AMERICAN JOURNAL OF PHYSIOLOGY
GASTROINTESTINAL AND LIVER PHYSIOLOGY

Am J Physiol Gastrointest Liver Physiol 326: G483–G494, 2024.
First published March 5, 2024; doi:10.1152/ajpgi.00281.2023

REVIEW

Interaction between fatty acid oxidation and ethanol metabolism in liver

Yongke Lu[1] and Joseph George[2]

[1]*Department of Biomedical Sciences, Joan C. Edwards College of Medicine, Marshall University, Huntington, West Virginia, United States and* [2]*Department of Hepatology, Kanazawa Medical University, Uchinada, Ishikawa, Japan*

Abstract

Fatty acid oxidation (FAO) releases the energy stored in fat to maintain basic biological processes. Dehydrogenation is a major way to oxidize fatty acids, which needs NAD^+ to accept the released H^+ from fatty acids and form NADH, which increases the ratio of NADH/NAD^+ and consequently inhibits FAO leading to the deposition of fat in the liver, which is termed fatty liver or steatosis. Consumption of alcohol (ethanol) initiates simple steatosis that progresses to alcoholic steatohepatitis, which constitutes a spectrum of liver disorders called alcohol-associated liver disease (ALD). ALD is linked to ethanol metabolism. Ethanol is metabolized by alcohol dehydrogenase (ADH), microsomal ethanol oxidation system (MEOS), mainly cytochrome P450 2E1 (CYP2E1), and catalase. ADH also requires NAD^+ to accept the released H^+ from ethanol. Thus, ethanol metabolism by ADH leads to increased ratio of NADH/NAD^+, which inhibits FAO and induces steatosis. CYP2E1 directly consumes reducing equivalent NADPH to oxidize ethanol, which generates reactive oxygen species (ROS) that lead to cellular injury. Catalase is mainly present in peroxisomes, where very long-chain fatty acids and branched-chain fatty acids are oxidized, and the resultant short-chain fatty acids will be further oxidized in mitochondria. Peroxisomal FAO generates hydrogen peroxide (H_2O_2), which is locally decomposed by catalase. When ethanol is present, catalase uses H_2O_2 to oxidize ethanol. In this review, we introduce FAO (including α-, β-, and ω-oxidation) and ethanol metabolism (by ADH, CYP2E1, and catalase) followed by the interaction between FAO and ethanol metabolism in the liver and its pathophysiological significance.

catalase; CYP2E1; hydrogen peroxide; NAD; peroxisomes

INTRODUCTION

Alcohol abuse is a major cause of advanced liver disease worldwide, and 50% of liver-related deaths are attributed to alcohol. Globally, around 35% of alcoholic patients develop various forms of alcohol-associated liver disease (ALD) (1). ALD shares multiple aspects of pathology with metabolic dysfunction-associated steatotic liver disease (MASLD): there is a progression from simple steatosis (fatty liver) to steatohepatitis, fibrosis, and cirrhosis leading to hepatocellular carcinoma (2). Adipose tissues are one of the major sources of fat in the liver (3). Ethanol enhances the mobilization of fat from adipose tissues to the liver. Adipose lipolysis increases serum levels of free fatty acids (FFA), which are absorbed by the liver and promote alcoholic steatosis (4, 5). Under obese conditions, binge alcohol consumption aggravates liver injury and promotes the pathogenesis of steatohepatitis from simple steatosis (6). High-fat diets (HFD) also promote the pathogenesis of ALD through enhanced intestinal absorption of fat and ethanol-induced steatosis (7). Impaired fatty acid oxidation (FAO) is one of the major reasons for the deposition of fat in the liver. Peroxisome proliferator-activated receptor α (PPARα) regulates mitochondrial, microsomal, and peroxisomal FAO. PPARα knockout ($ppar\alpha^{-/-}$) mice developed more severe steatohepatitis in ALD model (8) and MASLD model (9). Treatment with PPARα agonist WY-14,643 reversed hepatic fat accumulation and steatohepatitis in ALD model (10) and MASLD model (11).

The radical difference in etiology between ALD and MASLD is the intake of alcohol in the former. The toxic effects of the metabolic products of ethanol have been linked to the pathogenesis of ALD (12, 13). Ethanol is a substantial source of energy, and ethanol metabolism produces 7.1 kcal (29.7 kJ) per gram, a value that exceeds the energy content of carbohydrates and proteins. On average, ethanol accounts for half of the caloric intake of alcoholic individuals (12). Ethanol consumption affects multiple aspects of hepatic lipid metabolism, including fatty acid uptake, FAO, lipogenesis, lipid secretion, lipid droplet formation, autophagy, circadian rhythm, etc., which have been reviewed recently (14, 15). Both fat and ethanol metabolisms are associated with the development of ALD. This review focuses on the molecular interaction between lipid metabolism and ethanol metabolism in the liver, with special emphasis on FAO and ethanol breakdown.

TYPES OF FATTY ACID OXIDATION

Fatty acids can be initially oxidized or hydroxylated in different carbon atoms. FAO can take place either on α, β,

Correspondence: Y. Lu (luy@marshall.edu).
Submitted 22 November 2023 / Revised 13 February 2024 / Accepted 26 February 2024

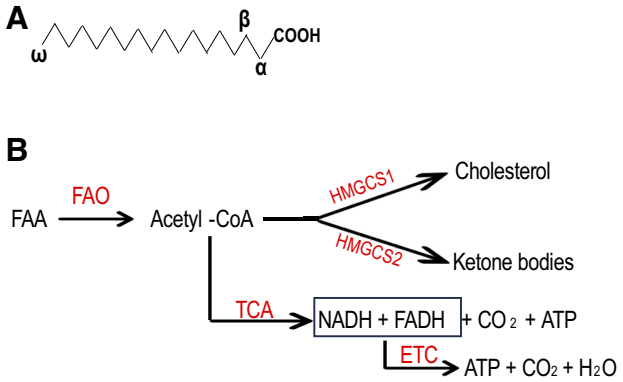

Figure 1. Fatty acid oxidation (FAO). *A*: FAO occurs at α-, β-, or ω–carbon atoms. *B*: FAO product acetyl-CoA is further oxidized as ketone bodies or carbon dioxide and water in mitochondria. Acetyl-CoA is also used for initial cholesterol synthesis in cytosol. ETC, electron transport chain; HMGCS, hydroxymethylglutaryl (HMG)-CoA synthase; TCA, tricarboxylic cycle.

or ω carbon atoms (Fig. 1*A*). Correspondingly, they are called α-oxidation, β-oxidation, and ω-oxidation. The most important and well-known FAO pathway is β-oxidation. ω-Oxidation is a subsidiary pathway of β-oxidation, and α-oxidation is only for branched-chain fatty acids like phytanic acid. Fatty acid products of α-oxidation and ω-oxidation finally enter β-oxidation for further oxidation.

Fatty Acid β-Oxidation

The β-oxidation of fatty acids leads to the oxidative removal of successive two-carbon units in the form of acetyl-CoA, which takes place in mitochondria and peroxisomes. Usually, very long-chain and branched-chain fatty acids are first activated by acyl-CoA synthetase (ACS) to generate fatty acyl-CoA for oxidation in peroxisomes, and the resultant short-chain fatty acyl-CoA will be further oxidized in mitochondria (16). There are four steps for β-oxidation in both peroxisomes and mitochondria, but there is a difference in step 1 between the peroxisomes and mitochondria, while the other three steps are the same. In peroxisomes, step 1 is catalyzed by acyl-CoA oxidase (ACOX), and hydrogen peroxide (H_2O_2) is produced as a byproduct. The produced H_2O_2 is locally decomposed by catalase immediately. In mitochondria, step 1 is catalyzed by acyl-CoA dehydrogenase (ACDH), and flavin adenine dinucleotide (FAD) serves as cofactor to accept the removed hydrogen from fatty acyl-CoA to form FADH. ACDH does not generate H_2O_2 (16). In step 3, in both peroxisomes and mitochondria, NADH is generated, and the NADH/NAD^+ ratio is increased. In mitochondria, electrons pass through electron respiratory chain accompanied by ATP synthesis. But in peroxisomes, due to the lack of electron transport chain (ETC), the released energy is not conserved as ATP but is dissipated as heat (17).

Complete oxidation of fatty acids takes place in three stages (18): *1*) β-oxidation to produce acetyl-CoA in peroxisomes and mitochondria. *2*) The tricarboxylic acid (TCA) cycle in mitochondria: the acetyl group of acetyl-CoA is oxidized to CO_2 in mitochondrial matrix, but peroxisomes lack the enzymes for the TCA cycle. Acetyl-CoA generated in peroxisomes needs to be transported to mitochondria and enter the TCA cycle. *3*) The ETC in mitochondria: the reduced electron carriers including NADH and FADH (mostly NADH) produced in the first two stages donate electrons to the ETC flowing through Complex I–V, through which the electrons pass to oxygen with concomitant oxidization of ADP to ATP and NADH being oxidized back to NAD^+ via oxidative phosphorylation in mitochondrial matrix.

Besides entering the TCA cycle, acetyl-CoA resulting from β-oxidation also undergoes conversion to the water-soluble ketone bodies (Fig. 1*B*), including acetone, acetoacetate, and D-β-hydroxybutyrate, which takes place in mitochondria. Liver does not further oxidize ketone bodies. Acetone is produced in smaller quantities and exhaled from lung, but acetoacetate and D-β-hydroxybutyrate are transported to extrahepatic tissues for conversion to acetyl-CoA. The brain preferentially uses glucose as a source of energy, but when glucose is unavailable (e.g., under starvation), the brain adapts to use acetoacetate and D-β-hydroxybutyrate but not fatty acids because fatty acids do not cross the blood–brain barrier (18). Ethanol enhances HFD-induced ketogenesis, but ethanol alone does not induce ketogenesis (19). Ketogenic diets could interfere with alcohol metabolism, as indicated in an experimental rat model of ethanol feeding in which a ketogenic diet reduced alcohol intake (20). Nutritional ketosis could serve as a potential treatment for alcohol abuse. For details, please refer to the recent review (21).

Fatty Acid ω-Oxidation

The ω-oxidation of fatty acids involves oxidation of the ω carbon that is most distant from the carboxyl group. Fatty acid ω-oxidation is an accessory pathway that accounts for less than 5% of the total FAO (22). Under conditions of increased flux of fatty acids to the liver (such as starvation and diabetes), ω-oxidation may increase to approximately 15% (23). In addition, when β-oxidation is decreased, ω-oxidation will increase (24). The ω-oxidation is a rescue pathway for FAO disorders in patients with peroxisome disorders like Refsum disease and X-linked adrenoleukodystrophy (25). The ω-oxidation takes place in the endoplasmic reticulum (ER) of the cell. The ω-carbon of fatty acids is oxidized by cytochrome P450 (CYP) enzymes to form ω-hydroxyl fatty acid, which is further oxidized to aldehyde fatty acid by alcohol dehydrogenase (ADH), a major enzyme for ethanol metabolism (ADH metabolism of ethanol will be elaborated in *Alcohol Dehydrogenase*). After ω-oxidation, fatty acids are finally transformed into dicarboxylic acids, which will be subjected to β-oxidation for complete oxidation (25).

