Review

A Unified Mechanistic Framework for Metabolism-Based Therapeutics in MASH: A Comprehensive Review of SGLT2 Inhibitors, GLP-1 Receptor Agonists, and Statins

Kangchan Choi , Seungchan Choi , Mi Ra Lee , Seul Ki Han , Jin Suk Lee * , Moon Young Kim *

Posted Date: 19 August 2025

doi: 10.20944/preprints202508.0991.v3

Keywords: SGLT2 inhibitor; LSEC; Liver fibrosis; GLP-1 analogue; statins

 doi:10.20944/preprints202508.0991.v3

Review

A Unified Mechanistic Framework for Metabolism-Based Therapeutics in MASH: A Comprehensive Review of SGLT2 Inhibitors, GLP-1 Receptor Agonists, and Statins

Kangchan Choi [1], Seungchan Choi [2], Mi Ra Lee [2], Seul Ki Han [2,3], Jin Suk Lee [2,3,*] and Moon Young Kim [2,3,*]

[1] School of Medicine, Trinity Medical Sciences University

[2] Regeneration Medicine Research Center, Yonsei University Wonju College of Medicine

[3] Department of Internal Medicine, Wonju Severance Christian Hospital

* Correspondence: cherry0613@yonsei.ac.kr (J.S.L.); drkimmy@yonsei.ac.kr (M.Y.K.)

Abstract

Metabolic dysfunction-associated steatohepatitis (MASH) represents a multifaceted and progressive hepatic disorder that poses considerable therapeutic challenges in contemporary clinical practice. The therapeutic landscape has undergone substantial transformation with the identification of three pharmacologically distinct classes of metabolism-targeting agents, sodium-glucose cotransporter-2 (SGLT2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), each demonstrating histologic or surrogate improvements with antifibrotic potential, albeit with varying levels of evidence. As of March 2024 and August 2025, respectively, resmetirom (Rezdiffra) and semaglutide (Wegovy) have received FDA accelerated approvals for adults with noncirrhotic MASH with F2–F3 fibrosis, sharpening the clinical focus on antifibrotic mechanisms. However, this breakthrough has simultaneously presented an intriguing mechanistic conundrum: how do these therapeutically disparate compounds achieve their shared objective of hepatic fibrosis resolution? This comprehensive review endeavors to construct an integrative theoretical framework addressing this fundamental question. We hypothesize that adenosine monophosphate-activated protein kinase (AMPK), functioning as the cellular energy homeostasis sentinel, constitutes the primary convergence point for these therapeutic interventions. Our proposed model suggests that each pharmacological class engages this central hub through distinct, non-redundant upstream signaling cascades: SGLT2 inhibitors are posited to induce an "energy-deficit" state with secondary AMPK activation (often LKB1-dependent in specific contexts), rather than uniformly direct hepatic signaling; GLP-1 receptor agonists act predominantly via systemic metabolic effects (weight loss and improvement in insulin resistance), while direct hepatic GLP-1R–CaMKKβ–AMPK signaling remains debated and, if present, may operate in restricted settings; and statins deplete isoprenoids (FPP/GGPP), reducing geranylgeranylation of small GTPases (e.g., RhoA/Rac) and thereby indirectly dampening profibrotic programs, with potential cross-talk to AMPK. Despite this upstream divergence, the pathways appear to converge on microvascular repair (e.g., the AMPK–eNOS/NO axis) and stellate-cell deactivation, yielding differential yet synergistic downstream responses. Additionally, we present a comprehensive multi-phase paradigm explaining the seemingly contradictory upregulation of SGLT2 expression within fibrotic hepatic tissue. This model encompasses an initial liver-specific "adaptive response" occurring in hepatocytes and liver sinusoidal endothelial cells, followed by a putative pathological "epigenetic lock-in" in activated hepatic stellate cells—plausibly involving hypoxia-inducible factor-1α (HIF-1α) and brahma-related gene 1 (BRG1) chromatin remodeling— that requires targeted validation and for which cell-type specificity remains to be resolved. This hypothesis-generating, unifying framework not only addresses existing mechanistic inconsistencies but also establishes a robust foundation for future

 doi:10.20944/preprints202508.0991.v3

MASH therapeutic development, particularly emphasizing personalized treatment approaches and rationally designed combination therapy strategies, while explicitly acknowledging remaining uncertainties in pathway directness and cell-type specificity.

