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Mitochondrial Dysfunction and Signaling in Chronic Liver Diseases

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Mitochondria regulate hepatic lipid metabolism and oxidative stress. Ultrastructural mitochondrial lesions, altered mitochondrial dynamics, decreased activity of respiratory chain complexes, and impaired ability to synthesize adenosine triphosphate are observed in liver tissues from patients with alcohol-associated and non-associated liver diseases. Increased lipogenesis with decreased fatty acid β-oxidation leads to the accumulation of triglycerides in hepatocytes, which, combined with increased levels of reactive oxygen species, contributes to insulin resistance in patients with steatohepatitis. Moreover, mitochondrial reactive oxygen species mediate metabolic pathway signaling; alterations in these pathways affect development and progression of chronic liver diseases. Mitochondrial stress and lesions promote cell death, liver fibrogenesis, inflammation, and the innate immune responses to viral infections. We review the involvement of mitochondrial processes in development of chronic liver diseases, such as nonalcoholic fatty, alcohol-associated, and drug-associated liver diseases, as well as hepatitis B and C, and discuss how they might be targeted therapeutically.

Keywords: NASH; NAFLD; HCV; HBV; ROS.

Alcoholic fatty liver disease (AFLD) and nonalcoholic fatty liver disease (NAFLD) can progress from benign steatosis to fibrosis, cirrhosis, and hepatocellular carcinoma. Obesity, alcohol, drug use, and chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) are all associated, to different degrees, with increased prevalence of steatosis, characterized by the accumulation of fat droplets within the hepatocytes.[1–7] Steatosis remains benign in most patients, but in some cases leads to hepatocyte necrosis, infiltration of inflammatory cells, and progressive development of fibrosis into cirrhosis.[1,2] The association of steatosis with these other liver lesions is called steatohepatitis.[3–5] Steatohepatitis associates with central obesity, steatosis, diabetes, hyperlipidemia, and insulin resistance (IR).[2,4] In addition to obesity-associated steatohepatitis, there are more-severe forms of fatty liver, associated with alcohol excess,[6–8] some drugs,[9–11] or chronic HBV or HCV infection.[3–6,12]

Mitochondrial dysfunction and oxidative stress have been detected in liver tissues from patients with steatosis and IR, diabetes, non-alcoholic steatohepatitis (NASH), or various stages of alcoholic steatohepatitis (ASH).[1–14] We review mitochondrial functions and oxidative stress, and summarize recent findings on the mechanisms by which mitochondrial dysfunction and altered mitochondrial reactive oxygen species (ROS) affect signaling pathways to contribute to AFLD, NALFD, and hepatitis B and C.

Structure and Dynamics

Mitochondria are organelles evolved from an ancestral bacterium engaged in an endosymbiotic process with an ancestral eukaryote.[15] They keep bacterial vestiges, such as N-formylated proteins, double-stranded circular mitochondrial DNA (mtDNA), and a double membrane—a mitochondrial outer membrane (MOM) delimiting intermembrane space and a mitochondrial inner membrane (MIM) delimiting the mitochondrial matrix. mtDNA encodes 13 polypeptides of mitochondrial respiratory chain (MRC) complexes and adenosine triphosphate (ATP) synthase, 22 transfer RNAs, and 2 ribosomal RNAs required for intra-mitochondrial translation.[16] All other mitochondrial proteins are encoded by nuclear DNA, synthesized in the cytoplasm, and imported into the mitochondria.

***Authors share co-first authorship.**

Abbreviations used in this paper: **AFLD, alcoholic fatty liver disease; AMPK, adenosine monophosate–activated protein kinase; ASH, alcoholic steatohepatitis; ASK1, mitogen-activated protein kinase kinase kinase 5; ATP, adenosine triphosphate; CoA, coenzyme A; CPT1, carnitine palmitoyl transferase 1; DAMP, damage-associated molecular pattern; FFA, free fatty acid; GSH, glutathione; HBV, hepatitis B virus; HCV, hepatitis C virus; IL, interleukin; IR, insulin resistance; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; mGSH, mitochondrial glutathione; MIM, mitochondrial inner membrane; MnSOD, manganese superoxide dismutase; MOM, mitochondrial outer membrane; MRC, mitochondrial respiratory chain; mtDNA, mitochondrial DNA; mtUPR, mitochondrial unfolded protein response; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; NAFLD, nonalcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NRF, nuclear respiratory factor; PGC-1α, peroxisome proliferator activated receptor γ–coactivator 1α; PPARα, peroxisome proliferator-activated receptor α; ROS, reactive oxygen species; SREBP-1c, sterol regulatory element binding transcription factor 1 variant 1-c; TNF, tumor necrosis factor; VLDL, very-low-density lipoprotein.**

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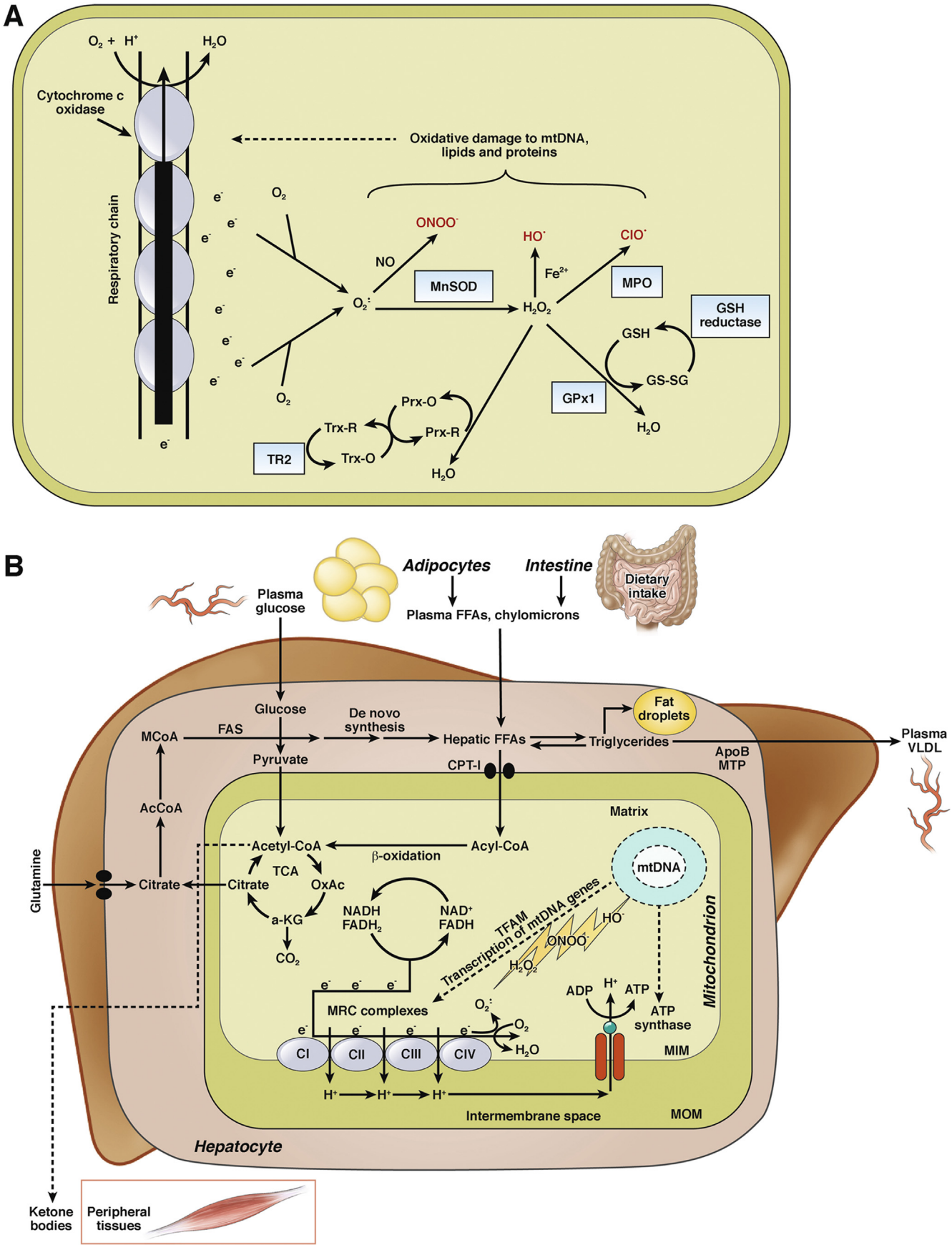
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O_2 + H^+
H_2O
Cytochrome c oxidase
Oxidative damage to mtDNA, lipids and proteins
Respiratory chain
e^-
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MnSOD
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H_2O_2
HO$^{\cdot}$
Fe^{2+}
ClO$^{\cdot}$
MPO
GSH reductase
GSH
GS-SG
GPx1
H_2O
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Trx-R
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Adipocytes
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Plasma FFAs, chylomicrons
Glucose
De novo synthesis
MCoA
FAS
Hepatic FFAs
Fat droplets
Triglycerides
Plasma VLDL
ApoB MTP
Pyruvate
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Citrate
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CO_2
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NAD^+ FADH
mtDNA
TFAM
Transcription of mtDNA genes
HO$^{\cdot}$
ONOO$^-$
H_2O_2
Mitochondrion
MRC complexes
CI
CII
CIII
CIV
ADP
H^+
ATP
ATP synthase
MIM
Intermembrane space
MOM
Hepatocyte
Ketone bodies
Peripheral tissues

Mitochondrial fusion, fission, biogenesis, and mitophagy determine mitochondrial morphology, quality, and abundance, and are tightly controlled in response to metabolic cues and stressors to ensure adaptation of mitochondrial function to cellular energetic and metabolic demands. Fusion of the MOM is mediated by mitofusins 1 and 2, whereas the fusion of MIM requires optic atrophy protein 1.[17] Mitochondrial fission requires dynamin-related protein 1, which is recruited by the mitochondrial fission 1 protein.[18]

Mitochondrial biogenesis maintains mitochondrial mass to restore energy homeostasis during energy deprivation or following mitochondrial insults. Mitochondrial biogenesis is regulated by peroxisome proliferator–activated receptor gamma coactivator 1α (PPARGC1A or PGC-1α) and nuclear respiratory factor 1 (NRF1), which control expression of mtDNA and nuclear DNA genes encoding subunits of the MRC complexes and mtDNA replication and transcription, respectively.[19–21]

Defective mitochondria are cleared via PTEN-induced putative kinase 1 and parkin-mediated mitophagy to control mitochondrial quality.[22,23] The mitochondrial unfolded protein response (mtUPR) maintains mitochondrial homeostasis, controls the stochiometric balance between proteins encoded by the nuclear and mitochondrial genomes, ensures mitochondrial quality and proteostasis, and senses mitochondrial protein misfolding.[24,25]

Mitochondria are required for fat metabolism and energy production, the urea cycle, and metabolism of amino acids and iron,[1,2,14] regulating signaling pathways that mediate these processes.[26–30] Mitochondria regulate the innate immune response to control inflammation and associated diseases.[20–23] Alterations in these processes can contribute to development and progression of liver diseases.[1,21,23,26,28–38]

Formation of Reactive Oxygen Species and Mitochondrial Antioxidant Defense

Mitochondria are the major site of ROS formation in the cell[5,39] (Figure 1*A*). Although most of the electrons donated to the MRC migrate down to cytochrome *c* oxidase, where they react with protons and oxygen to form water, some of these electrons react directly with oxygen to form the superoxide anion ($O_2^{\cdot-}$) radical, leading to formation of hydrogen peroxide (H_2O_2).

