S, Saito H, Kato S and Ishii H (2004) Bax interacts with the voltage-dependent anion channel and mediates ethanol-induced apoptosis in rat hepatocytes. Am J Physiol Gastrointest Liver Physiol 287:G695–G705. [PubMed: 15044178]

Adachi M and Ishii H (2002) Role of mitochondria in alcoholic liver injury. Free Radic Biol Med 32:487–491. [PubMed: 11958949]

Ajmo JM, Liang X, Rogers CQ, Pennock B and You M (2008) Resveratrol alleviates alcoholic fatty liver in mice. Am J Physiol Gastrointest Liver Physiol 295:G833–G842. [PubMed: 18755807]

Anandatheerthavarada HK, Addya S, Dwivedi RS, Biswas G, Mullick J and Avadhani NG (1997) Localization of multiple forms of inducible cytochromes P450 in rat liver mitochondria: immunological characteristics and patterns of xenobiotic substrate metabolism. Arch Biochem Biophys 339:136–150. [PubMed: 9056243]

Anders LC, Lang AL, Anwar-Mohamed A, Douglas AN, Bushau AM, Falkner KC, Hill BG, Warner NL, Arteel GE, Cave M, McClain CJ and Beier JI (2016) Vinyl Chloride Metabolites Potentiate Inflammatory Liver Injury Caused by LPS in Mice. Toxicol Sci 151:312–323. [PubMed: 26962056]

Anuka E, Gal M, Stocco DM and Orly J (2013) Expression and roles of steroidogenic acute regulatory (StAR) protein in 'non-classical', extra-adrenal and extra-gonadal cells and tissues. Mol Cell Endocrinol 371:47–61. [PubMed: 23415713]

Aoyama T, Paik YH and Seki E (2010) Toll-like receptor signaling and liver fibrosis. Gastroenterol Res Pract 2010:2010. pii: 192543. doi: 10.1155/2010/192543.

Apte UM, Banerjee A, McRee R, Wellberg E and Ramaiah SK (2005) Role of osteopontin in hepatic neutrophil infiltration during alcoholic steatohepatitis. Toxicol Appl Pharmacol 207:25–38. [PubMed: 15885730]

Arnoult D, Soares F, Tattoli I and Girardin SE (2011) Mitochondria in innate immunity. EMBO Rep 12:901–910. [PubMed: 21799518]

Arteel GE, Raleigh JA, Bradford BU and Thurman RG (1996) Acute alcohol produces hypoxia directly in rat liver tissue *in vivo*: Role of Kupffer cells. Am J Physiol 271:G494–G500. [PubMed: 8843775]

Bailey SM and Cunningham CC (2002) Contribution of mitochondria to oxidative stress associated with alcoholic liver disease. Free Radic Biol Med 32:11–16. [PubMed: 11755312]

Bailey SM, Pietsch EC and Cunningham CC (1999) Ethanol stimulates the production of reactive oxygen species at mitochondrial complexes I and III. Free Radic Biol Med 27:891–900. [PubMed: 10515594]

Bala S, Marcos M, Gattu A, Catalano D and Szabo G (2014) Acute binge drinking increases serum endotoxin and bacterial DNA levels in healthy individuals. PLoS One 9:e96864. [PubMed: 24828436]

Bansal S, Liu CP, Sepuri NB, Anandatheerthavarada HK, Selvaraj V, Hoek J, Milne GL, Guengerich FP and Avadhani NG (2010) Mitochondria-targeted cytochrome P450 2E1 induces oxidative damage and augments alcohol-mediated oxidative stress. J Biol Chem 285:24609–24619. [PubMed: 20529841]

Barnard JA, Lyons RM and Moses HL (1990) The cell biology of transforming growth factor beta. Biochim Biophys Acta 1032:79–87. [PubMed: 2194569]

Bataller R and Gao B (2015) Liver fibrosis in alcoholic liver disease. Semin Liver Dis 35:146–156. [PubMed: 25974900]

Bayrhuber M, Meins T, Habeck M, Becker S, Giller K, Villinger S, Vonrhein C, Griesinger C, Zweckstetter M and Zeth K (2008) Structure of the human voltage-dependent anion channel. Proc Natl Acad Sci U S A 105:15370–15375. [PubMed: 18832158]

Beier JI, Arteel GE and McClain CJ (2011) Advances in alcoholic liver disease. Curr Gastroenterol Rep 13:56–64. [PubMed: 21088999]

Bernstein J, Videla L and Israel Y (1975) Hormonal influences in the development of the hypermetabolic state of the liver produced by chronic administration of ethanol. J Pharmacol Exp Therapeut 192:583–591.

Bhagwandeen B, Apte M, Manwarring L and Dickeson J (1987) Endotoxin induced hepatic necrosis in rats on an alcohol diet. J Pathol 151:47–53.

Bhattacharya A, Prakash YS and Eissa NT (2014) Secretory function of autophagy in innate immune cells. Cell Microbiol 16:1637–1645. [PubMed: 25237740]

Bode CH, Kugler V and Bode JCH (1987) Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcohol excess. J Hepatol 4:8–14. [PubMed: 3571935]

Bouillaud F, Alves-Guerra MC and Ricquier D (2016) UCPs, at the interface between bioenergetics and metabolism. Biochim Biophys Acta 1863:2443–2456. [PubMed: 27091404]

Bradford BU, Enomoto N, Ikejima K, Rose ML, Bojes HK, Forman DT and Thurman RG (1999) Peroxisomes are involved in the swift increase in alcohol metabolism. J Pharmacol Exp Ther 288:254–259. [PubMed: 9862778]

Bradford BU, Misra UK and Thurman RG (1993) Kupffer cells are required for the swift increase in alcohol metabolism. Res Commun Subst Abuse 14:1–6.

Bradford BU and Rusyn I (2005) Swift increase in alcohol metabolism (SIAM): understanding the phenomenon of hypermetabolism in liver. Alcohol 35:13–17. [PubMed: 15922133]

Bradford BU, Wall CA, Ikejima K, Rossignol DP, Christ WJ and Thurman RG. The endotoxin antagonist B-464 and antibiotics block the swift increase in alcohol metabolism. Hepatology 22, 550 1995.

Brand MD (2016) Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. Free Radic Biol Med 100:14–31. [PubMed: 27085844]

Bunik VI and Brand MD (2018) Generation of superoxide and hydrogen peroxide by side reactions of mitochondrial 2-oxoacid dehydrogenase complexes in isolation and in cells. Biol Chem. 399: 407–420. [PubMed: 29337692]

Cave M, Falkner KC, Ray M, Joshi-Barve S, Brock G, Khan R, Bon HM and McClain CJ (2010) Toxicant-associated steatohepatitis in vinyl chloride workers. Hepatology 51:474–481. [PubMed: 19902480]

Chacko KR and Reinus J (2016) Spectrum of Alcoholic Liver Disease. Clin Liver Dis 20:419–427. [PubMed: 27373606]

Chao X, Wang S, Zhao K, Li Y, Williams JA, Li T, Chavan H, Krishnamurthy P, He XC, Li L, Ballabio A, Ni HM and Ding WX (2018) Impaired TFEB-mediated Lysosome Biogenesis and Autophagy Promote Chronic Ethanol-induced Liver Injury and Steatosis in Mice. *Gastroenterology*pii: S0016–5085(18)34560–8. doi: 10.1053/j.gastro.2018.05.027.

Chavin KD, Yang S, Lin HZ, Chatham J, Chacko VP, Hoek JB, Walajtys-Rode E, Rashid A, Chen CH, Huang CC, Wu TC, Lane MD and Diehl AM (1999) Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. J Biol Chem 274:5692–5700. [PubMed: 10026188]

Chistiakov DA, Shkurat TP, Melnichenko AA, Grechko AV and Orekhov AN (2017) The role of mitochondrial dysfunction in cardiovascular disease: a brief review. Ann Med 1–7.

Colombini M (2012) VDAC structure, selectivity, and dynamics. Biochim Biophys Acta 1818:1457–1465. [PubMed: 22240010]

Correnti JM, Juskeviciute E, Swarup A and Hoek JB (2014) Pharmacological ceramide reduction alleviates alcohol-induced steatosis and hepatomegaly in adiponectin knockout mice. Am J Physiol Gastrointest Liver Physiol 306:G959–G973. [PubMed: 24742988]

Das S, Wong R, Rajapakse N, Murphy E and Steenbergen C (2008) Glycogen synthase kinase 3 inhibition slows mitochondrial adenine nucleotide transport and regulates voltage-dependent anion channel phosphorylation. Circ Res 103:983–991. [PubMed: 18802025]

Demori I, Burlando B, Gerdoni E, Lanni A, Fugassa E and Voci A (2008) Uncoupling protein-2 induction in rat hepatocytes after acute carbon tetrachloride liver injury. J Cell Physiol 216:413–418. [PubMed: 18314881]

Diehl AM (2005) Recent events in alcoholic liver disease V. effects of ethanol on liver regeneration. Am J Physiol Gastrointest Liver Physiol 288:G1–G6. [PubMed: 15591584]

Diehl AM and Hoek JB (1999) Mitochondrial uncoupling: role of uncoupling protein anion carriers and relationship to thermogenesis and weight control “the benefits of losing control”. J Bioenerg Biomembr 31:493–506. [PubMed: 10653477]

Ding WX, Li M, Chen X, Ni HM, Lin CW, Gao W, Lu B, Stolz DB, Clemens DL and Yin XM (2010) Autophagy reduces acute ethanol-induced hepatotoxicity and steatosis in mice. Gastroenterology 139:1740–1752. [PubMed: 20659474]

Dou X, Xia Y, Chen J, Qian Y, Li S, Zhang X and Song Z (2014) Rectification of impaired adipose tissue methylation status and lipolytic response contributes to hepatoprotective effect of betaine in a mouse model of alcoholic liver disease. Br J Pharmacol 171:4073–4086. [PubMed: 24819676]

Eid N, Ito Y, Horibe A and Otsuki Y (2016) Ethanol-induced mitophagy in liver is associated with activation of the PINK1-Parkin pathway triggered by oxidative DNA damage. Histol Histopathol 31:1143–1159. [PubMed: 26935412]

Eid N, Ito Y, Maemura K and Otsuki Y (2013) Elevated autophagic sequestration of mitochondria and lipid droplets in steatotic hepatocytes of chronic ethanol-treated rats: an immunohistochemical and electron microscopic study. J Mol Histol 44:311–326. [PubMed: 23371376]

El-Assal O, Hong F, Kim WH, Radaeva S and Gao B (2004) IL-6-deficient mice are susceptible to ethanol-induced hepatic steatosis: IL-6 protects against ethanol-induced oxidative stress and

mitochondrial permeability transition in the liver. Cell Mol Immunol 1:205–211. [PubMed: 16219169]

Elmore SP, Qian T, Grissom SF and Lemasters JJ (2001) The mitochondrial permeability transition initiates autophagy in rat hepatocytes. FASEB J 15:2286–2287. [PubMed: 11511528]