It is generally accepted that ω-oxidation is mainly catalyzed by CYP4As and CYP4Fs (25). CYP2E1 is also important for ω-oxidation (26). In CYP2E1 knockout (*cyp2e1*$^{-/-}$) mice, CYP4As were upregulated to compensate for the loss of CYP2E1 (27). Previously, we found that chronic ethanol feeding suppressed hepatic PPARα expression, which was observed in the WT mice but not in the *cyp2e1*$^{-/-}$ mice (28). Recently, it was reported that three polyunsaturated fatty acids (PUFA), including docosahexaenoic acid (DHA), 9,12-linoleic acid (LCA), and arachidonic acid (AA), were directly metabolized by CYP2E1, and these PUFAs can serve as PPARα agonists to activate FAO, which explains why

CYP2E1 deficiency causes PPARα activation (29). CYP2E1 is an alcohol-inducible enzyme and plays an important role in ethanol metabolism, which will be discussed in *Cytochrome P450 2E1*.

Fatty Acid α-Oxidation

Unlike β-oxidation, which removes two carbons at a time, fatty acid α-oxidation removes only one carbon atom at a time, which is also from the carboxyl end of the fatty acid chain. The α-oxidation takes place only in peroxisomes, it is especially important for the metabolism of branched-chain fatty acids such as phytanic acid. Phytanic acid is derived from the side chain of phytol group of chlorophyll. Humans obtain phytanic acid primarily from dairy products and from fats of ruminant animals. Bacteria in the rumen of these animals produce phytanic acid as they digest plant chlorophyll to release phytol (30, 31). Phytanic acid is 3,7,11,15-tetramethylhexadecanoic acid with four side methyl groups in C3,7,11, and 15. The C3 methyl group is in β-carbon, so β-oxidation is blocked. If it undergoes α-oxidation to make α-carbon (C2) be oxidized and released as CO_2, then phytanic acid is metabolized to pristanic acid, i.e., 2,6,10,14-tetramethylpentadecanoic acid; thus, the original C3 methyl group will become C2 methyl group and there will be no methyl group in β-carbon to block β-oxidation (31, 32). Unlike straight-chain fatty acid β-oxidation, which only produces acetyl-CoA, pristanic acid β-oxidation produces both acetyl-CoA and propionyl-CoA. Phytanic acid and its metabolite pristanic acid activate PPARα in a concentration-dependent manner via the ligand-binding domain of PPARα as well as via a PPAR response element (PPRE) (33). Phytanic acid precursor phytol conversion to phytanic acid was also in a PPARα-dependent manner (34).

ETHANOL METABOLISM

Ethanol is metabolized to acetaldehyde by three enzyme systems in the liver: *1*) alcohol dehydrogenase (ADH); *2*) microsomal ethanol-oxidizing system (MEOS), which contains CYP2E1; and *3*) catalase (12, 13, 35). The resultant acetaldehyde is further metabolized to acetate by acetaldehyde dehydrogenase (ALDH) (12, 13). Figure 2 demonstrates the biochemical reactions and the intracellular locations of ethanol metabolism. In addition, ethanol in a small fraction is esterized with fatty acids to form fatty acid ethyl ester (FAEE) by carboxylesterase or carboxylester lipase in the liver or pancreas (36, 37). FAEE accumulation causes inflammation and injury in the pancreas (38). Recently, it was reported that FAEEs also mediated binge alcohol-induced liver injury (39).

Alcohol Dehydrogenase

ADH is localized in the cytosolic fraction of cells. The highest amount of ADH is present in the liver, followed by gastrointestinal tract. The primary function of ADH is to oxidize the endogenous alcohol produced by microorganisms in the gut and the exogenous ethanol and other alcohols consumed with the diet. In humans, multiple forms of ADH exist in the liver and are encoded by at least seven genes (40). They catalyze the removal of hydrogen atom from ethanol to NAD^+ and form acetaldehyde and NADH (Fig. 2), which results in a strikingly increased ratio of NADH/NAD^+ and the associated redox changes. The increased ratio of NADH/NAD^+ inhibits FAO, which contributes to the development of alcoholic steatosis (12, 13).

ADH has a broad spectrum of substrate specificity. As we discussed in *Fatty Acid ω-Oxidation*, ADH also has

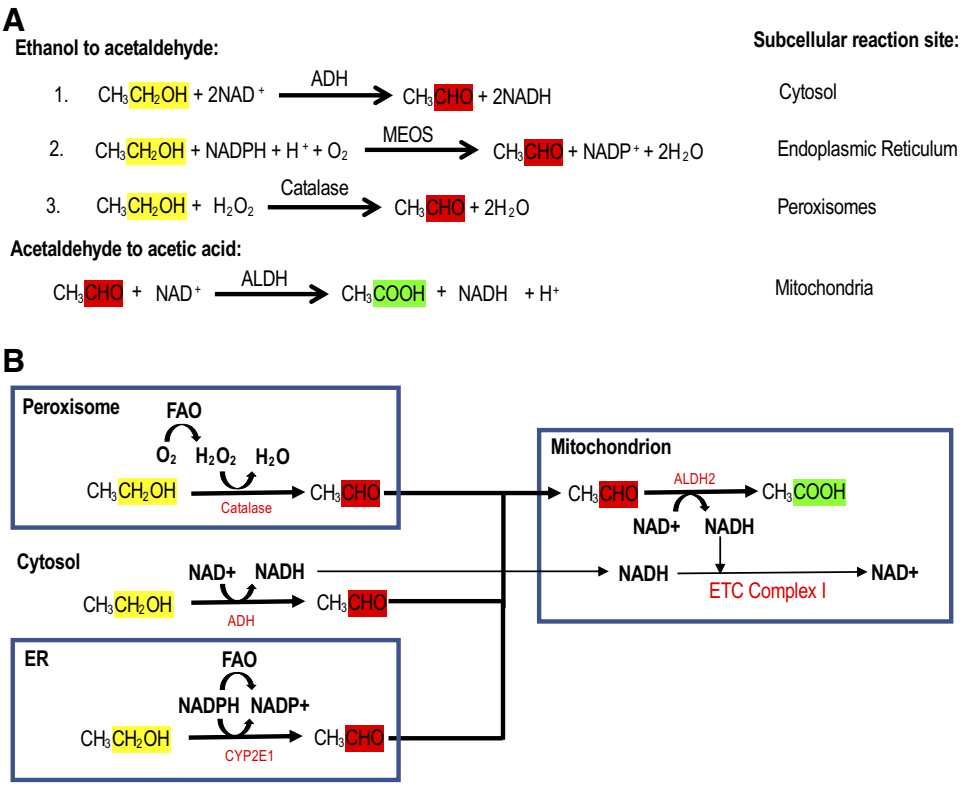

Figure 2. Alcohol metabolism in the liver. *A*: biochemical reaction. *B*: interaction of the three ethanol metabolism pathways and mitochondrial acetaldehyde metabolism. CH_3CHO, acetaldehyde; CH_3COOH, acetic acid; CH_3CH_2OH, ethanol; ETC Complex I, electron transport chain Complex I.

ω-hydroxy fatty acid dehydrogenase activity in the process of fatty acid ω-oxidation (25). The K_m values for the oxidation of ω-hydroxy fatty acids by ADH (10–40 μM) are lower than the K_m values for the oxidation of ethanol by ADH (0.2–2 mM). Therefore, higher concentration of ethanol (5.6 mM) inhibits the oxidation of ω-hydroxy fatty acids by rat liver ADH. In contrast, a lower concentration of ω-hydroxy fatty acids (17 μM of 18-hydroxystearic acid) is enough to inhibit the oxidation of ethanol by rat liver ADH (41). These results indicate that the oxidation of ω-hydroxy fatty acids is a more important physiological function of ADH than ethanol metabolism.

In addition, ADH also metabolizes retinol (Vitamin A) to retinal, and the latter is further oxidized to retinoic acid (42). Retinol, retinal, and retinoic acid are collectively called retinoids. Ethanol may compete with retinol for ADH to affect retinoid homeostasis. Acute ethanol administration reduces retinoic acid levels, which is primarily due to a decrease in ADH-initiated retinoic acid synthesis (43). The effects of ethanol on retinoid metabolism have been extensively reviewed by Clugston and Blaner (44) as well as Napoli (45).

Cytochrome P450 2E1

In 1966, it was reported that ethanol feeding through newly designed Lieber-DeCarli liquid diets induced proliferation of smooth endoplasmic reticulum (SER) in rats (46). In 1968, Lieber and Decarli reported that microsomal fractions derived from the ethanol-induced SER contain MEOS, and activity of the MEOS requires O_2 and NADPH but partially inhibited by carbon monoxide, a characteristic feature of CYP (47). Now it is well established that CYP2E1 is a major component of MEOS and involved in the metabolic degradation of ethanol. CYP2E1 has a relatively higher K_m for ethanol than ADH (8–10 mM vs. 0.2–2 mM) and only approximately 10% ethanol in the body is metabolized by CYP2E1 (12, 13, 35). CYP2E1 is an active generator of reactive oxygen species (ROS) and has a loose association with cytochrome P450 reductase. So, it generates ROS even in the absence of substrates (48). Therefore, CYP2E1-mediated cytotoxicity or liver injury is associated with oxidative stress (12, 13, 35). For more details, please see historical review early by Lieber (49) and recently by Teschke (50).

The Laboratories of Arthur Cederbaum carried out a series of studies on CYP2E1 in different systems, including isolated microsomes, human hepatoma HepG2 cells overexpressing CYP2E1, the *cyp2e1*$^{-/-}$ mice, and *cyp2e1*$^{-/-}$ mice with human CYP2E1 knock-in (*cyp2e1*$^{-/-}$ KI mice). The microsomes isolated from ethanol-fed rats containing higher levels of CYP2E1 metabolize more ethanol, which was inhibited by hydroxyl radical (·OH) scavengers (51, 52), CYP2E1 inhibitors, or neutralizing antibodies against CYP2E1 (53), suggesting the essential role of CYP2E1 and CYP2E1-mediated ROS in microsomal oxidation of ethanol. Interestingly, when the HepG2 cells constitutively express CYP2E1, antioxidant genes and their encoded antioxidant enzymes were upregulated, which reflects an adaptive mechanism to remove CYP2E1-derived oxidants, but these CYP2E1 overexpressing HepG2 cells were still more sensitive to ethanol-induced cytotoxicity than the HepG2 cells expressing only empty plasmid (54). These studies suggest that upregulation of CYP2E1 could produce ROS, leading to cellular injury. There was no difference in alcoholic liver injury between *cyp2e1*$^{-/-}$ mice and WT mice in a surgical intragastric infusion model (55). However, in a voluntary oral feeding model, Cederbaum group observed less alcoholic steatosis and inflammation in the *cyp2e1*$^{-/-}$ mice than in the WT mice (28). Furthermore, in the *cyp2e1*$^{-/-}$ KI mice that human CYP2E1 was reconstituted into the *cyp2e1*$^{-/-}$ mice, more severe alcoholic liver injury was observed (56). These results suggest that CYP2E1 contributes to the development of ALD *in vivo*.

