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# Research Advances in the Pathogenesis of Non-Alcoholic Fatty Liver Disease

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**Abstract:** ***Non-alcoholic fatty liver disease (NAFLD) is a chronic metabolic disorder characterized by hepatic steatosis. Its prevalence continues to rise against the backdrop of metabolic syndrome and obesity, posing a significant public health threat. Recent advancements in metabolomics, single-cell sequencing, and gene-editing technologies have greatly enhanced our understanding of NAFLD pathogenesis. This article reviews the latest research progress on NAFLD mechanisms, focusing on metabolic dysregulation, inflammatory responses, endoplasmic reticulum (ER) stress, and gut microbiota dysbiosis.***

**Keywords:** Non-alcoholic fatty liver disease (NAFLD), Pathogenesis, Insulin resistance, Lipid metabolism disorder, Gut microbiota**.**

## 1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases worldwide [1]. It is defined by the presence of ≥5% hepatic steatosis in the absence of excessive alcohol consumption or other liver disease etiologies. The global prevalence of NAFLD has increased from 25.3% (1990–2006) to 38.2% (2016–2019), representing a 50.4% rise [2] .In China, rapid socioeconomic development and lifestyle changes have driven a surge in NAFLD prevalence, making it the most prevalent chronic liver disease [2,3], NAFLD is closely associated with metabolic comorbidities, including obesity (51.34%), type 2 diabetes (22.51%), dyslipidemia (69.16%), hypertension (39.34%), and metabolic syndrome (42.54%). Fibrosis progression and annual progression rates in NASH are 40.76% and 0.09, respectively, while the hepatocellular carcinoma incidence among NAFLD patients is 0.44 per 1000 person-years [4].

NAFLD encompasses non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), and advanced stages such as liver fibrosis (LF) and cirrhosis [5]. NAFL is histologically characterized by simple steatosis, whereas NASH involves hepatocyte injury, inflammation, and possible fibrosis. Cirrhosis arising from NASH is termed NASH cirrhosis. NAFLD is strongly linked to obesity, insulin resistance, type 2 diabetes, hypertension, dyslipidemia, and metabolic syndrome [6]. The pathogenesis of NAFLD is complex, involving metabolic, inflammatory, and fibrotic processes. The classical "two-hit hypothesis" proposed by Day and James in 1998 has been increasingly recognized as oversimplified [7]. Emerging evidence supports a "multiple parallel hits" model, where diverse factors and mechanisms synergistically drive disease progression [8]. This review summarizes recent advances in NAFLD pathogenesis.

## 2. Metabolic Imbalance and Lipotoxicity

Non-alcoholic fatty liver disease (NAFLD) exhibits a pathophysiological association with metabolic syndrome components, notably dyslipidemia, obesity, and insulin-resistant type 2 diabetes mellitus. Substantial clinical evidence has established that systemic insulin resistance constitutes a hallmark metabolic perturbation in NAFLD patients. The hepatic triglyceride (TAG) accumulation central to NAFLD pathogenesis originates from three principal sources: dietary chylomicron remnants, adipose tissue-derived lipolytic free fatty acids (FFAs), and hepatic de novo lipogenesis (DNL). Following cellular uptake, FFAs undergo esterification via acyl-CoA synthetase catalysis to form fatty acyl-CoA derivatives. These metabolites are subsequently partitioned into mitochondrial β-oxidation pathways, endoplasmic reticulum-mediated TAG synthesis for cytosolic lipid droplet (LD) storage, or apolipoprotein B100-coupled assembly into very-low-density lipoprotein (VLDL) particles for hepatic secretion [9]. The insulin-resistant state drives NAFLD progression through dual mechanisms: transcriptional upregulation of DNL enzymes and diminished suppression of adipocyte lipolysis, collectively resulting in pathological FFA flux to hepatocytes. At the molecular level, PPARγ signaling axis dysfunction in adipose tissue exacerbates metabolic dysregulation, manifesting as endocrine dysfunction characterized by hypoadiponectinemia and elevated secretion of proinflammatory cytokines including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [10,11]. Concurrently, impaired mitochondrial β-oxidation capacity contributes to the accrual of lipotoxic species, particularly ceramides and diacylglycerols. These metabolites activate protein kinase C isoforms while synergizing with lysophosphatidylcholine derivatives and oxysterols (e.g., 27-hydroxycholesterol) to establish a hepatotoxic microenvironment through sustained endoplasmic reticulum stress and inflammasome activation [12–14].

