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Lipid peroxidation derived reactive aldehydes in alcoholic liver disease

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Abstract

Lipid peroxidation is a known consequence of oxidative stress and is thought to play a key role in numerous disease pathologies, including alcoholic liver disease (ALD). The over-accumulation of lipid peroxidation products during chronic alcohol consumption results in pathogenic lesions on protein, DNA, and lipids throughout the cell. Molecular adducts due to secondary end products of lipid peroxidation impact a host of biochemical processes, including inflammation, antioxidant defense, and metabolism. The aggregate burden of lipid peroxidation which occurs due to chronic alcohol metabolism, including downstream signaling events, contributes to the development and progression of ALD. In this current opinion we highlight recent studies and approaches relating cellular mechanisms of lipid peroxidation to the pathogenesis of alcoholic liver disease.

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Keywords

Alcoholic liver disease, Lipid peroxidation, Oxidative stress.

Abbreviations

ALD, alcoholic liver disease; CYP2E1, cytochrome P450 2E1; ADH, alcohol dehydrogenase; ALDH2, aldehyde dehydrogenase 2; NAD^+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; ROS, reactive oxygen species; LC-MS/MS, liquid chromatography tandem mass spectrometry.

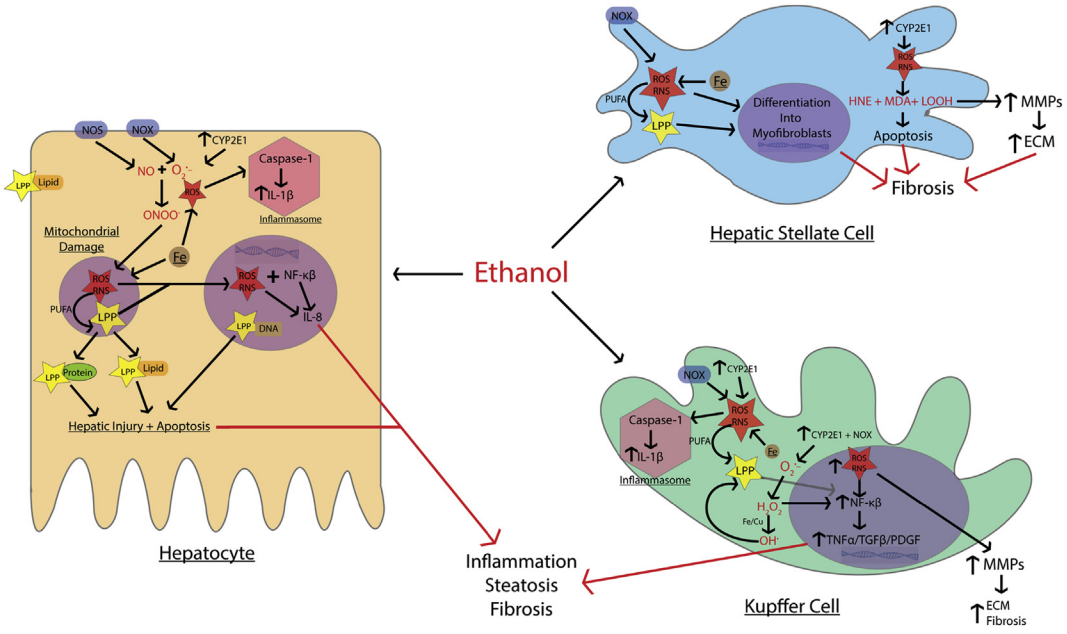
1. Introduction: pathogenesis of alcoholic liver disease

Alcohol abuse is the third most common preventable cause of death in the United States and is a significant source of morbidity and mortality worldwide, accounting for approximately six percent of all global deaths [1,2]. Alcoholic liver disease (ALD) is a complex disease arising from chronic alcohol consumption, which develops through a number of progressive stages [3]. The initial stage is alcoholic fatty liver (hepatic steatosis), which develops in the vast majority of chronic drinkers due to augmented metabolism, synthesis, transportation, and deposition of fatty acids and triglycerides [4]. The next pathological stage primarily includes alcoholic steatohepatitis (hepatic inflammation and injury), with a subset of patients developing a more severe form of liver damage termed alcoholic hepatitis, with very poor prognosis [5,6]. Patients demonstrating chronic liver injury may further progress to hepatic fibrosis and cirrhosis, developing end-stage liver disease, and can ultimately lead to the development of hepatocellular carcinoma [6] (see Fig. 1).

The hepatotoxicity of alcohol is largely due to its oxidative metabolism which produces a host of toxic intermediates and by-products [3]. When alcohol enters the liver, it is oxidized to acetaldehyde via hepatic alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1) [7]. The second, and rate-limiting step, is the oxidation of acetaldehyde to acetate via the aldehyde dehydrogenase family of enzymes (ALDH). During periods of excessive alcohol consumption, ALDH detoxification capacity becomes saturated, resulting in the induction of secondary metabolizing pathways, namely CYP2E1. The induction of CYP2E1 leads to the increased generation and accumulation of superoxide and hydrogen peroxide, which can lead to persistent damage of DNA, protein, and lipids [8,9]. In addition to the generation of radical and non-radical species, many other etiologies are implicated in ALD, including acetaldehyde toxicity [8,10].