Manganese superoxide dismutase allows the spontaneous dismutation of the $O_2^{\cdot-}$ into oxygen and H_2O_2, which is then detoxified into water by mitochondrial glutathione (GSH) peroxidase and peroxiredoxins.[39,40] Alternatively, H_2O_2 reacts with iron to form the highly reactive hydroxyl radical[39–41] and active myeloperoxidase forms the hypochloride radical[42] (Figure 1*A*).

Although there has been debate over the presence of nitric oxide synthase within mitochondria, the freely diffusible nitric oxide formed elsewhere can cross mitochondrial membranes to react with superoxide and form peroxynitrite within mitochondria.[31,43] Peroxynitrite causes formation of 3-nitrotyrosine residues on several proteins, including complex I and V subunits.[31,44] Peroxiredoxins and selenium-containing glutathione peroxidase catalytically detoxify peroxynitrite.[45] These oxygen and nitrogen radicals damage mtDNA, proteins, and lipids, increasing further ROS production in a vicious cycle.

Signal Transduction by Mitochondrial Reactive Oxygen Species

Beside their damaging effects, mitochondrial ROS are also signal-transducing molecules under physiological and pathological conditions, depending on the intensity and

Figure 1. (*A*) Mitochondrial Formation of ROS. Most of the electrons donated to the respiratory chain react safely with protons and oxygen to form water at the level of cytochrome *c* oxidase (or complex IV). However, some of these electrons rather react directly with oxygen at the level of complex I and complex III of the respiratory chain to form the superoxide anion radical ($O_2^{\cdot-}$), which, in turn, generates hydrogen peroxide (H_2O_2). Then, H_2O_2 can react with ferrous iron (Fe^{2+}) to form hydroxyl radical ($^{\cdot}OH$). The freely diffusible nitric oxide (NO) formed elsewhere by inducible NO synthase (iNOS) or endothelial NO synthase (eNOS) can cross mitochondrial membranes to react with superoxide and form peroxynitrite within mitochondria. Myeloperoxidase can also form hypochlorite radical ($CLO^{\cdot}$) from H_2O_2. These ROS damage mtDNA, proteins, and lipids, increasing further mitochondrial ROS production in a vicious cycle. Mitochondria contain several antioxidant enzymes that safely scavenge these ROS. Manganese superoxide dismutase dismutates $O_2^{\cdot-}$ into oxygen and H_2O_2, which is then detoxified into water by mitochondrial glutathione peroxidase (GPx1), leading to the oxidation of reduced GSH into glutathione disulfide (GSSG). GSSG then reduced back into GSH by GSH reductase. The detoxification of H_2O_2 also involves peroxiredoxins, thioredoxin, and thioredoxin reductase 2 (TR2). The detoxification of peroxynitrite is mediated by its interaction with proteins, such as peroxiredoxins, oxyhemoglobin, selenium-containing glutathione peroxidase, or by different pharmacologic approaches, such as manganese or iron porphyrins, glutathione peroxidase mimetics, flavonoids, nitroxides, and tyrosine-containing peptides. (*B*) Fatty acid metabolism and ATP formation in hepatocytes. Plasma FFAs released from adipose tissue or from hydrolysis of intestinal chylomicrons are taken up by the liver. Liver FFAs are also synthesized de novo by hepatocytes. Liver FFAs undergo mitochondrial β-oxidation or are esterified into triglycerides that accumulate as cytoplasmic fat deposits or interacted with cholesterol esters, phospholipids and apolipoprotein B (ApoB) and microsomal triglyceride transfer protein (MTP) and secreted as VLDLs. CPT1 allows the entry of FFAs into mitochondria, where they are degraded by β-oxidation into acetyl-CoA that can be completely degraded to CO_2 by the tricarboxylic acid cycle (TCA) or mostly condensed into ketone bodies that are secreted by the liver to be re-oxidized in muscles and other peripheral tissues. The formed NADH and reduced flavine-adenine dinucleotide ($FADH_2$) transfer their electrons to the MRC complexes. Although these electrons migrate along the MRC, protons are extruded from the mitochondrial matrix into the intermembrane space, creating an electrochemical gradient a cross the MIM, the energy of which is then used by ATP synthase to generate ATP. FAS, fatty acid synthase; MCoA, malonyl-CoA; OxA, oxaloacetate; α-KG, α-ketoglytarate.

duration of oxidative stress.[27,29] Low-intensity production of ROS is important in metabolic adaptation, moderate ROS release may be involved in regulating inflammatory mediators, and high levels of ROS activate pathways, such as apoptosis or autophagy. In each case, different H_2O_2-sensitive pathways are mobilized.[27–29]

Mitochondria-derived ROS activate adenosine monophosate–activated protein kinase (AMPK)[26,28] and mitogen-activated protein kinases (MAPKs), such as c-Jun N-terminal kinase (JNK).[29] Their substrate phosphorylation has direct consequences on diverse metabolic pathways, regulation of gene expression by transcription factors, and direct activation or inhibition of specific target proteins, such as protein tyrosine phosphatases and protein kinases. AMPK and MAPK signaling pathways are activated in response to different stresses, including alterations in nutrients, cytokines, growth factors, drugs, and toxins—these signaling pathways have important roles in development of liver diseases and injuries, such as NAFLD, ALD, viral hepatitis, fibrosis, inflammation, carcinogenesis, and drug-induced hepatotoxicity.[26,28,29]

Role of Mitochondria in Fatty Acid Metabolism and Energy Supply

The β-oxidation of fatty acids into acetyl-coenzyme A (CoA), and its oxidation by the Krebs cycle, lead to reduced forms of reduced nicotinamide adenine dinucleotide (NADH) and reduced flavine-adenine dinucleotide, which transfer their electrons to the MRC[46] (Figure 1*B*). This transfer of electrons is coupled to the extrusion of protons from the mitochondrial matrix to the intermembrane space, creating a large electrochemical gradient across the MIM.[9] The energy released, when these protons re-enter into the matrix through ATP synthase, drives ATP synthesis.[1,3,13]

AMPK stimulates glucose and fatty acid oxidation, and activates PGC-1α.[26,28] PGC-1α interacts with peroxisome proliferator–activated receptor α (PPARα) to induce the expression of several fatty acid-metabolizing enzymes, including carnitine palmitoyltransferase 1 (CPT1) and acyl-CoA dehydrogenases, consequently increasing mitochondrial β-oxidation of fatty acid.[9,46] PGC-1α also induces the expression of, and bind to NRF1 to increase the expression of TFAM.[4,14,47] NRF1 and TFAM regulate the transcription and the replication of mtDNA, whereas NRF1 regulates expression of nuclear DNA-encoded MRC proteins. PGC-1α thereby increases the mitochondrial mass, as well as the mitochondrial oxidative phosphorylation capacities.[20]

Liver free fatty acids (FFAs) arise from plasma FFAs released by adipose tissue, and from intestine chylomicrons, or are synthesized de novo within the hepatocytes.[13,14] These FFAs either enter mitochondria to undergo β-oxidation or are esterified and stored as triglycerides, which either accumulate as fat droplets within hepatocytes or are condensed with cholesterol esters, phospholipids, and apolipoprotein B and then secreted as very-low-density lipoproteins (VLDLs)[13,14] (Figure 1*B*).

CPT1 mediates the import of FFAs into the mitochondria (Figure 1*B*). CPT1 is inhibited by malonyl-CoA,[1,14] formed by acetyl-CoA carboxylase during the first step of the synthesis of FFAs from acetyl-CoA.[14,28] Ingestion of carbohydrates causes high glucose and insulin levels, leading to hepatic FFAs synthesis.[3,48] High malonyl-CoA levels inhibit CPT1 and consequently β-oxidation.[14,46] These FFAs are then esterified into triglycerides, which are secreted as VLDLs.[1,14] In the fasting state, however, FFAs are released by adipose tissue and taken up by the liver. Hepatic FFA synthesis and malonyl-CoA levels are low in this case, allowing massive mitochondrial import of FFAs and extensive β-oxidation. β-oxidation degrades FFAs into acetyl-CoA molecules that can be completely degraded to CO_2 by the Krebs cycle or condensed into ketone bodies, which are re-oxidized in peripheral tissues during fasting conditions.[9,14] This entire process carefully regulates energy storage and disposal under healthy conditions, but is compromised in patients with obesity or NASH.[4,9,10,14]

Alterations in Fatty Acids and Glucose Metabolism in Patients With Non-Alcoholic Steatohepatitis

Hypercaloric diet increases adipocyte triglycerides, plasma and liver FFAs, and triglycerides, and cause resistance to insulin in adipose tissue and muscles, consequently compromising the cellular uptake and use of glucose by these tissues[2,3,13] (Figure 2). In obese individuals, IR tends to increase glycemia, leading to an increase in blood insulin. Sometimes, however, this compensatory increase fails and diabetes and NASH develop.[2,4,13] IR itself can cause fatty liver. High levels of insulin during IR allow the sustained activity of the hormone-sensitive lipase after a meal, thereby increasing adipocyte lipolysis. The resulting increase of the plasma FFAs could facilitate their absorption by the liver.