Engen PA, Green SJ, Voigt RM, Forsyth CB and Keshavarzian A (2015) The Gastrointestinal Microbiome: Alcohol Effects on the Composition of Intestinal Microbiota. Alcohol Res 37:223–236. [PubMed: 26695747]

Enomoto N, Ikejima K, Bradford BU, Rivera CA, Kono H, Goto M, Yamashina S, Schemmer P, Kitamura T, Oide H, Takei Y, Hirose M, Shimizu H, Miyazaki A, Brenner DA, Sato N and Thurman RG (2000a) Role of Kupffer cells and gut-derived endotoxins in alcoholic liver injury. J Gastroenterol Hepatol **15 Suppl**:D20–D25. [PubMed: 10759216] **15 Suppl**

Enomoto N, Ikejima K, Kitamura T, Oide H, Takei Y, Sato N and Thurman RG (2000b) Alcohol enhances lipopolysaccharide-induced increases in nitric oxide production by Kupffer cells via mechanisms dependent on endotoxin. Alcohol Clin Exp Res 24:55S–58S. [PubMed: 10803781]

Enomoto N, Ikejima K, Yamashina S, Enomoto A, Nishiura T, Nishimura T, Brenner DA, Schemmer P, Bradford BU, Rivera CA, Zhong Z and Thurman RG (2000c) Kupffer cell-derived prostaglandin E(2) is involved in alcohol-induced fat accumulation in rat liver. Am J Physiol Gastrointest Liver Physiol 279:G100–G106. [PubMed: 10898751]

Enomoto N, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura T, Takei Y, Sato AN and Thurman RG (2001) Kupffer cell sensitization by alcohol involves increased permeability to gut-derived endotoxin. Alcohol Clin Exp Res 25:51S–54S. [PubMed: 11410742]

Enomoto N, Takei Y, Kitamura T, Hirose M, Ikejima K and Sato N (2002) Estriol enhances lipopolysaccharide-induced increases in nitric oxide production by Kupffer cells via mechanisms dependent on endotoxin. Alcohol Clin Exp Res 26:66S–69S. [PubMed: 12198378]

Evans ZP, Ellett JD, Schmidt MG, Schnellmann RG and Chavin KD (2008) Mitochondrial uncoupling protein-2 mediates steatotic liver injury following ischemia/reperfusion. J Biol Chem 283:8573–8579. [PubMed: 18086675]

Fernandez A, Matias N, Fucho R, Ribas V, Von MC, Nuno N, Baulies A, Martinez L, Tarrats N, Mari M, Colell A, Morales A, Dubuquoy L, Mathurin P, Bataller R, Caballeria J, Elena M, Balsinde J, Kaplowitz N, Garcia-Ruiz C and Fernandez-Checa JC (2013) ASMase is required for chronic alcohol induced hepatic endoplasmic reticulum stress and mitochondrial cholesterol loading. J Hepatol 59:805–813. [PubMed: 23707365]

Fernandez-Checa JC and Kaplowitz N (2005) Hepatic mitochondrial glutathione: transport and role in disease and toxicity. Toxicol Appl Pharmacol 204:263–273. [PubMed: 15845418]

Fesus L, Demeny MA and Petrovski G (2011) Autophagy shapes inflammation. Antioxid Redox Signal 14:2233–2243. [PubMed: 20812858]

Forman DT, Bradford BU, Handler JA, Glassman EB and Thurman RG (1988) Involvement of hormones in the swift increase in alcohol metabolism. Ann Clin Lab Sci 18:318–325. [PubMed: 3044269]

Fromenty B, Grimbert S, Mansouri A, Beaugrand M, Erlinger S, Rotig A and Pessayre D (1995) Hepatic mitochondrial DNA deletion in alcoholics: association with microvesicular steatosis. Gastroenterology 108:193–200. [PubMed: 7806041]

Fromenty B and Pessayre D (1995) Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. Pharmacol Ther 67:101–154. [PubMed: 7494860]

Fukui H, Brauner B, Bode JCH and Bode CH (1991) Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. J Hepatol 12:162–169. [PubMed: 2050995]

Fung P and Pyrsopoulos N (2017) Emerging concepts in alcoholic hepatitis. World J Hepatol 9:567–585. [PubMed: 28515843]

Gabele E, Muhlbauer M, Dorn C, Weiss TS, Froh M, Schnabl B, Wiest R, Scholmerich J, Obermeier F and Hellerbrand C (2008) Role of TLR9 in hepatic stellate cells and experimental liver fibrosis. Biochem Biophys Res Commun 376:271–276. [PubMed: 18760996]

Gao B and Bataller R (2011) Alcoholic Liver Disease: Pathogenesis and New Therapeutic Targets. Gastroenterology. 141:1572–1585. [PubMed: 21920463]

Garcia-Ruiz C, Kaplowitz N and Fernandez-Checa JC (2013) Role of Mitochondria in Alcoholic Liver Disease. Curr Pathobiol Rep 1:159–168. [PubMed: 25343061]

Garcia-Villafranca J, Guillen A and Castro J (2008) Ethanol consumption impairs regulation of fatty acid metabolism by decreasing the activity of AMP-activated protein kinase in rat liver. Biochimie 90:460–466. [PubMed: 17997005]

Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ and Springer W (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol 12:119–131. [PubMed: 20098416]

Glassman EB, McLaughlin GA, Forman DT, Felder MR and Thurman RG (1985) Role of alcohol dehydrogenase in the swift increase in alcohol metabolism (SIAM). Studies with deer mice deficient in alcohol dehydrogenase. Biochem Pharmacol 34:3523–3526. [PubMed: 2932115]

Gonzalez FJ, Ueno T, Umeno M, Song BJ, Veech RL and Gelboin HV (1991) Microsomal ethanol oxidizing system: transcriptional and posttranscriptional regulation of cytochrome P450, CYP2E1. *Alcohol Alcohol Suppl* 1:97–101. [PubMed: 1845601]

Groebner JL and Tuma PL (2015) The Altered Hepatic Tubulin Code in Alcoholic Liver Disease. Biomolecules 5:2140–2159. [PubMed: 26393662]

Gupta R (2017) Phosphorylation of rat brain purified mitochondrial Voltage-Dependent Anion Channel by c-Jun N-terminal kinase-3 modifies open-channel noise. Biochem Biophys Res Commun 490:1221–1225. [PubMed: 28676395]

Gupta R and Ghosh S (2015) Phosphorylation of voltage-dependent anion channel by c-Jun N-terminal Kinase-3 leads to closure of the channel. Biochem Biophys Res Commun 459:100–106. [PubMed: 25721670]

Gurung P, Lukens JR and Kanneganti TD (2015) Mitochondria: diversity in the regulation of the NLRP3 inflammasome. Trends Mol Med. 21:193–201. [PubMed: 25500014]

Han D, Johnson HS, Rao MP, Martin G, Sancheti H, Silkwood KH, Decker CW, Nguyen KT, Casian JG, Cadenas E and Kaplowitz N (2017) Mitochondrial remodeling in the liver following chronic alcohol feeding to rats. Free Radic Biol Med 102:100–110. [PubMed: 27867097]

Han D, Ybanez MD, Johnson HS, McDonald JN, Mesropyan L, Sancheti H, Martin G, Martin A, Lim AM, Dara L, Cadenas E, Tsukamoto H and Kaplowitz N (2012) Dynamic adaptation of liver mitochondria to chronic alcohol feeding in mice: biogenesis, remodeling, and functional alterations. J Biol Chem 287:42165–42179. [PubMed: 23086958]

Hariharan N, Zhai P and Sadoshima J (2011) Oxidative stress stimulates autophagic flux during ischemia/reperfusion. Antioxid Redox Signal 14:2179–2190. [PubMed: 20812860]

Higgins DF, Kimura K, Iwano M and Haase VH (2008) Hypoxia-inducible factor signaling in the development of tissue fibrosis. Cell Cycle 7:1128–1132. [PubMed: 18418042]

Hoek JB, Cahill A and Pastorino JG (2002) Alcohol and mitochondria: a dysfunctional relationship. Gastroenterology 122:2049–2063. [PubMed: 12055609]

Hoek JB and Pastorino JG (2004) Cellular signaling mechanisms in alcohol-induced liver damage. Semin Liver Dis 24:257–272. [PubMed: 15349804]

Holmuhamedov E and Lemasters JJ (2009) Ethanol exposure decreases mitochondrial outer membrane permeability in cultured rat hepatocytes. Arch Biochem Biophys 481:226–233. [PubMed: 19014900]

Holmuhamedov EL, Czerny C, Beeson CC and Lemasters JJ (2012) Ethanol suppresses ureagenesis in rat hepatocytes: role of acetaldehyde. J Biol Chem 287:7692–7700. [PubMed: 22228763]

Holmuhamedov EL, Lovelace G and Lemasters JJ. (2011) Aldehyde products of ethanol oxidation and oxidative stress suppress ureagenic but not basal respiration of cultured hepatocytes. Biophys.J. 100, 460a.

Hu Q, Wood CR, Cimen S, Venkatachalam AB and Alwayn IP (2015) Mitochondrial Damage-Associated Molecular Patterns (MTDs) Are Released during Hepatic Ischemia Reperfusion and Induce Inflammatory Responses. PLoS One 10:e0140105. [PubMed: 26451593]

Hughes MM and O'Neill LAJ (2018) Metabolic regulation of NLRP3. Immunol Rev 281:88–98. [PubMed: 29247992]

Ikejima K, Enomoto N, Iimuro Y, Ikejima A, Fang D, Xu J, Forman DT, Brenner DA and Thurman RG (1998) Estrogen increases sensitivity of hepatic Kupffer cells to endotoxin. Am J Physiol 274:G669–G676. [PubMed: 9575848]

Imajo K, Fujita K, Yoneda M, Nozaki Y, Ogawa Y, Shinohara Y, Kato S, Mawatari H, Shibata W, Kitani H, Ikejima K, Kirikoshi H, Nakajima N, Saito S, Maeyama S, Watanabe S, Wada K and Nakajima A (2012) Hyperresponsivity to low-dose endotoxin during progression to nonalcoholic steatohepatitis is regulated by leptin-mediated signaling. Cell Metab 16:44–54. [PubMed: 22768838]

Iracheta-Vellve A, Petrasek J, Satishchandran A, Gyongyosi B, Saha B, Kodys K, Fitzgerald KA, Kurt-Jones EA and Szabo G (2015) Inhibition of sterile danger signals, uric acid and ATP, prevents inflammasome activation and protects from alcoholic steatohepatitis in mice. J Hepatol 63:1147–1155. [PubMed: 26100496]

Ishak KG, Zimmerman HJ and Ray MB (1991) Alcoholic liver disease: pathologic, pathogenetic and clinical aspects. Alcohol Clin Exp Res 15:45–66. [PubMed: 2059245]

Israel Y, Videla L and Bernstein J (1975a) Liver hypermetabolic state after chronic ethanol consumption: Hormonal interrelations and pathogenic implications. Fed Proc 34:2052–2059. [PubMed: 1100436]

