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**Role of mitochondria in alcoholic liver disease**

Nassir F *et al.* Mitochondria and alcoholic liver disease

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**Abstract**

Alcohol abuse is the leading cause of liver related morbidity and mortality. Chronic or binge alcohol drinking causes hepatic steatosis which can develop to steatohepatitis, cirrhosis and ultimately hepatocellular carcinoma. The pathogenesis of alcoholic liver disease (ALD) is poorly characterized, however several recent studies point to a major role of mitochondria in this process. Mitochondria play a crucial role in cellular energy metabolism and in reactive species formation. Alcohol treatment causes mitochondrial DNA damage, lipid accumulation and oxidative stress. Studies in both animal models and in humans showed that alcohol administration causes changes in the mitochondrial morphology and function suggesting a role of these changes in the pathogenesis of ALD. We review recent findings on mechanisms by which alcohol negatively impacts mitochondrial biogenesis and function and we will discuss the specific intracellular pathways affected by alcohol consumption. Interestingly, recent findings indicate that a large number of mitochondrial proteins are acetylated and that mitochondrial proteins acetylation and sirtuins are modulated by alcohol. Understanding the mechanisms behind alcohol mediated impaired mitochondrial biogenesis and function may help identify potential therapeutic targets for treating ALD in humans.

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**Key words:** Liver; Alcoholic liver disease; Mitochondria; Alcohol; Sirtuins

**Core tip:** Excessive chronic or binge alcohol consumption causes alcoholic liver disease (ALD) with a spectrum ranging from simple steatosis to steatohepatitis and cirrhosis. One of the characteristics of ALD is the alteration in mitochondrial structure and function. This review summarizes some of the recent findings of the molecular mechanisms involved in the modulation of mitochondrial function and their implication in the development of ALD.

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**INTRODUCTION**

Alcohol is widely consumed in most parts of the world and has long been associated with various liver diseases accounting for about 4% of all deaths[[1](#_ENREF_1)]. The 2011 global status report on alcohol and health (World Health Organization) indicated that 4.5% of the global burden of disease and injury was attributed to alcohol with 7.4% for men and 1.4% for women. In the United States, 50% of the adult population (aged 18 and over) consumed alcohol on regular basis in 2011 (Summary Health Statistics for U.S. Adults: National Health Interview Survey, 2011). Excessive chronic or binge (acute large doses) of alcohol consumption causes hepatic steatosis, which can progress, if drinking continues, to more advanced form of alcoholic liver disease (ALD) such as alcoholic steatohepatitis (SH), hepatic fibrosis and cirrhosis and ultimately hepatocellular carcinoma[[2-4](#_ENREF_2)]. The pathogenesis of ALD is still poorly understood making the progress in finding treatment slow. One of the characteristics of ALD both in animal models and in patients is the perturbation in the morphology and function of mitochondria. Abnormal mitochondrial and cellular redox homeostasis has been documented in alcoholic steatohepatitis and results in alterations of multiple redox-sensitive signaling cascades[[5](#_ENREF_5)]. We will review current understandings of the role of alcohol metabolism in the pathogenesis of liver disease and the recent mechanisms involved in ALD with special focus on the mitochondrial changes associated with alcohol consumption and their potential implications in ALD.

**SPECTRUM OF ALCOHOLIC LIVER DISEASE**

ALD is a multistage disease consisting of hepatic steatosis (fatty liver), alcoholic hepatitis, and chronic hepatitis (inflammation) with hepatic fibrosis (development of scar tissue) or cirrhosis[[5](#_ENREF_5), [6](#_ENREF_6)]. The different stages of ALD are not mutually exclusive and may be present simultaneously in certain individual[[7](#_ENREF_7)]; alcoholic cirrhosis can develop without precedent of hepatic steatosis or alcoholic hepatitis. Hepatic steatosis is the earliest response to alcohol consumption and develops in 90% of heavy alcohol drinkers[[8-10](#_ENREF_8)]. Simple hepatic steatosis is usually asymptomatic, reversible and resolve after 4-6 weeks of abstinence[[11](#_ENREF_11)]. With continuous alcohol intake, 20%-30% of patients with steatosis develop alcoholic hepatitis and 16% of patients with steatohepatitis will develop cirrhosis[[12](#_ENREF_12),[13](#_ENREF_13)]. Fibrosis of the liver, a consequence of inflammation, infection or injury, results from excessive accumulation of collagen and other extracellular matrix proteins in the liver which impedes normal function of the liver leading to the development of cirrhosis. Alcohol associated cirrhosis is a consequence of sustained alcohol intake and is characterized by both steatosis and hepatitis with fibrosis[[4](#_ENREF_4)]. Alcoholic cirrhosis is irreversible and is among the top ten causes of death worldwide. According to the National Institute on Alcohol Abuse and Alcoholism, liver cirrhosis is the 12th leading cause of death in the United States with 29925 death in 2010, about 50% of which are alcohol related[[14](#_ENREF_14)]. Liver cirrhosis predisposes the HCC, it is seen in about 80% of HCC patients[[15](#_ENREF_15),[16](#_ENREF_16)]. In the US, HCC is the most rapidly growing cause of cancer-related mortality, particularly among men ages 40 to 60[[17](#_ENREF_17),[18](#_ENREF_18)].