Another important mechanism of steatosis in the metabolic syndrome involves increases in hepatic FFAs synthesis.[2,49] IR in the muscle results in hyperinsulinemia and hyperglycemia. In obese patients, high levels of insulin increase the expression of sterol regulatory element binding transcription factor 1, variant 1-c (SREBP-1c) in the liver, which increases expression of lipogenic enzymes, thereby increasing the hepatic synthesis of FFAs[13,14,50] (Figure 2). It is not clear how these changes occur in in obese individuals, despite information from animal models. For example, lipoatrophic transgenic mice that lack adipose tissue have features observed in obese patients, such as steatosis, increased plasma FFAs and triglycerides, hyperinsulinemia, and hyperglycemia.[6,51] These mice have decreased adipocyte-storing capacities.[3]

In lean people, the adipocytes store fat in the postprandial period to release FFAs during fasting. High levels of insulin would block adipocyte lipolysis in these lean individuals.[3] In obese persons, however, fat-overloaded adipocytes may continue to release FFAs after meals, despite high insulin levels,[3] thereby increasing plasma FFAs and their uptake by the liver (Figure 2). In addition, the high insulin and glucose levels associated with IR might increase hepatic FFA synthesis from glucose.[2] So,

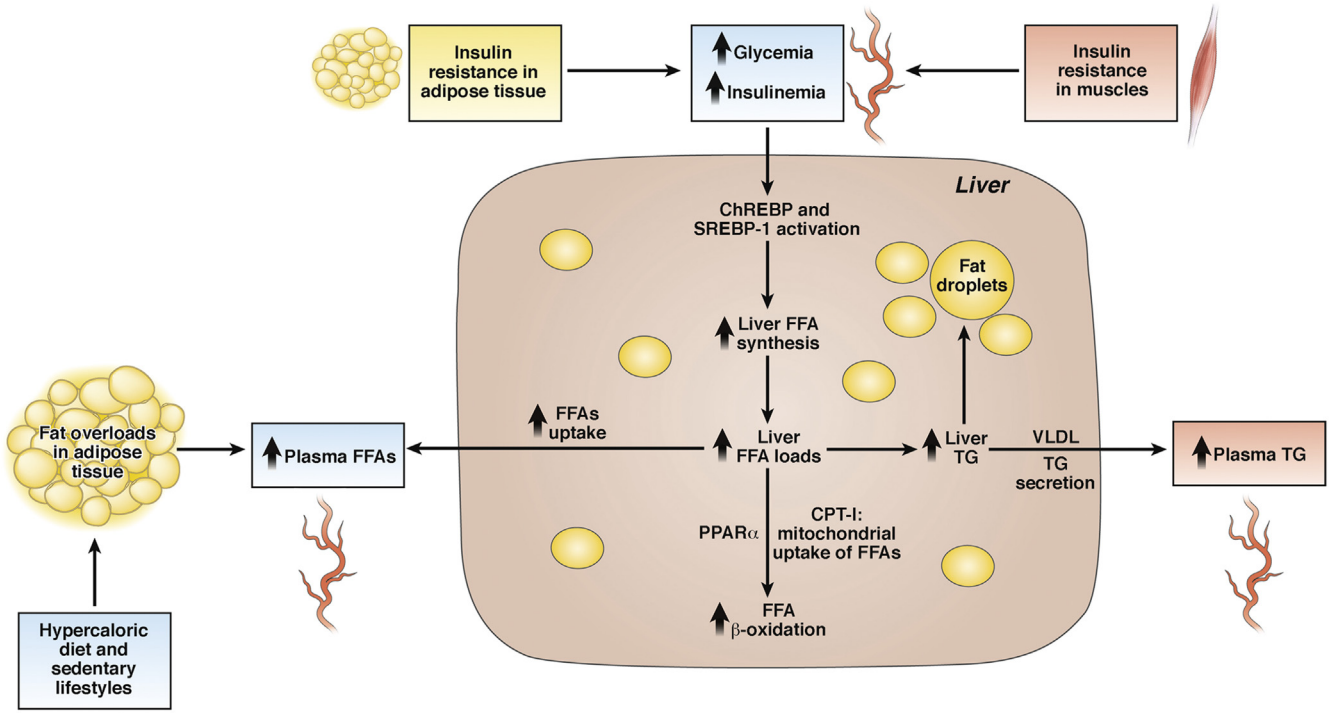


Figure 2. Alterations in fat and glucose metabolism in patients with obesity-associated NASH. Fat-engorged adipocytes (due to hypercaloric diet and lack of exercise) release FFAs increasing plasma FFAs and their uptake by the liver. The triglycerides-engorged adipocytes and myocytes could play a role in decreasing the synthesis of glucose transporters or their transport to the plasma membrane, causing resistance to the insulin. The resulting high glucose and insulin levels induce the expression of SREBP-1c and activation of CHREBP in the liver, which increase the expression of several lipogenic enzymes, thereby increasing the hepatic synthesis of the FFAs from glucose. Increased liver FAAs causes their uptake by mitochondria through CPT1, activates PPARα, which increases β-oxidation enzymes and thus FFAs oxidation. Although mitochondrial β-oxidation is increased in obese patients with NASH, this may not be sufficient to handle with high loads of hepatic FFAs because of the sustained release of FFAs by adipose tissue due to insulin resistance, which increases adipocyte lipolysis. These excess FAAs are instead converted into triglycerides (TG), causing steatosis and increased hepatic secretion of TGs, leading to hypertriglyceridemia.

increased uptake of FFAs from the adipose tissue (associated with an increased synthesis of FFAs in the liver) increases hepatic FFAs loads. The increased FFAs in liver leads to an increase in mitochondrial β-oxidation.[2,3,52] Although insulin and malonyl-CoA tend to decrease CPT1 activity and thereby FFA uptake by mitochondria in lean persons, these effects might not occur in insulin-resistant obese persons; diabetic BB Wistar rats have significant increases in CPT1 activity, with decreased sensitivity to the inhibitory effect of malonyl-CoA.[53] If similar effects occur in insulin-resistant obese persons, these CPT1 changes might permit an increase in mitochondrial import of FFAs and β-oxidation, despite high levels of insulin and malonyl-CoA. Although mitochondrial β-oxidation is increased in obese patients with NASH,[4,14] this is not enough to handle the increased hepatic FFA loads.[2,4] These FFAs are instead converted into triglycerides, which cause steatosis, and partly secreted into the plasma as VLDLs, which cause hypertriglyceridemia[13] (Figure 2). Whereas insulin tends to decrease hepatic VLDL secretion in healthy persons, this effect does not occur in individuals with obesity-associated diabetes because of the IR.

Mechanisms

Liver tissues from patients with obesity and NASH have ultrastructural damage to the mitochondria.[3,52] MRC is uncoupled and the activity of MRC complexes is decreased, causing hepatic depletion of ATP.[4,54] High mitochondrial levels of ROS and ROS-mediated mtDNA damage are observed in liver tissues from patients with NASH.[4,13]

Similarly, hepatic tissues from obese (*ob/ob*) mice have increased synthesis of FFAs from glucose,[55,56] increased triglycerides,[57,58] increased mitochondrial ROS production, and higher oxidative stress, documented by increased lipid peroxidation, decreased hepatic mitochondrial components of MRC,[44] and decreased levels of ATP.[56,58,59] Livers from *ob/ob* mice, compared with control mice, have higher levels of tumor necrosis factor (TNF)[57,58] and FFAs,[56] and increased proton leakage, which decreases ATP formation. The mechanisms for these mitochondrial changes involve alterations in mitochondrial ROS formation and ROS-signaling pathways, changes in mitochondrial biogenesis and mitophagy, and modifications in mitochondrial levels of cholesterol and GSH, as well as in FFAs, lipid peroxidation products, and TNF.

Compromised Mitochondrial Adaptation and Flexibility in Non-Alcoholic Steatohepatitis

Liver tissues from patients with early-stage NAFLD have higher mitochondrial biogenesis and mitochondrial mass than liver tissues from healthy individuals. Similarly, liver tissues from patients with NASH have higher mitochondrial biogenesis and mitochondrial mass, but lower maximal respiration and mitochondrial uncoupling, which is associated with greater hepatic IR.[4] Compared to early stages of obesity-related IR, patients with NASH have increased oxidative stress, increased lipid peroxidation, and oxidative DNA damage associated with reduced antioxidant defense capacities and increased inflammatory activities.[4] These findings indicate liver adaptation and mitochondrial flexibility at early stages of NAFLD development, which are subsequently lost in NASH. Alterations in mitochondrial biogenesis and accumulation of damaged mitochondria might therefore be secondary to defects in the mitophagy pathway observed in liver tissues from patients with NASH.[35]

Altered Mitophagy in Non-Alcoholic Steatohepatitis

Removal of excessively damaged mitochondria, by mitophagy, protects against development of NAFLD.[22,55] Mitophagy regulates liver metabolism and prevents cell death by reducing oxidative stress and preserving mitochondrial bioenergetics.[14] Changes that characterize the metabolic syndrome, including obesity and hyperglycemia, alter mitophagy in patients with NAFLD and NASH and in animal models of ASH.[22,23,35] Lipids as well as IR and hyperinsulinemia are associated with loss of expression of genes that regulate autophagy.[22] Autophagy is inhibited in liver tissues from patients with NASH and correlates with its severity.[22] Moreover, alterations in mitophagy lead to accumulation of severely damaged and dysfunctional mitochondria, resulting in cell necrosis, which releases bacterial vestiges retained in mitochondria (hypomethylated CpG motifs and formyl-peptides)—these might promote liver inflammation and NASH development.

Altered Mitochondrial Cholesterol and Mitochondrial Levels of Glutathione

Increased cholesterol synthesis and levels have been observed in livers of patients with NASH, and mitochondrial GSH (mGSH) depletion has been observed in animal models and patients with NASH.[60,61] High levels of cholesterol within mitochondria deplete mGSH from livers with steatosis,[60–62] and primary mouse hepatocytes incubated with free cholesterol undergo apoptosis and necrosis.[61] These effects are accompanied by the opening of mitochondrial permeability transition pore, cytochrome *c* release, liver oxidative stress, and ATP depletion. Other studies have demonstrated that free cholesterol sensitizes hepatocytes to TNF- and Fas-induced steatohepatitis.[61,62] The mechanism involves cholesterol-mediated mGSH depletion because replenishment of mGSH by S-adenosyl-L-methionine or GSH ethyl ester protects mice fed a high-cholesterol diet from lipopolysaccharide (LPS)-induced liver injury.[61]

Uncoupling of Mitochondrial Respiration by Free Fatty Acids

Increased hepatic FFAs in obesity uncouple respiration from ATP formation. Uncoupling proteins are increased in *ob/ob* mice[59] and promote recycling of FFAs across the MIM, via the dicarboxylate carrier and uncoupling proteins.[3,63] The forward and backcross of FFAs through the MIM cause the re-entry of protons from the intermembrane space into the matrix.[3,63] Consequently, ATP synthase is bypassed and ATP falls. This mechanism has been proposed to occur in patients with obesity and NASH.[1,4]

Impairment of Respiration by Lipid Peroxidation Products and Tumor Necrosis Factor

Mitochondria-derived ROS oxidize the unsaturated lipids to cause lipid peroxidation.[1] Extensive lipid peroxidation was observed in rats with steatohepatitis caused by a methionine- and choline-deficient diet.[64] Lipid peroxidation products alter mtDNA[4,41] and react with mitochondrial proteins, including MRC complexes.[41,44,65] These effects tend to partially block the transfer of electrons in the MRC, increasing the formation of $O_2^{\cdot-}$ and other ROS, although adaptive changes such as mitochondrial biogenesis occur. Moreover, lipid peroxidation and ROS consume antioxidant enzymes and vitamins,[4,13] thereby compromising ROS scavenging, causing further lipid peroxidation, and increasing mitochondrial damage and ROS formation.