CYP2E1 may also affect retinoid homeostasis by degrading retinoic acid and retinol into polar metabolites. Metabolism of retinoic acid results in the loss of retinoic acid, promoting carcinogenesis (57). In addition, CYP2E1 metabolically activates a large number of carcinogens that could lead to the development of cancer (58). Therefore, alcohol-induced CYP2E1 plays an important role in alcohol-mediated carcinogenesis.

Catalase

In 1936, Keilin and Hartree first reported that catalase promotes the oxidation of ethanol to acetaldehyde in the presence of a H_2O_2-generating system (59). Catalase is the enzyme that decomposes high concentrations of H_2O_2, so it was surprising that catalase catalyzes the H_2O_2 oxidation of ethanol instead of preventing it. The reaction mainly involves two steps: H_2O_2 production, a primary oxidation system, and ethanol oxidation by H_2O_2 (catalyzed by catalase), a coupled secondary oxidation (60). As shown in Fig. 3, a free catalase binds with one molecule of H_2O_2 to form a catalase-H_2O_2 complex. Second, the catalase-H_2O_2 complex reacts with a second molecule of H_2O_2 to decompose both the two molecules of H_2O_2 to two molecules of H_2O and one molecule of O_2, which was called "catalatic" reaction (60). Third, if ethanol is present, then the H_2O_2 in the catalase-H_2O_2 complex will be used for the oxidation of ethanol, which was called "peroxidatic" reaction. Although the ethanol is oxidized to acetaldehyde, H_2O_2 in the catalase-H_2O_2 complex is decomposed to H_2O, but no O_2 is released (60, 61).

Catalase is mainly located in the peroxisomes. Peroxisomes contain multiple oxidases that make electrons pass directly to O_2 generating H_2O_2 (62). Therefore, peroxisomes are good sites for catalase to oxidize ethanol. The peroxisomal H_2O_2 production by oxidases and catalase-catalyzed ethanol oxidation by H_2O_2 forms a "coupling ethanol oxidation" system (59). The rate of "coupling ethanol oxidation" is mainly limited by the ratio of the H_2O_2 generation rate to the catalase concentration (60). However, when H_2O_2 concentration is too high, catalase will have a priority to decompose H_2O_2, and

1. $\text{Catalase} + H_2O_2 \longrightarrow \text{Catalase-}H_2O_2$
2. $\text{Catalase-}H_2O_2 + H_2O_2 \longrightarrow \text{Catalase} + 2H_2O + O_2$
3. $\text{Catalase-}H_2O_2 + CH_3CH_2OH \longrightarrow \text{Catalase} + 2H_2O + CH_3CHO$

Figure 3. Reactions of catalase oxidizing H_2O_2 and ethanol. CH_3CHO, acetaldehyde; CH_3CH_2OH, ethanol.

only those H_2O_2 gradually and continuously released from a H_2O_2 generation system (H_2O_2 will not be accumulated to reach higher concentrations) is effective for the oxidation of ethanol (59–61, 63). The catalase K_m value for ethanol could not be measured because it depends on the continuous generation of H_2O_2 (60).

Acetaldehyde Dehydrogenase

Acetaldehyde dehydrogenase (ALDH) oxidizes acetaldehyde (64). Acetaldehyde is mainly oxidized by mitochondrial ALDH2 rather than by cytosolic ALDH1 because ALDH2 has a much lower K_m (0.2 μM) than ALDH1 (180 μM) (65). ALDH also needs NAD^+, so ALDH competes with ADH for the availability of NAD^+. In the cytosol, xanthine oxidase (XOD) and aldehyde oxidase (AOD) can also oxidize acetaldehyde. Like other oxidases, XOD and AOD also produce H_2O_2 (66), which will be used for the oxidation of ethanol by catalase (Fig. 4). In an *in vitro* biochemical reaction system, the addition of Schardinger enzyme (the old name for XOD) made the catalase metabolism of ethanol continuously go on until all the ethanol was metabolized, which was called "cyclic ethanol oxidation" (59). However, the K_m values of AOD and XOD for acetaldehyde are 1 mM and 30 mM, respectively (66), which are much higher than the K_m values of ALDH1 and ALDH2. It is unclear whether *in vivo* XOD and AOD play a significant role in the oxidation of acetaldehyde. If yes, under HFD plus ethanol, fatty acid β-oxidation and ADH oxidation of ethanol consume more NAD^+, and consequently, NAD^+ availability for ALDH will be limited, and thus ALDH oxidation of acetaldehyde will slow down, and XOD/AOD oxidation of acetaldehyde and "cyclic ethanol oxidation" will be increased.

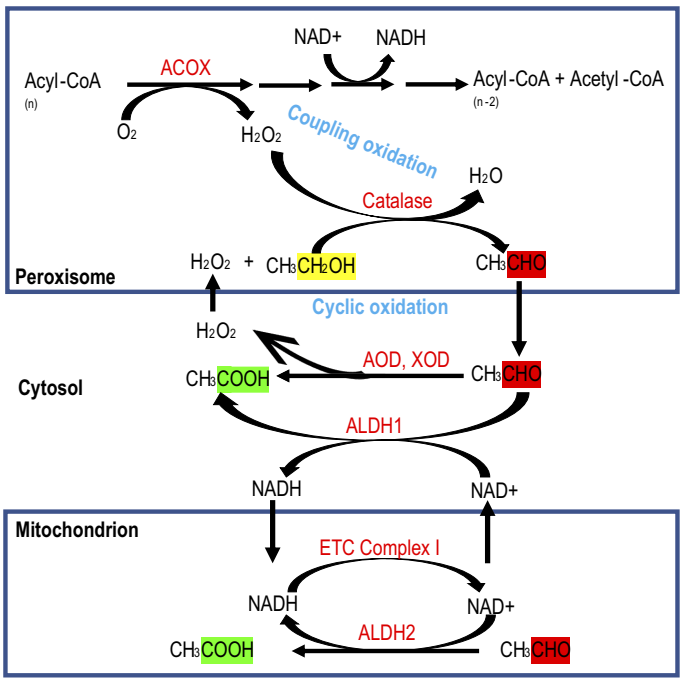

Figure 4. H_2O_2 generation and ethanol-acetaldehyde oxidation. AOD, aldehyde oxidase; CH_3CHO, acetaldehyde; CH_3COOH, acetic acid; CH_3CH_2OH, ethanol; ETC Complex I, electron transport chain Complex I; XOD, xanthine oxidase. *n*, carbon number in acyl-CoA; n-2, 2 carbons are removed after a cycle of oxidation.

INTERACTION OF FAO AND ETHANOL-ACETALDEHYDE METABOLISM

There are no efficient feedback mechanisms to regulate ethanol metabolism (13, 40). However, ethanol-acetaldehyde metabolism in the cytosol, ER, peroxisomes, and mitochondria can change the redox state of the liver. Ethanol metabolism could lead to alterations in hepatic lipid, carbohydrate, protein, lactate, and uric acid metabolism, which may affect ethanol metabolism. Here, we focus on the molecular interactions between FAO and ethanol metabolism.

Peroxisomal Fatty Acid β-Oxidation and Ethanol Metabolism

As discussed in *Fatty Acid β-Oxidation*, four steps of fatty acid β-oxidation take place in the peroxisomes and mitochondria, but step 1 is different in the peroxisomes and mitochondria (Fig. 5). In peroxisomes, ACOX catalyzes the step 1 reaction and produces H_2O_2, which is detoxified by catalase or is used for ethanol oxidation by catalase if ethanol is present.

Generally, catalase is considered to play a minor or insignificant role in ethanol metabolism *in vivo*. Under normal physiological conditions, peroxisomes are responsible for approximately 20% of oxygen consumption in the liver (17). Recently, Zhong and colleagues reported that after 8 wk of ethanol feeding, $Cat^{-/-}$ mice exhibited higher levels of ethanol in the liver and blood than the WT mice (67), suggesting that catalase may also play a significant role in ethanol metabolism *in vivo*. Furthermore, Zhong and colleagues fed male mice with the Lieber-DeCarli ethanol diet for 7 wk, followed by 1 wk of the ethanol diet supplied with 25 mg/L WY-14,643. They noticed that serum levels of ethanol were decreased dramatically by WY-14,643, which was paralleled with the induced catalase and suppressed CYP2E1, while ADH1 and ALDH2 were not altered, indicating that the induced catalase is responsible for the decrease in serum levels of ethanol (67).

Alternatively, we fed female mice with the Lieber-DeCarli ethanol diet containing 0–10 mg/L WY-14,643 for 3 wk. Serum levels of ethanol were strikingly decreased with WY-14,643, which was not observed in the $ppar\alpha^{-/-}$ mice but noticed in the mice lacking liver fatty acid binding protein (L-FABP). This suggests that PPARα regulates this process, but L-FABP has no effect. Similarly, catalase was induced but ADH and CYP2E1 were not induced by WY-14,643, which was not observed in the $ppar\alpha^{-/-}$ mice, either (68, 69). The catalase inhibitor 3-AT reversed the WY-14,643-decreased levels of ethanol in the serum (68). In a binge alcohol model, we treated WY-14,643-fed mice with 2.5 g/kg ethanol by gavage and collected the blood at different time points for ethanol assay. The results depicted that blood ethanol clearance was escalated with WY-14,643 treatment, which was not observed in $Cat^{-/-}$ mice (70), suggesting that catalase is essential for the WY-14,643 to enhance ethanol metabolism.

As discussed before, the catalase oxidation of ethanol needs H_2O_2. Peroxisomal ACOX1 generates H_2O_2 when it oxidizes fatty acids. Ethanol oxidation by catalase can be stimulated by fatty acids because the peroxisomal FAO

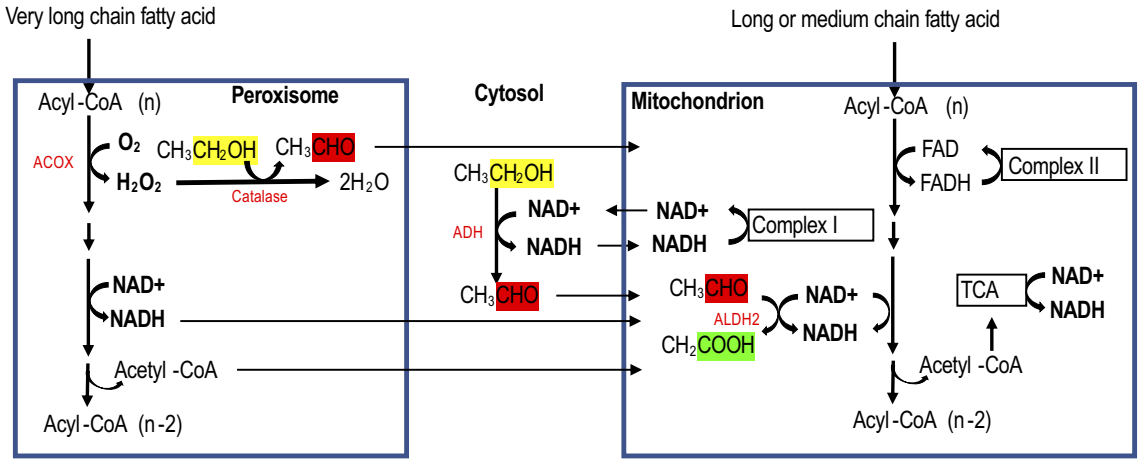

Figure 5. Fatty acid β-oxidation and ethanol-acetaldehyde oxidation. CH_3CHO, acetaldehyde; CH_3COOH, acetic acid; CH_3CH_2OH, ethanol; TCA, tricarboxylic acid cycle. *n*, carbon number in acyl-CoA; n-2, 2 carbons are removed after a cycle of oxidation.