Keywords: SGLT2 inhibitor; LSEC; Liver fibrosis; GLP-1 analogue; statins

1. Introduction

Metabolic dysfunction-associated steatohepatitis (MASH) constitutes the progressive inflammatory manifestation within the broader continuum of hepatic pathology that originates from lipid accumulation (hepatic steatosis). This condition has emerged as a predominant etiology of chronic liver disease globally [1,2], reflecting the concurrent epidemiological trends of obesity and type 2 diabetes mellitus [3]. Although simple hepatic steatosis frequently follows a benign clinical course [4–6], the pathological transition to MASH, distinguished by characteristic hepatocellular damage including ballooning degeneration and lobular inflammatory infiltrates [7], represents a pivotal pathophysiological threshold.

This sustained inflammatory milieu initiates a persistent wound-healing response that subsequently becomes dysregulated, culminating in excessive deposition of extracellular matrix components, predominantly collagen fibers, with consequent formation of fibrotic tissue architecture [8,9]. Hepatic fibrosis, as this process is termed, serves as the predominant prognostic indicator for long-term clinical outcomes and survival in MASH patients [10]. The fibrotic progression follows a well-established staging system ranging from F0 (absence of fibrosis) through F4 (established cirrhosis) [11], with each advancing stage conferring exponentially escalating risks for hepatic decompensation, portal hypertension development, and hepatocellular carcinoma emergence [12]. Historically, no therapies were approved [13,14]; however, this has shifted with FDA accelerated approvals—resmetirom (March 2024) [15] and semaglutide (August 2025)[16]—for adults with noncirrhotic MASH with F2–F3 fibrosis, underscoring a transitional moment for the field.

Within this therapeutically constrained environment, three pharmacologically distinct drug categories, each originally conceptualized for alternative metabolic disorders, have shown signals of efficacy against MASH pathophysiology and associated fibrotic processes. These therapeutic classes encompass sodium-glucose cotransporter-2 (SGLT2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins). Across studies, improvements in histology or validated surrogates have been reported, albeit with varying levels of evidence and patient selection, including fibrosis-related endpoints in selected cohorts [15,17–20].

This convergent therapeutic efficacy appears particularly intriguing considering their fundamentally disparate primary pharmacological mechanisms, subsequently generating several pivotal mechanistic inquiries that form the foundation of this comprehensive review. These include the “SGLT2 regulatory paradox,” in which hepatic expression appears context-dependent and may be induced in disease states [21], the mechanistic convergence phenomenon whereby pharmacologically heterogeneous agents achieve unified therapeutic objectives [22], and the conditions under which combination treatment strategies yield additive or synergistic benefit [23].

The present analysis endeavors to establish a coherent and mechanistically detailed theoretical framework addressing these complex questions. We shall develop a sophisticated multi-stage, multi-cellular paradigm elucidating the paradoxical SGLT2 regulatory mechanisms. Subsequently, we position adenosine monophosphate-activated protein kinase (AMPK) as the fundamental convergence nexus and advance a unifying hypothesis explaining how each therapeutic class engages this central hub through distinct upstream signaling pathways—SGLT2 inhibitors by inducing an energy-deficit state with secondary, often LKB1-dependent, AMPK activation [24,25]; GLP-1 receptor agonists predominantly via systemic metabolic effects [26,27], with any direct hepatic CaMKKβ–AMPK signaling remaining debated[28]; and statins through isoprenoid depletion that

 doi:10.20944/preprints202508.0991.v3

limits geranylgeranylation of small GTPases (e.g., RhoA/Rac) and indirectly dampens profibrotic programs [29]—ultimately generating complementary downstream effects on microvascular function (e.g., the AMPK–eNOS/NO axis) and stellate-cell activation status [30]. Where appropriate, we explicitly acknowledge remaining uncertainties in pathway directness and cell-type specificity, while outlining rational criteria for combination therapy design.