Israel Y, Videla L, Fernandez-Videla V and Bernstein J (1975b) Effects of chronic ethanol treatment and thyroxine administration on ethanol metabolism and liver oxidative capacity. J Pharmacol Exp Ther 192:565–574. [PubMed: 1120957]

Israel Y, Videla L, MacDonald A and Bernstein J (1973) Metabolic alterations produced in the liver by chronic alcohol administration: Comparison between the effects produced by ethanol and by thyroid hormones. Biochem J 134:523–529. [PubMed: 16742813]

Iyer SS, He Q, Janczy JR, Elliott EI, Zhong Z, Olivier AK, Sadler JJ, Knepper-Adrian V, Han R, Qiao L, Eisenbarth SC, Nauseef WM, Cassel SL and Sutterwala FS (2013) Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. Immunity 39:311–323. [PubMed: 23954133]

Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D and Lemasters JJ (2002) Mechanisms of hepatotoxicity. Toxicol Sci 65:166–176. [PubMed: 11812920]

Jang ZH, Chung HC, Ahn YG, Kwon YK, Kim JS, Ryu JH, Ryu DH, Kim CH and Hwang GS (2012) Metabolic profiling of an alcoholic fatty liver in zebrafish (Danio rerio). Mol Biosyst 8:2001–2009. [PubMed: 22532405]

Jennett RB, Tuma DJ and Sorrell MF (1980) Effect of ethanol and its metabolites on microtubule formation. Pharmacology 21:363–368. [PubMed: 7433516]

Jhangiani SS, Agarwal N, Holmes R, Cayten CG and Pitchumoni CS (1986) Energy expenditure in chronic alcoholics with and without liver disease. Am J Clin Nutr 44:323–329. [PubMed: 3092630]

Ji C and Kaplowitz N (2003) Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice. Gastroenterology 124:1488–1499. [PubMed: 12730887]

Ji C and Kaplowitz N (2006) ER stress: can the liver cope? J Hepatol 45:321–333. [PubMed: 16797772]

Ji S, Christenson VR, Lemasters JJ and Thurman RG (1983) Selective increase in pericentral oxygen gradient in perfused rat liver following ethanol treatment. Pharmacol Biochem Behav 18:439–442. [PubMed: 6685302]

Ji S, Lemasters JJ, Christenson VR and Thurman RG (1982a) Periportal and pericentral pyridine nucleotide fluorescence from the surface of the perfused liver: Evaluation of the hypothesis that chronic treatment with ethanol produces pericentral hypoxia. Proc Natl Acad Sci U S A 79:5415–5419. [PubMed: 6957871]

Ji S, Lemasters JJ and Thurman RG (1982b) Intralobular hepatic pyridine nucleotide fluorescence: Evaluation of the hypothesis that chronic treatment with ethanol produces pericentral hypoxia. Proc Natl Acad Sci 80:5415–5419.

Jin SM and Youle RJ (2012) PINK1- and Parkin-mediated mitophagy at a glance. J Cell Sci 125:795–799. [PubMed: 22448035]

Khanova E, Wu R, Wang W, Yan R, Chen Y, French SW, Llorente C, Pan SQ, Yang Q, Li Y, Lazaro R, Ansong C, Smith RD, Bataller R, Morgan T, Schnabl B and Tsukamoto H (2018) Pyroptosis by

caspase11/4-gasdermin-D pathway in alcoholic hepatitis in mice and patients. Hepatology. 67:1737–1753. [PubMed: 29108122]

Kharbanda KK, McVicker DL, Zetterman RK and Donohue TM, Jr. (1995) Ethanol consumption reduces the proteolytic capacity and protease activities of hepatic lysosomes. Biochim Biophys Acta 1245:421–429. [PubMed: 8541322]

Kim I, Rodriguez-Enriquez S and Lemasters JJ (2007) Selective degradation of mitochondria by mitophagy. Arch Biochem Biophys 462:245–253. [PubMed: 17475204]

Kim JS, Nitta T, Mohuczy D, O'Malley KA, Moldawer LL, Dunn WA, Jr. and Behrns KE (2008) Impaired autophagy: A mechanism of mitochondrial dysfunction in anoxic rat hepatocytes. Hepatology 47:1725–1736. [PubMed: 18311843]

Kim WR, Brown RS, Jr, Terrault NA and El-Serag H (2002) Burden of liver disease in the United States: summary of a workshop. Hepatology 36:227–242. [PubMed: 12085369]

King AL, Swain TM, Mao Z, Udoh US, Oliva CR, Betancourt AM, Griguer CE, Crowe DR, Lesort M and Bailey SM (2014) Involvement of the mitochondrial permeability transition pore in chronic ethanol-mediated liver injury in mice. Am J Physiol Gastrointest Liver Physiol 306:G265–G277. [PubMed: 24356880]

Koch OR, De Leo ME, Borrello S, Palombini G, Galeotti T. (1994) Ethanol treatment up-regulates the expression of mitochondrial manganese superoxide dismutase in rat liver. Biochem Biophys Res Commun 201:1356–1365. [PubMed: 8024580]

Kong X, Yang Y, Ren L, Shao T, Li F, Zhao C, Liu L, Zhang H, McClain CJ and Feng W (2017) Activation of autophagy attenuates EtOH-LPS-induced hepatic steatosis and injury through MD2 associated TLR4 signaling. Sci Rep 7:9292. [PubMed: 28839246]

Kono A, Enomoto N, Takei Y, Hirose M, Ikejima K and Sato N (2002) Oral contraceptives worsen endotoxin-induced liver injury in rats. Alcohol Clin Exp Res 26:70S–74S. [PubMed: 12198379]

Korshunov SS, Skulachev VP and Starkov AA (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett 416:15–18. [PubMed: 9369223]

Kovacic P, Pozos RS, Somanathan R, Shangari N and O'Brien PJ (2005) Mechanism of mitochondrial uncouplers, inhibitors, and toxins: focus on electron transfer, free radicals, and structure-activity relationships. Curr Med Chem 12:2601–2623. [PubMed: 16248817]

Krishnasamy Y, Ramshesh VK, Gooz M, Schnellmann RG, Lemasters JJ and Zhong Z (2016) Ethanol and High Cholesterol Diet Causes Severe Steatohepatitis and Early Liver Fibrosis in Mice. PLoS One 11:e0163342. [PubMed: 27676640]

Kubli DA and Gustafsson AB (2012) Mitochondria and mitophagy: the yin and yang of cell death control. Circ Res 111:1208–1221. [PubMed: 23065344]

Lapaquette P, Guzzo J, Bretillon L and Bringer MA (2015) Cellular and Molecular Connections between Autophagy and Inflammation. Mediators Inflamm 2015:398483. [PubMed: 26221063]

Larosche I, Letteron P, Berson A, Fromenty B, Huang TT, Moreau R, Pessayre D and Mansouri A (2010) Hepatic mitochondrial DNA depletion after an alcohol binge in mice: probable role of peroxynitrite and modulation by manganese superoxide dismutase. J Pharmacol Exp Ther 332:886–897. [PubMed: 20016022]

Lemasters JJ (2004) Rusty notions of cell injury. J Hepatol 40:696–698. [PubMed: 15030988]

Lemasters JJ (2013) Hepatotoxicity due to mitochondrial injury, in Drug-Induced Liver Disease (Kaplowitz N and L DeLeve eds) pp 85–100, Elsevier, Amsterdam.

Lemasters JJ (2014) Variants of mitochondrial autophagy: Types 1 and 2 mitophagy and micromitophagy (Type 3). Redox Biol 2:749–754. [PubMed: 25009776]

Lemasters JJ (2017) Evolution of Voltage-Dependent Anion Channel Function: From Molecular Sieve to Governator to Actuator of Ferroptosis. Front Oncol 7:303. [PubMed: 29312883]

Lemasters JJ and Holmuhamedov E (2006) Voltage-dependent anion channel (VDAC) as mitochondrial governator--thinking outside the box. Biochim Biophys Acta 1762:181–190. [PubMed: 16307870]

Leung TM and Nieto N (2013) CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease. J Hepatol 58:395–398. [PubMed: 22940046]

Levine JA, Harris MM and Morgan MY (2000) Energy expenditure in chronic alcohol abuse. Eur J Clin Invest 30:779–786. [PubMed: 10998077]

Li YH, Woo SH, Choi DH and Cho EH (2015) Succinate causes alpha-SMA production through GPR91 activation in hepatic stellate cells. Biochem Biophys Res Commun 463:853–858. [PubMed: 26051274]

Lieber CS (2004) Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol 34:9–19. [PubMed: 15670660]

Lieber CS (2005) Metabolism of alcohol. Clin Liver Dis 9:1–35. [PubMed: 15763227]

Lieber CS, Jones DP, LOSOWSKY MS and DAVIDSON CS (1962) Interrelation of uric acid and ethanol metabolism in man. J Clin Invest 41:1863–1870. [PubMed: 13930523]

Lieber CS, Leo MA, Wang X and DeCarli LM (2008a) Alcohol alters hepatic FoxO1, p53, and mitochondrial SIRT5 deacetylation function. Biochem Biophys Res Commun 373:246–252. [PubMed: 18555008]

Lieber CS, Leo MA, Wang X and DeCarli LM (2008b) Effect of chronic alcohol consumption on Hepatic SIRT1 and PGC-1alpha in rats. Biochem Biophys Res Commun 370:44–48. [PubMed: 18342626]

Lin CW, Zhang H, Li M, Xiong X, Chen X, Chen X, Dong XC and Yin XM (2013) Pharmacological promotion of autophagy alleviates steatosis and injury in alcoholic and non-alcoholic fatty liver conditions in mice. J Hepatol 58:993–999. [PubMed: 23339953]

Lu Y and Cederbaum AI (2008) CYP2E1 and oxidative liver injury by alcohol. Free Radic Biol Med 44:723–738. [PubMed: 18078827]

Magdaleno F, Blajszczak CC and Nieto N (2017) Key Events Participating in the Pathogenesis of Alcoholic Liver Disease. Biomolecules 7:pii: E9. doi: 10.3390/biom7010009. [PubMed: 28134813]

Magnusson B, Asp L, Bostrom P, Ruiz M, Stillemark-Billton P, Linden D, Boren J and Olofsson SO (2006) Adipocyte differentiation-related protein promotes fatty acid storage in cytosolic triglycerides and inhibits secretion of very low-density lipoproteins. Arterioscler Thromb Vasc Biol 26:1566–1571. [PubMed: 16627799]

Maldonado EN and Lemasters JJ (2012) Warburg revisited: regulation of mitochondrial metabolism by voltage-dependent anion channels in cancer cells. J Pharmacol Exp Ther 342:637–641. [PubMed: 22700429]

Maldonado EN, Patnaik J, Mullins MR and Lemasters JJ (2010) Free tubulin modulates mitochondrial membrane potential in cancer cells. Cancer Res 70:10192–10201. [PubMed: 21159641]

Maldonado EN, Sheldon KL, DeHart DN, Patnaik J, Manevich Y, Townsend DM, Bezrukov SM, Rostovtseva TK and Lemasters JJ (2013) Voltage-dependent anion channels modulate mitochondrial metabolism in cancer cells: regulation by free tubulin and erastin. J Biol Chem 288:11920–11929. [PubMed: 23471966]

Malhi H, Gores GJ and Lemasters JJ (2006) Apoptosis and necrosis in the liver: a tale of two deaths? Hepatology 43:S31–S44. [PubMed: 16447272]

Mansouri A, Demeilliers C, Amsellem S, Pessayre D and Fromenty B (2001) Acute ethanol administration oxidatively damages and depletes mitochondrial dna in mouse liver, brain, heart, and skeletal muscles: protective effects of antioxidants. J Pharmacol Exp Ther 298:737–743. [PubMed: 11454938]

Mansouri A, Gattolliat CH and Asselah T (2018) Mitochondrial Dysfunction and Signaling in Chronic Liver Diseases. Gastroenterology pii: S0016–5085(18)34770–X. doi: 10.1053/j.gastro.2018.06.083.