produces H_2O_2. It had been shown that in perfused livers, supplying fatty acids increased H_2O_2 production by eightfold, which was in parallel with the increased ethanol metabolism (71). Consistently, under fasting conditions, serum fatty acids are elevated, and hepatic ethanol metabolism is mediated predominantly by catalase-H_2O_2 (72). It has been demonstrated that HFD can induce peroxisomal FAO (73–75). Interestingly, the Lieber-DeCarli diets used for the experimental ALD model in rodents are designed to be HFD (40% calories from fat vs. 12% calories from fat in the normal chow). ACOX1-mediated H_2O_2 production is such an important event that WY-14,643 could enhance ethanol metabolism by inducing ACOX1 alone even though catalase was not induced (70). Most of the very long-chain fatty acids are directly oxidized by ACOX1 in peroxisomes. However, arachidonic acid (AA) is usually esterified to phospholipids and does not undergo FAO (76). Released from phospholipids by phospholipase A2 (PLA2), AA is converted by cyclooxygenase 2 (COX-2) to prostaglandins, which are ACOX1 substrates (77, 78). Thus, PLA2, COX-2, and ACOX1 were induced by WY-14,643 to attenuate fat accumulation in the liver in the Lieber-DeCarli model of ALD, which was not observed in the *ppar*$\alpha^{-/-}$ mice (69).

It is well known that WY-14,643 induces peroxisome proliferation (62), which is also a PPARα-dependent process (79). Peroxisomal biogenesis factor peroxin 16 (PEX16) is a key regulator for the biogenesis of peroxisomes (80, 81). Humans with mutation of PEX16 lack structurally intact peroxisomes (82). PEX16 was also involved in the WY-14,643 induction of peroxisome proliferation. Liver-specific PEX16 knockout mice (*Pex16*$^{Alb\text{-}Cre}$) displayed increased hepatic ACOX1 and catalase, which were in the cytoplasm due to lack of peroxisomes. However, the increased ACOX1 and catalase did not increase ethanol metabolism, suggesting that intact peroxisomes provide a favorable intracellular environment for the catalase metabolism of ethanol (70). It is well known that PPARα regulates peroxisomal FAO (62, 83), and PPARα activation by WY-14,643 prevents hepatic TG accumulation in the experimental ALD model (10). Thus, in the peroxisomes, both lipid metabolism and ethanol metabolism are enhanced by PPARα activation, and an interaction between lipid metabolism and ethanol metabolism is present in peroxisomes.

Mitochondrial Fatty Acid β-Oxidation and Acetaldehyde Metabolism

Mitochondria are the most important cellular compartment for acetaldehyde oxidation because mitochondrial ALDH2 has the lowest K_m value for acetaldehyde, i.e., ALDH2 has the highest affinity for acetaldehyde among the acetaldehyde metabolism enzymes, including ALDH1, XOD, and AOD as we discussed in *Catalase*. ALDH2 needs NAD^+ to accept the hydrogen removed from acetaldehyde and form NADH. Mitochondria are active in complete FAO, including mitochondrial fatty acid β-oxidation, TCA cycle, and oxidative phosphorylation in the ETC. Mitochondrial fatty acid β-oxidation and TCA cycle also consume NAD^+ to form NADH. Thus, within mitochondria, ALDH2 competes with fatty acid β-oxidation and TCA cycle for the availability of NAD^+. Oxidation of NADH back to NAD^+, an essential factor in FAO and ethanol metabolism, is predominant in the liver (40). Through the ETC, NADH is converted back to NAD^+ for reuse as the acceptor for hydrogen (Figs. 4 and 5). Acetaldehyde dehydrogenation to acetic acid in mitochondria will also contribute to the production of ATP as a form of energy. Ethanol oxidation may release 7 kcal/g of energy, which is higher than glucose and protein (both 4 kcal/g) but lower than fat (9 kcal/g).

Mitochondrial fatty acid β-oxidation does not generate H_2O_2, but the mitochondrial ETC will generate superoxide anion, which will be converted to H_2O_2 by superoxide dismutase (SOD). Catalase was also reported to be detected in mitochondria (84), but it is unclear whether mitochondrial catalase directly metabolizes ethanol at a significant rate. Mitochondria also express CYP2E1, and ethanol treatment induces mitochondrial CYP2E1-mediated ROS production and oxidative stress (85). It is speculated that mitochondria are potential sites for ethanol metabolism, which needs to be directly measured to prove it. If mitochondria metabolize ethanol, then the produced acetaldehyde will be further locally oxidized by ALDH2 to acetic acid,

which will be further locally oxidized to produce ATP in mitochondria.

Interaction of Microsomal Fatty Acid ω-Oxidation and Ethanol Metabolism

It is well established that CYP2E1 metabolically oxidizes ethanol into acetaldehyde (35). CYP2E1 also catalyzes ω-oxidation of fatty acids to produce dicarboxylic acids (26). Interestingly, under pathological conditions such as type II diabetes and obesity with elevated serum ketone bodies and/or fatty acids, liver CYP2E1 is also upregulated (86). Thus, ethanol-induced CYP2E1 will promote both ethanol metabolism and ω-oxidization of HFD-derived fatty acids. If the fatty acid ω-oxidation substrate is medium chain like lauric acid (12-C), the resultant dicarboxylic acid will be β-oxidized in mitochondria. If the ω-oxidation substrates are PUFAs like DHA (22-C; rich in fish oil) and AA or its derived eicosanoids (20-C), the final dicarboxylic acids will be initially β-oxidized in peroxisomes, which will generate H_2O_2 to promote the development of ALD. Indeed, when the rats were fed with the Lieber-DeCarli liquid ethanol diets supplemented with fish oil containing very long-chain PUFAs, severe alcoholic liver injury was developed, but if the ethanol diets were supplemented with medium-chain triglycerides, the rats displayed normal liver (87).

The ω-oxidization of fatty acids produces ω-hydroxy fatty acids, which will be further oxidized by ADH (Fig. 6). The ADH K_m values for oxidation of the ω-hydroxy fatty acids are lower than the K_m for ADH oxidation of ethanol. Therefore, in *in vitro* studies, higher concentration of ethanol is needed to inhibit ADH oxidation of ω-hydroxy fatty acids, but lower concentration of ω-hydroxy fatty acids is enough to inhibit ADH oxidation of ethanol (41). However, in rats, ethanol feeding significantly increased but did not inhibit microsomal fatty acid ω-oxidation (88), which might be a secondary reaction to the total inhibited FAO by ethanol because ω-oxidation is increased in response to a decreased β-oxidation (24).

Interaction of Peroxisomal Fatty Acid α-Oxidation and Ethanol Metabolism

As a branched-chain fatty acid, phytanic acid undergoes α-oxidization followed by β-oxidation, both of which take place in peroxisomes. Published reports are not available about the interaction of peroxisomal fatty acid α-oxidation and catalase metabolism of ethanol. We are investigating the role of phytanic acid precursor phytol on ethanol metabolism. Phytol has been mixed with the Lieber-DeCarli ethanol diet at a concentration of 1 mL/L (0.1%, vol/vol). After 2 wk of feeding, serum ethanol was lower in the mice fed with the ethanol diet containing phytol than in the mice fed with the ethanol diet without phytol, implicating that phytol enhances ethanol metabolism (Fig. 7*A*). Phytol induced catalase but did not induce CYP2E1, ADH1, and ALDH (Fig. 7*D*), suggesting that phytol lowering serum ethanol is due to the induction of catalase. The catalase was found to coordinate with ACOX1 for ethanol metabolism (70). Indeed, ACOX1 was also induced by phytol (Fig. 7*D*). Mild hepatomegaly as indicated by increased liver index (liver wt/body wt × 100) was also observed after the phytol treatment (Fig. 7*B*). In addition, phytol also induced the proliferation of peroxisomes, as indicated by the upregulation of PMP70, a peroxisome marker (Fig. 7*D*). Phytol also suppressed serum levels of TG (Fig. 7*C*). Initially, we hypothesized that the phytol metabolite phytanic acid undergoes α-oxidization to generate pristanic acid, which undergoes β-oxidation in peroxisomes, producing H_2O_2 to promote the catalase metabolism of ethanol. The effects of phytol we observed are similar to the effects of WY-14,643, implicating that phytol exerts effects via PPARα. Indeed, in the liver, phytol is metabolized to phytanic acid in a PPARα-dependent manner (34). Phytanic acid and pristanic acid were also demonstrated to be naturally occurring ligands of PPARα (33). Thus, phytol activation of PPARα pathway may play an important role in the phytol enhancing effect on ethanol metabolism.

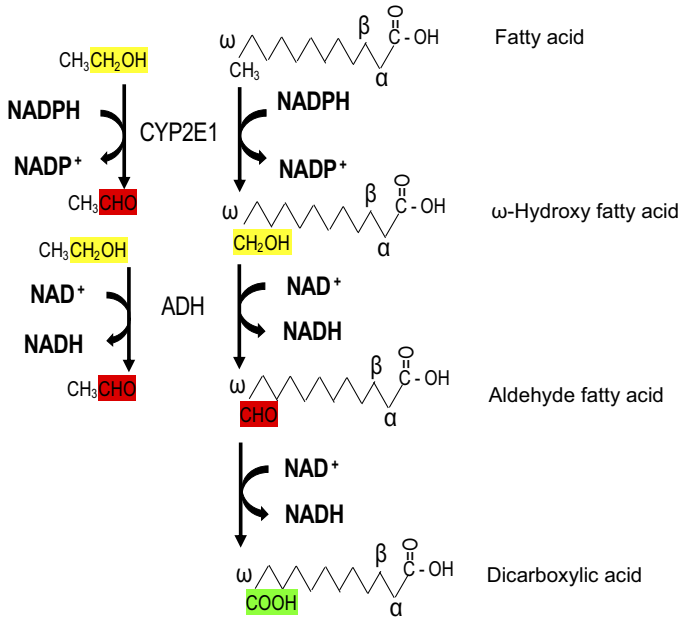

Figure 6. Interaction of fatty acid ω–oxidation and ethanol oxidation.

ETHANOL METABOLISM: DETOXIFICATION OR BIOACTIVATION?