Another mechanism impairing mitochondrial respiration involves ROS-mediated release of TNF in the liver.[3,5] TNF impairs MRC and causes opening of mitochondrial permeability transition pore, uncoupling oxidative phosphorylation. Both effects further increase mitochondrial ROS formation and lipid peroxidation.[1] Added mechanisms of mitochondrial dysfunction in steatohepatitis progression include alcohol abuse, Wilson disease, certain drugs, HBV, and HCV.[5-7,9,11,12,21,46,66–71]

Alcoholic Steatohepatitis

Ethanol metabolism consumes nicotinamide adenine dinucleotide $(NAD)^+$ equivalents, increasing the NADH/NAD^+ ratio (Figure 3), which inhibits mitochondrial β-oxidation, leading to steatosis.[1,6,7,46] This process inhibits sirtuin deacetylases and histone deacetylation, compromising epigenetic mechanisms that regulate fat and glucose metabolism.[72–74] Specific post-translational protein modifications are risk factors for ASH development and progression.[73,74] An increased NADH/NAD^+ ratio also causes the reduction of ferric iron to ferrous iron, a potent generator of the hydroxyl radicals.[3,5] Moreover, levels of cytochrome P-450 2E1 are greatly increased in liver tissues from patients with ASH[72]—an-alcohol-inducible isoform of cytochrome P-450 2E1, which leaks ROS and forms the 1-hydroxyethyl radical, is expressed in mitochondria.[75]

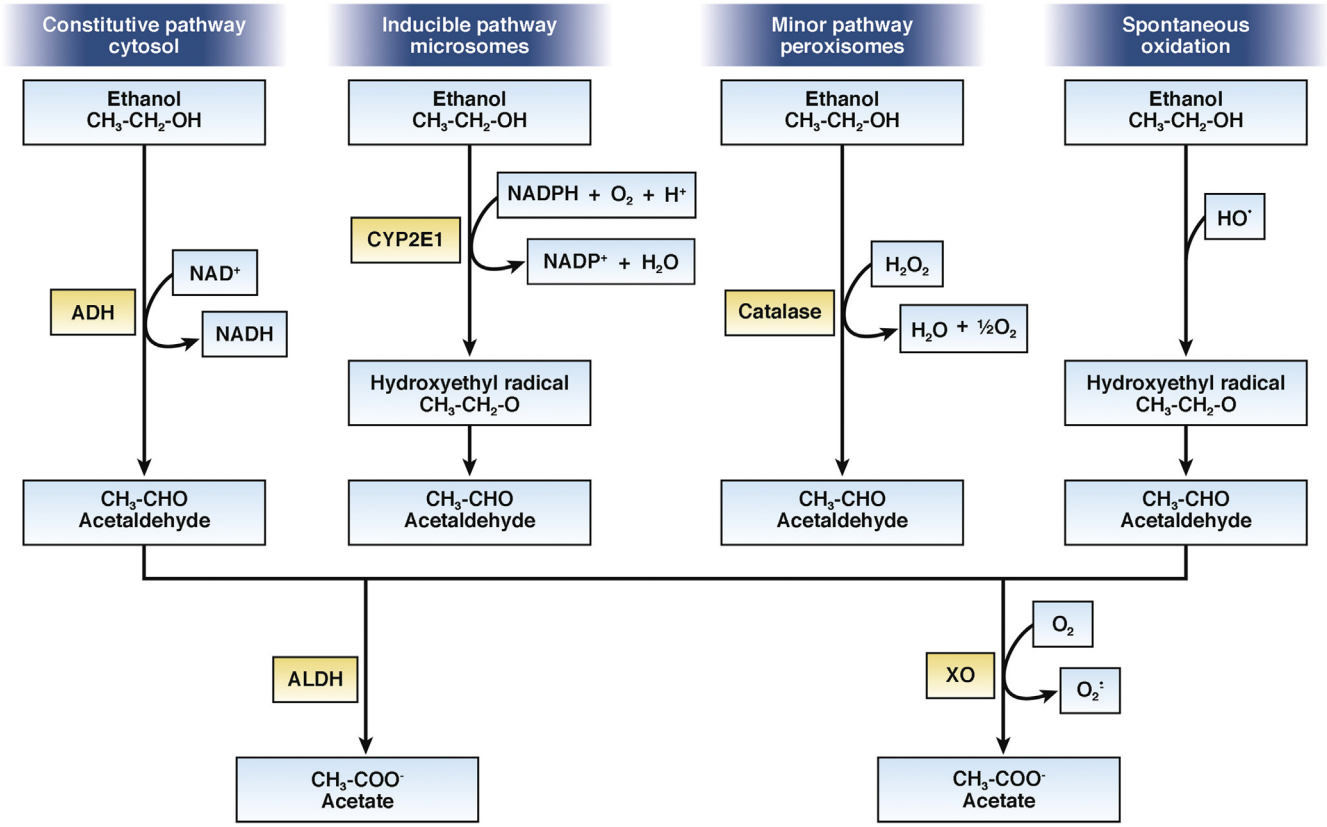
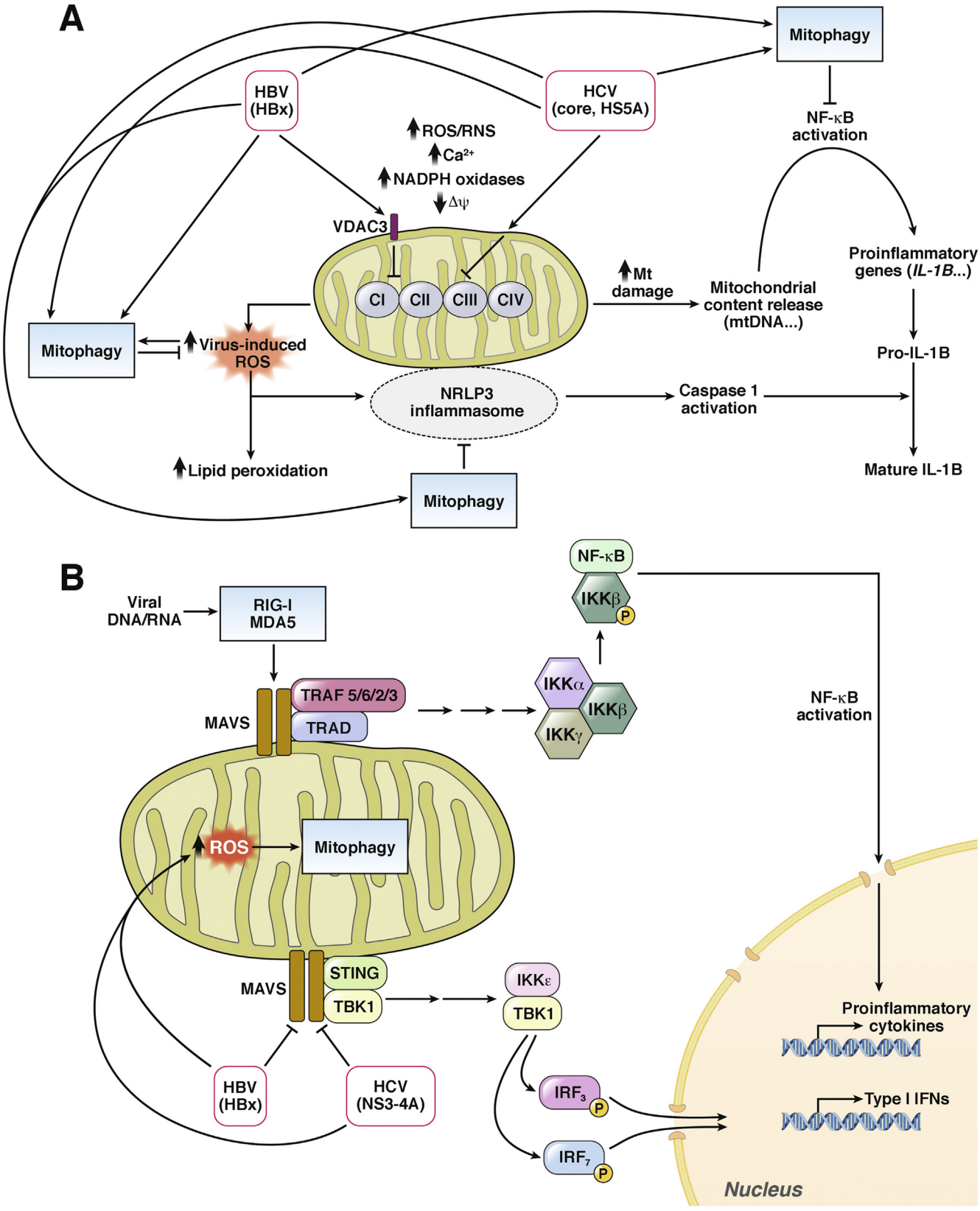


Figure 3. Metabolism of ethanol into acetaldehyde via oxidative pathways. Cytosolic metabolism of ethanol involves ADH, the microsomal inducible pathway involving cytochrome P-450 2E1 (CYP2E1), the peroxisomal pathway involving catalase, and a spontaneous oxidation of ethanol mediated by hydroxyl radical (·OH). The resulting acetaldehyde is oxidized into acetate by mitochondrial and cytosolic isoforms of ALDH and by cytosolic xanthine oxidase (OX). Ethanol metabolism is associated with ROS production, as well as the formation of hydroxyethyl and ethoxy radicals. Alcohol dehydrogenase and aldehyde dehydrogenase consume NAD^+ equivalents, thereby inhibiting β-oxidation of FFAs causing steatosis.