Mari M, Colell A, Morales A, Caballero F, Moles A, Fernandez A, Terrones O, Basanez G, Antonsson B, Garcia-Ruiz C and Fernandez-Checa JC (2008) Mechanism of mitochondrial glutathione-dependent hepatocellular susceptibility to TNF despite NF-kappaB activation. Gastroenterology 134:1507–1520. [PubMed: 18343380]

Matsuhashi T, Karbowski M, Liu X, Usukura J, Wozniak M and Wakabayashi T (1998) Complete suppresion of ethanol-induced formation of megamitochondria by 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (4-OH-TEMPO). Free Radic Biol Med 24:139–147. [PubMed: 9436623]

McClain CJ, Cohen DA, Dinarello CA, Cannon JG, Shedlofsky SI and Kaplan AM (1986) Serum interleukin 1 (IL-1) activity in alcoholic hepatitis. Life Sci 3:1479–1485.

McLelland GL, Soubannier V, Chen CX, McBride HM and Fon EA (2014) Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. EMBO J 33:282–295. [PubMed: 24446486]

Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T and Akira S (2013) Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat Immunol 14:454–460. [PubMed: 23502856]

Miura K, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, Olefsky JM, Brenner DA and Seki E (2010) Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. Gastroenterology 139:323–334. [PubMed: 20347818]

Morales-Ibanez O, Dominguez M, Ki SH, Marcos M, Chaves JF, Nguyen-Khac E, Houchi H, Affo S, Sancho-Bru P, Altamirano J, Michelena J, Garcia-Pagan JC, Abraldes JG, Arroyo V, Caballeria J, Laso FJ, Gao B and Bataller R (2013) Human and experimental evidence supporting a role for osteopontin in alcoholic hepatitis. Hepatology 58:1742–1756. [PubMed: 23729174]

Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, Kwasny M, Lau CK and Keshavarzian A (2012) Colonic microbiome is altered in alcoholism. Am J Physiol Gastrointest Liver Physiol 302:G966–G978. [PubMed: 22241860]

Nakahira K, Hisata S and Choi AM (2015) The Roles of Mitochondrial Damage-Associated Molecular Patterns in Diseases. Antioxid Redox Signal 23:1329–1350. [PubMed: 26067258]

Nanji AA (2004) Role of different dietary fatty acids in the pathogenesis of experimental alcoholic liver disease. Alcohol 34:21–25. [PubMed: 15670661]

Nanji AA, Griniuviene B, Sadrzadeh SM, Levitsky S and McCully JD (1995) Effect of type of dietary fat and ethanol on antioxidant enzyme mRNA induction in rat liver. J Lipid Res 36:736–744. [PubMed: 7616120]

Narendra D, Tanaka A, Suen DF and Youle RJ (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 183:795–803. [PubMed: 19029340]

Nath B, Levin I, Csak T, Petrasek J, Mueller C, Kodys K, Catalano D, Mandrekar P and Szabo G (2011) Hepatocyte-specific hypoxia-inducible factor-1alpha is a determinant of lipid accumulation and liver injury in alcohol-induced steatosis in mice. Hepatology 53:1526–1537. [PubMed: 21520168]

Neumann D, Buckers J, Kastrup L, Hell SW and Jakobs S (2010) Two-color STED microscopy reveals different degrees of colocalization between hexokinase-I and the three human VDAC isoforms. PMC Biophys 3:4. doi: 10.1186/1757-5036-3-4. [PubMed: 20205711]

Nicholas DG and Ferguson SJ (2013) *Bioenergetics* Academic Press, AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD • PARIS • SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO.

Nieminen AL, Byrne AM, Herman B and Lemasters JJ (1997) Mitochondrial permeability transition in hepatocytes induced by *t*-BuOOH: NAD(P)H and reactive oxygen species. Am J Physiol 272:C1286–C1294. [PubMed: 9142854]

Nieminen AL, Saylor AK, Tesfai SA, Herman B and Lemasters JJ (1995) Contribution of the mitochondrial permeability transition to lethal injury after exposure of hepatocytes to t-butylhydroperoxide. Biochem J 307 **(** Pt 1**)**:99–106. [PubMed: 7718000] **()**

Nolan JP (2010) The role of intestinal endotoxin in liver injury: a long and evolving history. Hepatology 52:1829–1835. [PubMed: 20890945]

Peng Y, French BA, Tillman B, Morgan TR and French SW (2014) The inflammasome in alcoholic hepatitis: Its relationship with Mallory-Denk body formation. Exp Mol Pathol 97:305–313. [PubMed: 25149528]

Pessayre D, Fromenty B, Berson A, Robin MA, Letteron P, Moreau R and Mansouri A (2012) Central role of mitochondria in drug-induced liver injury. Drug Metab Rev 44:34–87. [PubMed: 21892896]

Petrasek J, Bala S, Csak T, Lippai D, Kodys K, Menashy V, Barrieau M, Min SY, Kurt-Jones EA and Szabo G (2012) IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice. J Clin Invest 122:3476–3489. [PubMed: 22945633]

Petrasek J, Iracheta-Vellve A, Saha B, Satishchandran A, Kodys K, Fitzgerald KA, Kurt-Jones EA and Szabo G (2015) Metabolic danger signals, uric acid and ATP, mediate inflammatory cross-talk between hepatocytes and immune cells in alcoholic liver disease. J Leukoc Biol 98:249–256. [PubMed: 25934928]

Pi H, Xu S, Zhang L, Guo P, Li Y, Xie J, Tian L, He M, Lu Y, Li M, Zhang Y, Zhong M, Xiang Y, Deng L, Zhou Z and Yu Z (2013) Dynamin 1-like-dependent mitochondrial fission initiates overactive mitophagy in the hepatotoxicity of cadmium. Autophagy 9:1780–1800. [PubMed: 24121705]

Pickles S, Vigie P and Youle RJ (2018) Mitophagy and Quality Control Mechanisms in Mitochondrial Maintenance. Curr Biol 28:R170–R185. [PubMed: 29462587]

Qu W, Zhong Z, Goto M and Thurman RG (1996) Kupffer cell prostaglandin E_2 stimulates parenchymal cell O_2 consumption: alcohol and cell-cell communication. Am J Physiol 270:G574–G580. [PubMed: 8928786]

Raoof M, Zhang Q, Itagaki K and Hauser CJ (2010) Mitochondrial peptides are potent immune activators that activate human neutrophils via FPR-1. J Trauma 68:1328–1332. [PubMed: 20539176]

Redmann M, Dodson M, Boyer-Guittaut M, Darley-Usmar V and Zhang J (2014) Mitophagy mechanisms and role in human diseases. Int J Biochem Cell Biol 57:127–133.

Rehm J, Samokhvalov AV and Shield KD (2013) Global burden of alcoholic liver diseases. J Hepatol 59:160–168. [PubMed: 23511777]

Rehman H, Liu Q, Krishnasamy Y, Ramshesh VK, Schnellmann RG, Lemaster JJ and Zhong Z. (2015) Activation of aldehyde dehydrogenase-2 attenuates chronic ethanol-induced steatohepatitis. Gastroenterology 148, S989–S990.

Ribiere C, Hininger I, Saffar-Boccara C, Sabourault D and Nordmann R (1994) Mitochondrial respiratory activity and superoxide radical generation in the liver, brain and heart after chronic ethanol intake. Biochem Pharmacol 47:1827–1833. [PubMed: 8204099]

Riew TR, Kim HL, Jin X, Choi JH, Shin YJ, Kim JS and Lee MY (2017) Spatiotemporal expression of osteopontin in the striatum of rats subjected to the mitochondrial toxin 3-nitropropionic acid correlates with microcalcification. Sci Rep 7:45173. doi: 10.1038/srep45173. [PubMed: 28345671]

Rivera CA, Bradford BU, Seabra V and Thurman RG (1998) Role of endotoxin in the hypermetabolic state following acute ethanol exposure. Am J Physiol 275:G1252–G1258. [PubMed: 9843760]

Robin MA, Sauvage I, Grandperret T, Descatoire V, Pessayre D and Fromenty B (2005) Ethanol increases mitochondrial cytochrome P450 2E1 in mouse liver and rat hepatocytes. FEBS Lett 579:6895–6902. [PubMed: 16337197]

Rodriguez-Enriquez S, Kai Y, Maldonado E, Currin RT and Lemasters JJ (2009) Roles of mitophagy and the mitochondrial permeability transition in remodeling of cultured rat hepatocytes. Autophagy 5:1099–1106. [PubMed: 19783904]

Rojas-Feria M, Castro M, Suarez E, Ampuero J and Romero-Gomez M (2013) Hepatobiliary manifestations in inflammatory bowel disease: the gut, the drugs and the liver. World J Gastroenterol 19:7327–7340. [PubMed: 24259964]

Rolla R, Vay D, Mottaran E, Parodi M, Vidali M, Sartori M, Rigamonti C, Bellomo G and Albano E (2001) Antiphospholipid antibodies associated with alcoholic liver disease specifically recognise oxidised phospholipids. Gut 49:852–859. [PubMed: 11709522]

Rostovtseva T and Colombini M (1997) VDAC channels mediate and gate the flow of ATP: implications for the regulation of mitochondrial function. Biophys J 72:1954–1962. [PubMed: 9129800]

Roth MJ, Baer DJ, Albert PS, Castonguay TW, Dorgan JF, Dawsey SM, Brown ED, Hartman TJ, Campbell WS, Giffen CA, Judd JT and Taylor PR (2003) Relationship between serum leptin levels and alcohol consumption in a controlled feeding and alcohol ingestion study. J Natl Cancer Inst 95:1722–1725. [PubMed: 14625264]

Rubio-Tapia A and Murray JA (2008) Liver involvement in celiac disease. Minerva Med 99:595–604. [PubMed: 19034257]

Scarpellini E, Forlino M, Lupo M, Rasetti C, Fava G, Abenavoli L and De SA (2016) Gut Microbiota and Alcoholic Liver Disease. Rev Recent Clin Trials 11:213–219. [PubMed: 27515958]