## 3. Inflammation and Immune Dysregulation

The inflammatory cascade within the hepatic microenvironment serves as a critical driver of NAFLD progression. Hepatic inflammation arises through synergistic interactions between extrahepatic mediators (notably dysfunctional adipose tissue and gut-derived signals) and intrahepatic triggers encompassing lipotoxic stress, pattern recognition receptor activation, and programmed cell death pathways [15]. Emerging evidence indicates that adipose tissue remodeling induces macrophage infiltration and crown-like structure formation in visceral fat depots, generating systemic inflammatory flux through elevated circulating levels of tumor necrosis factor-α(TNF-α),

interleukin-1β (IL-1β), and interleukin-6 (IL-6)–cytokines that directly promote hepatic injury while exacerbating insulin resistance through JNK-1 and SOCS3-dependent mechanisms [16]. Notably, clinical studies demonstrate significant positive correlations between serum IL-6 concentrations, histological NASH severity, fibrosis staging, and the degree of insulin resistance [17]. In addition, fat accumulation in hepatocytes triggers oxidative stress and endoplasmic reticulum stress, which in turn activate inflammatory signaling pathways such as nuclear factor-κB (NF-κB) and NLRP3 inflammatory vesicles. Activation of these signaling pathways leads to the release of pro-inflammatory cytokines, further exacerbating liver inflammation and fibrosis [18] Immune cells in the liver, particularly macrophages (Kupffer cells) and T cells, are critical in the inflammatory response in NAFLD. Kupffer cells are the major immune cells in the liver and are able to regulate hepatic inflammation by phagocytosis of exogenous substances, presentation of antigens, and triggering of immune responses [19]. CD8+ T cells exert cytotoxic effects through perforation, granzyme and pro-inflammatory cytokine secretion [20]. With regard to the pathogenesis of NASH, studies have shown that there is an increase in the number of activated cytotoxic CD8+ T cells in the liver, accompanied by elevated levels of inflammatory mediators. This enhanced CD8+ T cell activity correlates with the progression of liver injury [21].

## 4. Endoplasmic Reticulum (ER) Stress

Under physiological conditions, the liver maintains transient endoplasmic reticulum (ER) stress responses that are rapidly resolved through adaptive homeostatic mechanisms. In NAFLD pathogenesis, however, persistent ER stress establishes a self-perpetuating cycle that drives disease progression toward cirrhosis and hepatocellular carcinoma via sustained inflammatory signaling and regulated cell death pathways [22,23]. ER stress activation in this context is principally induced by three metabolic perturbations: lipid overload (particularly free fatty acid flux), proteostatic imbalance, and redox dysregulation. Preclinical studies utilizing high-fat diet (HFD) models demonstrate significant upregulation of canonical ER stress markers including glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP) [24,25]. Notably, ER stress exhibits bidirectional crosstalk with hepatic lipid metabolism. Experimental evidence reveals that ER stress upregulates diacylglycerol acyltransferase 1 (DGAT1)-mediated triglyceride synthesis, thereby exacerbating steatosis [26]. Mechanistically, ER stress modulates lipid homeostasis through transcriptional regulation of metabolic genes, such as sterol regulatory element-binding protein 1c (SREBP-1c), whose activation promotes de novo lipogenesis and intrahepatic lipid accumulation [27]. The transition from steatosis to steatohepatitis involves ER stress-mediated activation of apoptotic cascades, predominantly through CHOP-dependent induction of pro-apoptotic factors. Han et al. [28] demonstrated that pharmacological attenuation of ER stress via inhibition of the IRE1α-TRAF2-JNK signaling axis significantly reduces hepatocyte apoptosis and ameliorates NAFLD progression in experimental models.