Over the past few decades researchers have elucidated numerous mechanisms contributing to the development of ALD, including oxidative stress [6,11,12]. Oxidative stress has recently been redefined as an imbalance between oxidants and antioxidants in favor of the former, leading to a disruption of redox signaling and control and/or molecular damage [9,13,14]. The increased generation of radical and non-radical species is known to

Figure 1



Illustrative diagram showing the involvement of hepatocytes, hepatic stellate cells, and Kupffer cells in ROS/RNS and LPP mediated pathophysiology of alcoholic liver disease. In hepatocytes, ethanol ingestion results in the activation of nitric oxide synthase (NOS) and NAD(P)H oxidase (NOX), which produce nitric oxide (NO) and the superoxide radical ($O_2^{\bullet-}$) respectively. NO and $O_2^{\bullet-}$ react to form peroxynitrite ($ONOO^-$), which causes mitochondrial damage through further production of reactive oxygen species (ROS) and lipid peroxidation products (LPP), which can form adducts with proteins, lipids, and DNA. Adduct formation then contributes to hepatic injury characteristic of ALD (inflammation, steatosis, and fibrosis). Ethanol mediated activation of NOX can also occur in hepatic stellate cells and Kupffer cells, which results in ROS/RNS production and the generation of LPP, leading to the differentiation of hepatic stellate cells into myofibroblasts and initiating inflammation through NF-κβ (nuclear factor kappa-light-chain-enhancer of activated B cells) activation in Kupffer cells. Lipid peroxidation products, like 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA), as well as lipid hydroperoxides (LOOH) can form adducts within hepatic stellate cells and Kupffer cells. Cytochrome P450 2E1 (CYP2E1) induction by alcohol results in $O_2^{\bullet-}$ production, and potentially hydroxyl radical ($OH^{\bullet}$) production from the reaction of hydrogen peroxide (H_2O_2) and iron (Fe), in all three cell types. ROS and RNS in hepatocytes and Kupffer cells stimulate interleukin-1 beta (IL-1β) production in the inflammasome, leading to inflammation and cell death. In Kupffer cells and hepatic stellate cells, ROS and RNS production results in activation of matrix metalloproteinases, increased deposition of extracellular matrix (ECM) proteins, and resultant fibrosis.

contribute to enhanced lipid peroxidation (LPO), which includes the overproduction of reactive aldehydes such as 4-hydroxynonenal (4-HNE), malondialdehyde (MDA), and acrolein. The accumulation of these reactive aldehydes leads to depletion of cellular antioxidant defenses and covalent adduction of DNA, lipids, and proteins [8,15]. Here, we focus on the generation and consequences of alcohol-induced reactive species and consequent lipid peroxidation, focusing on the disruption of redox signaling and control through proteomic and metabolic signaling, thus disrupting normal hepatic function and contributing to the pathogenesis of ALD.

2. Redox imbalance and lipid peroxidation

The recently revised definition of oxidative stress accounts for the downstream consequences of radical and non-radical species, including a disruption in thiol/disulfide systems such as thioredoxin-1, cysteine/cystine (Cys/CySS), and glutathione (GSH/GSSG) [16–18]. Oxidative stress can impact separate and distinct redox pathways as opposed to the less nuanced global thiol/disulfide balance [13]. The term redox regulation may refer to two distinct functions. In the more traditional sense, it refers to the elimination or generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [19]. In a more nuanced context it also applies to the reversible oxidation of cysteine thiols and methionine thioethers of proteins to disulfides or sulfenic acid groups, which contribute to the regulation of biological function. Free radical initiation events leading to the generation of hydrogen peroxide and peroxynitrite from superoxide and nitric oxide pose a significant oxidative burden [20]. The accumulation of these reactive species and resulting generation of lipid peroxidation products disrupts critical cellular signaling and control pathways. Specifically, the thiol containing proteome is highly susceptible to modification and is a critical aspect of redox regulation [16]. Furthermore, these protein thiols are prone to two-electron oxidation by nonradical oxidants like hydrogen peroxide, disulfides, quinones, aldehydes, and lipid hydroperoxides [21].

The generation of excess radical and nonradical species facilitates hepatic injury through a variety of mechanisms in ALD, including mitochondrial damage, activating hepatic stellate cells resulting in transformation into myofibroblasts, activating periportal fibroblasts and fibrocytes, remodeling of the extracellular matrix (ECM), stimulating immune responses to ROS, and by exacerbating inflammation through activation of the nuclear factor NF-κB [8,22–36]. The major sources of normal physiological oxidants in the human body are the mitochondrial respiratory chain and the cytochrome P450 mixed-function oxidases. Redox imbalances during the initiation and progression of ALD arise due to many factors. The metabolism of alcohol by ALD and ALDH both result in the formation of NADH, which increases respiratory chain activity and superoxide radical production [22,37]. Chronic alcohol consumption results in oxidative damage to mitochondrial respiratory complexes, eventually resulting in further ROS production [38]. Alcohol has been shown to induce $p66^{SHC}$, a protein known to generate ROS through electron transfer from cytochrome *c*, causing mitochondrial damage in mice [39,40]. Inducible CYP2E1 not only reduces O_2 to the superoxide radical and hydrogen peroxide, but also produces the hydroxyl radical in the presence of iron catalysts, which are increased in response to alcohol consumption [41,42]. Alcohol has been shown to facilitate xanthine dehydrogenase conversion to xanthine oxidase, resulting in superoxide radical and hydrogen peroxide production [43]. NADPH oxidase enzymes (NOX) and inducible nitric oxide synthase (iNOS) are activated in hepatocytes in response to alcohol ingestion, resulting in superoxide and nitric oxide formation, the combination of which produces peroxynitrite, which is a powerful oxidant leading to mitochondrial damage in ALD [44–49]. Apart from hepatocytes, infiltrating monocytes and lymphocytes also contribute to oxidative stress. Activated Kupffer cells produce TNFα in response to free radical-activated NF-κB, contributing to injury [6,25–30,50–57]. Increased iron levels and Fenton chemistry also contribute to Kupffer cell activation [58,59]. Animal studies involving treatment with antioxidant compounds, depletion of endogenous antioxidants, and both overexpression and knockout of endogenous antioxidant genes (thioredoxin, SOD, catalase, glutathione peroxidase-1, metallothionein, sulfiredoxin, peroxiredoxin-1, and Nrf2) have provided evidence for the causal role of oxidative stress in ALD [60–72]. Additionally, the effect of alcohol on the antioxidant glutathione continues to be debated as a critical factor in redox imbalances [61,73–76]. Interestingly, the efficacy of antioxidant therapy in humans remains uncertain, which supports the idea that two-electron nonradical oxidants may present an equal if not more significant role than radical species in terms of redox imbalance [35,65–71,77–85].