Scheer MA, Schneider KJ, Finnigan RL, Maloney EP, Wells MA and Clemens DL (2016) The Involvement of Acetaldehyde in Ethanol-Induced Cell Cycle Impairment. Biomolecules 6: pii: E17. doi: 10.3390/biom6020017. [PubMed: 27043646]

Schroder K, Zhou R and Tschopp J (2010) The NLRP3 inflammasome: a sensor for metabolic danger? Science 327:296–300. [PubMed: 20075245]

Schweers RL, Zhang J, Randall MS, Loyd MR, Li W, Dorsey FC, Kundu M, Opferman JT, Cleveland JL, Miller JL and Ney PA (2007) NIX is required for programmed mitochondrial clearance during reticulocyte maturation. Proc Natl Acad Sci U S A 104:19500–19505. [PubMed: 18048346]

Seth D, Duly A, Kuo PC, McCaughan GW and Haber PS (2014) Osteopontin is an important mediator of alcoholic liver disease via hepatic stellate cell activation. World J Gastroenterol 20:13088–13104. [PubMed: 25278703]

Seth D, Gorrell MD, Cordoba S, McCaughan GW and Haber PS (2006) Intrahepatic gene expression in human alcoholic hepatitis. J Hepatol 45:306–320. [PubMed: 16797773]

Sheldon KL, Maldonado EN, Lemasters JJ, Rostovtseva TK and Bezrukov SM (2011) Phosphorylation of voltage-dependent anion channel by serine/threonine kinases governs its interaction with tubulin. PLoS One 6:e25539. [PubMed: 22022409]

Shepard BD and Tuma PL (2009) Alcohol-induced protein hyperacetylation: mechanisms and consequences. World J Gastroenterol 15:1219–1230. [PubMed: 19291822]

Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, Chen S, Ramanujan VK, Wolf AJ, Vergnes L, Ojcius DM, Rentsendorj A, Vargas M, Guerrero C, Wang Y, Fitzgerald KA, Underhill DM, Town T and Arditi M (2012) Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. Immunity 36:401–414. [PubMed: 22342844]

Shimamoto A, Liu JY and Fujimiya T (2010) SIAM-Like phenomenon caused by low doses of alcohol. Alcohol Clin Exp Res 34 Suppl 1:S2–S6. [PubMed: 19382898]

Shoshan-Barmatz V and Gincel D (2003) The voltage-dependent anion channel: characterization, modulation, and role in mitochondrial function in cell life and death. Cell Biochem Biophys 39:279–292. [PubMed: 14716081]

Shoshan-Barmatz V and Golan M (2012) Mitochondrial VDAC1: function in cell life and death and a target for cancer therapy. Curr Med Chem 19:714–735. [PubMed: 22204343]

Sies H, Haussinger D and Grosskopf M (1974) Mitochondrial nicotinamide nucleotide systems: ammonium chloride responses and associated metabolic transitions in hemoglobin-free perfused rat liver. Hoppe Seylers Z Physiol Chem 355:305–320. [PubMed: 4154891]

Song Z, Zhou Z, Deaciuc I, Chen T and McClain CJ (2008) Inhibition of adiponectin production by homocysteine: a potential mechanism for alcoholic liver disease. Hepatology 47:867–879. [PubMed: 18167065]

Soubannier V, McLelland GL, Zunino R, Braschi E, Rippstein P, Fon EA and McBride HM (2012) A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. Curr Biol 22:135–141. [PubMed: 22226745]

Starkov AA and Fiskum G (2003) Regulation of brain mitochondrial H_2O_2 production by membrane potential and NAD(P)H redox state. J Neurochem 86:1101–1107. [PubMed: 12911618]

Stiburkova B, Pavlikova M, Sokolova J and Kozich V (2014) Metabolic syndrome, alcohol consumption and genetic factors are associated with serum uric acid concentration. PLoS One 9:e97646. [PubMed: 24827988]

Sun Y, Vashisht AA, Tchieu J, Wohlschlegel JA and Dreier L (2012) Voltage-dependent anion channels (VDACs) recruit Parkin to defective mitochondria to promote mitochondrial autophagy. J Biol Chem 287:40652–40660. [PubMed: 23060438]

Suter PM, Jequier E and Schutz Y (1994) Effect of ethanol on energy expenditure. Am J Physiol 266:R1204–R1212. [PubMed: 8184963]

Szabo G and Petrasek J (2017) Gut-liver axis and sterile signals in the development of alcoholic liver disease. Alcohol Alcohol. 52:414–424. [PubMed: 28482064]

Tan W and Colombini M (2007) VDAC closure increases calcium ion flux. Biochim Biophys Acta 1768:2510–2515. [PubMed: 17617374]

Thomes PG, Trambly CS, Fox HS, Tuma DJ and Donohue TM, Jr. (2015) Acute and Chronic Ethanol Administration Differentially Modulate Hepatic Autophagy and Transcription Factor EB. Alcohol Clin Exp Res 39:2354–2363. [PubMed: 26556759]

Thurman RG, Bradford BU, Iimuro Y, Knecht KT, Arteel GE, Yin M, Connor HD, Wall C, Raleigh JA, Frankenberg Mv, Adachi Y, Forman DT, Brenner D, Kadiiska M and Mason RP (1998) The role of gut-derived bacterial toxins and free radicals in alcohol- induced liver injury. J Gastroenterol Hepatol 13 Suppl:S39–50:S39–50.

Thurman RG, Cheren I, Forman D, Ewing JA and Glassman E (1989) Swift increase in alcohol metabolism in humans. Alcohol Clin Exp Res 13:572–576. [PubMed: 2679213]

Thurman RG, Gao W, Connor HD, Adachi Y, Stachlewitz RF, Zhong Z, Knecht KT, Bradford BU, Currin RT, Mason RP and Lemasters JJ (1995) Role of Kupffer cells in liver transplantation and alcoholic liver injury: 1994 update, in *Cells of the Hepatic Sinusoid, Volume* 5 (Wisse E, Knook DL and Wake K eds) pp 219–227, The Kupffer Cell Foundation, Leiden, The Netherlands.

Thurman RG, Paschal DL, Abu-Murad C, Pekkanen L, Bradford BU, Bullock KA and Glassman EB (1982) Swift increase in alcohol metabolism (SIAM) in the mouse: Comparison of the effect of short-term ethanol treatment on ethanol elimination in four inbred strains. J Pharmacol Exp Therapeut 223:45–52.

Thurman RG and Scholz R (1977) Interaction of glycolysis and respiration in perfused rat liver: Changes in oxygen uptake following the addition of ethanol. Eur J Biochem 75:13–21. [PubMed: 862614]

Tilg H, Moschen AR and Szabo G (2016) Interleukin-1 and inflammasomes in ALD/AAH and NAFLD/NASH. Hepatology 64:955–965. [PubMed: 26773297]

Tsukamoto H, French SW, Benson N, Delgado G, Rao GA, Larkin EC and Largman C (1985) Severe and progressive steatosis and focal necrosis in rat liver induced by continuous intragastric infusion of ethanol and low fat diet. Hepatology 5:224–232. [PubMed: 3979954]

Tsukamoto H, Machida K, Dynnyk A and Mkrtchyan H (2009) “Second hit” models of alcoholic liver disease. Semin Liver Dis 29:178–187. [PubMed: 19387917]

Tuma DJ, Smith SL and Sorrell MF (1991) Acetaldehyde and microtubules. Ann N Y Acad Sci 625:786–792. [PubMed: 2058934]

Vander Heiden MG, Chandel NS, Li XX, Schumacker PT, Colombini M and Thompson CB (2000) Outer mitochondrial membrane permeability can regulate coupled respiration and cell survival. Proc Natl Acad Sci U S A 97:4666–4671. [PubMed: 10781072]

Videla L, Bernstein J and Israel Y (1973) Metabolic alteration produced in the liver by chronic alcohol administration. Increased oxidative capacity. Biochem J 134:507–514. [PubMed: 16742811]

Videla L and Israel Y (1970) Factors that modify the metabolism of ethanol in rat liver and adaptive changes produced by its chronic administration. Biochem J 118:275–281. [PubMed: 5484675]

Wang S, Pacher P, De Lisle RC, Huang H and Ding WX (2016a) A Mechanistic Review of Cell Death in Alcohol-Induced Liver Injury. Alcohol Clin Exp Res 40:1215–1223. [PubMed: 27130888]

Wang Y, Tong J, Chang B, Wang B, Zhang D and Wang B (2014) Effects of alcohol on intestinal epithelial barrier permeability and expression of tight junction-associated proteins. Mol Med Rep 9:2352–2356. [PubMed: 24718485]

Wang ZG, Dou XB, Zhou ZX and Song ZY (2016b) Adipose tissue-liver axis in alcoholic liver disease. World J Gastrointest Pathophysiol 7:17–26. [PubMed: 26909225]

Watanabe A, Sohail MA, Gomes DA, Hashmi A, Nagata J, Sutterwala FS, Mahmood S, Jhandier MN, Shi Y, Flavell RA and Mehal WZ (2009) Inflammasome-mediated regulation of hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 296:G1248–G1257. [PubMed: 19359429]

Wen Y, Jeong S, Xia Q and Kong X (2016) Role of Osteopontin in Liver Diseases. Int J Biol Sci 12:1121–1128. [PubMed: 27570486]

Wendell GD and Thurman RG (1979) Effect of ethanol concentration on rates of ethanol elimination in normal and alcohol-treated rats *in vivo*. Biochem Pharmacol 28:273–279. [PubMed: 426842]

West AP, Shadel GS and Ghosh S (2011) Mitochondria in innate immune responses. Nat Rev Immunol 11:389–402. [PubMed: 21597473]

Wheeler MD, Kono H, Yin M, Nakagami M, Uesugi T, Arteel GE, Gabele E, Rusyn I, Yamashina S, Froh M, Adachi Y, Iimuro Y, Bradford BU, Smutney OM, Connor HD, Mason RP, Goyert SM, Peters JM, Gonzalez FJ, Samulski RJ and Thurman RG (2001a) The role of Kupffer cell oxidant production in early ethanol-induced liver disease. Free Radic Biol Med 31:1544–1549. [PubMed: 11744328]

Wheeler MD, Kono H, Yin M, Rusyn I, Froh M, Connor HD, Mason RP, Samulski RJ and Thurman RG (2001b) Delivery of Cu/Zn-superoxide dismutase gene with adenovirus reduces early alcohol-induced liver injury in rats. Gastroenterology 120:1241–1250. [PubMed: 11266387]

Williams JA, Manley S and Ding WX (2014) New advances in molecular mechanisms and emerging therapeutic targets in alcoholic liver diseases. World J Gastroenterol 20:12908–12933. [PubMed: 25278688]

Williams JA, Ni HM, Ding Y and Ding WX (2015) Parkin regulates mitophagy and mitochondrial function to protect against alcohol-induced liver injury and steatosis in mice. Am J Physiol Gastrointest Liver Physiol 309:G324–G340. [PubMed: 26159696]

Wong YC and Holzbaur EL (2014) Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. Proc Natl Acad Sci U S A 111:E4439–E4448. [PubMed: 25294927]