2. The SGLT2 Inhibitor Axis: A Story of Cell-Specific Adaptation and Pathology

To understand the action of SGLT2 inhibitors, we must first understand how their target becomes pathologically relevant in the diseased liver. We propose that this is a dynamic, two-stage process involving different cell types and regulatory mechanisms. In what follows, we outline a hypothesis-generating framework that is consistent with emerging tissue evidence while explicitly noting remaining uncertainties in cell-type specificity.

2.1. Stage 1: The Adaptive Response in Hepatocytes and LSECs

In the initial phase of hepatic injury, we propose that upregulation of SGLT2 represents an essential, liver-specific adaptive mechanism rather than a maladaptive error. The liver's primary function is not glucose reabsorption but the role of a central glucostat that senses and responds to fluctuations in systemic glucose levels [31,32]. Inflammatory and hypoxic stress impair this glucoregulatory capacity. We therefore suggest that, within hepatocytes and possibly liver sinusoidal endothelial cells (LSECs), intersecting pathological cues converge to induce context-dependent expression of SGLT2.

Cell type specificity is critical to this response and likely tissue-context dependent. Although HIF-1α may suppress SGLT2 expression in renal tissue [33], the distinct transcriptional apparatus of hepatic cells and the inflammatory milieu may permit cooperative activation by NF-κB together with HIF-1α. Such induced expression of SGLT2 offers the stressed liver an additional mechanism for modulating glucose flux at the parenchymal–sinusoidal interface and may support LSEC energetics and microvascular function (e.g., via AMPK–eNOS/NO coupling), which is vital for overall hepatic homeostasis.

2.2. Stage 2: The Pathological Lock-in in Hepatic Stellate Cells

As chronic hepatic injury endures and fibrosis progresses under the influence of activated hepatic stellate cells (HSCs) [34], the regulatory response shifts from a reversible adaptation to a pathologically stabilized state through epigenetic modification—that is, the covalent alteration of DNA and histones to regulate chromatin accessibility [35]. The phenotypic conversion of quiescent HSCs into proliferative myofibroblasts capable of extensive collagen synthesis demands a substantial and sustained energy supply [36,37]. To meet this demand HSCs reprogram their metabolism toward glycolysis in a durable manner.

We propose that this metabolic reprogramming is secured by an "epigenetic lock-in".

Specifically, we hypothesize that hypoxia-inducible factor-1α (HIF-1α) cooperatively recruits the BRG1 chromatin remodeling complex to the SGLT2 regulatory locus.

The BRG1 complex then uses ATP hydrolysis to reposition nucleosomes, thereby exposing the promoter region and facilitating the recruitment of histone acetyltransferases that deposit active histone marks such as H3 acetylation [38]. This sequence of events would predict increased chromatin accessibility and active promoter/enhancer marks and, if confirmed, could explain a stably elevated SGLT2 transcriptional state in activated HSCs. Importantly, this model remains to be validated directly in primary human cells (e.g., by ChIP/ATAC-seq and perturbation of HIF-1α or BRG1) and should be regarded as a testable working hypothesis rather than a definitive conclusion.

Within this two-stage framework, SGLT2 inhibition is expected to alleviate pathological glucose flux and to induce an "energy-deficit" signal with secondary AMPK activation [39–41], thereby

 doi:10.20944/preprints202508.0991.v3

complementing microvascular repair in LSECs and attenuating profibrotic drive in HSCs [42–44]; such effects provide a mechanistic basis for synergy with other metabolism-targeting agents [45].

3. The Unified Therapeutic Framework: AMPK as the Central Convergent Node

The therapeutic benefits of all three drug classes can be unified by designating AMP-activated protein kinase (AMPK) as the principal convergence point for metabolic restoration. Each class engages this key energy sensor via a distinct upstream signaling cascade, thereby eliciting a unique modality of protective stress. Accordingly, we present a hypothesis-generating framework in which upstream diversity yields downstream convergence, while acknowledging cell-type specificity and context dependence.