Lipid Peroxidation (LPO) is initiated by the free radical or non-radical abstraction of hydrogen from polyunsaturated fatty acids (PUFAs), resulting in decreased membrane fluidity, increased membrane permeability, and loss of membrane-bound protein function [86,87]. The initiation step generates a carbon-centered lipid radical, which reacts with oxygen to form a lipid peroxyl radical. This lipid peroxyl radical abstracts another electron from a nearby lipid to generate a new lipid radical and lipid hydroperoxide, termed the propagation step. Importantly, this propagation step can result in hundreds of lipid peroxidation products (LPP) generated from a single initiation event [88]. The termination step of lipid peroxidation involves the self-quenching of the lipid peroxy radical or through antioxidants like vitamin E, vitamin C, SOD, catalase, and peroxidase. Hydroperoxide degradation occurs through two-electron reduction, carried out primarily by glutathione peroxidases and selenoprotein P, or through one-electron reduction, which leads to continued lipid peroxidation [86]. When a lipid peroxyl radical is not neutralized it can fragment and decompose to form secondary products, including Di-aldehydes (MDA), α,β-unsaturated aldehydes [acrolein, 4-hydroxynonenal (HNE), 4-hydroxyhexenal (HHE), 4-oxononenal (4-ONE)], and acetaldehyde. These reactive aldehydes, in particular, play a central role in signaling, autophagy, senescence, the cell cycle and proliferation, and apoptosis [89].

3. Lipid peroxidation and alcoholic liver disease

Recent studies using murine models of ALD indicate that when combined with a diet rich in polyunsaturated fatty acids (PUFAs), chronic alcohol consumption results in enhanced lipid peroxidation and significant liver injury [90–93]. Importantly, substitution of PUFAs with saturated fatty acids significantly reduced accumulation of LPP as well as ameliorated hepatic injury. It is thought that the increase in dietary polyunsaturated fat provides a pool of lipids that are readily peroxidizable, serving as a source for the production of reactive aldehydes. Numerous key studies demonstrate an enhanced production of reactive aldehydes in ALD. First, using immunohistochemistry (IHC), increased periportal post-translational modification by 4-HNE, MDA, 4-ONE and acrolein occurs following chronic alcohol consumption [94–98]. This is further supported by data demonstrating co-localization of 4-HNE with the lipid vesicle protein adipophilin (Perilipin 2, PLIN2) on the outer coat of lipid vesicles in a murine model of ALD [99]. Reactive aldehyde accumulation has been reported in cytosolic, microsomal, mitochondrial, and nuclear fractions following chronic alcohol consumption, impacting biochemical processes across multiple cellular compartments [97,98,100–103].

Lipid peroxidation occurs in patients with ALD, making a compelling argument for a causal role in ALD. Studies have shown increases in LPP and protein adducts in

blood, urine, and tissues of ALD patients, including 4-HNE, 8-OHdG, 8-isoprostane, and MDA [104–113]. Studies in both mouse and human liver tissue have also demonstrated antibody production against oxidized phospholipids, MDA, and 4-HNE, which lead to TNFα production and hepatic injury [114–119]. Alcohol-induced LPP adducts disrupt protein function, promote fibrosis, and induce humoral and cell-mediated immune responses [114–118,120–128]. Indeed, many of the same elevated LPP detected in humans have been identified in animal models of ALD, including MDA, 4-HNE, and F_2-isoprostanes [11,31]. Animal models have also demonstrated lipid peroxidation-derived 4-HNE stimulation of stellate cells resulting in collagen production [129]. Intragastric infusion models of ALD have demonstrated a correlation with increased LPP, LPP-protein adducts, and lipid radicals [61,68,79,130]. An increase in mitochondrial LPP-protein adducts in chronic alcohol models, specifically carbonylated proteins, parallels mitochondrial dysregulation resulting from chronic alcohol metabolism [73,94,119,131,132].

A central question concerning the appearance of aldehyde-modified proteins in the liver due to chronic alcohol consumption revolves around the association of these molecular lesions with development of histopathology or impairment of hepatocellular function. Recently, the contribution of reactive aldehydes to alcohol-induced injury has been explored using the 10-day chronic-plus-binge model of alcohol consumption, where supplementation with the acrolein scavenger hydralazine significantly decreased the accumulation of acrolein adducts and hepatic injury [133]. In addition, supplementation with the herb Danshen, resulted in an increase in PPAR activation and GSTA4 expression, resulting in the increased clearance of 4-HNE and reducing oxidative injury [134]. A recent report demonstrated that PPAR knockout increased 4-HNE accumulation when compared to alcohol-fed WT mice, providing evidence that both GSTA4 and β-oxidation are contributing factors in the mitigation of lipid peroxidation [94,119].

Significant progress has been made in the identification of proteins modified by LPP in ALD. Early proteomic approaches used to identify modified proteins in ALD used 2-dimensional electrophoresis followed by protein identification [135,136]. Interestingly, the majority of identified proteins were involved in either protein folding and oxidative stress responses. In a recent study using 4-HNE antibody conjugated co-immunoprecipitation, elevated mitochondrial adducts were identified in a 5-week murine model of ALD [97,103]. Advances in biotin hydrazide chemistry and in the sensitivity of mass spectrometry have permitted a more in-depth proteomic approach to identify less abundant proteins modified due to alcohol metabolism. To date, global proteomic approaches applied to various models of ALD have identified over 2000 proteins that undergo adduction in either murine models or in human hepatic tissue isolated from patients with end-stage ALD [94,97,113,137]. Additionally, recent reports have identified which amino acids are modified by reactive aldehydes on over 70 proteins [94,97,113,137]. Utilizing GSTA4-4 knockout mice it was determined that protein adduction by LPP was increased in mitochondrial fractions in pathways regulating oxidative stress, fatty acid metabolism, and amino acid metabolism, supporting the contribution of GSTA4-4 in protecting mitochondria from reactive aldehydes [119,137]. Another report demonstrated that lipid peroxidation derived protein adduct formation is increased in tissue obtained from end-stage alcoholics [113]. Not surprisingly, modification of proteins regulating oxidative stress, metabolic, and cytoskeletal processes was increased [119]. Protein-specific approaches have also been utilized in models of ALD to identify low abundant molecular targets including L-FABP, GRP78, PDI, PTEN, AMPK, Akt, and Sirtuin 3 in the liver after chronic alcohol treatment using co-immunoprecipitation techniques and antibodies directed against each protein [95,135,138–142]. In each report, protein adduction was identified to contribute to altered cellular signaling. A recent cutting-edge approach utilized an aldehyde reactive probe to enrich 4-HNE modified proteins for identification and label-free quantitation by LC-MS/MS [143]. Further scrutinizing protein targets, the authors applied a peptide-specific assay to reveal distinct reactivity profiles for 4-HNE protein adduction while identifying 31 protein targets with a total of 61 modification sites in an early-stage model of ALD. In aggregate, these findings support the hypothesis that alcohol-induced production of reactive aldehydes exacerbates hepatocyte injury by directly impacting cellular metabolism and anti-oxidant responses through protein-specific modifications.