Although hepatic steatosis is found in 90% of heavy alcohol drinkers, the severe forms of ALD such as fibrosis and cirrhosis develop only in 30% of individuals with heavy alcohol intake suggesting that other factors are involved in the progression of the disease. The possible risk factors that can affect the development of liver injury include the dose[[19](#_ENREF_19)], duration[[20](#_ENREF_20)], type of alcohol consumed[[21](#_ENREF_21)], drinking patterns[[22](#_ENREF_22)], gender[[4](#_ENREF_4),[23](#_ENREF_23)], ethnicity[[4](#_ENREF_4),[24](#_ENREF_24)], and associated risk factors, including obesity[[25](#_ENREF_25),[26](#_ENREF_26)] and genetic factors[[27](#_ENREF_27),[28](#_ENREF_28)].

**METABOLISM OF ALCOHOL IN THE LIVER**

When consumed, 90% of ingested alcohol is absorbed in the upper GI tract and diffuse throughout the body[[29](#_ENREF_29),[30](#_ENREF_30)]. Studies both in humans[[31](#_ENREF_31),[32](#_ENREF_32)] and animals[[33-35](#_ENREF_33)] have shown that both short and long term ethanol treatment can disrupt the epithelial barrier of the GI tract which results in increased intestinal permeability and enhanced movement of luminal antigens such as bacteria and endotoxins into the portal circulation[[36](#_ENREF_36),[37](#_ENREF_37)]. This can lead to Kupffer cells activation in the liver and cytokine release which may consequently results in liver injury and ALD[[38](#_ENREF_38),[39](#_ENREF_39)]. The liver is the main organ responsible for metabolizing ingested alcohol; therefore it is more susceptible to alcohol related injury. Alcohol is metabolized in the liver by both oxidative and non-oxidative pathways. Briefly, the oxidative pathways of alcohol metabolism involve three enzymes, alcohol dehydrogenase (ADH) in the cytosol, cytochrome P450 2E1 (CYP2E1) in the peroxisomes and catalase in the microsomes[[29](#_ENREF_29), [40](#_ENREF_40)]. ADH is present in the cytosol where it converts alcohol to acetaldehyde and other metabolites. In this reaction nicotinamide adenine dinucleotide (NAD+) is reduced by two electrons to NADH generating a highly reduced environment in hepatocytes. The increased NADH/NAD ratio favors hepatic triglyceride accumulation. In addition, excess NADH may promote fatty acid synthesis. Acetaldehyde inhibits protein synthesis and may be linked to tumor development[[41](#_ENREF_41)]. Cytochrome P450 enzymes (Cyp2E1, 1A2, and 3A4) present mainly in microsomes and endoplasmic reticulum also contribute to alcohol metabolism into acetaldehyde at high concentration of ethanol. Catalase in the peroxisomal pathway requires hydrogen peroxidase (H2O2) to oxidize alcohol into acetaldehyde and water. Acetaldehyde is a highly reactive and toxic byproduct to hepatocytes that may contribute to tissue damage because it forms a variety of protein and DNA adducts that promote glutathione depletion, lipid peroxidation, and mitochondrial damage[[40](#_ENREF_40),[41](#_ENREF_41)]. It also contributes to the changes in the redox state of the cell and the formation of reactive oxygen species (ROS)[[41](#_ENREF_41)]. The acetaldehyde produced from alcohol oxidation is rapidly metabolized into NADH and acetate by aldehyde dehydrogenase (ALDH) in mitochondria. The product of acetaldehyde breakdown is rapidly removed from the liver and is metabolized into CO2 via the TCA cycle in the heart, skeletal muscle and the brain. Genetic variations in ADH and ALDH influence the susceptibility of developing alcoholism and alcohol related injury. The nonoxidative pathway is minor in normal conditions and leads to the formation of fatty acid ethyl ester (FAEE) and phosphatidyl ethanol (PEth). The products of the nonoxidative pathway have pathological and diagnostic significance. Both PEth and FAEE are poorly metabolized; they accumulate in the liver and interfere with cell signaling. Because of their intermediate half-life and tendency to accumulate, non-oxidative ethanol metabolites can be used as biomarkers for alcohol consumption[[42](#_ENREF_42)]. A second nonoxidative pathway occurs at high circulating levels of alcohol and involves phospholipase D (PLD), which converts phosphatidylcholine to generate phosphatidic acid (PA) and subsequently phosphatidyl ethanol[[40](#_ENREF_40)]. Phosphatidyl ethanol is poorly metabolized and its effects on the cell are unknown, however it might interfere with the production of PA and disrupt cell signaling[[40](#_ENREF_40)].