ROS-mediated mtDNA strand breaks, lipid peroxidation-generated mtDNA adducts, oxidized mtDNA bases, mtDNA depletion and deletions, and oxidation of mitochondrial proteins and lipids cause mitochondrial dysfunction and increase further mitochondrial ROS formation in alcoholics[7] and ethanol-fed mice.[41,44,76,77]

In mice, a single dose of ethanol causes massive mtDNA degradation and depletion,[44,76,77] which are prevented by genetic overexpression of manganese superoxide dismutase,[44] 4-methylpyrazole (ethanol metabolism inhibitor), or melatonin (an antioxidant).[76] Ethanol-mediated mtDNA depletion is transient and followed by resynthesis of mtDNA, which restores normal levels of mtDNA.[44,76,77] This observation indicates that severely damaged mitochondria might be cleaned up by mitophagy and mitochondrial mass might be restored by mitochondrial biogenesis. Replication of damaged mtDNA molecules during this adaptive biogenesis, together with mtDNA strand breaks, causes mtDNA deletion in liver tissues from patients with ASH.[7] Multiple mtDNA deletions were detected in liver tissues from patients with alcohol abuse and severe microvesicular steatosis.[7] Whereas only 3% of individuals without alcohol abuse (controls) had a single mitochondrial DNA deletion, 24% of all patients who abuse alcohol and 85% of those with severe alcoholic microvesicular steatosis had multiple heteroplasmic 4977-, 5385-, 5039-, and 5556-base pair mtDNA deletions.[7] Even though hepatocytes contain hundreds of copies of mtDNA, it is possible that the combination of diverse mtDNA deletions and point mutations, together with mtDNA strand breaks and adducts, could reach a threshold sufficient to reduce mitochondrial respiration and ATP synthesis, resulting in mitochondrial dysfunction in patients with ASH.[7]

Because cholesterol regulates levels of mGSH, and mGSH is important for maintaining the mitochondrial redox homeostasis and as a cofactor for a several antioxidant enzymes, mGSH and mitochondrial cholesterol are important factors in the pathogenesis of ASH.[60,61] mGSH originates exclusively from cytosol via mitochondrial carriers sensitive to inhibitory effects of cholesterol.[61,62] Alcohol intake depletes mGSH via cholesterol overload in the mitochondria; strategies are aimed at correcting the loss of mitochondrial membrane fluidity to replenish mGSH pools.[60,61] Importantly, alcohol-induced endoplasmic reticulum stress increases cholesterol synthesis and alters its trafficking into mitochondria, thereby indirectly altering mGSH levels.[61] Accordingly, preventing endoplasmic reticulum stress by tauroursodeoxycholic acid restored the mGSH pool in ethanol-fed rats.[61,62] Furthermore, ethanol-induced endoplasmic reticulum stress leads to activation of the


A
Mitophagy
HBV (HBx)
HCV (core, HS5A)
NF-κB activation
ROS/RNS
Ca2+
NADPH oxidases
Δψ
VDAC3
CI
CII
CIII
CIV
Mt damage
Mitochondrial content release (mtDNA...)
Proinflammatory genes (IL-1B...)
Mitophagy
Virus-induced ROS
Pro-IL-1B
NRLP3 inflammasome
Caspase 1 activation
Lipid peroxidation
Mature IL-1B
Mitophagy
B
NF-κB
IKKβ
P
Viral DNA/RNA
RIG-I MDA5
TRAF 5/6/2/3
TRAD
MAVS
IKKα
IKKβ
IKKγ
NF-κB activation
ROS
Mitophagy
MAVS
STING
TBK1
IKKε
TBK1
Proinflammatory cytokines
HBV (HBx)
HCV (NS3-4A)
IRF3
P
Type I IFNs
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transcription factors SREBP-1c and SREBP2, resulting in de novo synthesis of FFAs and cholesterol.[14,28,61,62]

Gut-derived endotoxin also alters the mitochondria and promotes inflammation and fibrosis, culminating in ASH.[8] LPS increases mitochondrial ROS formation, causes mtDNA damage, and decreases complex I activity in mice.[31] LPS also increases plasma levels of TNF and concentrations of interferon-beta, decreases mtDNA-encoded mRNAs and *TFAM* messenger RNA, and increases plasma levels of nitrites, nitrates, and inducible nitric oxide synthase.[31]

Hepatocyte apoptosis promotes progression of AFLD, and mitophagy protects against apoptosis and AFLD.[23] PTEN-induced putative kinase 1 and parkin-mediated mitophagy are increased in mitochondria of ethanol-fed rats, and activation of mitophagy reduces alcohol-induced liver injury in ethanol-fed mice (Lieber DeCarli diet).[23] In contrast, inhibition of mitophagy alters liver metabolism[22] and exacerbates ethanol-induced liver injury.[23] Furthermore, mtDNA damage associated with AFLD pathogenesis can induce mitophagy. Mitochondria that contain mutant mtDNA are specifically selected for degradation by mitophagy.[23]

Ethanol also alters the mtUPR and protein homeostasis.[36] Induction of the mtUPR requires mitochondria to nucleus retrograde communications, which involve the transcription factors CHOP[24] and ATFS5,[25] promoting transcription of mitochondrial chaperones and proteases. The stoichiometric balance of mtDNA-encoded proteins and their nuclear DNA-encoded partners is important—any stress condition that disturbs this balance can cause protein aggregation within mitochondria, which presumably activates mtUPR.[24] There is evidence for alterations in the mtUPR in mouse models of ASH.[36]

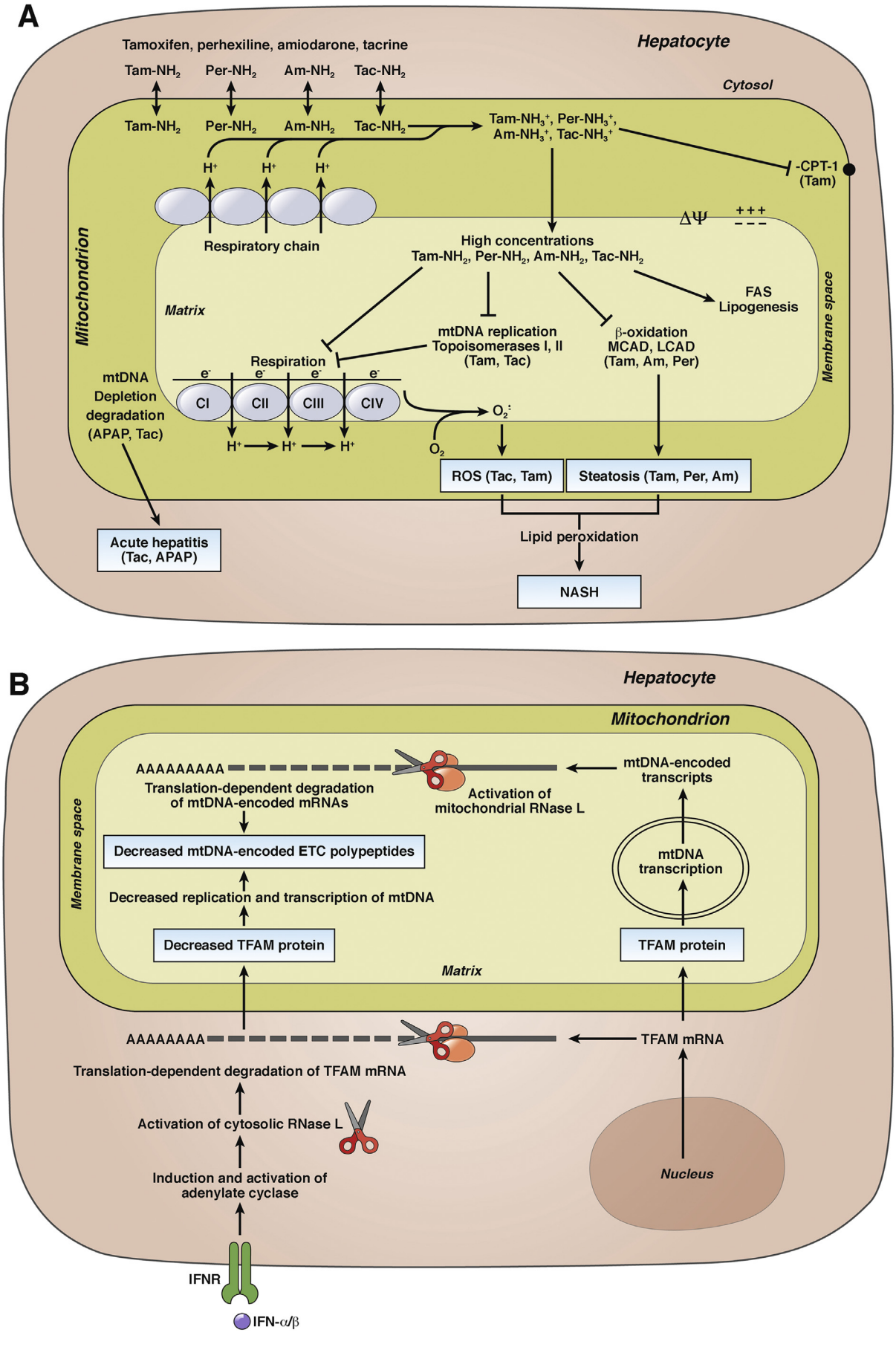
Chronic Hepatitis B Virus or Hepatitis C Virus Infection

Alterations of mitochondrial oxidative metabolism and oxidative/nitrosative stress contribute to initiation and progression of chronic HBV or HCV infection.[78–87] HCV induces oxidative and nitrosative stress in cell lines and in livers from infected patients[78,88–97] and this involves the HCV core,[12,90] NS5A,[90–93] E1,[91] E2,[91,94] NS3/4A,[92] and NS4B proteins[91,95] (Figure 4*A*). Some of these proteins localize to mitochondria, where they interact with MRC.[96] Liver biopsies from patients with chronic HCV infection contain DNA damage and high levels of 4-hydroxynonenal and malondialdehyde,[98,99] which correlate with the severity of inflammation and fibrosis.[99,100] Moreover, the mitochondrial cytochrome P-450 2E1 isoform, which generates ROS and lipoperoxidation products, is induced by HCV.[101,102] Furthermore, mitochondrial dysfunction is accompanied by massive ROS production due to inhibition of complex I activity[103] in hepatocytes of transgenic mice overexpressing HCV core protein.[104,105] Overexpression of HCV core or NS4A proteins collapse the mitochondrial membrane potential, followed by mitochondrial swelling and release of cytochrome *c*, resulting in excess ROS formation and apoptosis in mice[104–106] (Figure 4*A*). HCV can also alter mitochondrial function by altering calcium (Ca^{2+}) homeostasis and redistribution within mitochondria (Figure 4*A*). Ca^{2+} chelators prevent induction of oxidative stress in HCV-infected cells.[93,107–111]

The effect of HCV on mitochondria is not limited to the induction of mitochondrial oxidative stress and dysfunction. HCV proteins inactivate also the mitochondrion-dependent innate immune antiviral signaling and inflammatory pathways.[112,113] One mechanism involves NS3/4A-mediated cleavage of the mitochondrial antiviral signaling protein. To escape innate immune surveillance, HCV stimulates mitophagy, resulting in the elimination of damaged organelles, preventing mitochondria-mediated hepatocyte apoptosis, and allowing the viral infection to persist[38,113] (Figure 4*B*). Liver tissues from patients with chronic HCV infection have a low mtDNA copy number.[99]

