

Oxidative Stress and Mitochondrial Dysfunction - Mediated NLRP3 Inflammasome Activation in MASLD Progression: Mechanisms and Therapeutic Implications

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Abstract: Metabolic dysfunction-associated steatotic liver disease (MASLD) is highly prevalent and may progress from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, cirrhosis, and hepatocellular carcinoma. Mitochondrial dysfunction and oxidative stress are central to this progression. Although increased mitochondrial oxidation may initially compensate for substrate overload, persistent lipotoxicity ultimately impairs respiratory capacity, redox homeostasis, and mitochondrial quality control. Damaged mitochondria generate mitochondrial reactive oxygen species and release danger signals, including oxidized mitochondrial DNA and cardiolipin, while defective mitophagy promotes their accumulation. Together with inflammatory priming signals, these changes facilitate NLRP3 inflammasome assembly, caspase-1 activation, IL-1 β and IL-18 maturation, and gasdermin D-mediated pyroptosis. Hepatocytes are important sources of mitochondrial danger signals, whereas myeloid cells appear to be major NLRP3-dependent inflammatory and fibrogenic effectors. Hepatic stellate cells subsequently translate inflammatory and pyroptotic signals into extracellular matrix deposition. Antioxidants, mitochondria-targeted agents, metabolic therapies, and selective NLRP3 inhibitors may modulate different nodes of this axis. However, the histological benefits of currently available metabolic therapies do not establish NLRP3 inhibition as their clinical mechanism, and direct NLRP3 inhibition remains preclinical in MASH, with efficacy varying across experimental models. Future studies should integrate human-relevant models, spatial and single-cell approaches, cell-specific causal validation, and mechanistic biomarkers to identify patients most likely to benefit from targeted intervention.

Keywords: Metabolic dysfunction-associated steatotic liver disease, Mitochondrial dysfunction, Oxidative stress, NLRP3 inflammasome, Mitophagy, Pyroptosis, Liver fibrosis.

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by hepatic steatosis with cardiometabolic risk and may progress to metabolic dysfunction-associated steatohepatitis (MASH), cirrhosis, and hepatocellular carcinoma [1]. Using the former NAFLD criteria, its global adult prevalence was estimated at approximately 32% [2], and fibrosis stage is a major determinant of liver-related outcomes [3].

Progression to MASH reflects interacting metabolic stress, mitochondrial dysfunction, inflammation, and cell death [4, 5]. Damaged mitochondria generate mitochondrial reactive oxygen species (mtROS) and release danger signals,

including oxidized mitochondrial DNA (ox-mtDNA), that promote NLRP3 inflammasome activation [6]. Activated NLRP3 drives caspase-1-dependent maturation of IL-1 β and IL-18 and gasdermin D-mediated pyroptosis [7]. Pyroptotic hepatocytes can activate hepatic stellate cells and promote fibrosis [8], while NLRP3 blockade reduces inflammation and fibrosis in experimental NASH [9].

This review examines the mechanisms, cell- and stage-specific effects, therapeutic implications, and translational uncertainties of this axis in MASLD. The overall framework linking metabolic overload to mitochondrial injury, NLRP3 activation, inflammation, and fibrosis is summarized in Figure 1.