**ROLE OF MITOCHONDRIA IN LIVER PHYSIOLOGY**

***Mitochondrial structure***

Mitochondria are organelles with double membrane structure. The outer membrane delimits the intermediate space while the inner membrane delimits the mitochondrial matrix. The structure of the inner membrane is highly complex and consists of the complexes of the electron transport system, the ATP synthetase complex, and transport proteins. The matrix contains a highly concentrated mixture of enzymes involved in the oxidation of pyruvate and fatty acids in TCA cycle. Eukaryotic cells contain nuclear and mitochondrial (mDNA) genomes sequestered into distinct subcellular compartments. Human mitochondrial DNA (mtDNA) is found in the matrix and consists of 13 structural genes that encode subunits essential for respiratory complexes I, III, IV, and V of the mitochondrial respiratory chain involved in the generation of ATP. Each mitochondrion contains 5-10 copies of mtDNA and each cell contains a high copy number of mtDNA. It is considered that the number of normal mtDNA copies must fall below 20%–40% of basal levels to induce mitochondrial dysfunction and the adverse effects. The mitochondrial matrix possesses an incomplete mitochondrial DNA repair system, and is highly sensitive to ROS-induced oxidative damage due to its proximity to the inner mitochondrial membrane where most of the ROS are produced[[43-45](#_ENREF_43)].

***Mitochondrial function***

Mitochondria are the power producer of the cell which plays a central role in the generation of energy from nutrient oxidation. Hepatocytes are rich in mitochondria; each hepatocyte contains about 800 mitochondria occupying about 18% of the entire cell volume. Mitochondria have a unique role in the liver compared to other organs as they participate in glucose, lipids and protein metabolism. Mitochondria play an essential role in the cell as they provide the majority of cellular energy in the form of ATP; generate and regulate ROS; buffer cytosolic Ca2+ and regulate apoptosis. Carbohydrates and fats are oxidized in the mitochondria to produce energy. Glucose is converted into pyruvate within the cytosol, transported to the mitochondria where it is converted to acetyl-CoA. Acetyl-CoA undergoes TCA cycle resulting in CO2, water and energy. Fatty acids in the liver originate from the diet, adipose tissue lipolysis, or hydrolysis of intracellular stores or *de novo* lipogenesis. These FFAs are metabolized through oxidation, ketogenesis or esterification into triglyceride. Together with the muscle, the liver is the main site for mitochondrial fatty acid oxidation. The oxidation of FFAs occurs mainly in the mitochondria of hepatocytes where FFAs are converted by carnitine palmitoyltransferase-1 (CPT1) into acyl-carnitine which is transported into the mitochondrial matrix. FFAs oxidation into acetyl-coenzyme A (acetyl-CoA) and its subsequent oxidation by the TCA cycle generates reduced nicotinimide-adenine dinucleotide (NADH) and reduced flavine-adenin dinucleotide (FADH2). NADH and FADH2 are the electron donor and transfer the hydrogen/electron to an oxygen molecule, via a variety of redox components in complex I through IV in the mitochondrial respiratory chain located in the inner mitochondrial membrane[[46](#_ENREF_46)]. The flow of electron in the respiratory chain is coupled with pumping of protons from the mitochondrial matrix into the inter-membrane space, thus creating an electrochemical gradient across the membrane[[46](#_ENREF_46)]. When ATP is low, protons re-enter the matrix trough ATP synthase and the energy released initiates ATP synthesis[[46](#_ENREF_46)]. Although most of the electrons end up in water, the insulation of complexes I and III of the respiratory chain from oxygen in not perfect[[46](#_ENREF_46)]. Excessive electron flow to the respiratory chain results in accumulation and leakage of electrons at these two sites which react with oxygen to produce ROS[[47](#_ENREF_47)]. About 1%-2% of mitochondrial oxygen consumption results in ROS production under normal condition[[48](#_ENREF_48)], 90% of cellular ROS are produced in the mitochondria[[49](#_ENREF_49)]. Mitochondria play an important role in ROS homeostasis as they are the main site for ROS generation but can also be a target for excessive ROS exposure. The ROS production occurs through reduction of oxygen to superoxide (O2-) by complex I and III of the electron transfer chain (ETC). Under normal conditions Mn-SOD located in the matrix of mitochondria converts the O2- to H2O2 which is subsequently reduced to H2O. ROS production in mitochondria is upregulated in conditions where increased NADH and increased membrane potential is not coupled with an increase in ATP production. Excessive production of ROS exceeding the cell’s antioxidant defenses can damage components of the cells such as lipids, proteins and nucleic acids (particularly mtDNA) leading to oxidative stress and ultimately apoptosis. This can be observed in conditions of increased oxidation of FFA such as in NASH[[50](#_ENREF_50)] and when levels of NADH are augmented due to alcohol metabolism in alcoholic steatohepatitis[[45](#_ENREF_45)].