3.1. Path 1: SGLT2 Inhibitors - The 'Energy Crisis' Pathway

SGLT2 inhibitors are proposed to block or limit glucose uptake in liver sinusoidal endothelial cells and hepatic stellate cells that, under disease conditions, may upregulate glucose import machinery (e.g., GLUT1 and, in specific contexts, SGLT2). This blockade induces an acute energy deficit reflected by a rise in the intracellular AMP/ATP ratio. Such a shift constitutes the canonical activation signal for the upstream kinase LKB1 [46]. As a result, SGLT2 inhibitors predominantly promote AMPK activation via LKB1 in many settings, although direct hepatic signaling may not be universal. Once stimulated by this bioenergetic stress, AMPK orchestrates protective cellular responses, including suppression of the NLRP3 inflammasome and enhancement of autophagic activity in a context-dependent manner [47].

Detailed Mechanism of Action

A. Creation of 'Metabolic Vulnerability'

The transition of liver sinusoidal endothelial cells (LSECs) to a capillarized phenotype represents an active process that demands extensive bioenergetic reprogramming [48]. Under quiescent conditions, healthy endothelial cells derive most of their ATP from fatty acid β-oxidation in mitochondria [49]. Upon activation, however, LSECs undergo a glycolytic switch driven by HIF-1α-mediated upregulation of key glycolytic enzymes.

This reprogramming shifts energy production from high-yield oxidative phosphorylation to rapid but less efficient glycolysis, maintaining ATP supply through high flux while increasing demand for extracellular glucose [50]. The resulting availability of biosynthetic precursors and rapid ATP turnover support cellular proliferation and extracellular matrix remodeling [51].

Consequently, activated LSECs become increasingly dependent on elevated glucose flux to sustain their pro-fibrotic activities. To satisfy this increased demand, these cells upregulate facilitative glucose transporters such as GLUT1 [52], and we hypothesize that, in disease-specific contexts, SGLT2 may be co-opted as a supplementary, sodium-coupled uptake mechanism. This metabolic rearrangement renders capillarized LSECs exquisitely vulnerable, since their continued survival and fibrogenic function become contingent on the integrity of these glucose import pathways.

B. The AMPK-Orchestrated Dual Response

Once activated within LSECs by the energy crisis, AMPK acts as a master switch, initiating two distinct but complementary restorative arms:

i) The Effector Arm (Restoring Endothelial Function to Suppress HSCs)

A key outcome of AMPK activation in endothelial cells is the phosphorylation and activation of endothelial nitric oxide synthase (eNOS) [53], which catalyzes the formation of nitric oxide (NO). NO serves as the principal paracrine mediator for preserving LSEC integrity and maintaining hepatic stellate cell (HSC) quiescence [54]. In fibrotic liver tissue, eNOS activity is markedly diminished [55]. By activating AMPK in LSECs, SGLT2 inhibitors reinstate eNOS phosphorylation and NO synthesis. The regenerated NO released by these rehabilitated endothelial cells then acts on nearby HSCs to inhibit their activation and encourage a reversion to a quiescent phenotype. This microvascular repair

 doi:10.20944/preprints202508.0991.v3

axis (AMPK–eNOS/NO) functionally addresses fibrotic progression by restoring the gatekeeper role of LSECs.

ii) The Reinforcing Arm (Dismantling the Metabolic Program in LSECs)

Simultaneously, AMPK acts to dismantle the underlying metabolic pathology within the LSEC. Activated AMPK is a well-recognized negative regulator of HIF-1α signaling, limiting its stability and transcriptional activity [56]. By blunting the master regulator of the 'glycolytic switch,' AMPK progressively reduces the LSEC's glucose addiction. This creates a self-reinforcing feedback loop: as HIF-1α activity falls, the cell's glycolytic program falters, increasing susceptibility to continued SGLT2 blockade. Together with inflammasome restraint and pro-autophagic effects, this may support sustained—potentially amplifying—therapeutic benefits over time, while the precise contribution of each component likely varies by disease stage and cell type.