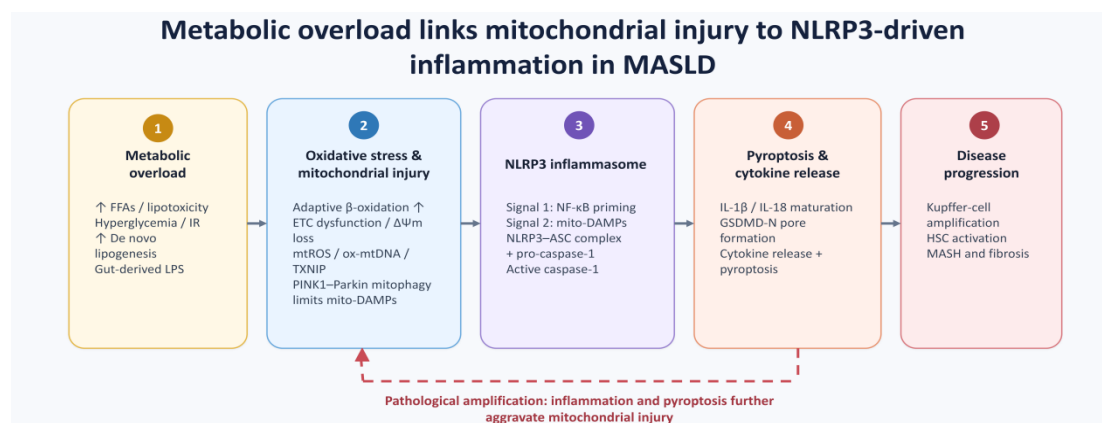


Figure 1: Integrated oxidative stress-mitochondrial injury-NLRP3 axis in MASLD

2. Metabolic Stress and Mitochondrial Redox Imbalance in MASLD

2.1 Lipotoxicity and Major Sources of Oxidative Stress

Triglyceride storage can initially buffer excess fatty acids, whereas saturated fatty acids, ceramides, free cholesterol, and oxidized lipids become hepatotoxic when uptake exceeds oxidation, export, and safe storage [10]. Mitochondrial electron leakage is a major source of reactive oxygen species (ROS); peroxisomal oxidation, CYP2E1, NADPH oxidases, and endoplasmic reticulum stress also contribute, promoting lipid peroxidation, respiratory-chain injury, loss of membrane potential, and mitochondrial DNA oxidation [11].

2.2 Mitochondrial Adaptation and Decompensation

Mitochondrial dysfunction is stage dependent. In early steatosis, increased tricarboxylic acid cycle flux and respiration may compensate for substrate excess, whereas this adaptation is lost in MASH, with impaired respiratory capacity and increased ROS [12, 13]. Thus, progression reflects loss of mitochondrial flexibility rather than a uniform decline in oxidative metabolism.

2.3 Impaired Mitophagy

Mitophagy removes damaged mitochondria and limits mitochondrial danger-signal accumulation. In experimental progression from NAFLD to NASH, impaired mitophagy increases mtROS and promotes NLRP3 activation [14], linking defective mitochondrial quality control to inflammatory progression.

These mitochondrial abnormalities generate the activation signals discussed in the following section.

3. Mitochondrial Danger Signals Promote NLRP3 Inflammasome Activation

3.1 Priming and Activation of the NLRP3 Inflammasome

Priming through Toll-like receptor or cytokine receptor signaling activates NF- κ B and increases NLRP3 and pro-IL-1 β expression [15]. Activation signals subsequently induce K⁺ efflux, NEK7–NLRP3 interaction, ASC assembly, and caspase-1 activation [15, 16]. Caspase-1 then processes pro-IL-1 β , pro-IL-18, and GSDMD [7]; therefore, increased NLRP3 expression alone indicates priming rather than functional inflammasome activation.

3.2 Mitochondrial ROS as an Upstream Signal

Mitochondrial ROS can promote NLRP3 activation by promoting oxidative mitochondrial damage and loss of mitochondrial integrity, and suppression of mitochondrial ROS reduces inflammasome activity in several experimental systems [17]. In MASLD-related experimental settings, mitochondrial ROS may link lipotoxic stress to NLRP3 activation [14]. However, mitochondrial support of NLRP3 activation is not universally ROS dependent; in macrophages, an intact electron transport chain can sustain NLRP3

activation through phosphocreatine-dependent ATP production independently of mitochondrial ROS [18].

3.3 Oxidized Mitochondrial DNA and Cytosolic mtDNA

Newly synthesized mtDNA can become oxidized, enter the cytosol, and bind NLRP3 [19]. Mitochondrial DNA may also engage inflammasome-independent innate immune pathways. In experimental NASH, STING signaling in Kupffer cells contributes to inflammation [20], whereas hepatocyte-derived mtDNA activates TLR9 [21]. Therefore, detection of cytosolic or extracellular mtDNA alone does not establish direct NLRP3 activation.

3.4 TXNIP, Cardiolipin, and Ion Fluxes

Oxidative stress-dependent TXNIP release and cardiolipin exposure can facilitate NLRP3 activation [22, 23], but K⁺ efflux–NEK7 is the more consistent convergence mechanism [16].

3.5 Mitophagy Constrains NLRP3 Activation

Mitophagy restrains NLRP3 by removing mitochondria that generate mtROS and release mtDNA. In macrophages, LC3B or Beclin 1 deficiency promotes the accumulation of damaged mitochondria and cytosolic mtDNA and enhances caspase-1 activation [24], whereas TNF- α -mediated suppression of hepatocyte mitophagy may reinforce inflammation in MASH [25]. Because LC3-II and p62 accumulation cannot distinguish increased autophagosome formation from impaired degradation, dynamic flux should be assessed [26].