## 5. The Gut-liver Axis Mechanism

### 5.1 Dysbiosis of the Gut Microbiota

The gut-liver axis constitutes a fundamental pathophysiological interface in hepatic disorders. Anatomically, the portal venous system establishes direct circulatory connectivity between intestinal and hepatic compartments, delivering approximately 70% of hepatic blood supply from splanchnic circulation [29]. Emerging evidence positions gut microbial dysbiosis as a critical modulator of NAFLD progression, with clinical studies demonstrating characteristic shifts in NAFLD/NASH patients' microbiota profiles—marked by enrichment of Proteobacteria and Prevotella phyla alongside Firmicutes depletion [30–32]. Bile acid (BA) metabolism exemplifies gut-liver crosstalk, with primary BAs synthesized from hepatic cholesterol undergoing microbial biotransformation into secondary bile acids (e.g., deoxycholic acid, DCA). This process is tightly regulated through farnesoid X receptor (FXR)-mediated negative feedback, where FXR activation suppresses cytochrome P450 7A1 (CYP7A1) expression to control BA synthesis [33,34]. Preclinical models reveal that microbial modulation of tauro-β-muricholic acid (TβMCA), an endogenous FXR antagonist, activates the FXR-fibroblast growth factor 15 (FGF15) axis to inhibit hepatic BA production [35]. Experimental investigations further elucidate environmental toxicant-microbiota interactions. Chi et al. [36] demonstrated that subtoxic PCB126 exposure induces gut dysbiosis in murine models, subsequently provoking hepatic lipid accumulation, dyslipidemia, and histopathological liver injury through gut-liver axis activation.

### 5.2 Increased Intestinal Permeability

The gut-liver axis operates through a multilayered barrier system, with intestinal barrier integrity serving as the primary gatekeeper against microbial translocation. This barrier comprises three synergistic components: (1) physical defenses (tight junction proteins including occludin and claudin-1), (2) biochemical protection (mucus layer and antimicrobial peptides), and (3) immunological surveillance (lamina propria immune cells and commensal microbiota) [37]. Pathological disruption of this tripartite barrier — manifested as intestinal hyperpermeability—enables portal translocation of microbial-associated molecular patterns (MAMPs) such as lipopolysaccharide (LPS), pathogen-derived toxins, and dietary antigens, which subsequently activate hepatic inflammatory cascades [38] Notably, both intestinal epithelial barrier dysfunction and gut vascular barrier (GVB) impairment constitute initiating events in NASH pathogenesis. Pharmacological activation of farnesoid X receptor (FXR) by obeticholic acid (OCA) demonstrates therapeutic potential through β-catenin stabilization in endothelial cells, effectively restoring GVB integrity and arresting NAFLD-to-NASH transition [39]. Complementary experimental evidence from Rahman et al. [40] reveals that genetic ablation of junctional adhesion molecule-A (JAM-A) in diet-induced obese mice exacerbates paracellular leakage, promoting hepatic LPS deposition. This process activates Toll-like receptor 4 (TLR4)-dependent innate immune signaling, thereby driving steatohepatitis and perisinusoidal fibrosis.

## 6. Summary and Future Directions

The conceptual framework of non-alcoholic fatty liver disease (NAFLD) pathogenesis has transitioned from the classical "two-hit" hypothesis to a contemporary "multiple parallel hits" paradigm, integrating multi-organ crosstalk and systemic metabolic-inflammatory interplay. This pathophysiological network coalesces four core mechanisms: (1) metabolic derangements (insulin resistance and lipotoxic stress), (2) chronic sterile inflammation (innate/adaptive immune activation with cytokine dysregulation), (3) endoplasmic reticulum (ER) stress-mediated proteostatic failure, and (4) gut-liver axis dysfunction (microbial dysbiosis, intestinal hyperpermeability, and endotoxin translocation). Notably, gut-derived lipopolysaccharide (LPS) activates dual pathogenic circuits through TLR4/NF-κB-driven inflammatory signaling and farnesoid X receptor (FXR)-mediated metabolic reprogramming, synergistically amplifying hepatic fibrogenesis. Genetic polymorphisms (e.g., PNPLA3 rs738409) and epigenetic modifiers (DNA methylation, histone acetylation) further modulate disease susceptibility and progression trajectories. [41,42]. Future investigations should employ multi-omics integration (metagenomic-metabolomic-proteomic triangulation) to deconvolute the dynamic "gut-liver-immune" interactome, prioritizing therapeutic nodes such as microbial-derived metabolites (e.g., secondary bile acids), exosomal non-coding RNAs, and inflammasome-ER stress interfaces. Rational drug development strategies targeting these pathways, combined with metabolic stabilization and microbiome-directed interventions, may establish precision medicine paradigms to intercept NAFLD-hepatocellular carcinoma transition.