Wong YC and Holzbaur EL (2015) Temporal dynamics of PARK2/parkin and OPTN/optineurin recruitment during the mitophagy of damaged mitochondria. Autophagy 11:422–424. [PubMed: 25801386]

Wood SL, Emmison N, Borthwick AC and Yeaman SJ (1993) The protein phosphatases responsible for dephosphorylation of hormone-sensitive lipase in isolated rat adipocytes. Biochem J 295 **(** Pt 2**)**: 531–535. [PubMed: 8240253] **()**

Wree A, Eguchi A, McGeough MD, Pena CA, Johnson CD, Canbay A, Hoffman HM and Feldstein AE (2014) NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice. Hepatology 59:898–910. [PubMed: 23813842]

Xu MJ, Zhou Z, Parker R and Gao B (2017) Targeting inflammation for the treatment of alcoholic liver disease. Pharmacol Ther 180:77–89. [PubMed: 28642119]

Yan AW, Fouts DE, Brandl J, Starkel P, Torralba M, Schott E, Tsukamoto H, Nelson KE, Brenner DA and Schnabl B (2011) Enteric dysbiosis associated with a mouse model of alcoholic liver disease. Hepatology 53:96–105. [PubMed: 21254165]

Yin H, Hu M, Zhang R, Shen Z, Flatow L and You M (2012) MicroRNA-217 promotes ethanol-induced fat accumulation in hepatocytes by down-regulating SIRT1. J Biol Chem 287:9817–9826. [PubMed: 22308024]

Yin M, Gabele E, Wheeler MD, Connor HD, Bradford BU, Dikalova A, Rusyn I, Mason RP and Thruman RG (2001) Alcohol-induced free radicals in mice: direct toxicants or signalling molecules? Hepatology 34:935–942. [PubMed: 11679964]

Young TA, Cunningham CC and Bailey SM (2002) Reactive oxygen species production by the mitochondrial respiratory chain in isolated rat hepatocytes and liver mitochondria: studies using myxothiazol. Arch Biochem Biophys 405:65–72. [PubMed: 12176058]

Yu HB and Finlay BB (2008) The caspase-1 inflammasome: a pilot of innate immune responses. Cell Host Microbe 4:198–208. [PubMed: 18779046]

Yue J and Mulder KM (2001) Transforming growth factor-beta signal transduction in epithelial cells. Pharmacol Ther 91:1–34. [PubMed: 11707292]

Yuki T, Bradford BU and Thurman RG (1980) Role of hormones in the mechanism of the swift increase in alcohol metabolism in the rat. Pharmacol Biochem Behav 13:67–71. [PubMed: 6113604]

Yuki T and Thurman RG (1980a) Metabolism of the swift increase in alcohol metabolism (SIAM) in the rat, in *Alcohol and Aldehyde Metabolizing Systems* (Thurman RG ed) pp 697–704, Plenum Press, New York.

Yuki T and Thurman RG (1980b) The swift increase in alcohol metabolism: Time course for the increase in hepatic oxygen uptake and the involvement of glycolysis. Biochem J 186:119–126. [PubMed: 6989357]

Zhang J, Culp ML, Craver JG and Darley-Usmar V (2018) Mitochondrial function and autophagy: integrating proteotoxic, redox, and metabolic stress in Parkinson's disease. J Neurochem 144:691–709. [PubMed: 29341130]

Zhang J, Wang Q, Xu C, Lu Y, Hu H, Qin B, Wang Y, He D, Li C, Yu X, Wang S and Liu J (2017) MitoTEMPO Prevents Oxalate Induced Injury in NRK-52E Cells via Inhibiting Mitochondrial Dysfunction and Modulating Oxidative Stress. *Oxid Med Cell Longev* 2017:7528090. doi: 10.1155/2017/7528090.

Zhang Q, Itagaki K and Hauser CJ (2010a) Mitochondrial DNA is released by shock and activates neutrophils via p38 map kinase. Shock 34:55–59. [PubMed: 19997055]

Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K and Hauser CJ (2010b) Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 464:104–107. [PubMed: 20203610]

Zhong W, Zhao Y, Tang Y, Wei X, Shi X, Sun W, Sun X, Yin X, Sun X, Kim S, McClain CJ, Zhang X and Zhou Z (2012) Chronic alcohol exposure stimulates adipose tissue lipolysis in mice: role of reverse triglyceride transport in the pathogenesis of alcoholic steatosis. Am J Pathol 180:998–1007. [PubMed: 22234172]

Zhong Z, Ramshesh VK, Rehman H, Liu Q, Theruvath TP, Krishnasamy Y and Lemasters JJ (2014) Acute ethanol causes hepatic mitochondrial depolarization in mice: role of ethanol metabolism. PLoS One 9:e91308. [PubMed: 24618581]

Zhong Z, Rehman H, Liu Q, Krishinasamy Y, Ramshesh VK, Mittler CS and Lemasters JJ. (2016) A unifying hypothesis linking ethanol-induced mitochondrial depolarization to mitophagy and the proinflammatory and early profibrotic events in alcoholic liver disease Program and Abstracts of ISBRA/ESBRA Congress on Alcohol and Alcoholism, Berlin, Germany http://isbra-esbra-2016.org/essential_grid/a-unifying-hypothesis-linking-ethanol-induced-mitochondrial-depolarization-to-mitophagy-and-the-proinflammatory-and-early-profibrotic-events-in-alcoholic-liver-disease/.

Zhou R, Yazdi AS, Menu P and Tschopp J (2011) A role for mitochondria in NLRP3 inflammasome activation. Nature 469:221–225 [PubMed: 21124315]

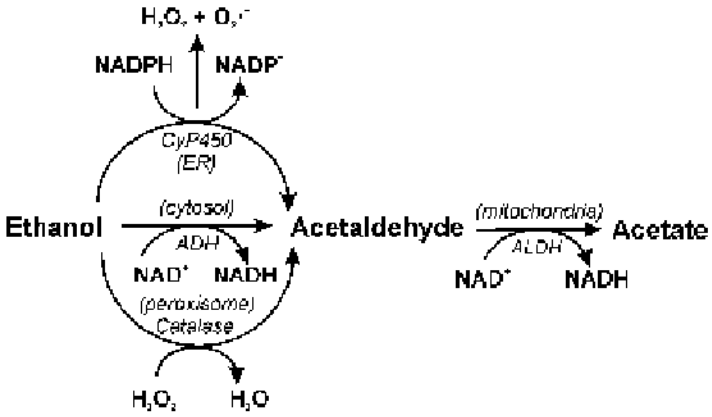

Fig. 1. Alcohol metabolism.
See text for details.

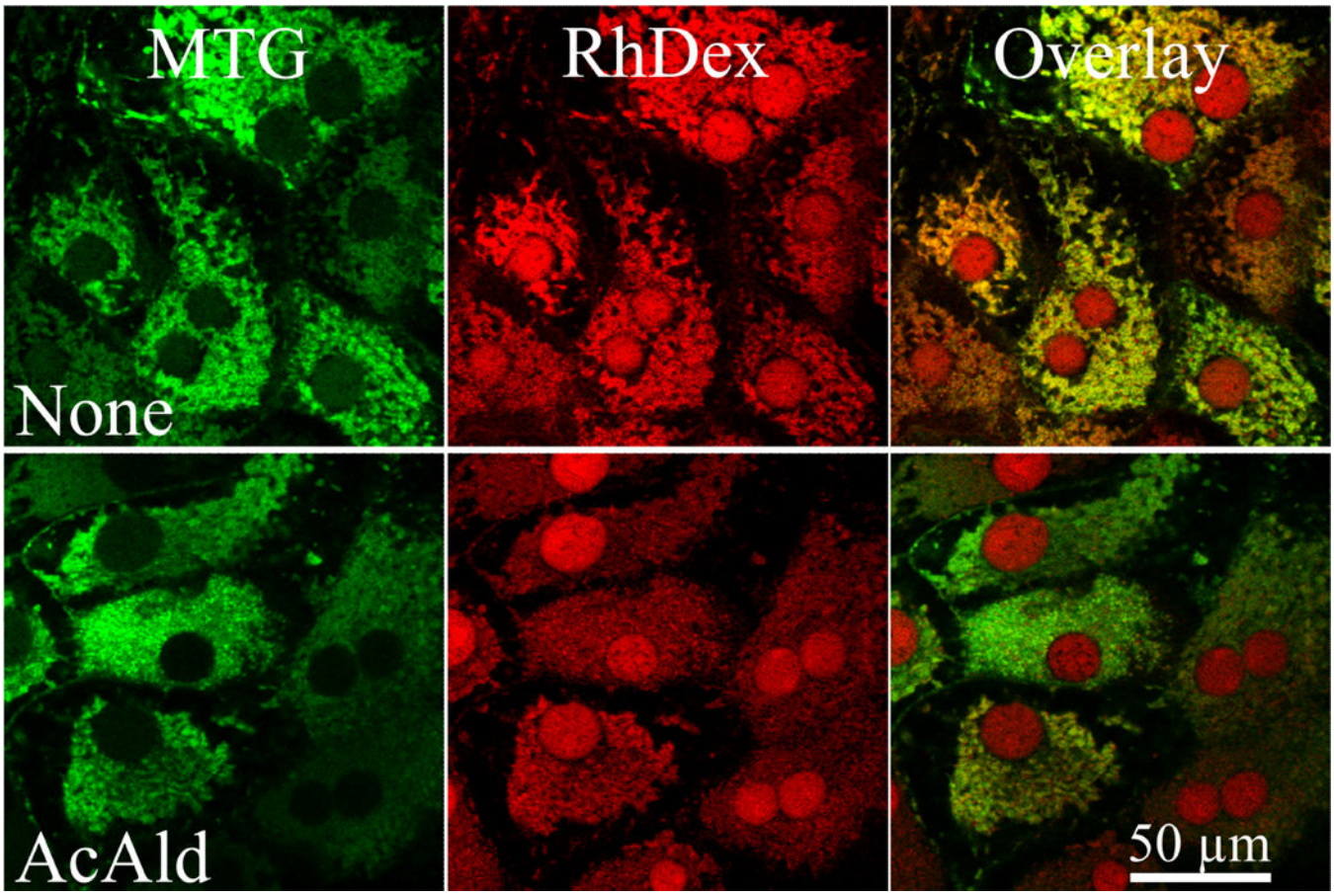

Fig. 2. Acetaldehyde suppression of rhodamine dextran entry into the mitochondrial intermembrane space of permeabilized rat hepatocytes.

Digitonin-permeabilized rat hepatocytes were briefly incubated with 3-kDa rhodamine dextran (RhDex) to allow the red-fluorescing marker to enter the mitochondrial intermembrane space through VDAC. The VDAC inhibitor, 4, 4-diisothiocyanatostilbene-2,2-disulfonic acid (DIDS), was added afterwards to “lock in” RhDex after subsequent RhDex washout. Green-fluorescing MitoTracker Green (MTG) marked the mitochondrial matrix. In comparison to untreated cells, pretreatment with AcAld (500 μM) decreased RhDex uptake. Nuclei are unspecifically stained by RhDex. Adapted from (Holmuhamedov, et al., 2012).