ALDH2 Detoxification of Acetaldehyde

Many drugs induce proliferation of SER, which contains drug-metabolizing enzymes including CYPs to biotransform the drugs to be more water-soluble for excretion and/or less toxic than the parent form, which is called detoxification. However, some chemical biotransformation may also lead to the formation of reactive metabolites that are more toxic, which is called bioactivation. Ethanol metabolism is a typical bioactivation process because ethanol metabolism forms acetaldehyde, a toxic metabolite. Fortunately, ALDH2 affinity for acetaldehyde is much higher (K_m = 0.2 μM) than ADH affinity for ethanol (K_m = 0.2–2 mM), so normally acetaldehyde is present in the body at a much lower concentration than ethanol. However, ethanol-induced cellular damage during chronic alcohol consumption is considered to be associated with biomacromolecular adducts formed with acetaldehyde (89, 90). The Laboratories of Bin Gao at NIAAA

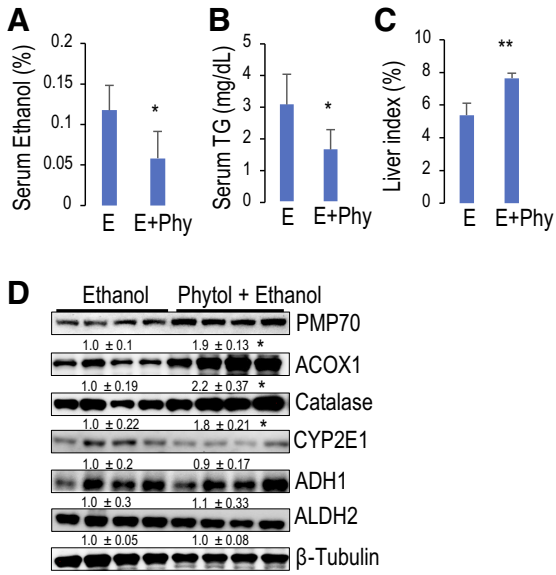

Figure 7. Phytol feeding lowered serum ethanol (*A*) and TG (*B*) but increased liver index (*C*) and induced PMP70, ACOX1, and catalase (*D*). *$P < 0.05$ and **$P < 0.01$, compared with Ethanol group. E, ethanol; E + Phy, ethanol + phytol.

carried out experiments using *Aldh2*$^{-/-}$ mice to examine the role of ALDH2 in the development of ALD. In the NIAAA model initially developed by the Bin Gao group (a binge-on-chronic model), the *Aldh2*$^{-/-}$ mice displayed higher serum levels of acetaldehyde and developed more severe liver inflammation than the WT mice, although the *Aldh2*$^{-/-}$ mice were resistant to alcoholic steatosis (91). When the ethanol-fed mice were injected with a low dose of carbon tetrachloride (CCl_4), the *Aldh2*$^{-/-}$ mice developed more severe fibrosis than the WT mice (91). However, in a binge model, after a binge ethanol administration (5 g/kg for 9 h) by gavage, a similar degree of liver injury in the *Aldh2*$^{-/-}$ mice and WT mice was observed, even when the binge ethanol was administrated after 3 mo of HFD feeding (39). In contrast, the Laboratory of B-J Song, another NIAAA group, observed a clear liver injury in the *Aldh2*$^{-/-}$ mice but not in the WT mice after a low dose of ethanol gavage (3.5 g/kg for 6 h) (92).

ADH and Alcoholic Steatosis

ADH oxidation of ethanol produces NADH and increases the ratio of NADH/NAD^+, which inhibits FAO and promotes steatosis (12, 13). Experiments in both chronic and acute models showed that the absence of ADH leads to elevated serum ethanol levels. In ADH-deficient deer mice, after chronic ethanol feeding, blood alcohol concentrations were 1.5 times higher than in normal deer mice; pancreatic FAEE, a nonoxidative ethanol metabolite, was remarkably increased, and significant pancreatic injury was detected only in the ADH-deficient deer mice but not in the normal deer mice (93). Similarly, after binge ethanol gavage, ADH1 knockout (*Adh1*$^{-/-}$) mice displayed higher levels of serum ethanol than the WT mice, and the *Adh1*$^{-/-}$ mice developed more severe liver injury than the WT mice, which was also mediated by FAEE (39).

CYP2E1-Induced Oxidative Liver Injury

Alcohol consumption also induces SER to metabolize ethanol via the upregulated CYP2E1, which contributes to the development of ALD in the Lieber-DeCarli model (28). In the *cyp2e1*$^{-/-}$ KI mice fed with the Lieber-DeCarli ethanol diet, when severe alcoholic liver injury was observed (56), an enhanced ethanol metabolism, as indicated by reduced serum ethanol levels, was also observed, which was in parallel with hypoxia induced by ethanol feeding (94). In binge models, CYP2E1 is not significantly induced, so ethanol gavage-induced alcoholic liver injury in WT mice was similar to the *cyp2e1*$^{-/-}$ mice (39). In contrast, in the *cyp2e1*$^{-/-}$ KI mice where CYP2E1 is highly expressed, binge ethanol (6 g/kg for 4 h) induced significant liver injury as indicated by the increased serum levels of ALT and AST, which was not observed in the *cyp2e1*$^{-/-}$ mice (94). Therefore, the elevated CYP2E1 (including ethanol-induced and constitutively highly expressed) but not the basal CYP2E1 plays a significant role in the development of ALD. Adaptive mechanisms have been developed to counteract the CYP2E1-mediated oxidative liver injury. As discussed in *Cytochrome P450 2E1,* antioxidant levels are increased to remove CYP2E1-derived ROS (54). In addition, CYP2E1-generated ROS activates Nrf2 to upregulate CYP2A5 (95, 96), and the induced CYP2A5 is protective against the development of ALD because ALD is more severe in the *cyp2a5*$^{-/-}$ mice than WT mice (97, 98). These protective mechanisms are developed to counteract the CYP2E1-mediated liver injury, implicating that CYP2E1-mediated ethanol metabolism is a process of bioactivation rather than detoxification.

Catalase Oxidation of Ethanol and Liver Injury

Unlike ADH and CYP2E1, catalase oxidizes ethanol without formation of large amounts of NADH (inhibiting fatty acid oxidation) or depletion of NADPH (inducing oxidative stress), catalase seems to provide an innocuous metabolic pathway for alcohol oxidation. When H_2O_2 concentrations are higher, catalase has a priority for the "catalatic" reaction to decompose H_2O_2. Only at lower H_2O_2 concentrations, "peroxidatic" reaction of catalase to oxidize ethanol is initiated, and only those H_2O_2 gradually and continually released from a H_2O_2 generation system is effective for the ethanol oxidation (59–61, 63). When the ratio of a H_2O_2 generation (μM/min) to catalase concentration (μM) was 10 and above, H_2O_2 was decomposed to H_2O and O_2. However, as the ratio decreased, although the H_2O_2 was still decomposed, production of O_2 was increasingly diminished, and at a ratio of 1, O_2 formation was no longer observed (99), and an alternate pathway was proposed that catalase degrades H_2O_2 and generates ·OH instead of O_2 and H_2O (100). Similarly, in the presence of ethanol, O_2 is not produced when catalase oxidizes ethanol and H_2O_2 to acetaldehyde and H_2O, respectively (Figs. 2 and 3). It is unclear whether catalase metabolism of ethanol also produces ·OH, which is more active and toxic. Catalase is an antioxidant enzyme, so it is not surprising that the *Cat*$^{-/-}$ mice developed more severe alcoholic liver injury than the WT mice (67). However, when catalase metabolism was enhanced by WY-14,643, liver injury was observed even though steatosis was ameliorated (68, 70). Under peroxisome proliferation, ACOX1 was induced to a greater extent than

catalase, which is considered a reason why PPARα agonists induce oxidative stress (62). Therefore, the markedly increased catalase metabolism of ethanol might be associated with the enhanced alcoholic liver injury. On the other hand, under peroxisome proliferation, the ratio of ACOX1-generated H_2O_2 to catalase might also be increased, which makes catalase prefer oxidizing H_2O_2, but not ethanol.

To summarize, different ethanol metabolism pathways could produce different effects. While ADH-mediated ethanol metabolism may alter the redox status of NAD^+/NADH, CYP2E1 mediates oxidative stress. CYP2E1 has a higher K_m value than ADH, but when ADH is absent and intracellular ethanol is increased, CYP2E1-mediated ethanol metabolism will be increased, and oxidative liver injury will be induced. Catalase oxidation of ethanol is dependent on peroxisomal fatty acid β-oxidation-generated H_2O_2. Both ethanol oxidation by ADH and peroxisomal fatty acid β-oxidation are NAD^+-dependent reactions (Fig. 5). NADH generated in peroxisomes (fatty acid β-oxidation) and cytosols (ethanol metabolism by ADH) would be reoxidized to NAD^+ in mitochondria to be reused as a coenzyme in the oxidation of fatty acids and ethanol (74). Therefore, increases in the NADH/NAD ratio caused by ADH ethanol metabolism may inhibit catalase oxidation of ethanol.

DIFFERENCE BETWEEN MASLD AND ALD: DUE TO ETHANOL METABOLISM?

Obesity and consumption of HFD are associated with MASLD. MASLD was previously referred to nonalcoholic fatty liver diseases (NAFLD), which shares with ALD many aspects of pathology and pathogenic mechanisms, and both MASLD and ALD are linked to the same risk factor: obesity (2). The comparison between ALD and MASLD, including their epidemiology, diagnosis, management, pathophysiology mechanisms, possible future treatments, and prevention, has been extensively reviewed recently (2). Generally, during the development of MASLD and ALD, there is a "fat flow" from adipose tissues to the liver. In either case, fat flows in free fatty acids. The radical difference in etiology between MASLD and ALD is ethanol consumption. In the presence of ethanol, liver injury is enhanced, which is associated with the interaction of FAO and ethanol metabolism in the liver.

Synergistic Effect between Obesity and Ethanol

Otsuka Long-Evans Tokushima fatty (OLETF) rats spontaneously develop obesity with simple steatosis in the liver. When the animals were administered 10 mL of 10% ethanol orally for 3 consecutive weeks, massive steatohepatitis and hepatocyte ballooning were observed, whereas the same courses of ethanol administration did not induce steatohepatitis in their wild-type control Otsuka Long-Evans Tokushima (OLET) rats (101). In another model of obesity, obese fa/fa rats and lean Fa/? rats were treated with six doses of ethanol (4 g/kg every 12 h for 3 days), liver injury was observed in the obese fa/fa rats but not in the lean Fa/? rats (102). In chronic rodent models, HFD induces MASLD with the co-occurrence of obesity. After 3 mo of HFD feeding, the mice became obese, and serum ALT increased, indicating that steatohepatitis (NASH) had developed. When a binge of 5 g/kg of ethanol was administered, the increased serum ALT was further elevated (103). Similarly, in another model, combination of ethanol with high-fructose, high-fat, high-cholesterol diet [FFC; 40% calories from fat, 20% calories from fructose, and 2% (wt/wt) of cholesterol] feeding caused an increase in serum ALT: after 4 wk of feeding, the mice were fed 5% ethanol in water plus weekly ethanol gavage at 2.5 g/kg for another 12 wk, while the FFC diet continued (104). These results suggest that ethanol promotes steatohepatitis in obese rodents.