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## References

[1] Me R. Nonalcoholic fatty liver disease: a systematic review[J]. JAMA, 2015, 313(22).

[2] Riazi K, Azhari H, Charette JH, et.al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis[J]. The Lancet. Gastroenterology & Hepatology, 2022, 7(9): 851-861.

[3] Fan JG, Kim SU, Wong VWS. New trends on obesity and NAFLD in Asia[J]. Journal of Hepatology, 2017, 67(4): 862-873.

[4] Zm Y, Ab K, D A, et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes[J]. Hepatology (Baltimore, Md.), 2016, 64(1).

[5] Govaere O, Cockell S, Tiniakos D, et al. Transcriptomic profiling across the nonalcoholic fatty liver disease spectrum reveals gene signatures for steatohepatitis and fibrosis[J]. Science Translational Medicine, 2020, 12(572): eaba4448.

[6] Younossi ZM. Non-alcoholic fatty liver disease – A global public health perspective[J]. Journal of Hepatology, 2019, 70(3): 531-544.

[7] Day CP, James OF. Steatohepatitis: a tale of two “hits”? [J]. Gastroenterology, 1998, 114(4): 842-845.

[8] Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis[J]. Hepatology (Baltimore, Md.), 2010, 52(5): 1836-1846.

[9] Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights[J]. Science (New York, N.Y.), 2011, 332(6037): 1519-1523.

[10] Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD)[J]. Metabolism, 2016, 65(8): 1038-1048.

[11] Guilherme A, Virbasius JV, Puri V, et al. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes[J]. Nature Reviews Molecular Cell Biology, 2008, 9(5): 367-377.

[12] Blüher M. Adipose tissue dysfunction contributes to obesity related metabolic diseases[J]. Best Practice & Research. Clinical Endocrinology & Metabolism, 2013, 27(2): 163-177.

[13] Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites[J]. Hepatology (Baltimore, Md.), 2010, 52(2): 774-788.

[14] Han MS, Park SY, Shinzawa K, et al. Lysophosphatidylcholine as a death effector in the lipoapoptosis of hepatocytes[J]. Journal of Lipid Research, 2008, 49(1): 84-97.

[15] Cebi M, Yilmaz Y. Immune system dysregulation in the pathogenesis of non-alcoholic steatohepatitis: unveiling the critical role of T and B lymphocytes[J]. FRONTIERS IN IMMUNOLOGY, 2024, 15: 1445634.

[16] Daryabor G, Atashzar MR, Kabelitz D, et al. The Effects of Type 2 Diabetes Mellitus on Organ Metabolism and the Immune System[J]. Frontiers in Immunology, 2020, 11.

[17] Song C, Long X, He J, et al. Recent evaluation about inflammatory mechanisms in nonalcoholic fatty liver disease[J]. Frontiers in Pharmacology, 2023, 14: 1081334.

[18] S Z, K C, Y P, et al. Regulation mechanism of endoplasmic reticulum stress on metabolic enzymes in liver diseases[J]. Pharmacological research, 2024, 207.

[19] Huang Q, Xin X, Sun Q, et al. Plant-derived bioactive compounds regulate the NLRP3 inflammasome to treat NAFLD[J]. Frontiers in Pharmacology, 2022, 13: 896899.

[20] Koh CH, Lee S, Kwak M, et al. CD8 T-cell subsets: heterogeneity, functions, and therapeutic potential[J]. Experimental & Molecular Medicine, 2023, 55(11): 2287-2299.

[21] Zhong X, Lv M, Ma M, et al. State of CD8+ T cells in progression from nonalcoholic steatohepatitis to hepatocellular carcinoma: From pathogenesis to immunotherapy[J]. Biomedicine & Pharmacotherapy, 2023, 165: 115131.

[22] Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response[J]. Nature Reviews Molecular Cell Biology, 2020, 21(8): 421-438.

[23] Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond[J]. Nature Reviews Molecular Cell Biology, 2012, 13(2): 89-102.