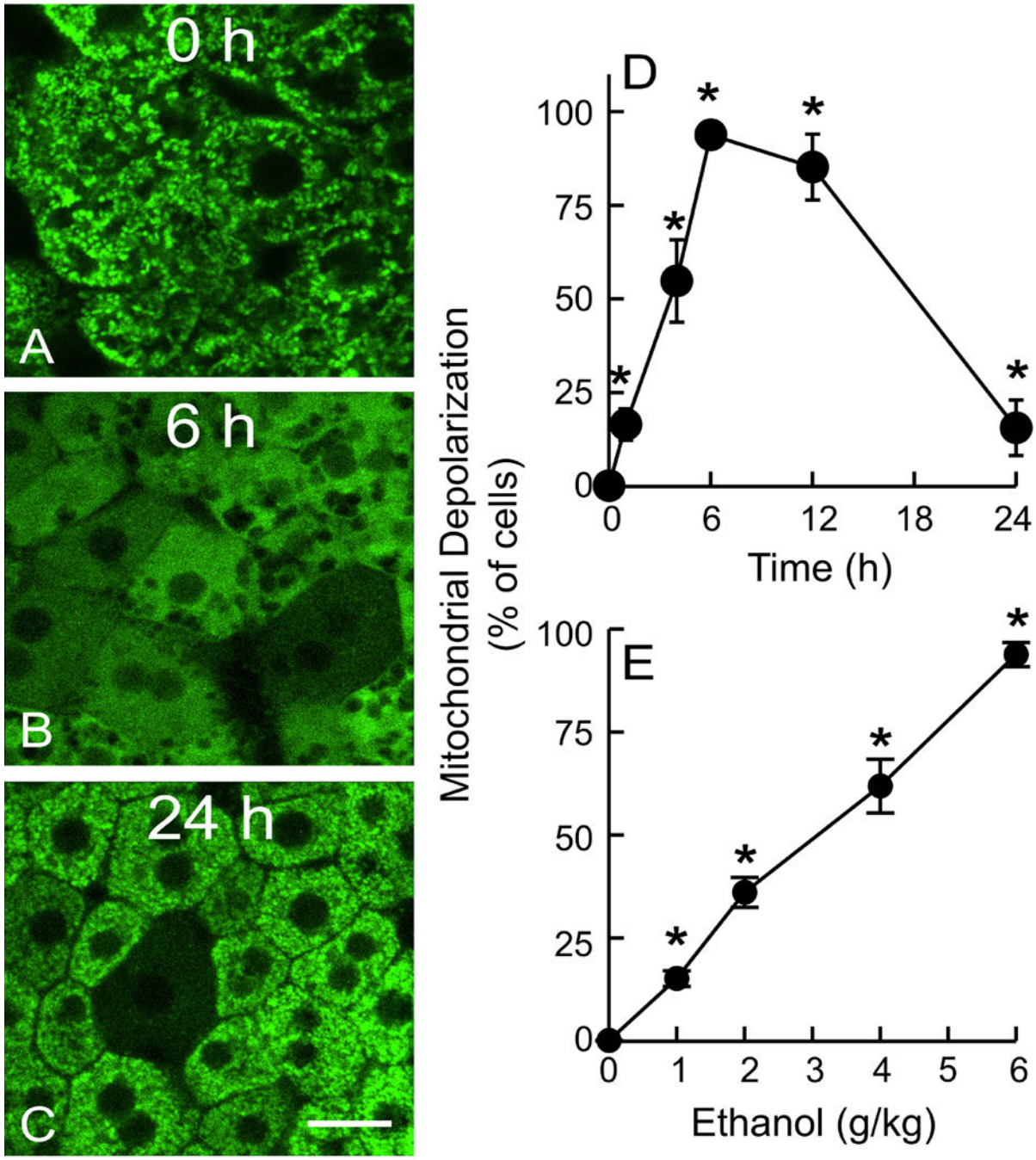

Fig. 3. Acute ethanol causes reversible mitochondrial depolarization in vivo in a time- and dose-dependent manner.

Mice were gavaged with one dose of ethanol (0–6 g/kg), and mitochondrial polarization was detected by intravital multiphoton microscopy of green-fluorescing rhodamine 123 at 0 to 24 h after treatment. Representative images after treatment with 6 g/kg ethanol are shown in **A**–**C**. Bar is 10 μm. **D** and **E** show, respectively, the time course of mtDepo after 6 g/kg ethanol and the dose-dependency of mtDepo after 6 h. Values are means ± SEM (n = 4–5 per group). *, $p<0.05$ vs no ethanol. Adapted from (Zhong, et al., 2014).

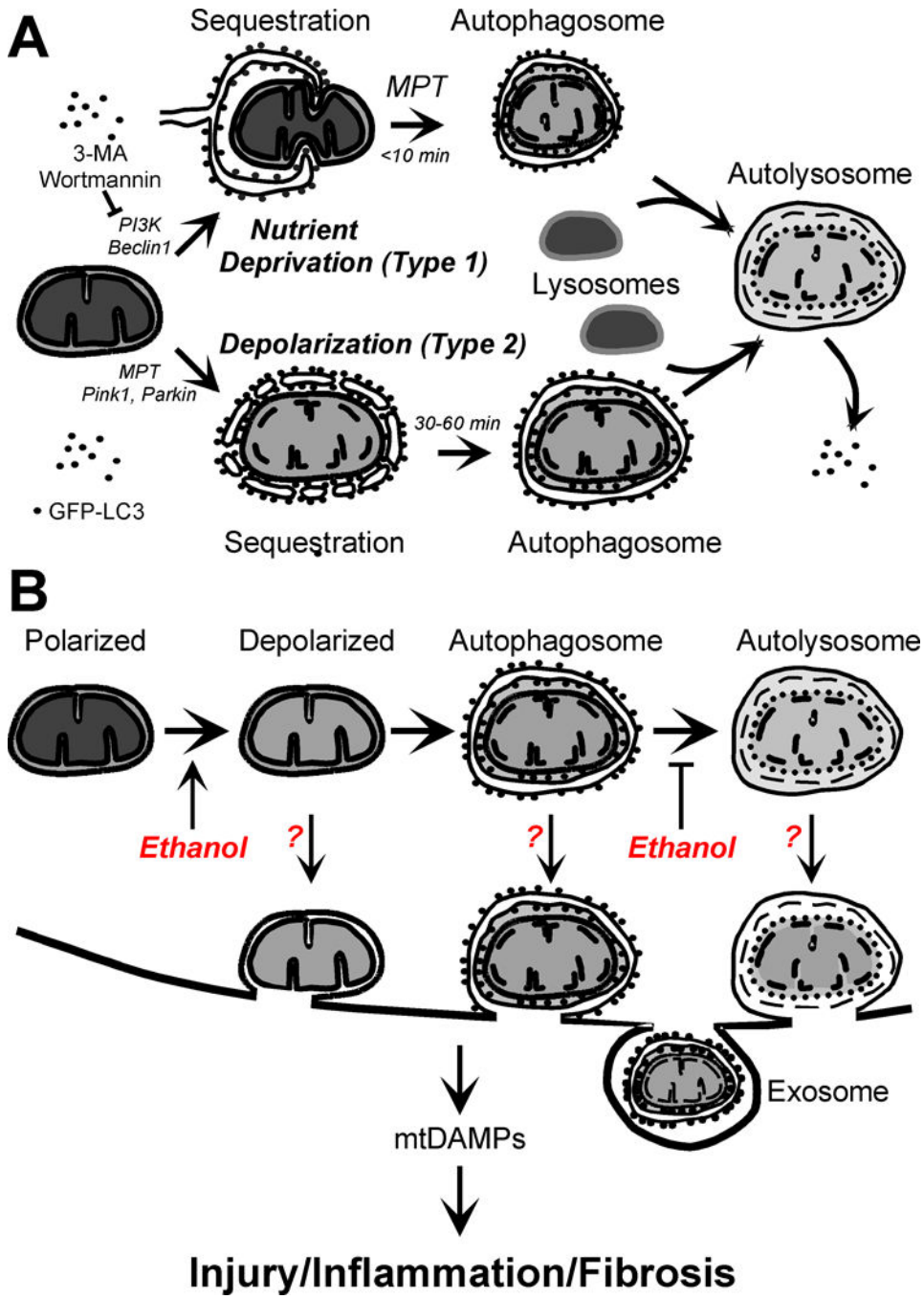

Fig. 4. Mitophagy types and the progression of ethanol-induced mitochondrial depolarization to mitophagy to release of mitochondrial damage-associated molecular pattern molecules in the pathogenesis of alcoholic liver disease.

A: In Type 1 mitophagy, PI3K, beclin-1 and other proteins initiate formation of cup-shaped LC3-containing sequestration membranes (phagophores) that fuse around individual mitochondria to form mitophagosomes, often with coordinated mitochondrial fission. PI3K inhibitors like 3-methyladenine (3-MA) and wortmannin block Type 1 mitophagy. In Type 1 mitophagy, mtDepo does not occur until after sequestration and acidification of mitophagosome outer compartments (space between the inner and outer autophagosomal

membranes). Subsequently mitophagosomes fuse with lysosomes to form autolysosomes in which mitochondrial digestion is complete within about 15 min. Type 1 mitophagy is typical of nutrient deprivation and removal of unneeded mitochondria. In Type 2 mitophagy, mtDepo initiates autophagic sequestration through association of Pink1 and Parkin and subsequent ubiquitination of mitochondrial proteins to recruit autophagy receptor proteins like p62/SQSTM-1. LC3-containing membrane vesicles then associate with the depolarized mitochondria and fuse to form autophagosomes. By contrast to Type 1 mitophagy, PI3K inhibitors do not block Type 2 mitophagy, and cup-shaped phagophores and mitochondrial fission are absent. **B:** We propose the hypothesis that ethanol-induced mtDepo stimulates Type 2 mitophagy. After high dose binge drinking, extensive mtDepo likely overwhelms cellular capacity to form mitophagosomes, whereas after chronic ethanol, capability for subsequent autophagic processing (delivery of mitophagosomes to lysosomes) becomes compromised. Such dysregulation of autophagy leads to extracellular release of damaged mitochondria, mitophagosomes and/or autolysosomes by fusion with the plasma membrane or exosome formation. mtDAMPs released in this way then promote liver injury, inflammation and fibrosis. mtDAMPs are also likely released internally to activate intracellular inflammasomes (not illustrated).