4. Concluding remarks

The production and accumulation of hepatocellular LPPs is a pathogenic hallmark of alcohol metabolism. These reactive species have been found to contribute to inflammation, metabolic perturbations, and redox signaling. While current studies continue to investigate the underlying mechanisms of LPP toxicity in ALD, further research in needed to develop specific assays that can identify and quantify these reactive aldehydes and their reactivity profiles. Antioxidants continue to lack effectiveness as ALD therapies due to a delicate balance that exists in redox regulatory signaling and control. Ultimately, an overwhelming amount of research supports the restriction of alcohol consumption as a means to prevent ALD, which coincides with minimizing the production and accumulation of LPPs.

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Conflicts of interest

The authors declare no conflict of interest.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. CDC. World health organization (WHO): *Global status report on alcohol and health*. 2014 ed. 2014 XIV Available from: http://www.who.int/substance_abuse/publications/global_alcohol_report/msb_gsr_2014_1.pdf?ua=1.

2. Mokdad AH, *et al.*: **Actual causes of death in the United States, 2000**. *J Am Med Assoc* 2004, **291**:1238–1245.

3. Seitz HK, *et al.*: **Alcoholic liver disease**. *Nat Rev Dis Primers* 2018, **4**:16.

4. Stickel F, *et al.*: **Pathophysiology and management of alcoholic liver disease: update 2016**. *Gut Liver* 2017, **11**:173–188.

5. Dunn W, Shah VH: **Pathogenesis of alcoholic liver disease**. *Clin Liver Dis* 2016, **20**:445–456.

6. Gao B, Bataller R: **Alcoholic liver disease: pathogenesis and new therapeutic targets**. *Gastroenterology* 2011, **141**:1572–1585.

7. Zeng T, *et al.*: **Impairment of Akt activity by CYP2E1 mediated oxidative stress is involved in chronic ethanol-induced fatty liver**. *Redox Biol* 2017, **14**:295–304.

8. Ceni E, Mello T, Galli A: **Pathogenesis of alcoholic liver disease: role of oxidative metabolism**. *World J Gastroenterol* 2014, **20**:17756–17772.

9. ** Sies H, Berndt C, Jones DP: **Oxidative stress**. *Annu Rev Biochem* 2017, **86**:715–748.

10. Doody EE, *et al.*: **Ethanol metabolism by alcohol dehydrogenase or cytochrome P450 2E1 differentially impairs hepatic protein trafficking and growth hormone signaling**. *Am J Physiol Gastrointest Liver Physiol* 2017, **313**:G558–G569.

11. * Cederbaum AI, Lu Y, Wu D: **Role of oxidative stress in alcohol-induced liver injury**. *Arch Toxicol* 2009, **83**:519–548.

12. Assiri MA, *et al.*: **Chronic ethanol metabolism inhibits hepatic mitochondrial superoxide dismutase via lysine acetylation**. *Alcohol Clin Exp Res* 2017 Oct, **41**:1705–1714.

13. Jones DP: **Redefining oxidative stress**. *Antioxidants Redox Signal* 2006, **8**:1865–1879.

14. Sies H: **Oxidative stress: a concept in redox biology and medicine**. *Redox Biol* 2015, **4**:180–183.

15. Fritz KS, Petersen DR: **Exploring the biology of lipid peroxidation-derived protein carbonylation**. *Chem Res Toxicol* 2011, **24**:1411–1419.

16. ** Jones DP, Sies H: **The redox code**. *Antioxidants Redox Signal* 2015, **23**:734–746.

17. Roede JR, *et al.*: **Characterization of plasma thiol redox potential in a common marmoset model of aging**. *Redox Biol* 2013, **1**:387–393.

18. Jones DP, *et al.*: **Cysteine/cystine couple is a newly recognized node in the circuitry for biologic redox signaling and control**. *Faseb J* 2004, **18**:1246–1248.

19. Kim HS, *et al.*: **SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress**. *Cancer Cell* 2010, **17**:41–52.

20. Marnett LJ: **Inflammation and cancer: chemical approaches to mechanisms, imaging, and treatment**. *J Org Chem* 2012, **77**:5224–5238.

21. * Jones DP: **Radical-free biology of oxidative stress**. *Am J Physiol Cell Physiol* 2008, **295**:C849–C868.

22. Boveris A, *et al.*: **Increased chemiluminescence and superoxide production in the liver of chronically ethanol-treated rats**. *Arch Biochem Biophys* 1983, **227**:534–541.

23. * Kukielka E, Dicker E, Cederbaum AI: **Increased production of reactive oxygen species by rat liver mitochondria after chronic ethanol treatment**. *Arch Biochem Biophys* 1994, **309**:377–386.

24. Sato N: **Central role of mitochondria in metabolic regulation of liver pathophysiology**. *J Gastroenterol Hepatol* 2007, **22**(Suppl 1):S1–S6.