Mitochondrial injury is involved in HBV-associated liver injury—the HBx protein is believed to contribute to mitochondrial dysfunction.[80] HBx is localized on the MOM of infected liver cell lines,[81–87] alters various mitochondrial relevant functions, increases mitochondrial ROS formation, decreases mitochondrial transmembrane potential,[84] and alters mitochondrial calcium homeostatsis[84,114,115] (Figure 4*A*). Mitophagy appears to be involved in the innate immune response to HBV, as a negative regulator of mitochondrial antiviral signaling-mediated innate immune signaling.[32,116] HBV alters mitochondrial dynamics[37] and activates autophagy to ensure survival of infected cells and thereby HBV replication.[37,117] Inhibition of autophagy greatly increased death of HBx-expressing hepatocyte cell lines and HBx protein reduced starvation-induced apoptosis through activation of mitophagy.[117] Consistently, HBx increases expression of parkin and recruitment to the mitochondria,

Figure 4. Mitochondria in HBV and HCV infection. (*A*) HBV and HCV promote oxidative stress, mitochondrial dysfunction, and a metabolic switch. HBV and HCV viral proteins bind to the mitochondrial membranes and lead to increased ROS and reactive nitrogen species (RNS), cellular calcium redistribution, induction of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and loss of mitochondrial membrane potential, resulting in mitochondrial respiratory chain dysfunction. Virus-induced ROS result in increased lipid peroxidation and activation of the inflammasome. Increased mitochondrial damage and inflammasome activation lead to expression of inflammatory cytokines. Viral proteins use mitochondria-based mechanisms to inhibit the ability of ROS, inflammasome, and cytokines to induce apoptosis to allow continued viral replication. (*B*) HBV and HCV proteins inactivate the mitochondrion-dependent innate immune antiviral signaling and inflammatory pathways. HBx or NS3/4A-mediated cleavage of the mitochondria-bound mitochondrial antiviral signaling, an integrating adaptor protein that senses viral DNA or RNA and coordinates the induction of type 1 interferons and interferon-stimulating genes via IRF3m IRF7 pathways, and expression of inflammatory cytokines by NF-κB. IRF, interferon regulatory factor.


A
Hepatocyte
Tamoxifen, perhexiline, amiodarone, tacrine
Tam-NH2
Per-NH2
Am-NH2
Tac-NH2
Cytosol
Tam-NH3+, Per-NH3+, Am-NH3+, Tac-NH3+
-CPT-1 (Tam)
H+
Respiratory chain
ΔΨ
+++

Mitochondrion
High concentrations Tam-NH2, Per-NH2, Am-NH2, Tac-NH2
FAS Lipogenesis
Matrix
Membrane space
mtDNA replication Topoisomerases I, II (Tam, Tac)
β-oxidation MCAD, LCAD (Tam, Am, Per)
Respiration
e-
CI
CII
CIII
CIV
O2
O2-
mtDNA Depletion degradation (APAP, Tac)
ROS (Tac, Tam)
Steatosis (Tam, Per, Am)
Lipid peroxidation
Acute hepatitis (Tac, APAP)
NASH
B
Hepatocyte
Mitochondrion
Membrane space
AAAAAAAAA
Translation-dependent degradation of mtDNA-encoded mRNAs
Activation of mitochondrial RNase L
mtDNA-encoded transcripts
Decreased mtDNA-encoded ETC polypeptides
mtDNA transcription
Decreased replication and transcription of mtDNA
Decreased TFAM protein
TFAM protein
Matrix
AAAAAAAA
TFAM mRNA
Translation-dependent degradation of TFAM mRNA
Activation of cytosolic RNase L
Nucleus
Induction and activation of adenylate cyclase
IFNR
IFN-α/β

culminating in mitophagy.[37] These findings indicate that HBV activates mitophagy to suppress virus-induced apoptosis, and inactivates the cellular antiviral response to promote viral persistence[37,118] (Figure 4*A*).

Mitochondrial Dysfunction in Drug-Induced Steatohepatitis

Several mitochondrial-damaging drugs can cause steatohepatitis.[9] Some drugs directly inhibit specific enzymes that mediate β-oxidation or deplete their cofactors—others directly interfere with MRC complexes or with mtDNA replication and transcription, whereas some drugs increase mitochondrial ROS and cause mtDNA damage[9,10,119] (Figure 5). For example, diethylaminoethoxyhexestrol, perhexiline, amiodarone, and tamoxifen selectively accumulate and reach high concentrations in the mitochondrial matrix[10,46,119] to inhibit β-oxidation (causing steatosis)[10] and block the transfer of electrons along the MRC, increasing production of ROS[11] (Figure 5*A*).

Other mitochondrial hepatotoxic drugs can sequester CoA (such as aspirin and valproic acid), directly inhibit mitochondrial β-oxidation enzymes (such as anti-inflammatory drugs, tetracyclines, 2-arylpropionate, aminoptine, tianeptine, amiodarone, perhexiline, tamoxifen), or inhibit β-oxidation enzymes and the transfer of electrons within MRC (such as perhexiline and amiodarone).[9,46] Some drugs destroy and deplete mtDNA (such as acetaminophen),[21,70] inhibit mtDNA replication (such as tamoxifen, tacrine, dideoxynucleosides, abacavir, and ganciclovir),[9,10,119] inhibit mitochondrial biogenesis (such as acetaminophen),[21] or impair mitochondrial protein synthesis (such as linezolid and erythromycins).[9] A single drug can have different effects on mitochondrial function (such as valproic acid)[9] (Figure 5*A*). Interferon-alfa, used to treat patients with chronic HBV infection, also alters mitochondrial transcription and translation by activating RNase L, which degrades *TFAM* messenger RNA and mtDNA-encoded mRNAs[9,31] (Figure 5*B*).

HCV, alcohol, and obesity-associated NASH have additive effects with drugs that impair mitochondrial β-oxidation to increase damage to mitochondria to aggravate steatosis and steatohepatitis. NASH may be an independent risk factor for drug-related acute hepatitis.[120,121] Diabetes could increase risk of acute drug-induced liver failure.[122] Obesity increases risk for tamoxifen-induced steatohepatitis in women[123]; obese patients with rheumatoid arthritis receiving methotrexate treatment have abnormal results from liver tests.[124]

Roles for Mitochondrial Stress in Liver Inflammatory Responses and Fibrogenesis

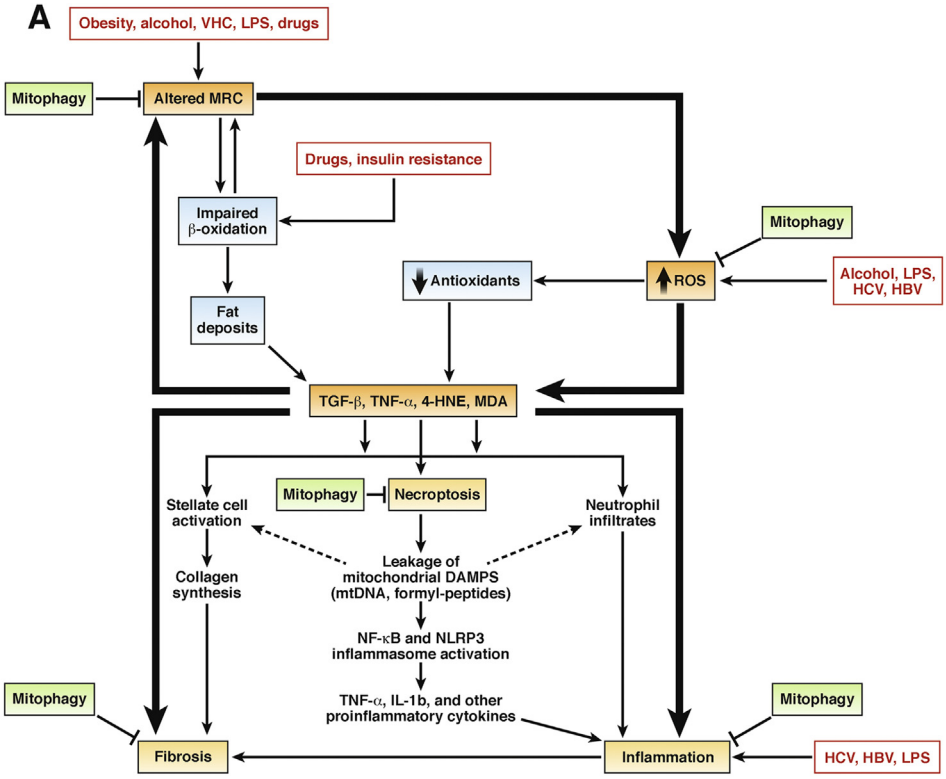
Steatohepatitis of all etiologies is associated with increased formation of ROS by mitochondria.[5] This could occur via vicious cycles that involve steatosis, lipid peroxidation, ROS formation, depletion of antioxidants, altered mitophagy, and mitochondrial danger signals-induced expression of inflammatory cytokines[5–13,23,33,34,125,126] (Figure 6*A*). Some factors that cause steatohepatitis can further increase mitochondrial ROS formation, such as alcohol abuse, HBV or HCV infection, or steatohepatitis-inducing drugs.[5–7] These second hits could account for the increased severity of necroinflammation and fibrosis in some patients.