3.6 A Self-amplifying Mitochondrial-inflammatory Loop

Mitochondrial injury and inflammasome activation form a positive feedback loop. Caspase-1 activation can increase mitochondrial permeability and inhibit Parkin-dependent mitophagy [27], whereas GSDMD pore formation promotes cytokine release and pyroptotic cell injury [28]. Persistent NLRP3–caspase-1–GSDMD signaling thereby expands intracellular injury into pyroptosis and hepatic inflammation [28, 29], while pyroptotic hepatocytes and released inflammasome particles can promote HSC activation and fibrosis [8].

Once released from injured or pyroptotic cells, these signals extend the response from intracellular inflammasome activation to multicellular liver injury.

4. Cellular Crosstalk and Stage-Specific Roles in MASLD Progression

4.1 Hepatocyte Injury Initiates Mitochondrial Danger Signaling

During metabolic stress, injured or dying hepatocytes release mtDNA and other mitochondrial danger signals. Hepatocyte-derived mtDNA activates TLR9 and promotes steatohepatitis [21], whereas hepatocyte-derived mitochondrial danger signals, with mtDNA as a major active component, can directly activate HSCs and promote fibrosis [30].

4.2 Macrophage NLRP3 Activation Amplifies Hepatic Inflammation

Kupffer cells and recruited macrophages integrate gut-derived inflammatory priming signals [10] with hepatocyte-derived mitochondrial danger signals [21, 30]. Myeloid-specific *Nlrp3* deletion reduces liver inflammation and fibrosis, identifying myeloid cells as major NLRP3 effectors in experimental MASH [31].

4.3 HSCs Translate Inflammation into Fibrosis

Hepatocyte pyroptosis and released inflammasome particles can activate HSCs [8], whereas HSC-intrinsic NLRP3 signaling may further promote fibrogenesis [32]. Available cell-specific evidence nevertheless favors myeloid NLRP3 as the major upstream driver [31].

4.4 Stage-specific Cellular Contributions

Simple steatosis may retain mitochondrial adaptation [13], MASH shows intensified hepatocyte-immune cell crosstalk [33], and advanced disease is increasingly dominated by fibrogenic gene programs [34].

5. Therapeutic Targeting of the Oxidative Stress-Mitochondrial Dysfunction-NLRP3 Axis

5.1 Antioxidant and Mitochondria-targeted Strategies

Vitamin E improved steatohepatitis activity but did not significantly improve fibrosis in the PIVENS trial [35]. The mitochondria-targeted antioxidant MitoQ attenuates experimental MASH but lacks human histological validation [36].

5.2 Direct Inhibition of NLRP3

MCC950 reduces inflammation and fibrosis in some experimental MASH models [9], although negative findings have been reported in a separate long-term HFHC model [37]. The selective NLRP3 inhibitor JT001 has also shown pharmacological activity in preclinical MASH models [38]. However, direct NLRP3 inhibition lacks human histological efficacy evidence in MASH and remains preclinical.

5.3 Indirect Metabolic Modulation of the Axis

Semaglutide improves MASH resolution without worsening of fibrosis and fibrosis improvement without worsening of MASH [39]. However, the trial did not establish NLRP3 inhibition as the mechanism underlying these clinical benefits.

5.4 Lifestyle Intervention

Lifestyle intervention remains foundational, and weight loss

of at least 10% is associated with MASH resolution and fibrosis regression [40].

5.5 Natural Products Targeting Multiple Nodes

Oridonin acts as a covalent NLRP3 inhibitor in preclinical inflammasome systems [41], whereas berberine suppresses ROS/TXNIP-associated NLRP3 activation in experimental NASH [42]. However, these mechanistic findings do not establish clinical efficacy; silymarin failed its primary histological endpoint [43].

5.6 Translational Challenges

Translation is limited by heterogeneous disease mechanisms [44], the incomplete correspondence between animal models and human MASLD [45], and liver-biopsy sampling variability [46]. In addition, validated biomarkers demonstrating engagement of the mitochondrial-NLRP3 axis are currently lacking. Resmetirom improves MASH resolution and fibrosis endpoints [47], but the trial did not determine whether these benefits involved modulation of this axis. Development should prioritize biomarker-defined patients and liver- or myeloid-targeted delivery.

Table 1 summarizes representative interventions targeting different nodes of the axis, distinguishing direct from indirect modulation and highlighting differences in evidence maturity and translational limitations. These limitations support the development of biomarker-defined patient populations and liver- or myeloid-targeted delivery strategies.