25. Bautista AP: **Neutrophilic infiltration in alcoholic hepatitis**. *Alcohol* 2002, **27**:17–21.

26. Cubero FJ, Urtasun R, Nieto N: **Alcohol and liver fibrosis**. *Semin Liver Dis* 2009, **29**:211–221.

27. Inokuchi S, *et al.*: **Toll-like receptor 4 mediates alcohol-induced steatohepatitis through bone marrow-derived and endogenous liver cells in mice**. *Alcohol Clin Exp Res* 2011, **35**:1509–1518.

28. Petrasek J, *et al.*: **IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice**. *J Clin Invest* 2012, **122**:3476–3489.

29. Purohit V, Brenner DA: **Mechanisms of alcohol-induced hepatic fibrosis: a summary of the Ron Thurman Symposium**. *Hepatology* 2006, **43**:872–878.

30. Seki E, *et al.*: **TLR4 enhances TGF-beta signaling and hepatic fibrosis**. *Nat Med* 2007, **13**:1324–1332.

31. Beier JI, McClain CJ: **Mechanisms and cell signaling in alcoholic liver disease**. *Biol Chem* 2010, **391**:1249–1264.

32. Nieto N, *et al.*: **CYP2E1-mediated oxidative stress induces collagen type I expression in rat hepatic stellate cells**. *Hepatology* 1999, **30**:987–996.

33. Svineng G, *et al.*: **The role of reactive oxygen species in integrin and matrix metalloproteinase expression and function**. *Connect Tissue Res* 2008, **49**:197–202.

34. Cohen JI, Nagy LE: **Pathogenesis of alcoholic liver disease: interactions between parenchymal and non-parenchymal cells**. *J Dig Dis* 2011, **12**:3–9.

35. Zhu H, *et al.*: **Oxidative stress and redox signaling mechanisms of alcoholic liver disease: updated experimental and clinical evidence**. *J Dig Dis* 2012, **13**:133–142.

36. Albano E: **Alcohol, oxidative stress and free radical damage**. *Proc Nutr Soc* 2006, **65**:278–290.

37. Cunningham CC, Ivester P: **Chronic ethanol, oxygen tension and hepatocyte energy metabolism**. *Front Biosci* 1999, **4**:D551–D556.

38. * Wu D, Cederbaum AI: **Oxidative stress and alcoholic liver disease**. *Semin Liver Dis* 2009, **29**:141–154.

39. Giorgio M, *et al.*: **Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis**. *Cell* 2005, **122**:221–233.

40. Koch OR, *et al.*: **Role of the life span determinant P66(shcA) in ethanol-induced liver damage**. *Lab Invest* 2008, **88**:750–760.

41. ** Lu Y, Cederbaum AI: **CYP2E1 and oxidative liver injury by alcohol**. *Free Radic Biol Med* 2008, **44**:723–738.

42. Wu D, Cederbaum AI: **Alcohol, oxidative stress, and free radical damage**. *Alcohol Res Health* 2003, **27**:277–284.

43. Sultatos LG: **Effects of acute ethanol administration on the hepatic xanthine dehydrogenase/oxidase system in the rat**. *J Pharmacol Exp Therapeut* 1988, **246**:946–949.

44. De Minicis S, Brenner DA: **Oxidative stress in alcoholic liver disease: role of NADPH oxidase complex**. *J Gastroenterol Hepatol* 2008, **23**(Suppl 1):S98–S103.

45. Larosche I, *et al.*: **Hepatic mitochondrial DNA depletion after an alcohol binge in mice: probable role of peroxynitrite and modulation by manganese superoxide dismutase**. *J Pharmacol Exp Therapeut* 2010, **332**:886–897.

46. Spitzer JA, *et al.*: **Ethanol and LPS modulate NF-kappaB activation, inducible NO synthase and COX-2 gene expression in rat liver cells in vivo**. *Front Biosci* 2002, **7**:a99–a108.

47. Yang ES, Lee JH, Park JW: **Ethanol induces peroxynitrite-mediated toxicity through inactivation of NADP+-dependent isocitrate dehydrogenase and superoxide dismutase**. *Biochimie* 2008, **90**:1316–1324.

48. Arteel GE, *et al.*: **Oxidative stress occurs in perfused rat liver at low oxygen tension by mechanisms involving peroxynitrite**. *Mol Pharmacol* 1999, **55**:708–715.

49. Wang JF, Greenberg SS, Spitzer JJ: **Chronic alcohol administration stimulates nitric oxide formation in the rat liver with or without pretreatment by lipopolysaccharide**. *Alcohol Clin Exp Res* 1995, **19**:387–393.

50. Thurman RG: **II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin**. *Am J Physiol* 1998, **275**: G605–G611.

51. Wheeler MD, *et al.*: **The role of Kupffer cell oxidant production in early ethanol-induced liver disease**. *Free Radic Biol Med* 2001, **31**:1544–1549.

52. Yoshiyuki T, *et al.*: **Roles of kupffer cells in alcoholic liver disease**. *Alcohol Clin Exp Res* 2005, **29**:1116–1120.

53. Zima T, Kalousova M: **Oxidative stress and signal transduction pathways in alcoholic liver disease**. *Alcohol Clin Exp Res* 2005, **29**(11 Suppl):110S–115S.

54. Jaruga B, *et al.*: **Chronic alcohol consumption accelerates liver injury in T cell-mediated hepatitis: alcohol disregulation of NF-kappaB and STAT3 signaling pathways**. *Am J Physiol Gastrointest Liver Physiol* 2004, **287**:G471–G479.

55. Voican CS, Perlemuter G, Naveau S: **Mechanisms of the inflammatory reaction implicated in alcoholic hepatitis: 2011 update**. *Clin Res Hepatol Gastroenterol* 2011, **35**:465–474.

56. Zhao XJ, *et al.*: **TRIF and IRF-3 binding to the TNF promoter results in macrophage TNF dysregulation and steatosis induced by chronic ethanol**. *J Immunol* 2008, **181**: 3049–3056.