Synergistic Effect between HFD and Ethanol

MASLD is correlated with increased energy intakes, especially in the form of added sugars, and HFD is another major risk factor for MASLD (105). When mice were fed HFD only for 3 days, no significant body weight gain was observed. However, a binge of 5 g/kg of ethanol induced liver injury in the mice fed with HFD for 3 days but not in mice fed with normal chow diet, suggesting that even in the absence of HFD-induced obesity, ethanol in combination with HFD feeding could still induce liver injury (103). Under normal chow with low-fat content (~10% calories from fat), ethanol mixed in drinking water at 10% (vol/vol) did not even induce simple steatosis, but the Lieber-DeCarli diets are designed to be HFD to facilitate the induction of experimental ALD, so the detected ALD in this model reflects a synergistic effect of HFD and ethanol (12). In binge models, when animals are fed normal chow with low fat, high doses of ethanol (5–7 g/kg) are required to induce liver injury. Binge ethanol induces lipolysis in adipose tissues to increase circulating FFA, which contributes to the development of acute liver injury (4). When the animals were fed a period of HFD feeding (3 days—3 mo), binge ethanol gavage induced much more severe liver injury, suggesting the important role of dietary fat in the binge-induced liver injury (103). In the chronic Lieber-DeCarli model with a high fat content, alcoholic liver injury was still developed even though serum fatty acids were increased to a lesser extent than in the acute models (5).

Synergistic Effect between Methionine-Choline-Deficient Diet and Ethanol

Unlike HFD, methionine-choline-deficient diet (MCD) induced MASLD with a body weight loss instead of obesity, but the combination of MCD and ethanol feeding still developed more severe liver injury than ethanol or MCD alone, and hepatic FFA contents in the combined feeding group were detected to be increased (106). Interestingly, PPARα activation by WY-14,643 has different effects on ALD and MCD-induced MASLD in mouse model. In the MCD-induced MASLD model, steatosis, steatohepatitis, and fibrosis were observed in mice. When WY-14,643 was added in the MCD diet (0.1% wt/wt), MCD-induced steatosis, steatohepatitis, and fibrosis were prevented (11). However, in the Lieber-DeCarli model of ALD, when WY-14,643 was added in ethanol liquid diet (10 mg/L, 0.001% wt/vol), catalase-mediated ethanol metabolism was enhanced and serum ALT was increased, suggesting that ethanol is synergistic with WY-14,643 to induce liver injury (68, 70). The different effect of WY-14,643 on liver pathology between ALD and MCD-induced MASLD could be attributed to ethanol.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y.L. conceived and designed research; Y.L. performed experiments; Y.L. analyzed data; Y.L. interpreted results of experiments; Y.L. prepared figures; Y.L. drafted manuscript; Y.L. and J.G. edited and revised manuscript; Y.L. approved final version of manuscript.

REFERENCES

1. **Bataller R**, **Arab JP**, **Shah VH.** Alcohol-associated hepatitis. *N Engl J Med* 387: 2436–2448, 2022. doi:10.1056/NEJMra2207599.
2. **Malnick SDH**, **Alin P**, **Somin M**, **Neuman MG.** Fatty liver disease-alcoholic and non-alcoholic: similar but different. *Int J Mol Sci* 23: 16226, 2022. doi:10.3390/ijms232416226.
3. **Donnelly KL**, **Smith CI**, **Schwarzenberg SJ**, **Jessurun J**, **Boldt MD**, **Parks EJ.** Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 115: 1343–1351, 2005. doi:10.1172/JCI23621.
4. **Horning MG**, **Williams EA**, **Maling HM**, **Brodie BB.** Depot fat as source of increased liver triglycerides after ethanol. *Biochem Biophys Res Commun* 3: 635–640, 1960. doi:10.1016/0006-291x(60)90077-2.
5. **Zhong W**, **Zhao Y**, **Tang Y**, **Wei X**, **Shi X**, **Sun W**, **Sun X**, **Yin X**, **Sun X**, **Kim S**, **McClain CJ**, **Zhang X**, **Zhou Z.** 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, 2012. doi:10.1016/j.ajpath.2011.11.017.
6. **Hwang S**, **Ren T**, **Gao B.** Obesity and binge alcohol intake are deadly combination to induce steatohepatitis: a model of high-fat diet and binge ethanol intake. *Clin Mol Hepatol* 26: 586–594, 2020. doi:10.3350/cmh.2020.0100.
7. **Lieber CS**, **DeCarli LM.** Quantitative relationship between amount of dietary fat and severity of alcoholic fatty liver. *Am J Clin Nutr* 23: 474–478, 1970. doi:10.1093/ajcn/23.4.474.
8. **Nakajima T**, **Kamijo Y**, **Tanaka N**, **Sugiyama E**, **Tanaka E**, **Kiyosawa K**, **Fukushima Y**, **Peters JM**, **Gonzalez FJ**, **Aoyama T.** Peroxisome proliferator-activated receptor α protects against alcohol-induced liver damage. *Hepatology* 40: 972–980, 2004. doi:10.1002/hep.20399.
9. **Ip E**, **Farrell GC**, **Robertson G**, **Hall P**, **Kirsch R**, **Leclercq I.** Central role of PPARα-dependent hepatic lipid turnover in dietary steatohepatitis in mice. *Hepatology* 38: 123–132, 2003. doi:10.1053/jhep.2003.50307.
10. **Fischer M**, **You M**, **Matsumoto M**, **Crabb DW.** Peroxisome proliferator-activated receptor alpha (PPARα) agonist treatment reverses PPARα dysfunction and abnormalities in hepatic lipid metabolism in ethanol-fed mice. *J Biol Chem* 278: 27997–28004, 2003. doi:10.1074/jbc.M302140200.
11. **Ip E**, **Farrell G**, **Hall P**, **Robertson G**, **Leclercq I.** Administration of the potent PPARα agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. *Hepatology* 39: 1286–1296, 2004. May doi:10.1002/hep.20170.
12. **Lieber CS.** Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. *Alcohol* 34: 9–19, 2004. doi:10.1016/j.alcohol.2004.07.008.
13. **Lieber CS.** Metabolism of alcohol. *Clin Liver Dis* 9: 1–35, 2005. doi:10.1016/j.cld.2004.10.005.
14. **Jeon S**, **Carr R.** Alcohol effects on hepatic lipid metabolism. *J Lipid Res* 61: 470–479, 2020. Apr doi:10.1194/jlr.R119000547.
15. **You M**, **Arteel GE.** Effect of ethanol on lipid metabolism. *J Hepatol* 70: 237–248, 2019. Feb doi:10.1016/j.jhep.2018.10.037.
16. **Reddy JK**, **Hashimoto T.** Peroxisomal β-oxidation and peroxisome proliferator-activated receptor α: an adaptive metabolic system. *Annu Rev Nutr* 21: 193–230, 2001. doi:10.1146/annurev.nutr.21.1.193.
17. **De Duve C**, **Baudhuin P.** Peroxisomes (microbodies and related particles). *Physiol Rev* 46: 323–357, 1966. doi:10.1152/physrev.1966.46.2.323.
18. **Nelson DL**, **Cox MM.** *Lehninger Principles of Biochemistry* (7th ed.). New York: W. H. Freeman and Company, 2017.
19. **Lefèvre A**, **Adler H**, **Lieber CS.** Effect of ethanol on ketone metabolism. *J Clin Invest* 49: 1775–1782, 1970. doi:10.1172/JCI106395.
20. **Wiers CE**, **Vendruscolo LF**, **van der Veen JW**, **Manza P**, **Shokri-Kojori E**, **Kroll DS**, **Feldman DE**, **McPherson KL**, **Biesecker CL**, **Zhang R**, **Herman K**, **Elvig SK**, **Vendruscolo JCM**, **Turner SA**, **Yang S**, **Schwandt M**, **Tomasi D**, **Cervenka MC**, **Fink-Jensen A**, **Benveniste H**, **Diazgranados N**, **Wang GJ**, **Koob GF**, **Volkow ND.** Ketogenic diet reduces alcohol withdrawal symptoms in humans and alcohol intake in rodents. *Sci Adv* 7: eabf6780, 2021. doi:10.1126/sciadv.abf6780.
21. **Mahajan VR**, **Elvig SK**, **Vendruscolo LF**, **Koob GF**, **Darcey VL**, **King MT**, **Kranzler HR**, **Volkow ND**, **Wiers CE.** Nutritional ketosis as a potential treatment for alcohol use disorder. *Front Psychiatry* 12: 781668, 2021. doi:10.3389/fpsyt.2021.781668.
22. **Antony GJ**, **Landau BR.** Relative contributions of α-, β-, and ω-oxidative pathways to in vitro fatty acid oxidation in rat liver. *J Lipid Res* 9: 267–269, 1968. doi:10.1016/S0022-2275(20)43128-1.
23. **Wada F**, **Usami M.** Studies on fatty acid ω-oxidation. Antiketogenic effect and gluconeogenicity of dicarboxylic acids. *Biochim Biophys Acta* 487: 361–368, 1977.
24. **Mortensen PB.** Formation and degradation of dicarboxylic acids in relation to alterations in fatty acid oxidation in rats. *Biochim Biophys Acta* 1124: 71–79, 1992. doi:10.1016/0005-2760(92)90128-i.
25. **Wanders RJ**, **Komen J**, **Kemp S.** Fatty acid ω-oxidation as a rescue pathway for fatty acid oxidation disorders in humans. *FEBS J* 278: 182–194, 2011. doi:10.1111/j.1742-4658.2010.07947.x.
26. **Laethem RM**, **Balazy M**, **Falck JR**, **Laethem CL**, **Koop DR.** Formation of 19(S)-, 19(R)-, and 18(R)-hydroxyeicosatetraenoic acids by alcohol-inducible cytochrome P450 2E1. *J Biol Chem* 268: 12912–12918, 1993. doi:10.1016/S0021-9258(18)31472-8.
27. **Leclercq IA**, **Farrell GC**, **Field J**, **Bell DR**, **Gonzalez FJ**, **Robertson GR.** CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. *J Clin Invest* 105: 1067–1075, 2000. doi:10.1172/JCI8814.
28. **Lu Y**, **Zhuge J**, **Wang X**, **Bai J**, **Cederbaum AI.** Cytochrome P450 2E1 contributes to ethanol-induced fatty liver in mice. *Hepatology* 47: 1483–1494, 2008. doi:10.1002/hep.22222.
29. **Zhang Y**, **Yan T**, **Wang T**, **Liu X**, **Hamada K**, **Sun D**, **Sun Y**, **Yang Y**, **Wang J**, **Takahashi S**, **Wang Q**, **Krausz KW**, **Jiang C**, **Xie C**, **Yang X**, **Gonzalez FJ.** Crosstalk between CYP2E1 and PPARα substrates and agonists modulate adipose browning and obesity. *Acta Pharm Sin B* 12: 2224–2238, 2022. doi:10.1016/j.apsb.2022.02.004.
30. **Mannaerts GP**, **Van Veldhoven PP**, **Casteels M.** Peroxisomal lipid degradation via β- and α-oxidation in mammals. *Cell Biochem Biophys* 32: 73–87, 2000. doi:10.1385/cbb:32:1-3:73.
31. **Verhoeven NM**, **Wanders RJ**, **Poll-The BT**, **Saudubray JM**, **Jakobs C.** The metabolism of phytanic acid and pristanic acid in man: a review. *J Inherit Metab Dis* 21: 697–728, 1998. doi:10.1023/a:1005476631419.
32. **Wanders RJ**, **Jansen GA**, **Skjeldal OH.** Refsum disease, peroxisomes and phytanic acid oxidation: a review. *J Neuropathol Exp Neurol* 60: 1021–1031, 2001. doi:10.1093/jnen/60.11.1021.
33. **Zomer AW**, **van Der Burg B**, **Jansen GA**, **Wanders RJ**, **Poll-The BT**, **van Der Saag PT.** Pristanic acid and phytanic acid: naturally occurring ligands for the nuclear receptor peroxisome proliferator-activated receptor α. *J Lipid Res* 41: 1801–1807, 2000. doi:10.1016/S0022-2275(20)31973-8.
34. **Gloerich J**, **van den Brink DM**, **Ruiter JP**, **van Vlies N**, **Vaz FM**, **Wanders RJ**, **Ferdinandusse S.** Metabolism of phytol to phytanic acid in the mouse, and the role of PPARα in its regulation. *J Lipid Res* 48: 77–85, 2007. doi:10.1194/jlr.M600050-JLR200.
35. **Lu Y**, **Cederbaum AI.** CYP2E1 and oxidative liver injury by alcohol. *Free Radic Biol Med* 44: 723–738, 2008. doi:10.1016/j.freeradbiomed.2007.11.004.
36. **Lange LG.** Nonoxidative ethanol metabolism: formation of fatty acid ethyl esters by cholesterol esterase. *Proc Natl Acad Sci USA* 79: 3954–3957, 1982. doi:10.1073/pnas.79.13.3954.
37. **Tsujita T**, **Okuda H.** Fatty acid ethyl ester synthase in rat adipose tissue and its relationship to carboxylesterase. *J Biol Chem* 267: 23489–23494, 1992. doi:10.1016/S0021-9258(18)35865-4.
38. **Huang W**, **Booth DM**, **Cane MC**, **Chvanov M**, **Javed MA**, **Elliott VL**, **Armstrong JA**, **Dingsdale H**, **Cash N**, **Li Y**, **Greenhalf W**, **Mukherjee**