**MITOCHONDRIAL ALTERATION IN ALD**

Recent studies have suggested that chronic ethanol administration causes changes in the mitochondrial morphology and function in both animal models and humans. The mitochondria are often enlarged and altered and these structural changes are associated with the development of fatty liver in the rat[[51](#_ENREF_51)] suggesting that chronic ethanol treatment affects hepatic energy metabolism. With the exception of one study that showed an increase in mitochondrial respiration in mice[[52](#_ENREF_52)], most studies in rats and in humans have shown altered mitochondrial respiration. The mechanism behind the species difference between the rat and mouse is unknown. Hepatocytes isolated from mice fed ethanol containing diet showed lower fatty acid oxidation and increased lipid synthesis. In the rat, chronic ethanol administration alters mitochondrial oxidative phosphorylation in the liver by inhibiting the synthesis of proteins of the respiratory complexes[[53](#_ENREF_53)]. Lower oxidation capacity in combination of the reducing environment induced by ethanol creates conditions of O2- formation and ROS production. As mentioned above, mtDNA, which encodes the subunits of the electron transport chain and the ATP synthase, is vulnerable to ROS due its proximity to the source (the inner membrane) of cellular ROS. Damage of mtDNA will in turn impair cellular energy metabolism and enhances ROS formation. Strong evidence indicates that oxidative stress and dysregulation of redox-sensitive signaling pathways are central to the pathobiology of ALD. It has been shown that a single dose of ethanol was able to damage mtDNA and cause cell toxicity[[45](#_ENREF_45)]. Other abnormalities that have been described as a result of ethanol treatment are decreases in ATP levels. Oxidative stress can cause cellular apoptosis via both mitochondria-independent (involving death receptor of the tumor necrosis factor (TNF) receptor gene family) and mitochondria-dependent (caused by intracellular stresses such DNA damage and ROS) pathways. Hepatocyte apoptosis has been observed in patients with alcoholic hepatitis[[54](#_ENREF_54)]. One of the mechanisms proposed to explain alcohol-induced hepatocyte apoptosis is the release of cytochrome C in the cytosol where it promotes caspases activation. The role of alcohol in ROS production, the induction of the mitochondrial cell death pathway and the possible mechanisms involved have been recently discussed in[[55](#_ENREF_55)].