57. Ajakaiye M, *et al.*: **Alcohol and hepatocyte-Kupffer cell interaction (review)**. *Mol Med Rep* 2011, **4**:597–602.

58. Tsukamoto H, *et al.*: **Iron primes hepatic macrophages for NF-kappaB activation in alcoholic liver injury**. *Am J Physiol* 1999, **277**:G1240–G1250.

59. Winterbourn CC: **Toxicity of iron and hydrogen peroxide: the Fenton reaction**. *Toxicol Lett* 1995, **82–83**:969–974.

60. Fernandez-Checa JC, *et al.*: **GSH transport in mitochondria: defense against TNF-induced oxidative stress and alcohol-induced defect**. *Am J Physiol* 1997, **273**:G7–G17.

61. Iimuro Y, *et al.*: **The glutathione precursor L-2-oxothiazolidine-4-carboxylic acid protects against liver injury due to chronic enteral ethanol exposure in the rat**. *Hepatology* 2000, **31**: 391–398.

62. Kaur J, Shalini S, Bansal MP: **Influence of vitamin E on alcohol-induced changes in antioxidant defenses in mice liver**. *Toxicol Mech Method* 2010, **20**:82–89.

63. Bae SH, *et al.*: **Concerted action of sulfiredoxin and peroxiredoxin I protects against alcohol-induced oxidative injury in mouse liver**. *Hepatology* 2011, **53**:945–953.

64. Cohen JI, *et al.*: **Exogenous thioredoxin prevents ethanol-induced oxidative damage and apoptosis in mouse liver**. *Hepatology* 2009, **49**:1709–1717.

65. Curry-McCoy TV, *et al.*: **Chronic ethanol consumption results in atypical liver injury in copper/zinc superoxide dismutase deficient mice**. *Alcohol Clin Exp Res* 2010, **34**:251–261.

66. Kessova IG, *et al.*: **Alcohol-induced liver injury in mice lacking Cu, Zn-superoxide dismutase**. *Hepatology* 2003, **38**: 1136–1145.

67. Kim SJ, *et al.*: **Ethanol-induced liver injury and changes in sulfur amino acid metabolomics in glutathione peroxidase and catalase double knockout mice**. *J Hepatol* 2009, **50**: 1184–1191.

68. Wheeler MD, *et al.*: **Delivery of the Cu/Zn-superoxide dismutase gene with adenovirus reduces early alcohol-induced liver injury in rats**. *Gastroenterology* 2001, **120**:1241–1250.

69. Wheeler MD, *et al.*: **Overexpression of manganese superoxide dismutase prevents alcohol-induced liver injury in the rat**. *J Biol Chem* 2001, **276**:36664–36672.

70. Zhou Z, Sun X, James Kang Y: **Metallothionein protection against alcoholic liver injury through inhibition of oxidative stress**. *Exp Biol Med* 2002, **227**:214–222.

71. Zhou Z, *et al.*: **Metallothionein-independent zinc protection from alcoholic liver injury**. *Am J Pathol* 2002, **160**:2267–2274.

72. Lamle J, *et al.*: **Nuclear factor-eythroid 2-related factor 2 prevents alcohol-induced fulminant liver injury**. *Gastroenterology* 2008, **134**:1159–1168.

73. ** Bailey SM, *et al.*: **Chronic ethanol consumption alters the glutathione/glutathione peroxidase-1 system and protein oxidation status in rat liver**. *Alcohol Clin Exp Res* 2001, **25**: 726–733.

74. Fernandez-Checa JC, Ookhtens M, Kaplowitz N: **Effects of chronic ethanol feeding on rat hepatocytic glutathione. Relationship of cytosolic glutathione to efflux and mitochondrial sequestration**. *J Clin Invest* 1989, **83**:1247–1252.

75. Oh SI, *et al.*: **Chronic ethanol consumption affects glutathione status in rat liver**. *J Nutr* 1998, **128**:758–763.

76. Deaciuc IV, *et al.*: **Modulation of caspase-3 activity and Fas ligand mRNA expression in rat liver cells in vivo by alcohol and lipopolysaccharide**. *Alcohol Clin Exp Res* 1999, **23**: 349–356.

77. Ajmo JM, *et al.*: **Resveratrol alleviates alcoholic fatty liver in mice**. *Am J Physiol Gastrointest Liver Physiol* 2008, **295**: G833–G842.

78. Bao W, *et al.*: **Curcumin alleviates ethanol-induced hepatocytes oxidative damage involving heme oxygenase-1 induction**. *J Ethnopharmacol* 2010, **128**:549–553.

79. Kono H, *et al.*: **Ebselen prevents early alcohol-induced liver injury in rats**. *Free Radic Biol Med* 2001, **30**:403–411.

80. Mandal P, *et al.*: **Adiponectin and heme oxygenase-1 suppress TLR4/MyD88-independent signaling in rat Kupffer cells and in mice after chronic ethanol exposure**. *J Immunol* 2010, **185**:4928–4937.

81. * Mantena SK, *et al.*: **Mitochondrial dysfunction and oxidative stress in the pathogenesis of alcohol- and obesity-induced fatty liver diseases**. *Free Radic Biol Med* 2008, **44**:1259–1272.

82. Ronis MJ, *et al.*: **Effects of N-acetylcysteine on ethanol-induced hepatotoxicity in rats fed via total enteral nutrition**. *Free Radic Biol Med* 2005, **39**:619–630.

83. Song Z, *et al.*: **S-adenosylmethionine (SAMe) protects against acute alcohol induced hepatotoxicity in mice**. *J Nutr Biochem* 2003, **14**:591–597.

84. Yanardag R, *et al.*: **Combined effects of vitamin C, vitamin E, and sodium selenate supplementation on absolute ethanol-induced injury in various organs of rats**. *Int J Toxicol* 2007, **26**: 513–523.

85. Galligan JJ, *et al.*: **Oxidative stress and the ER stress response in a murine model for early-stage alcoholic liver disease**. *J Toxicol* 2012, **2012**:207594.