3.2. Path 2: GLP-1 Receptor Agonists - The 'Hormonal Signal' Pathway

GLP-1 receptor agonists operate through endocrine signaling by engaging their specific receptors and elevating intracellular calcium levels [57]. In clinical practice, their predominant benefits in MASH are thought to arise from systemic metabolic effects—weight reduction and improved insulin sensitivity—that secondarily relieve hepatic lipotoxic/inflammatory stress [58,59]. This calcium influx activates the kinase CaMKKβ, which in turn phosphorylates and activates AMPK. In selected hepatic contexts, GLP-1R–CaMKKβ coupling may contribute to AMPK activation; more broadly, cAMP–PKA–LKB1 crosstalk and adipose–hepatic energy deficit can also feed into AMPK [60,61]. The CaMKKβ-dependent activation of AMPK preferentially modulates both anabolic and catabolic pathways, including suppression of de novo lipogenesis and enhancement of fatty acid oxidation. Moreover, the pronounced weight loss associated with GLP-1 receptor agonist therapy induces a systemic state akin to caloric restriction. This state activates the AMPK/SIRT1 axis and favors a unique, partially reversible, epigenetic reprogramming of HSCs. Importantly, the extent of direct hepatic GLP-1R signaling remains debated; where present, it likely operates alongside these dominant systemic mechanisms.

3.3. Path 3: Statins - The 'Cellular Stress' Pathway

Statins initiate a third and distinct mechanism of AMPK activation. By inhibiting HMG-CoA reductase, statins deprive the cell of essential, non-sterol isoprenoids (e.g., GGPP, FPP) that are used in protein prenylation and function. A principal consequence is reduced geranylgeranylation of small GTPases—especially RhoA and Rac—which remodel cytoskeletal/mechanotransduction pathways implicated in fibrogenesis [62,63]. Depleting these critical building blocks of the cell will provoke a form of 'cellular stress', principally to the mitochondria and endoplasmic reticulum. We posit that this cellular stress signal is also detected through the LKB1 pathway to activate AMPK, rendering statin-induced AMPK activation mechanistically distinct from the SGLT2 inhibitor–induced energy crisis. Furthermore, we propose a second, AMPK-independent mechanism: isoprenoid depletion blunts prenylation-dependent RhoA/ROCK and related signaling, which can indirectly attenuate HIF-1α transcriptional output and diminish BRG1-facilitated chromatin accessibility at profibrotic loci. This constitutes a 'dual attack'—an AMPK-mediated metabolic check plus a prenylation-dependent signaling brake—on the fibrogenic machinery, without invoking direct prenylation of BRG1 itself.

4. Discussion

This integrated framework provides an optimistic vision for the future of MASH therapy, most likely characterized by precision-based medicine and synergistic combination therapies. While our model is hypothesis-generating, it yields concrete, testable predictions for patient selection, sequencing, and combination design.

 doi:10.20944/preprints202508.0991.v3

4.1. A Case for Precision Medicine

The distinct downstream actions of these pharmacological classes support a personalized therapeutic strategy. Individuals exhibiting a predominantly inflammatory MASH phenotype may benefit more from the intrinsic anti-inflammatory effects of SGLT2 inhibitors. Those presenting with a metabolic MASH phenotype driven by obesity or dyslipidemia may achieve greater improvement through the systemic and lipogenesis-inhibiting properties of GLP-1 receptor agonists. Finally, patients with MASH complicated by dyslipidemia and a pronounced fibrogenic tendency might respond optimally to statins, which exert a dual mechanism targeting both lipid metabolism and fibrogenesis. In practice, stratification should incorporate readily obtainable markers—e.g., BMI/waist circumference, HbA1c/insulin resistance indices, triglycerides/LDL, non-invasive fibrosis scores and elastography/MRE, and microvascular surrogates—together with disease stage (F2–F3 vs cirrhosis) and comorbidities (renal function for SGLT2 inhibitors; gallbladder/pancreatobiliary risk and GI tolerance for GLP-1 RAs; myopathy risk and drug–drug interactions for statins). Such phenotype-guided matching aligns with our framework of non-redundant upstream cues converging on shared antifibrotic nodes.