[24] Shreya S, Grosset CF, Jain BP. Unfolded Protein Response Signaling in Liver Disorders: A 2023 Updated Review[J]. International Journal of Molecular Sciences, 2023, 24(18): 14066.
[25] Jiang Y, Gong Q, Gong Y, et al. Vitexin Attenuates Non-alcoholic Fatty Liver Disease Lipid Accumulation in High Fat-Diet Fed Mice by Activating Autophagy and Reducing Endoplasmic Reticulum Stress in Liver[J]. Biological & Pharmaceutical Bulletin, 2022, 45(3): 260-267.
[26] Zhou L, Shen H, Li X, et al. Endoplasmic reticulum stress in innate immune cells - a significant contribution to non-alcoholic fatty liver disease[J]. Frontiers in Immunology, 2022, 13: 951406.
[27] Arroyave-Ospina JC, Wu Z, Geng Y, et al. Role of Oxidative Stress in the Pathogenesis of Non-Alcoholic Fatty Liver Disease: Implications for Prevention and Therapy[J]. Antioxidants (Basel, Switzerland), 2021, 10(2): 174.
[28] Han H, Xue T, Li J, et al. Plant sterol ester of α-linolenic acid improved non-alcoholic fatty liver disease by attenuating endoplasmic reticulum stress-triggered apoptosis via activation of the AMPK[J]. Journal of Nutritional Biochemistry, 2022, 107: 109072.
[29] Bashiardes S, Shapiro H, Rozin S, et al. Non-alcoholic fatty liver and the gut microbiota[J]. Molecular Metabolism, 2016, 5(9): 782-794.
[30] Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH[J]. Hepatology (Baltimore, Md.), 2013, 57(2): 601-609.
[31] Michail S, Lin M, Frey MR, et al. Altered gut microbial energy and metabolism in children with non-alcoholic fatty liver disease[J]. FEMS Microbiology Ecology, 2015, 91(2): 1-9.
[32] Wong VWS, Wong GLH, Chan HY, et al. Bacterial endotoxin and non-alcoholic fatty liver disease in the general population: a prospective cohort study[J]. Alimentary Pharmacology & Therapeutics, 2015, 42(6): 731-740.
[33] Li T, Chiang JYL. Bile Acid Signaling in Metabolic and Inflammatory Diseases and Drug Development[J]. Pharmacological Reviews, 2024, 76(6): 1221-1253.
[34] Li T, Chiang JYL. Bile acid signaling in metabolic disease and drug therapy[J]. Pharmacological Reviews, 2014, 66(4): 948-983.
[35] Sayin SI, Wahlström A, Felin J, et al. Gut Microbiota Regulates Bile Acid Metabolism by Reducing the Levels of Tauro-beta-muricholic Acid, a Naturally Occurring FXR Antagonist[J]. Cell Metabolism, 2013, 17(2): 225-235.
[36] Chi Y, Lin Y, Lu Y, et al. Gut microbiota dysbiosis correlates with a low-dose PCB126-induced dyslipidemia and non-alcoholic fatty liver disease[J]. Science of The Total Environment, 2019, 653: 274-282.
[37] Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy[J]. Journal of Hepatology, 2020, 72(3): 558-577.
[38] Liu L, Yin M, Gao J, et al. Intestinal Barrier Function in the Pathogenesis of Nonalcoholic Fatty Liver Disease[J]. Journal of Clinical and Translational Hepatology, 2023, 11(2): 452-458.
[39] Mouries J, Brescia P, Silvestri A, et.al. Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development[J]. Journal of Hepatology, 2019, 71(6): 1216-1228.
[40] Rahman K, Desai C, Iyer SS, et.al. Loss of Junctional Adhesion Molecule A Promotes Severe Steatohepatitis in Mice on a Diet High in Saturated Fat, Fructose, and Cholesterol[J]. Gastroenterology, 2016, 151(4): 733-746.e12.
[41] Wang SW, Wang C, Cheng YM, et.al. Genetic predisposition of metabolic dysfunction-associated steatotic liver disease: a population-based genome-wide association study[J]. Hepatology International, 2025.
[42] Mansour RM, Abdel Mageed SS, Abulsoud AI, et.al. From fatty liver to fibrosis: the impact of miRNAs on NAFLD and NASH[J]. Functional & Integrative Genomics, 2025, 25(1): 30.