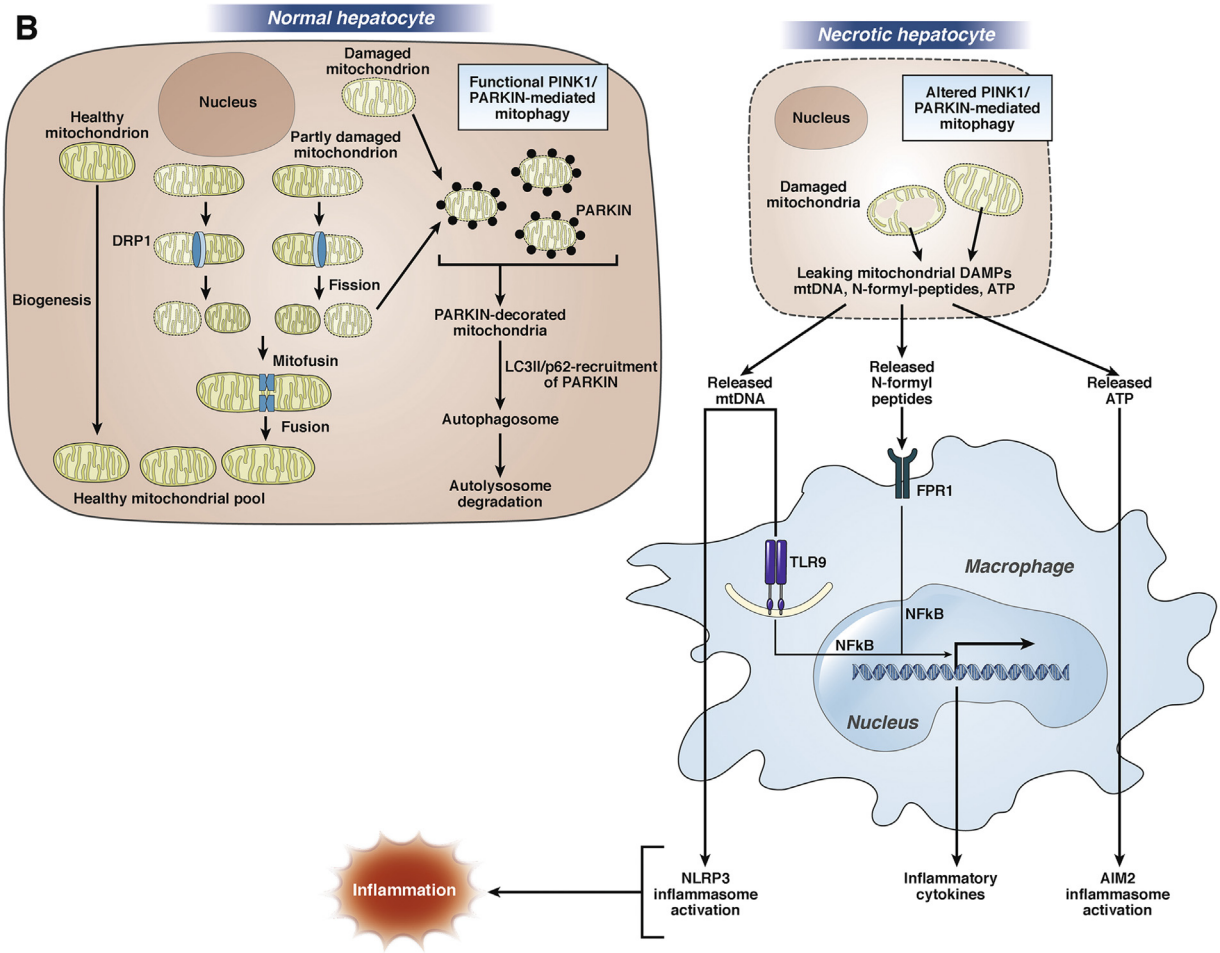
In patients with NASH, and especially ASH, production of ROS contributes to necroinflammation. ROS increase expression and synthesis of cytokines including interleukin (IL) 1β, IL8, and TNF, which cause apoptosis and necrosis of hepatocytes[1,3,5] (Figure 6*A*). Damaged mitochondria and the resulting necrosis of hepatocytes provide damage-associated molecular patterns (DAMPs) of mitochondrial origin. Mitochondria maintain bacterial-like vestiges, including hypomethylated CpG motifs in mtDNA, formyl-peptides, and other danger signals.[33,34,125,126] These mitochondrial DAMPs stimulate the innate immune response via pattern recognition receptors.[33,34,126] Once released, mtDNA and mitochondrial formyl-peptides interact with Toll-like receptor 9 and formyl-peptide receptor-1, respectively, to stimulate innate immunity and inflammatory cytokines expression resulting in chronic liver inflammation, as has been suggested in acute liver injury and other conditions[33,34,126] (Figures 6*A* and *B*). Finally, ROS-associated lipid peroxidation, transforming growth factor β1, and mitochondrial DAMPs are all involved in the inflammatory cell infiltrates by attracting neutrophils[3,5,34] (Figures 6*A* and *B*).

Mitochondrial Reactive Oxygen Species Signaling

Because of the broad nature of this subject, we have selected specific areas that illustrate important

Figure 5. Mitochondria in drug-induced steatoheptitis and acute hepatitis. (*A*) Alterations of mitochondria by tamoxifen (Tam), perhexiline (Per), amiodarone (Am), tacrine (Tac), and acetaminophen (APAP). Unprotonated, lipophilic forms of the drug cross the mitochondrial outer membrane and is protonated in the acidic intermembrane space. The positively charged, protonated form is electrophoretically pushed inside the mitochondrial matrix, where it achieves higher concentrations than in the cytosol. These high concentrations of the drug inhibit mitochondrial fatty acid β-oxidation causing steatosis, and mitochondrial respiration increasing ROS formation. These high concentrations also interfere with mtDNA maintenance, hampering mitochondrial respiration and ROS formation. ROS, combined with steatosis, increases lipid peroxidation and might be involved in drug-induced steatohepatitis. (*B*) Alterations of mitochondrial transcripts by interferon alfa or interferon beta. Interferons alfa and beta activate RNase L through the interferon receptor (IFNR). RNase L degrades *TFAM* messenger RNA, reducing levels of TFAM protein and transcription of mtDNA-encoded mitochondrial mRNAs. RNase L is also present within mitochondria, where it cleaves the mDNA-encoded mRNAs. Therefore, interferons alfa and beta reduce the synthesis and stability of mitochondrial transcripts.


A
Obesity, alcohol, VHC, LPS, drugs
Mitophagy
Altered MRC
Drugs, insulin resistance
Impaired β-oxidation
Fat deposits
Antioxidants
ROS
Mitophagy
Alcohol, LPS, HCV, HBV
TGF-β, TNF-α, 4-HNE, MDA
Stellate cell activation
Mitophagy
Necroptosis
Neutrophil infiltrates
Collagen synthesis
Leakage of mitochondrial DAMPS (mtDNA, formyl-peptides)
NF-κB and NLRP3 inflammasome activation
TNF-α, IL-1b, and other proinflammatory cytokines
Mitophagy
Mitophagy
Fibrosis
Inflammation
HCV, HBV, LPS


B
Normal hepatocyte
Damaged mitochondrion
Functional PINK1/ PARKIN-mediated mitophagy
Nucleus
Healthy mitochondrion
Partly damaged mitochondrion
PARKIN
DRP1
Fission
Biogenesis
PARKIN-decorated mitochondria
Mitofusin
LC3II/p62-recruitment of PARKIN
Fusion
Autophagosome
Healthy mitochondrial pool
Autolysosome degradation
Necrotic hepatocyte
Nucleus
Altered PINK1/ PARKIN-mediated mitophagy
Damaged mitochondria
Leaking mitochondrial DAMPs mtDNA, N-formyl-peptides, ATP
Released mtDNA
Released N-formyl peptides
Released ATP
FPR1
TLR9
Macrophage
NFkB
NFkB
Nucleus
Inflammation
NLRP3 inflammasome activation
Inflammatory cytokines
AIM2 inflammasome activation

recent conceptual advances. More detailed mechanisms and additional references are reported in previous reviews.[28,29]

AMPK and MAPK signaling pathways are altered in liver cells in response to stresses such as alterations in nutrients, cytokines, growth factors, and toxins. Altered AMPK and MAPK signaling play important roles in the development of liver diseases and injuries such as NAFLD, ALD, viral hepatitis, inflammation, fibrosis, carcinogenesis, and drug-induced hepatotoxicity.[28,29]

Adenosine Monophosate–Activated Protein Kinase

An increased ratio of AMP/ATP results in activation of AMPK,[28] which activates fatty acid β-oxidation and inhibits lipogenesis.[14,28] H_2O_2 production by mitochondria activates AMPK to regulate expression of antioxidant enzymes, via activation of NRF2.[28] A high-fat diet induced an antioxidant response in livers of wild-type mice but not NRF2 knockout ($Nrf2^{-/-}$) mice, which develop more severe NASH.[28] Livers from $Nrf2^{-/-}$ mice on a high-fat diet had decreased levels of AMPK, greater induction of lipogenic genes, and reduced expression of β-oxidation genes than wild-type mice. The reduced level of AMPK increased activity of SREBP-1c and SREBP-2, which increase lipogenesis.[28] Interestingly, fatty acid β-oxidation is restored by AMPK activators.[28] Therefore, H_2O_2-mediated AMPK activation protects against NASH by supporting mitochondrial function and suppressing lipogenesis. Importantly, AMPK has been shown to also stimulate glucose uptake and glycolysis, which may be relevant in preventing NASH-associated IR.[28]

Extracellular Signal-Regulated Kinase

Mitochondrial ROS-mediated activation of extracellular signal-regulated kinase controls mitochondrial mass by mitophagy and biogenesis, and regulates mitochondrial metabolic pathways and gluconeogenesis.[30] Extracellular signal-regulated kinase regulates these processes via general control of amino acid synthesis 5-like 1 (GCN5L1), which regulates FOXO1 at the post-transcriptional level. FOXO1 regulates hepatic gluconeogenesis, mitochondrial protein acetylation, and mitochondrial content during prolonged fasting.[30]

c-Jun N-Terminal Kinase

Activation of JNK and phosphorylation of its substrates lead to activation of the AP1 transcription factor, which regulates expression of other protein kinases and phosphatases. In the liver, the effects of JNK signaling vary with the duration and the extent of JNK activation—JNK is minimally or transiently activated in normal liver. Phosphorylated JNK translocates to mitochondria where it binds to MOM and MIM scaffold proteins to inhibit MRC, increasing ROS formation.[29,127,128] ROS then activate mitogen-activated protein kinase kinase kinase 5 (MAP3K5 or ASK1) and MKK4, which maintain activation of JNK.[29] Inhibiting interactions between phosphorylated JNK and mitochondria, and reducing production of mitochondrial ROS, reduce liver injury in mouse models.[29]

The roles for mitochondrial ROS-induced MAPK signaling pathways in the development of NAFLD, NASH, fibrosis, and IR have been investigated in animal models—sustained activation and mitochondrial translocation of JNK mediate liver injury.[29] Interfering with mitochondrial translocation of phosphorylated JNK prevents its sustained