Acetylation is an important posttranslational modification that regulates proteins. Recently, sirtuins, a family of NAD+ dependent deacetylases have been identified. In mammals, sirtuins are a family of seven proteins (SIRT1-7) that have been shown to be involved in longevity, DNA repair and the control of metabolic enzymes. Three sirtuins SITR3, SIRT4 and SIRT5 are localized within the mitochondrial matrix[[56](#_ENREF_56)]. Interestingly, SIRT3-deficient mice show increased mitochondrial protein hyperacetylation but not SIRT4 and SIRT5 deficient mice suggesting that SIRT3 is a major mitochondrial deacetylase[[57](#_ENREF_57), [58](#_ENREF_58)]. Chronic alcohol consumption induces a global acetylation of proteins mainly mitochondrial proteins indicating a role of the acetylation of mitochondrial proteins in mitochondrial biology. At least 20% of mitochondrial proteins are acetylated including proteins of TCA cycle, oxidative phosphorylation, β-oxidation and the urea cycle[[59](#_ENREF_59)]. Therefore, Sirtuins have been implicated in the regulation of mitochondrial number, turnover and activity and have been proposed to play a role in the pathogenesis of alcoholic liver disease[[60](#_ENREF_60)]. Chronic ethanol administration impairs hepatic lipid metabolism pathways largely by modulating SIRT1 (a nuclear sirtuin) and causes development of fatty liver. Ethanol decreases hepatic SIRT1 in rodent models suggesting a role of ethanol and SIRT1 in the regulation of mitochondrial energy metabolism and mitochondrial biogenesis. SIRT1 regulates lipid metabolism by deacetylating the sterol regulatory element-binding protein-1c (SREB-1c) and PPARγ coactivator 1 (PGC-1); thus it increases fatty acid synthesis and decreases fatty acid β-oxidation (deacetylation of PGC-1 increases its activity; deacetylation of SREBP-1c decreases its activity). In addition, evidence suggests that mitochondrial biogenesis is regulated at least in part by PGC-1 suggesting that ethanol mediated reduced deacetylation of PGC-1 would inhibit mitochondrial biogenesis[[61](#_ENREF_61)]. More recent studies, have demonstrated that chronic alcohol treatment specifically induced a small noncoding micro RNA (miRNA) (miR-217) in AML-12 hepatocytes and in mouse livers[[62](#_ENREF_62)]. Ethanol induced expression of miR-217 reduced SIRT1 expression which in turn results in an increase in lipogenic enzymes (acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-coenzyme A desaturase 1 (SCD1) and a decrease in genes involved in fatty acid oxidation (PPAR, PGC-1, and acyl-CoA oxidase (*AOX*))[[60](#_ENREF_60),[62](#_ENREF_62)]. In addition miR-217 modulation of SIRT1 has been recently shown to regulate lipin-1 which is a crucial regulator of hepatic lipid metabolism[[62](#_ENREF_62)]. Lipin-1 deficiency aggravated the defect in fatty acid oxidation and lipoprotein secretion induced by alcohol in mice fed the modified Lieber-DeCarli ethanol-containing low-fat diets for 4 weeks[[62](#_ENREF_62)] suggesting a role of this microRNA in the development of ALD. It is not known how alcohol induces miR-217 but it is suggested that the products of ethanol metabolism, acetaldehyde and acetate, may play a role in this process[[62](#_ENREF_62)].

Recent studies suggest that sirtuins can modulate ROS levels. As mentioned above, mitochondria are involved in the generation of ROS as well as in the defense against ROS. In addition, mitochondria are themselves target for ROS damage and cell fate. Mitochondrial SIRT3 deacetylates and activates enzymes involved in maintaining physiological ROS levels. SIRT3 reduces ROS species levels through deacetylation and activation of the antioxidant enzyme superoxide dismutase (SOD)[[63](#_ENREF_63), [64](#_ENREF_64)]. SIRT1 is essential for ROS-mediated apoptosis in embryonic stem cells by facilitating mitochondrial localization of p53[[65](#_ENREF_65)]. SIRT1 also inactivates the P65 subunit of NF-KB through direct acetylation, NFKB inhibition suppresses the inducible nitric oxide synthase (iNOS) and nitrous acid production and thus may lower cellular ROS levels[[66](#_ENREF_66)].

Together the recent findings point to a major role of mitochondria in alcohol induced hepatic fat accumulation. In summary, alcohol treatment inhibits adenosine monophoshate activated kinase activity and SIRT1 thereby regulating their target genes. Alcohol consumption results in inhibition of PPAR- and stimulation of SREBP-1C and the transformation of liver from an oxidizing to a fat-storing organ. Alcohol also induces reactive oxygen species levels and causes liver injury and apoptosis in part by regulating sirtuins levels and enzymes of the antioxidant defense.

**CONCLUSION**

Alcohol has deleterious effects on liver function. Ethanol and its metabolites destroy the liver over time. Mitochondria play an important role both in hepatic alcohol metabolism and in the bioenergetics of the hepatocyte. In this review we have outline the mechanisms by which alcohol negatively impacts mitochondrial function. The observed changes in mitochondrial function and the alteration in redox status and their implication in alcohol induced hepatic steatosis and the progression of liver disease have been discussed. We also reviewed some of the recent pathways involved in the development of ALD. Future research into the mechanism by which alcohol modulates mitochondrial biogenesis and function are critical for the development of biomarkers and the identification of potential therapeutic targets for the prevention and treatment of human ALD.

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