6. Controversies and Future Perspectives

6.1 Context-dependent Roles of NLRP3

Key controversies concern cellular source, mitochondrial dependence, and disease context. Hepatocytes are major sources of mitochondrial danger signals, whereas myeloid cells appear to be major NLRP3-dependent inflammatory and fibrogenic effectors [30, 31]. Mitochondrial injury is an important but not exclusive route to NLRP3 activation [7]. Moreover, MCC950 is protective in some MASH models [9], whereas neither genetic deletion of *Nlrp3* nor pharmacological inhibition of NLRP3 ameliorates disease in some models [37, 52]. NLRP3 is therefore a context-dependent amplifier rather than a universal driver.

6.2 Limitations of Current Evidence

Most evidence directly linking mitochondrial injury to NLRP3 activation in MASLD remains preclinical, and available models reproduce different components of human disease [45]. Studies should validate the source and specificity of ROS and cytosolic mtDNA measurements [53, 54] and distinguish NLRP3 priming from activation using cleaved caspase-1, mature IL-1 β , or GSDMD cleavage [7].

Table 1: Representative pharmacological interventions targeting the oxidative stress–mitochondrial damage–NLRP3 axis

Intervention	Primary node	Representative evidence	Main efficacy signal	Axis engagement	Evidence level	Key limitation / status
A. Human interventional evidence in MASH						
Vitamin E [35]	Oxidative stress	PIVENS; 96-week multicenter randomized, double-blind, placebo-controlled trial	Improved the prespecified composite histologic outcome and steatosis/inflammation; fibrosis was not significantly improved.	Proximal; antioxidant effect, but mitochondrial/NLRP3 markers were not tested	Level I—positive	Population excluded diabetes; long-term safety and antifibrotic efficacy remain uncertain.
Selonsertib [48]	ASK1 stress kinase	STELLAR-3/4; two 48-week phase 3 RCTs in F3 or F4 NASH	Showed pharmacodynamic p38 suppression but did not improve fibrosis or prespecified secondary efficacy endpoints versus placebo.	Proximal; oxidative-stress signaling, not NLRP3-specific	Level I—negative	Demonstrates that target engagement upstream of inflammation does not guarantee histologic benefit.
Resmetirom [47]	Hepatic THR-β	MAESTRO-NASH; phase 3 biopsy RCT in noncirrhotic NASH with F1B–F3 fibrosis	Improved MASH/NASH resolution and ≥1-stage fibrosis improvement; reduced liver fat and atherogenic lipids.	Indirect; reduces lipotoxic load and may lessen downstream mitochondrial danger signaling	Level I—positive	No direct demonstration that clinical benefit is mediated through mtROS or NLRP3.
Semaglutide [39]	GLP-1 receptor	ESSENCE part 1; 72-week phase 3 biopsy RCT in F2–F3 MASH	Improved MASH resolution without worsening of fibrosis and ≥1-stage fibrosis improvement without worsening of MASH at week 72.	Indirect; weight loss and metabolic improvement may reduce lipotoxicity and inflammatory priming	Level I—positive	Axis biomarkers were not causal endpoints.
Tirzepatide [49]	GIP/GLP-1 receptors	SYNERGY-NASH; 52-week phase 2 biopsy RCT in F2–F3 MASH	Produced dose-related MASH resolution; a higher proportion of participants achieved ≥1-stage fibrosis improvement without worsening of MASH than with placebo.	Indirect; reduces adiposity, insulin resistance and substrate overload	Level II—positive	Phase 2 duration and sample size; no direct mitochondrial or inflammasome target-engagement data.
B. Axis-directed preclinical pharmacology						
MCC950 [9, 37]	Direct NLRP3 inhibition	Atherogenic-diet-fed obese diabetic foz/foz mice and MCD-fed mice; separate 6-month HFHC study	Reduced caspase-1/IL-1β, inflammation and fibrosis in two models, but Nlrp3 deletion or MCC950 was ineffective in HFHC NASH.	Direct pharmacologic NLRP3 inhibition with pathway readouts	Level III—mixed	Marked model, diet, strain and treatment-window dependence; no human MASH biopsy trial.
JT001 [38]	Direct NLRP3 inhibition	Cell assays; biopsy-confirmed AMLN diet-induced obese NASH mice; choline-deficient NASH and Nlrp3 gain-of-function models	Inhibited NLRP3 assembly, IL-1β release and pyroptosis and reduced hepatic inflammation. Fibrosis fell significantly in the choline-deficient and gain-of-function models but showed only a trend in DIO-NASH.	Direct; pharmacokinetic/pharmacodynamic linkage reported	Level III—positive	Multiple murine models but one preclinical development program; human efficacy and chronic safety unknown.
MitoQ [36]	Mitochondrial redox stress	Four-week methionine- and choline-deficient high-fat diet (MCDHFD) mouse model	Reduced histologic steatosis and necroinflammation, increased catalase/GPx-1 expression and decreased selected inflammatory and profibrogenic transcripts.	Proximal/indirect; mitochondria-targeted antioxidant, but mtROS, mitochondrial function and NLRP3 were not directly measured	Level IV—positive	Single short-term dietary model; gene-expression changes do not establish antifibrotic efficacy or NLRP3 mediation.
Sulforaphane [50]	AMPK–autophagy-associated NLRP3 regulation	Nine-week HFD mice with concurrent sulforaphane; palmitate-treated primary mouse hepatocytes	Reduced steatosis, ALT/AST, caspase-1 activity and IL-1β in mice; restored mitochondrial function and suppressed NLRP3 activation via AMPK–autophagy in hepatocytes.	Multinodal; antioxidant/autophagy effects converge on NLRP3	Level IV—positive	Preventive concurrent-treatment design; mitochondrial rescue was shown mainly in hepatocytes, with no substantive fibrosis assessment.
Sweroside [51]	NLRP3 activation	Two-week MCD-fed mice with concurrent intraperitoneal treatment; bone-marrow-derived macrophages	Reduced IL-1β/caspase-1 production, immune-cell infiltration, triglyceride accumulation, fibrosis-associated markers, and ASC-speck formation.	Multinodal; associated with suppression of inflammasome activation	Level IV—positive	Short preventive MCD model; direct NLRP3 binding, pharmacokinetic exposure and clinical feasibility remain unresolved.