Figure 6. Mechanisms of fibrosis and necroinflammation in steatohepatitis. (*A*) ROS can oxidize fat deposits, releasing lipid peroxidation reactive aldehydes malonedialdehyde and 4-hydroxynonenal, which damage mitochondrial DNA and MRC proteins to further block flow of electrons along the MRC—this increases mitochondrial ROS formation. ROS deplete antioxidants and cause the formation of TNF. These events further impair the flow of electrons and increase mitochondrial ROS formation. These vicious cycles are aggravated by factors such as excessive alcohol intake, some drugs, chronic HBV or HCV infection, and LPS, which all impair mitochondrial respiration and increase ROS formation. Lipid peroxidation products increase hepatic production of transforming growth factor β1 (TGFBI), which activates conversion of hepatic stellate cells into collagen-secreting myofibroblasts, leading to fibrosis. Mitochondria-derived ROS also increase the synthesis of TNF and several other cytokines in the liver, which can cause apoptosis and necrosis. Necrosis produces mitochondrial DAMPS (formyl peptides and mtDNA CpG motifs), which stimulate the innate immune system and production of inflammatory cytokines to promote liver inflammation. ROS-associated lipid peroxidation and cytokines contribute to the inflammatory cell infiltrate, because 4-hydroxynonenal and TGFB1, are chemo-attractants for neutrophils. Different steps for possible preventive roles of mitophagy are indicated. (*B*) Role of mitochondrial lesions in liver inflammation and preventive roles of mitophagy. Excessively damaged and dysfunctional mitochondria are degraded by mitophagy preventing them from releasing their danger signals into cytosol. Targeting mitochondria by mitophagy is associated with mitochondrial fusion and fission. Mitochondrial fission isolates damaged mitochondria (usually with low membrane potential) to initiate their degradation. Partially altered mitochondrion undergoes dynamic-related protein-1–mediated mitochondrial fission, resulting in a healthy small mitochondrion and a small, altered mitochondrion. The altered parts of mitochondria are targeted to autophagosome for their final degradation by mitophagy. The resulting healthy mitochondria undergo mitofusine 1– and 2–mediated mitochondrial fusion to restore functional mitochondria. Mitochondrial biogenesis then restores mitochondrial mass. However, in conditions that alter mitophagy pathway, damaged mitochondria accumulate within hepatocyte resulting in cell necrosis and the release of mitochondrial DAMPs, such as mtDNA, formylated proteins, and ATP. These danger signals then activate Toll-like receptor 9 and formyl peptide receptor 1, resulting in the translocation and activation of NF-κB, and thereby the expression of inflammatory cytokines. mtDNA and ATP also activate the inflammasomes nucleotide-binding oligomerization domain-like receptor NLRP3 and absent in melanoma 2-like receptor AIM2, respectively. The synthesized inflammatory cytokines and the activation of inflammasomes result in chronic liver inflammation, which allows steatohepatitis development.

activation, suppresses IR, and thereby prevents development of fatty liver in mice fed a high-fat diet.[128] Sustained activation of JNK is linked to IR in the liver via FGF21.[129] FGF21 is produced at highest levels in the liver, where it increases expression of PGC-1α to regulate glucose and lipid metabolism and insulin sensitivity in adipose tissue.[130] Hepatic deletion of JNK decreases hepatic *Fgf21* messenger RNA and reduces adipocyte hypertrophy and macrophage infiltration, reducing fatty liver and body weight gain in mice on a high-fat diet.[129] Sustained activity of hepatic JNK reduces expression of genes regulated by PPARα and decreases fatty acid β-oxidation.[130] Therefore, hepatic JNK decreases fatty acid β-oxidation by reducing expression of PPARα-target genes, increasing ketogenesis and FGF21 expression, and promoting development of IR.

ASK1 and MAPK311 (or MLK3) regulate hepatic JNK in response to diet. JNK is not activated in MLK3-knockout mice, which have reduced hepatic steatosis and liver triglyceride when they are fed a high-fat diet.[29] Liver-specific deletion of ASK1 reduces IR, fatty liver, and obesity in mice on high-fat diets.[29]

We have discussed alterations in metabolism and mitochondrial ROS-mediated signaling pathways in chronic liver diseases at the mitochondrial levels. However, in hepatocytes, metabolism is regulated by many transcription factors and nuclear receptors. These include the liver X receptor and SREBP-1c, farnesoid X receptor, PGC-1α, SREBP-1c, MLXIPL or CHREBP, pregnane X receptor, and HNF4A.[14]

Applying Findings to Clinical Research

Patients with obesity must lose weight, but rapid weight loss with severe dieting paradoxically increases liver inflammation and fibrosis.[1] Fasting also causes antioxidant depletion, which increases ROS formation.[3] Instead, physical exercise and a moderate hypocaloric diet can progressively decrease adipose tissue fat stores, improve liver tests, and stop fibrogenesis.[3]

Given the ability of AMPK to increase β-oxidation and glycolysis, it is a therapeutic target for patients with NASH. The AMPK activator A-769662 increases fatty acid oxidation, reduces mechanisms of lipogenesis, and reduces plasma triglyceridemia and glycemia.[28] The hepatoprotective protein cardiotrophin activates AMPK, decreasing liver triglycerides and reducing inflammation in animal models of NAFLD.[28] Another activator of AMPK, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside, increases insulin sensitivity and improves glucose uptake, so it might benefit patients with NAFLD.[28] Moreover, AMPK activation also prevents and reverses drug-induced mitochondrial damage and hepatocyte injury by promoting mitochondrial fusion and function.[122]

Inhibitors of JNK would interfere with the physiological effects of JNK signaling, so they might have many side effects. In contrast, approaches to block interaction of JNK with mitochondria to prevent sustained activation of JNK in these organelles might be developed for treatment of chronic liver diseases. Inhibitors of ASK1, which activates JNK and mediates its translocation to mitochondria to increase the production of ROS, have been developed. The ASK1 inhibitors GS-4997 and GS-444217 prevented diet-induced fatty liver in mice.[132] A phase 2 study reported that the ASK1 inhibitors reduces liver fibrosis in humans with NASH.[132] Because ROS activate ASK1, up-regulation of antioxidant genes and antioxidant supplementation are other strategies to prevent sustained activation of JNK.

Findings from pilot studies of α-tocopherol (vitamin E) and other antioxidants have been summarized in previous reviews.[14,133] Six months of supplementation with vitamin E and vitamin C improved results from liver tests and reduced fibrosis but not inflammation in patients with NASH.[14] The PIVENS (Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis) trial tested the effects of vitamin E or pioglitazone in non-diabetic patients with NAFLD and found these to reduce steatohepatitis.[134] The GSH precursor N-acetylcysteine prevents development of steatosis induced by choline- and methionine-deficient diet in rats[14] and obese *ob/ob* mice.[135] MitoQ, which specifically scavenges superoxide anion produced within mitochondria, restores immune functions in patients with chronic HBV infection.[66]

Strategies to reduce ROS production are more complex, especially in patients with NASH and IR. Low concentrations of ROS can increase insulin signaling by inhibiting the protein-tyrosine phosphatase PTP1B.[136] Additionally, ROS up-regulates expression of PGC-1α and antioxidant enzymes.[136] Studies of antioxidants as treatments for NAFLD should consider not only the deleterious effects of ROS, but also their signaling functions. Further complicating the use of antioxidants is the specificity of ROS scavengers. Scavenging superoxide with SOD mimetics produces H_2O_2 and exacerbates steatohepatitis unless GSH was replenished.[60,136] It might be more effective to boost mitochondrial redox homeostasis using NAD^+ precursors. For example, resveratrol increases the NAD^+/NADH ratio and decreases oxidative stress, reduces hepatic lipogenesis, and reduces steatosis through activation of SIRT1 and AMPK in animal models of NAFLD[14] and in obese persons.[137]

In obese rats and mice, metformin increases insulin sensitivity and reduces hepatic steatosis and ROS formation, likely via its ability to regulate AMPK signaling.[58,138] The benefit of metformin has been demonstrated in a randomized controlled trial and in a meta-analysis.[14] Pharmacologic approaches that aimed to increase insulin signaling, via stimulation of fatty acid β-oxidation or mitochondrial uptake of fatty acids through CPT1, have also been tested. For example, etomoxir blocks CPT1 and decreases plasma glucose, increasing expression of the GLUT4 receptor in human myocytes.[137] Hydroxytyrosol inhibits the SREBP-1c pathway, prevents liver steatosis, and reduces IR in mice on a high-fat diet.[136]

Other pharmacologic strategies act broadly to alter energy expenditure or influence pathways that contribute to NAFLD, such as uncoupling compounds or agonists for PGC-1α, PPARγ, and PPARα/δ and their coactivators.[2,14,133] Alternatives to activate mitophagy, such as autophagy activators, have reduced fatty liver in mice.[22,23] Strategies to restore mitochondrial function can provide antiviral

immune activity to patients with chronic HBV infection. MitoQ protects the liver and restores $CD8^+$ T cells, which have been exhausted, to these patients.[66]

Future Directions

Unhealthy lifestyles (rich diet, lack of exercise, alcohol and drug intake) lead to fatty liver, which can develop into steatohepatitis in some patients. Oxidative stress, mitochondrial DAMPs, lipid peroxidation, drugs, alcohol, and HBV and HCV infection might promote progression of AFLD and NAFLD toward more severe forms of steatohepatitis.

The relationships among obesity, onset of IR, and NAFLD have been widely characterized. Impaired mitochondrial function contributes to hepatic fat accumulation, defective insulin signaling, and dysregulated lipoprotein trafficking. A severe impairment of mitochondrial oxidative phosphorylation decreases ATP, leading to cell dysfunction, and can thereby inhibit β-oxidation, leading to steatosis. Mitochondria adapt to increasing hepatic lipid content by increasing rates of β-oxidation and MRC activities. However, this leads to the increased production of ROS, which likely contributes to development of IR and reduces activity of the MRC, observed in the pronounced stages of NAFLD. Mitochondrial ROS lead to the accumulation of mitochondrial oxidized components, which further exacerbate IR and lead to NASH.

Despite the high prevalence of AFLD, targeted therapies have not been developed and we have a poor understanding of its pathogenesis. Most mechanisms of AFLD have been studied in animal models, which do not have all the human features of the disease. There are some targeted strategies to manage mitochondrial disorders in patients with liver diseases, but these can have many nonspecific effects. It will be important to develop agents with greater specificity for targets involved in pathogenesis of AFLD and NAFLD. Developing and testing molecules that target specific steps in mitochondrial ROS-mediated signaling pathways will be important for treatment of chronic liver diseases. With the exception of chronic HBV and HCV infection, during which the viruses induce mitophagy to escape immune system surveillance, strategies to activate mitophagy could be effective in reducing progression of liver injuries that involve mitochondrial damage and dysfunction. Correlation of plasma levels of DAMPs of mitochondrial origin (such as mtDNA) with stages of fibrosis, liver inflammation, or cirrhosis might be used in diagnosis, monitoring of disease progression, or identifying patients who require surveillance.

We also need to learn more about the long-term progression of NASH, in large prospective cohort studies. Treatments developed for HBV should include strategies to reduce mitochondrial dysfunction and autophagy. In addition, more relevant animal models of chronic liver diseases are needed to study mitochondrial dysfunction. Transcriptome, proteome, and genome-wide association studies might identify new mitochondrial mechanisms and targets, and identify genetic variants that affect risk for or outcomes of liver diseases. It is also important to remember to screen drugs for mitochondrial toxicity before testing them in patients with liver diseases.

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Conflicts of interest
The authors disclose no conflicts.