86. Ayala A, Munoz MF, Arguelles S: **Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal**. *Oxid Med Cell Longev* 2014, **2014**:360438.

87. Fritz KS, Petersen DR: **An overview of the chemistry and biology of reactive aldehydes**. *Free Radic Biol Med* 2013, **59**: 85–91.

88. ** Esterbauer H, Schaur RJ, Zollner H: **Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes**. *Free Radic Biol Med* 1991, **11**:81–128.

89. Redza-Dutordoir M, Averill-Bates DA: **Activation of apoptosis signalling pathways by reactive oxygen species**. *Biochim Biophys Acta* 2016, **1863**:2977–2992.

90. Shearn CT, *et al.*: **Increased dietary fat contributes to dysregulation of the LKB1/AMPK pathway and increased damage in a mouse model of early-stage ethanol-mediated steatosis**. *J Nutr Biochem* 2013, **24**:1436–1445.

91. Ronis MJ, *et al.*: **Dietary fat source alters hepatic gene expression profile and determines the type of liver pathology in rats overfed via total enteral nutrition**. *Physiol Genom* 2012, **44**:1073–1089.

92. Ronis MJ, *et al.*: **Dietary saturated fat reduces alcoholic hepatotoxicity in rats by altering fatty acid metabolism and membrane composition**. *J Nutr* 2004, **134**:904–912.

93. Korourian S, *et al.*: **Diet and risk of ethanol-induced hepatotoxicity: carbohydrate-fat relationships in rats**. *Toxicol Sci* 1999, **47**:110–117.

94. ** Shearn CT, *et al.*: **Deletion of GSTA4-4 results in increased mitochondrial post-translational modification of proteins by reactive aldehydes following chronic ethanol consumption in mice**. *Redox Biol* 2016, **7**:68–77.

95. Shearn CT, *et al.*: **Identification of 5' AMP-activated kinase as a target of reactive aldehydes during chronic ingestion of high concentrations of ethanol**. *J Biol Chem* 2014, **289**: 15449–15462.

96. Smathers RL, *et al.*: **Susceptibility of L-FABP-/- mice to oxidative stress in early-stage alcoholic liver**. *J Lipid Res* 2013, **54**:1335–1345.

97. Galligan JJ, *et al.*: **Protein carbonylation in a murine model for early alcoholic liver disease**. *Chem Res Toxicol* 2012, **25**: 1012–1021.

98. Chen J, *et al.*: **Formation of malondialdehyde adducts in livers of rats exposed to ethanol: role in ethanol-mediated inhibition of cytochrome c oxidase**. *Alcohol Clin Exp Res* 2000, **24**: 544–552.

99. Orlicky DJ, *et al.*: **Chronic ethanol consumption in mice alters hepatocyte lipid droplet properties**. *Alcohol Clin Exp Res* 2011, **35**:1020–1033.

100. Osna NA, *et al.*: **Aberrant post-translational protein modifications in the pathogenesis of alcohol-induced liver injury**. *World J Gastroenterol* 2016, **22**:6192–6200.

101. Smathers RL, *et al.*: **Overview of lipid peroxidation products and hepatic protein modification in alcoholic liver disease**. *Chem Biol Interact* 2011, **192**:107–112.

102. Sampey BP, *et al.*: **Immunohistochemical characterization of hepatic malondialdehyde and 4-hydroxynonenal modified proteins during early stages of ethanol-induced liver injury**. *Alcohol Clin Exp Res* 2003, **27**:1015–1022.

103. Andringa KK, *et al.*: **Proteomic analysis of 4-hydroxynonenal (4-HNE) modified proteins in liver mitochondria from chronic ethanol-fed rats**. *Redox Biol* 2014, **2C**:1038–1047.

104. Adachi J, *et al.*: **Plasma phosphatidylcholine hydroperoxide as a new marker of oxidative stress in alcoholic patients**. *J Lipid Res* 2004, **45**:967–971.

105. * Fink R, *et al.*: **Increased free-radical activity in alcoholics**. *Lancet* 1985, **2**:291–294.

106. Letteron P, *et al.*: **Increased ethane exhalation, an in vivo index of lipid peroxidation, in alcohol-abusers**. *Gut* 1993, **34**: 409–414.

107. Meagher EA, *et al.*: **Alcohol-induced generation of lipid peroxidation products in humans**. *J Clin Invest* 1999, **104**: 805–813.

108. Ohhira M, *et al.*: **Immunohistochemical detection of 4-hydroxy-2-nonenal-modified-protein adducts in human alcoholic liver diseases**. *Alcohol Clin Exp Res* 1998, **22**(3 Suppl): 145S–149S.

109. Pemberton PW, Smith A, Warnes TW: **Non-invasive monitoring of oxidant stress in alcoholic liver disease**. *Scand J Gastroenterol* 2005, **40**:1102–1108.

110. Seki S, *et al.*: **Pathological significance of oxidative cellular damage in human alcoholic liver disease**. *Histopathology* 2003, **42**:365–371.

111. Paradis V, *et al.*: **In situ detection of lipid peroxidation by-products in chronic liver diseases**. *Hepatology* 1997, **26**: 135–142.

112. Paradis V, *et al.*: **Cellular and subcellular localization of acetaldehyde-protein adducts in liver biopsies from alcoholic patients**. *J Histochem Cytochem* 1996, **44**:1051–1057.

113. Shearn CT, *et al.*: **Increased hepatocellular protein carbonylation in human end-stage alcoholic cirrhosis**. *Free Radic Biol Med* 2015, **89**:1144–1153.

114. Rolla R, *et al.*: **Antiphospholipid antibodies associated with alcoholic liver disease specifically recognise oxidised phospholipids**. *Gut* 2001, **49**:852–859.

115. Vidali M, *et al.*: **Immune responses against oxidative stress-derived antigens are associated with increased circulating tumor necrosis factor-alpha in heavy drinkers**. *Free Radic Biol Med* 2008, **45**:306–311.