Notes: Evidence hierarchy: Level I, phase 3 randomized biopsy trial with histological endpoints; Level II, phase 2 randomized biopsy trial; Level III, in vivo MASLD/MASH evidence with pharmacologic target engagement; Level IV, single-model or predominantly in vitro evidence. Level reflects study maturity—not effect size or axis specificity.

Translational interpretation: Phase 3 trials have demonstrated histologic efficacy of resmetirom and semaglutide in noncirrhotic MASH with fibrosis; however, neither trial establishes that NLRP3 inhibition mediates the clinical benefit. Selective NLRP3 inhibitors remain at the preclinical stage for MASH, and their reported efficacy varies across experimental models.

Abbreviations: ALT, alanine aminotransferase; AMLN, Amylin liver NASH diet; AMPK, AMP-activated protein kinase; ASC, apoptosis-associated speck-like protein containing a CARD; ASK1, apoptosis signal-regulating kinase 1; AST, aspartate aminotransferase; DIO, diet-induced obesity; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GPx-1, glutathione peroxidase 1; HFD, high-fat diet; HFHC, high-fat, high-cholesterol diet; IL, interleukin; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; MCD, methionine- and choline-deficient diet; MCDHFD, methionine- and choline-deficient high-fat diet; mtROS, mitochondrial reactive oxygen species; NASH, nonalcoholic steatohepatitis; NLRP3, NLR family pyrin domain containing 3; RCT, randomized controlled trial; THR-β, thyroid hormone receptor β.

6.3 Priorities for Future Research

Spatial multi-omics has identified disease-associated cellular states and intercellular programs in human MASLD [55]. Future studies should integrate such approaches with functional assays to determine whether candidate mitochondrial–NLRP3 signals are spatially and causally linked to disease progression. Human hepatocyte organoids can support causal target testing and drug screening [56], although multicellular models are still needed. Circulating biomarker panels can support noninvasive identification of steatohepatitis [57], but axis-specific biomarkers will be required to identify patients with a mitochondrial injury-high/NLRP3-high phenotype. Future trials should prioritize biomarker-enriched populations and liver- or myeloid-targeted interventions.

7. Conclusions

The oxidative stress–mitochondrial damage–NLRP3 axis links metabolic overload to inflammation and fibrosis in MASLD. Persistent lipotoxicity generates mitochondrial danger signals that promote NLRP3–caspase-1–GSDMD signaling and pyroptosis [6, 7, 28]. Hepatocytes may provide these danger signals, whereas myeloid cells are major NLRP3-dependent effectors [30, 31]. However, divergent experimental findings indicate that NLRP3 is a context-dependent amplifier rather than a universal driver [9, 37, 52]. Translation will require human-relevant models, cell-specific validation, mechanistic biomarkers, and biomarker-guided patient selection.

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