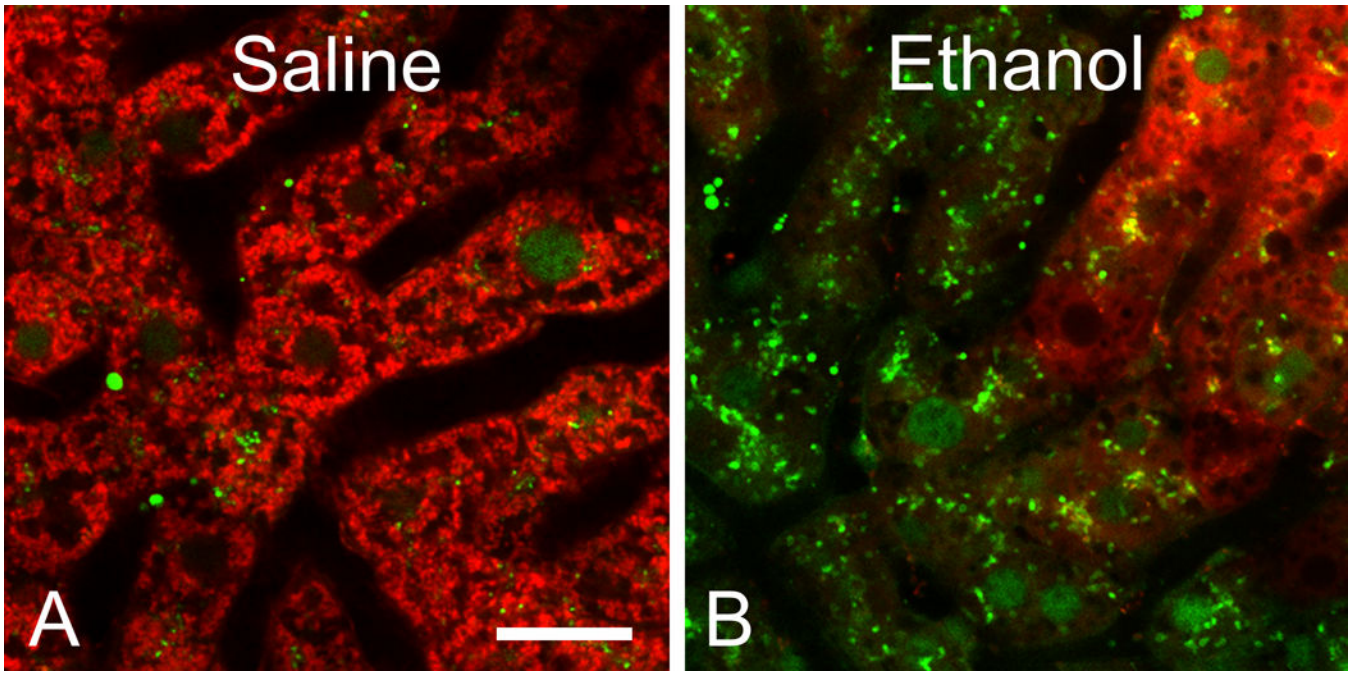

Fig. 5. Mitophagy is associated with mitochondrial depolarization after acute ethanol treatment.
Male LC3-GFP transgenic mice were gavaged with ethanol (4 g/kg) or saline, and intravital multiphoton microscopy was performed after 4 h of red-fluorescing TMRM, which accumulates into polarized mitochondria, and green-fluorescing LC3-GFP, a marker of forming and newly formed autophagosomes. After ethanol, note an increase of green LC3-GFP puncta in hepatocytes with mtDepo. Bar is 10 μm (Zhong, et al., 2016).

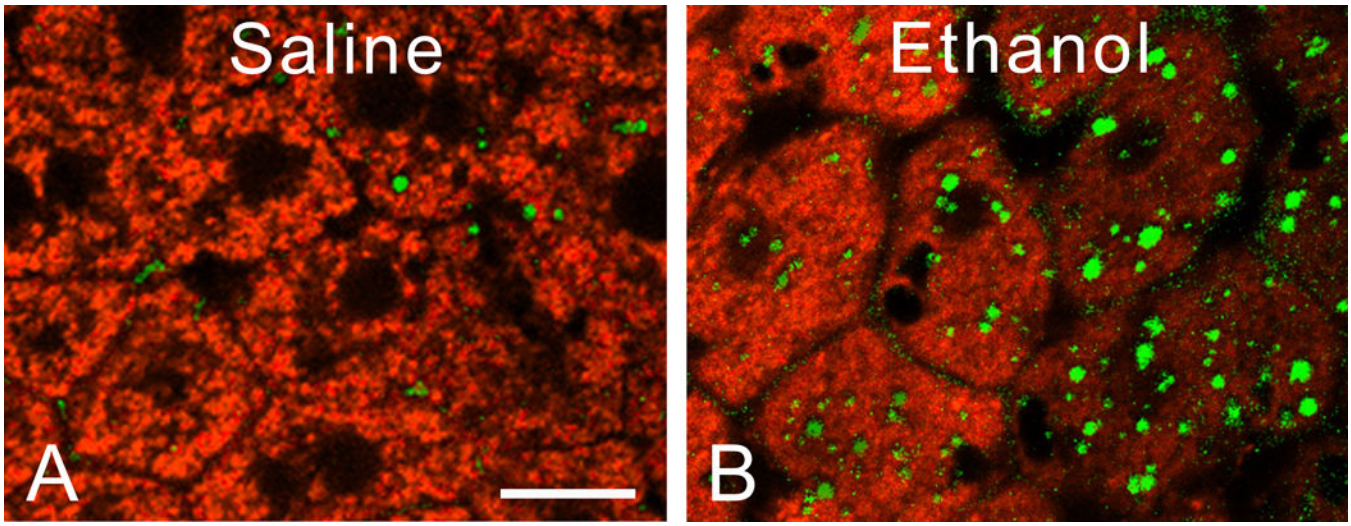

Fig. 6. Ethanol causes steatosis in hepatocytes with depolarized mitochondria.

Mice were gavaged with saline or ethanol (6 g/kg), and intravital microscopy of TMRM (red) and the lipid droplet-labeling fluorophore, BODIPY 493/503 (green), was performed after 2 h. Note presence of lipid droplets predominantly in hepatocytes with mtDepo. Bar is 10 μm. Adapted from (Zhong, et al., 2014).

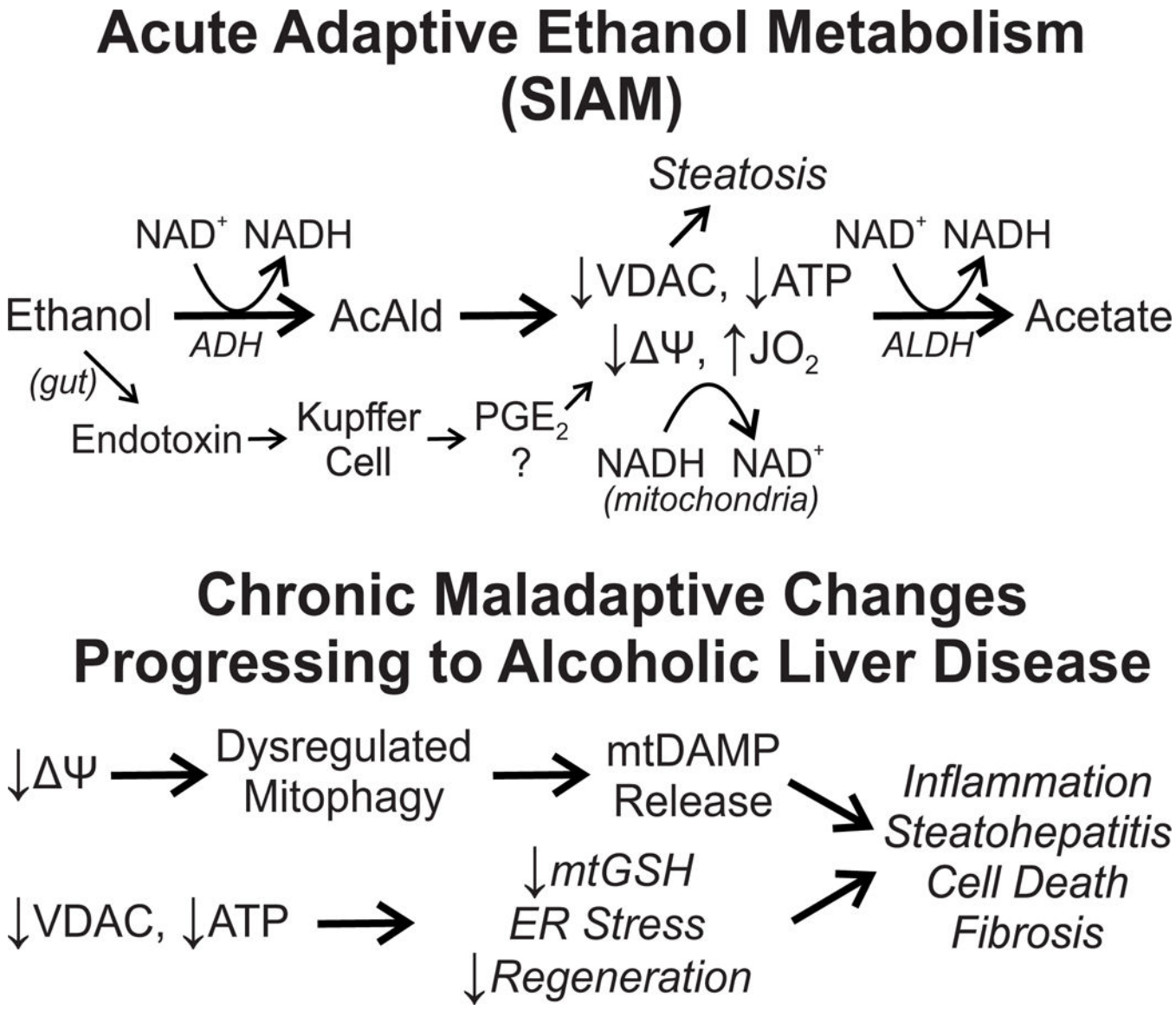

Fig. 7. Working hypothesis for how ethanol metabolism-associated mitochondrial alterations contribute to development of alcoholic liver disease

After ethanol consumption, a respiratory burst ($\uparrow J_{02}$) develops due to mitochondrial uncoupling and mtDepo ($\downarrow\Delta\Psi$) in the liver, and VDAC closure occurs. AcAld and gut-derived endotoxin acting through Kupffer cells and possibly PGE_2 promote mtDepo, whereas VDAC closure is promoted by AcAld. VDAC blocks uptake of fatty acyl-CoA for β-oxidation, which leads to acute steatosis. These adaptive mitochondrial alterations promote more rapid and selective oxidation of toxic AcAld by inhibiting oxidation of competing respiratory substrates and by more rapidly regenerating NAD^+ needed for ethanol oxidation to acetate. Depolarization in turn activates mitophagy. Although adaptive alcohol metabolism detoxifies ethanol more rapidly, the same metabolism occurring chronically becomes a tipping point from adaptation to maladaptation, leading to the pathogenic consequences of ALD. With chronic ethanol, excessive mitophagic burden leads to dysfunctional autophagic processing and release of mtDAMPs intracellularly and

extracellularly. VDAC closure also blocks mitochondrial glutathione uptake, leading to oxidative and ER stress. Decreased ATP also promotes ER stress and possibly impaired hepatic regeneration. Together these maladapative changes culminate inflammation, steatohepatitis, cell death and fibrosis.