116. Israel Y, *et al.*: **Monoclonal and polyclonal antibodies against acetaldehyde-containing epitopes in acetaldehyde-protein adducts**. In *Proc Natl Acad Sci U S A*, **83**; 1986:7923–7927.

117. Niemela O, *et al.*: **Antibodies against acetaldehyde-modified protein epitopes in human alcoholics**. *Hepatology* 1987, **7**: 1210–1214.

118. Viitala K, *et al.*: **Autoimmune responses against oxidant stress and acetaldehyde-derived epitopes in human alcohol consumers**. *Alcohol Clin Exp Res* 2000, **24**:1103–1109.

119. * Ronis MJ, *et al.*: **Increased 4-hydroxynonenal protein adducts in male GSTA4-4/PPAR-alpha double knockout mice enhance injury during early stages of alcoholic liver disease**. *Am J Physiol Gastrointest Liver Physiol* 2015, **308**: G403–G415.

120. Fraenkel-Conrat H, Singer B: **Nucleoside adducts are formed by cooperative reaction of acetaldehyde and alcohols: possible mechanism for the role of ethanol in carcinogenesis**. In *Proc Natl Acad Sci U S A*, **85**; 1988:3758–3761.

121. Sorrell MF, Tuma DJ: **The functional implications of acetaldehyde binding to cell constituents**. *Ann N Y Acad Sci* 1987, **492**:50–62.

122. * Niemela O: **Aldehyde-protein adducts in the liver as a result of ethanol-induced oxidative stress**. *Front Biosci* 1999, **4**: D506–D513.

123. Clot P, *et al.*: **Detection of antibodies against proteins modified by hydroxyethyl free radicals in patients with alcoholic cirrhosis**. *Gastroenterology* 1995, **108**:201–207.

124. Clot P, *et al.*: **Plasma membrane hydroxyethyl radical adducts cause antibody-dependent cytotoxicity in rat hepatocytes exposed to alcohol**. *Gastroenterology* 1997, **113**:265–276.

125. Viitala K, *et al.*: **Serum IgA, IgG, and IgM antibodies directed against acetaldehyde-derived epitopes: relationship to liver disease severity and alcohol consumption**. *Hepatology* 1997, **25**:1418–1424.

126. Worrall S, *et al.*: **Antibodies against acetaldehyde-modified epitopes: an elevated IgA response in alcoholics**. *Eur J Clin Invest* 1991, **21**:90–95.

127. Xu D, *et al.*: **Detection of circulating antibodies to malondialdehyde-acetaldehyde adducts in ethanol-fed rats**. *Gastroenterology* 1998, **115**:686–692.

128. * Rolla R, *et al.*: **Detection of circulating antibodies against malondialdehyde-acetaldehyde adducts in patients with alcohol-induced liver disease**. *Hepatology* 2000, **31**:878–884.

129. Zamara E, *et al.*: **4-Hydroxynonenal as a selective pro-fibrogenic stimulus for activated human hepatic stellate cells**. *J Hepatol* 2004, **40**:60–68.

130. Nanji AA, *et al.*: **Medium chain triglycerides and vitamin E reduce the severity of established experimental alcoholic liver disease**. *J Pharmacol Exp Therapeut* 1996, **277**: 1694–1700.

131. Cunningham CC, Coleman WB, Spach PI: **The effects of chronic ethanol consumption on hepatic mitochondrial energy metabolism**. *Alcohol Alcohol* 1990, **25**:127–136.

132. Sastre J, *et al.*: **Chronic ethanol feeding causes oxidative stress in rat liver mitochondria. Prevention by S-adenosyl methionine**. *Free Radic Res* 1999, **30**:325–327.

133. Chen WY, *et al.*: **Acrolein is a pathogenic mediator of alcoholic liver disease and the scavenger hydralazine is protective in mice**. *Cell Mol Gastroenterol Hepatol* 2016, **2**:685–700.

134. Ding L, *et al.*: **Danshen protects against early-stage alcoholic liver disease in mice via inducing PPARalpha activation and subsequent 4-HNE degradation**. *PloS One* 2017, **12**: e0186357.

135. Carbone DL, *et al.*: **Cysteine modification by lipid peroxidation products inhibits protein disulfide isomerase**. *Chem Res Toxicol* 2005, **18**:1324–1331.

136. Newton BW, *et al.*: **Liver proteome analysis in a rodent model of alcoholic steatosis**. *J Proteome Res* 2009, **8**:1663–1671.

137. Shearn CT, *et al.*: **Knockout of the Gsta4 gene in male mice leads to an altered pattern of hepatic protein carbonylation and enhanced inflammation following chronic consumption of an ethanol diet**. *Alcohol Clin Exp Res* 2018, **42**:1192–1205.

138. Shearn CT, *et al.*: **Increased carbonylation of the lipid phosphatase PTEN contributes to Akt2 activation in a murine model of early alcohol-induced steatosis**. *Free Radic Biol Med* 2013, **65**:680–692.

139. Fritz KS, *et al.*: **4-Hydroxynonenal inhibits SIRT3 via thiol-specific modification**. *Chem Res Toxicol* 2011, **24**:651–662.

140. Zeng T, *et al.*: **Impairment of Akt activity by CYP2E1 mediated oxidative stress is involved in chronic ethanol-induced fatty liver**. *Redox Biol* 2018, **14**:295–304.

141. Smathers RL, *et al.*: **Characterization of 4-HNE modified L-FABP reveals alterations in structural and functional dynamics**. *PloS One* 2012, **7**:e38459.

142. Galligan JJ, *et al.*: **Oxidative stress-mediated aldehyde adduction of GRP78 in a mouse model of alcoholic liver disease: functional independence of ATPase activity and chaperone function**. *Free Radic Biol Med* 2014, **73**:411–420.

143. ** Tzeng SC, Maier CS: **Label-free proteomics assisted by affinity enrichment for elucidating the chemical reactivity of the liver mitochondrial proteome toward adduction by the lipid electrophile 4-hydroxy-2-nonenal (HNE)**. *Front Chem* 2016, **4**:2.