R, **Kaphalia BS**, **Jaffar M**, **Petersen OH**, **Tepikin AV**, **Sutton R**, **Criddle DN.** Fatty acid ethyl ester synthase inhibition ameliorates ethanol-induced Ca^{2+}-dependent mitochondrial dysfunction and acute pancreatitis. *Gut* 63: 1313–1324, 2014. doi:10.1136/gutjnl-2012-304058.

39. **Park SH**, **Seo W**, **Xu MJ**, **Mackowiak B**, **Lin Y**, **He Y**, **Fu Y**, **Hwang S**, **Kim SJ**, **Guan Y**, **Feng D**, **Yu L**, **Lehner R**, **Liangpunsakul S**, **Gao B.** Ethanol and its nonoxidative metabolites promote acute liver injury by inducing ER stress, adipocyte death, and lipolysis. *Cell Mol Gastroenterol Hepatol* 15: 281–306, 2023. [36243320]doi:10.1016/j.jcmgh.2022.10.002.
40. **Cederbaum AI.** Alcohol metabolism. *Clin Liver Dis* 16: 667–685, 2012.doi:10.1016/j.cld.2012.08.002.
41. **Björkhem I.** On the role of alcohol dehydrogenase in ω-oxidation of fatty acids. *Eur J Biochem* 30: 441–451, 1972. doi:10.1111/j.1432-1033.1972.tb02116.x.
42. **Duester G.** Alcohol dehydrogenase as a critical mediator of retinoic acid synthesis from vitamin A in the mouse embryo. *J Nutr* 128: 459S–462S, 1998. doi:10.1093/jn/128.2.459S.
43. **Molotkov A**, **Duester G.** Retinol/ethanol drug interaction during acute alcohol intoxication in mice involves inhibition of retinol metabolism to retinoic acid by alcohol dehydrogenase. *J Biol Chem* 277: 22553–22557, 2002. doi:10.1074/jbc.M201603200.
44. **Clugston RD**, **Blaner WS.** The adverse effects of alcohol on vitamin A metabolism. *Nutrients* 4: 356–371, 2012. doi:10.3390/nu4050356.
45. **Napoli JL.** Physiological insights into all-trans-retinoic acid biosynthesis. *Biochim Biophys Acta* 1821: 152–167, 2012. doi:10.1016/j.bbalip.2011.05.004.
46. **Iseri OA**, **Lieber CS**, **Gottlieb LS.** The ultrastructure of fatty liver induced by prolonged ethanol ingestion. *Am J Pathol* 48: 535–555, 1966.
47. **Lieber CS**, **DeCarli LM.** Ethanol oxidation by hepatic microsomes: adaptive increase after ethanol feeding. *Science* 162: 917–918, 1968. doi:10.1126/science.162.3856.917.
48. **Ekström G**, **Ingelman-Sundberg M.** Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIE1). *Biochem Pharmacol* 38: 1313–1319, 1989. doi:10.1016/0006-2952(89)90338-9.
49. **Lieber CS.** Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968-1998)–a review. *Alcoholism Clin Exp Res* 23: 991–1007, 1999. doi:10.1111/j.1530-0277.1999.tb04217.x.
50. **Teschke R.** Microsomal ethanol-oxidizing system: success over 50 years and an encouraging future. *Alcohol Clin Exp Res* 43: 386–400, 2019.[30667528] doi:10.1111/acer.13961.
51. **Cederbaum AI**, **Dicker E**, **Cohen G.** Effect of hydroxyl radical scavengers on microsomal oxidation of alcohols and on associated microsomal reactions. *Biochemistry* 17: 3058–3064, 1978. doi:10.1021/bi00608a018.
52. **Klein SM**, **Cohen G**, **Lieber CS**, **Cederbaum AI.** Increased microsomal oxidation of hydroxyl radical scavenging agents and ethanol after chronic consumption of ethanol. *Arch Biochem Biophys* 223: 425–432, 1983. doi:10.1016/0003-9861(83)90606-9.
53. **Kukiełka E**, **Cederbaum AI.** Ferritin stimulation of lipid peroxidation by microsomes after chronic ethanol treatment: role of cytochrome P4502E1. *Arch Biochem Biophys* 332: 121–127, 1996. doi:10.1006/abbi.1996.0323.
54. **Cederbaum AI**, **Wu D**, **Mari M**, **Bai J.** CYP2E1-dependent toxicity and oxidative stress in HepG2 cells. *Free Radic Biol Med* 31: 1539–1543, 2001. doi:10.1016/s0891-5849(01)00743-2.
55. **Kono H**, **Bradford BU**, **Yin M**, **Sulik KK**, **Koop DR**, **Peters JM**, **Gonzalez FJ**, **McDonald T**, **Dikalova A**, **Kadiiska MB**, **Mason RP**, **Thurman RG.** CYP2E1 is not involved in early alcohol-induced liver injury. *Am J Physiol* 277: G1259–G1267, 1999. doi:10.1152/ajpgi.1999.277.6.G1259.
56. **Lu Y**, **Wu D**, **Wang X**, **Ward SC**, **Cederbaum AI.** Chronic alcohol-induced liver injury and oxidant stress are decreased in cytochrome P4502E1 knockout mice and restored in humanized cytochrome P4502E1 knock-in mice. *Free Radic Biol Med* 49: 1406–1416, 2010. doi:10.1016/j.freeradbiomed.2010.07.026.
57. **Seitz HK**, **Wang XD.** The role of cytochrome P450 2E1 in ethanol-mediated carcinogenesis. *Subcell Biochem* 67: 131–143, 2013. doi:10.1007/978-94-007-5881-0_3.
58. **Gonzalez FJ.** The 2006 Bernard B. Brodie Award Lecture. Cyp2e1. *Drug Metab Dispos* 35: 1–8, 2007. [17020953] doi:10.1124/dmd.106.012492.
59. **Keilin D**, **Hartree EF.** Coupled oxidation of alcohol. *Proc R Soc B* 119: 141–159, 1936. doi:10.1098/rspb.1936.0003.
60. **Oshino N**, **Oshino R**, **Chance B.** The characteristics of the "peroxidatic" reaction of catalase in ethanol oxidation. *Biochem J* 131: 555–563, 1973. doi:10.1042/bj1310555.
61. **Keilin D**, **Hartree EF.** Properties of catalase. Catalysis of coupled oxidation of alcohols. *Biochem J* 39: 293–301, 1945. doi:10.1042/bj0390293.
62. **Reddy JK.** Peroxisome proliferators and peroxisome proliferator-activated receptor α: biotic and xenobiotic sensing. *Am J Pathol* 164: 2305–2321, 2004. doi:10.1016/s0002-9440(10)63787-x.
63. **Kirkman HN**, **Gaetani GF.** Mammalian catalase: a venerable enzyme with new mysteries. *Trends Biochem Sci* 32: 44–50, 2007. doi:10.1016/j.tibs.2006.11.003.
64. **Zakhari S**, **Li TK.** Determinants of alcohol use and abuse: Impact of quantity and frequency patterns on liver disease. *Hepatology* 46: 2032–2039, 2007. [18046720] doi:10.1002/hep.22010.
65. **Klyosov AA.** Kinetics and specificity of human liver aldehyde dehydrogenases toward aliphatic, aromatic, and fused polycyclic aldehydes. *Biochemistry* 35: 4457–4467, 1996. doi:10.1021/bi9521102.
66. **Shaw S**, **Jayatilleke E.** The role of aldehyde oxidase in ethanol-induced hepatic lipid peroxidation in the rat. *Biochem J* 268: 579–583, 1990. doi:10.1042/bj2680579.
67. **Yue R**, **Chen GY**, **Xie G**, **Hao L**, **Guo W**, **Sun X**, **Jia W**, **Zhang Q**, **Zhou Z**, **Zhong W.** Activation of PPARα-catalase pathway reverses alcoholic liver injury via upregulating NAD synthesis and accelerating alcohol clearance. *Free Radic Biol Med* 174: 249–263, 2021. doi:10.1016/j.freeradbiomed.2021.08.005.
68. **Chen X**, **Xu Y**, **Denning KL**, **Grigore A**, **Lu Y.** PPARα agonist WY-14,643 enhances ethanol metabolism in mice: role of catalase. *Free Radic Biol Med* 169: 283–293, 2021. doi:10.1016/j.freeradbiomed.2021.04.018.
69. **Xu Y**, **Denning KL**, **Lu Y.** PPARα agonist WY-14,643 induces the PLA2/COX-2/ACOX1 pathway to enhance peroxisomal lipid metabolism and ameliorate alcoholic fatty liver in mice. *Biochem Biophys Res Commun* 613: 47–52, 2022. doi:10.1016/j.bbrc.2022.04.132.
70. **Chen X**, **Denning KL**, **Mazur A**, **Lawrence LM**, **Wang X**, **Lu Y.** Under peroxisome proliferation acyl-CoA oxidase coordinates with catalase to enhance ethanol metabolism. *Free Radic Biol Med* 208: 221–228, 2023. doi:10.1016/j.freeradbiomed.2023.08.016.
71. **Handler JA**, **Thurman RG.** Catalase-dependent ethanol oxidation in perfused rat liver. Requirement for fatty-acid-stimulated H2O2 production by peroxisomes. *Eur J Biochem* 176: 477–484, 1988. doi:10.1111/j.1432-1033.1988.tb14305.x.
72. **Handler JA**, **Thurman RG.** Hepatic ethanol metabolism is mediated predominantly by catalase-H2O2 in the fasted state. *FEBS Lett* 238: 139–141, 1988. doi:10.1016/0014-5793(88)80243-6.
73. **Neat CE**, **Thomassen MS**, **Osmundsen H.** Induction of peroxisomal β-oxidation in rat liver by high-fat diets. *Biochem J* 186: 369–371, 1980. doi:10.1042/bj1860369.
74. **Handler JA**, **Thurman RG.** Redox interactions between catalase and alcohol dehydrogenase pathways of ethanol metabolism in the perfused rat liver. *J Biol Chem* 265: 1510–1515, 1990. doi:10.1016/S0021-9258(19)40046-X.
75. **Thomassen MS**, **Christiansen EN**, **Norum KR.** Characterization of the stimulatory effect of high-fat diets on peroxisomal β-oxidation in rat liver. *Biochem J* 206: 195–202, 1982. doi:10.1042/bj2060195.
76. **Irvine RF.** How is the level of free arachidonic acid controlled in mammalian cells? *Biochem J* 204: 3–16, 1982. doi:10.1042/bj2040003.
77. **Veldhoven PP**, **Vanhove G**, **Assselberghs S**, **Eyssen HJ**, **Mannaerts GP.** Substrate specificities of rat liver peroxisomal acyl-CoA oxidases: palmitoyl-CoA oxidase (inducible acyl-CoA oxidase), pristanoyl-CoA oxidase (non-inducible acyl-CoA oxidase), and trihydroxycoprostanoyl-CoA oxidase. *J Biol Chem* 267: 20065–20074, 1992.
78. **Schepers L**, **Casteels M**, **Vamecq J**, **Parmentier G**, **Van Veldhoven PP**, **Mannaerts GP.** β-Oxidation of the carboxyl side chain of prostaglandin E2 in rat liver peroxisomes and mitochondria. *J Biol Chem* 263: 2724–2731, 1988. doi:10.1016/S0021-9258(18)69128-8.
79. **Lee SS**, **Pineau T**, **Drago J**, **Lee EJ**, **Owens JW**, **Kroetz DL**, **Fernandez-Salguero PM**, **Westphal H**, **Gonzalez FJ.** Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects

of peroxisome proliferators. *Mol Cell Biol* 15: 3012–3022, 1995. doi:10.1128/MCB.15.6.3012.
80. **Kim PK**, **Mullen RT**, **Schumann U**, **Lippincott-Schwartz J.** The origin and maintenance of mammalian peroxisomes involves a de novo PEX16-dependent pathway from the ER. *J Cell Biol* 173: 521–532, 2006. doi:10.1083/jcb.200601036.
81. **Lodhi IJ**, **Semenkovich CF.** Peroxisomes: a nexus for lipid metabolism and cellular signaling. *Cell Metab* 19: 380–392, 2014. doi:10.1016/j.cmet.2014.01.002.
82. **Honsho M**, **Tamura S**, **Shimozawa N**, **Suzuki Y**, **Kondo N**, **Fujiki Y.** Mutation in PEX16 is causal in the peroxisome-deficient Zellweger syndrome of complementation group D. *Am J Hum Genet* 63: 1622–1630, 1998. doi:10.1086/302161.
83. **Pawlak M**, **Lefebvre P**, **Staels B.** Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J Hepatol* 62: 720–733, 2015. doi:10.1016/j.jhep.2014.10.039.
84. **Salvi M**, **Battaglia V**, **Brunati AM**, **La Rocca N**, **Tibaldi E**, **Pietrangeli P**, **Marcocci L**, **Mondovì B**, **Rossi CA**, **Toninello A.** Catalase takes part in rat liver mitochondria oxidative stress defense. *J Biol Chem* 282: 24407–24415, 2007. doi:10.1074/jbc.M701589200.
85. **Knockaert L**, **Fromenty B**, **Robin MA.** Mechanisms of mitochondrial targeting of cytochrome P450 2E1: physiopathological role in liver injury and obesity. *FEBS J* 278: 4252–4260, 2011. doi:10.1111/j.1742-4658.2011.08357.x.
86. **Caro AA**, **Cederbaum AI.** Oxidative stress, toxicology, and pharmacology of CYP2E1. *Annu Rev Pharmacol Toxicol* 44: 27–42, 2004. doi:10.1146/annurev.pharmtox.44.101802.121704.
87. **Polavarapu R**, **Spitz DR**, **Sim JE**, **Follansbee MH**, **Oberley LW**, **Rahemtulla A**, **Nanji AA.** Increased lipid peroxidation and impaired antioxidant enzyme function is associated with pathological liver injury in experimental alcoholic liver disease in rats fed diets high in corn oil and fish oil. *Hepatology* 27: 1317–1323, 1998. doi:10.1002/hep.510270518.
88. **Ma X**, **Baraona E**, **Lieber CS.** Alcohol consumption enhances fatty acid ω-oxidation, with a greater increase in male than in female rats. *Hepatology* 18: 1247–1253, 1993. doi:10.1002/hep.1840180534.
89. **Setshedi M**, **Wands JR**, **Monte SM.** Acetaldehyde adducts in alcoholic liver disease. *Oxid Med Cell Longev* 3: 178–185, 2010. doi:10.4161/oxim.3.3.12288.
90. **Hayashi N**, **George J**, **Takeuchi M**, **Fukumura A**, **Toshikuni N**, **Arisawa T**, **Tsutsumi M.** Acetaldehyde-derived advanced glycation end-products promote alcoholic liver disease. *PLoS One* 8: e70034, 2013. doi:10.1371/journal.pone.0070034.
91. **Kwon HJ**, **Won YS**, **Park O**, **Chang B**, **Duryee MJ**, **Thiele GE**, **Matsumoto A**, **Singh S**, **Abdelmegeed MA**, **Song BJ**, **Kawamoto T**, **Vasiliou V**, **Thiele GM**, **Gao B.** Aldehyde dehydrogenase 2 deficiency ameliorates alcoholic fatty liver but worsens liver inflammation and fibrosis in mice. *Hepatology* 60: 146–157, 2014. doi:10.1002/hep.27036.
92. **Rungratanawanich W**, **Lin Y**, **Wang X**, **Kawamoto T**, **Chidambaram SB**, **Song BJ.** ALDH2 deficiency increases susceptibility to binge alcohol-induced gut leakiness, endotoxemia, and acute liver injury in mice through the gut-liver axis. *Redox Biol* 59: 102577, 2023. doi:10.1016/j.redox.2022.102577.
93. **Kaphalia BS**, **Bhopale KK**, **Kondraganti S**, **Wu H**, **Boor PJ**, **Ansari GA.** Pancreatic injury in hepatic alcohol dehydrogenase-deficient deer mice after subchronic exposure to ethanol. *Toxicol Appl Pharmacol* 246: 154–162, 2010. doi:10.1016/j.taap.2010.05.002.
94. **Lu Y**, **Cederbaum AI.** Cytochrome P450s and alcoholic liver disease. *Curr Pharm Des* 24: 1502–1517, 2018. [29637855] doi:10.2174/1381612824666180410091511.
95. **Lu Y**, **Zhang XH**, **Cederbaum AI.** Ethanol induction of CYP2A5: role of CYP2E1-ROS-Nrf2 pathway. *Toxicol Sci* 128: 427–438, 2012. doi:10.1093/toxsci/kfs164.
96. **Lu Y**, **Zhuge J**, **Wu D**, **Cederbaum AI.** Ethanol induction of CYP2A5: permissive role for CYP2E1. *Drug Metab Dispos* 39: 330–336, 2011. doi:10.1124/dmd.110.035691.
97. **Chen X**, **Ward SC**, **Cederbaum AI**, **Xiong H**, **Lu Y.** Alcoholic fatty liver is enhanced in CYP2A5 knockout mice: the role of the PPARα-FGF21 axis. *Toxicology* 379: 12–21, 2017. doi:10.1016/j.tox.2017.01.016.
98. **Hong F**, **Liu X**, **Ward SS**, **Xiong H**, **Cederbaum AI**, **Lu Y.** Absence of cytochrome P450 2A5 enhances alcohol-induced liver injury in mice. *Dig Liver Dis* 47: 470–477, 2015. doi:10.1016/j.dld.2015.02.012.
99. **de Groot H**, **Auferkamp O**, **Bramey TD**, **Groot K**, **Kirsch M**, **Korth HG**, **Petrat F**, **Sustmann R.** Non-oxygen-forming pathways of hydrogen peroxide degradation by bovine liver catalase at low hydrogen peroxide fluxes. *Free Radic Res* 40: 67–74, 2006. doi:10.1080/10715760500381029.
100. **Goyal MM**, **Basak A.** Hydroxyl radical generation theory: a possible explanation of unexplained actions of mammalian catalase. *Int J Biochem Mol Biol* 3: 282–289, 2012. doi:10.1016/j.freeradbiomed.2012.10.050.
101. **Minato T**, **Tsutsumi M**, **Tsuchishima M**, **Hayashi N**, **Saito T**, **Matsue Y**, **Toshikuni N**, **Arisawa T**, **George J.** Binge alcohol consumption aggravates oxidative stress and promotes pathogenesis of NASH from obesity-induced simple steatosis. *Mol Med* 20: 490–502, 2014. doi:10.2119/molmed.2014.00048.
102. **Carmiel-Haggai M**, **Cederbaum AI**, **Nieto N.** Binge ethanol exposure increases liver injury in obese rats. *Gastroenterology* 125: 1818–1833, 2003. doi:10.1053/j.gastro.2003.09.019.
103. **Chang B**, **Xu MJ**, **Zhou Z**, **Cai Y**, **Li M**, **Wang W**, **Feng D**, **Bertola A**, **Wang H**, **Kunos G**, **Gao B.** Short-or long-term high-fat diet feeding plus acute ethanol binge synergistically induce acute liver injury in mice: an important role for CXCL1. *Hepatology* 62: 1070–1085, 2015. [26033752] doi:10.1002/hep.27921.
104. **Buyco DG**, **Dempsey JL**, **Scorletti E**, **Jeon S**, **Lin C**, **Harkin J**, **Bayen S**, **Furth EE**, **Martin J**, **Delima M**, **Hooks R**, **Sostre-Colón J**, **Gharib SA**, **Titchenell PM**, **Carr RM.** Concomitant western diet and chronic-binge alcohol dysregulate hepatic metabolism. *PLoS One* 18: e0281954, 2023. doi:10.1371/journal.pone.0281954.
105. **Yki-Järvinen H**, **Luukkonen PK**, **Hodson L**, **Moore JB.** Dietary carbohydrates and fats in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 18: 770–786, 2021. doi:10.1038/s41575-021-00472-y.
106. **Gyamfi MA**, **Damjanov I**, **French S**, **Wan YJ.** The pathogenesis of ethanol versus methionine and choline deficient diet-induced liver injury. *Biochem Pharmacol* 75: 981–995, 2008. doi:10.1016/j.bcp.2007.09.030.