4.2. The Compelling Case for a 'Triple Therapy' Cocktail

The most striking implication is the prospect of synergy. Each drug class converges on the central AMPK axis via a distinct upstream cue—an energy deficit, a hormonal messenger, or a metabolic stressor. Their combined engagement of this master regulator may produce a more potent, durable, and comprehensive anti-fibrotic and anti-inflammatory outcome than could be achieved with single or dual regimens. This unified mechanism thus offers a robust rationale for conducting future clinical trials of combination therapy. A pragmatic development path could test (i) sequential add-on (run-in with one agent, staged layering of the others), (ii) factorial designs to quantify non-redundancy, and (iii) adaptive enrichment using early mechanistic readouts (e.g., weight-loss trajectories, ketone dynamics, non-invasive fibrosis panels, MRI-PDFF/MRE, and portal pressure surrogates). Safety will require equal emphasis: monitor for volume depletion or ketoacidosis signals with SGLT2 inhibition (especially with aggressive caloric restriction), GI intolerance with GLP-1 RAs, and myopathy or transaminase elevations with statins; staged titration and dose-sparing synergy are desirable.

4.3. The Concept of Differential Epigenetic Memory

The distinct mechanisms may also result in divergent long-term outcomes based on their durability. The direct chromatin-modifying actions of SGLT2 inhibitors and statins mediated by beta-hydroxybutyrate and BRG1 inhibition respectively could establish a structural and enduring anti-fibrotic epigenetic memory. In contrast, the benefits conferred by GLP-1 receptor agonists which depend largely on weight loss and systemic metabolic changes may create a functional and reversible therapeutic memory. These differences carry important implications for determining optimal treatment durations and designing follow-up maintenance strategies. Refining this concept, we posit that SGLT2 inhibition can raise β-hydroxybutyrate and thereby *indirectly* promote class I HDAC inhibition (an epigenetic mechanism consistent with durable transcriptional shifts), whereas statins *primarily* reduce isoprenoid-dependent prenylation (notably RhoA/Rac), which may secondarily diminish HIF-1α transcriptional drive and BRG1-facilitated chromatin accessibility at profibrotic loci. By contrast, GLP-1 RA benefits are often mediated by systemic weight loss and insulin-sensitization and may be more reversible; nevertheless, sustained weight reduction can imprint longer-lasting hepatic improvements. Clinically, this argues for stage- and phenotype-specific treatment durations (e.g., longer maintenance when epigenetic features are engaged), consideration of sequential de-escalation strategies, and periodic reassessment with non-invasive tests to balance durability against adverse-effect risk.

 doi:10.20944/preprints202508.0991.v3

5. Future Directions

Validating this comprehensive model requires a comparative approach that dissects the unique and shared mechanisms of these three drug classes. Accordingly, we prioritize human-relevant systems, explicit cell-type resolution, and pre-specified readouts that map upstream triggers to AMPK engagement and downstream fibrogenic programs.

5.1. Phase 1 & 2 (Validating the SGLT2 Axis)

Experiments confirming the cell-type-specific regulation of SGLT2 and the HIF-1α-BRG1 epigenetic lock-in in HSCs remain critical foundational steps. In practice, this entails: (i) cell-resolved localization of SGLT2 (e.g., IHC/IF, RNAscope, and single-cell/nuclei datasets) across hepatocytes, LSECs, and HSCs; (ii) perturbation of hypoxia/inflammation cues to test inducibility; (iii) CRISPR/siRNA SGLT2 knockdown with functional glucose-uptake and metabolic-flux assays (e.g., 2-NBDG, Seahorse) in each cell type; and (iv) ChIP-qPCR/ChIP-seq for HIF-1α and BRG1 at SGLT2 regulatory elements, coupled with ATAC-seq to quantify chromatin accessibility and reversibility. Where feasible, LSEC–HSC co-culture or liver-on-chip models can be used to measure NO bioavailability and paracrine control of HSC activation

5.2. Phase 3 (Validating the Unified AMPK Model)

A. Upstream Dependency: Apply selective inhibitors or employ genetic knockdown of LKB1 and CaMKKβ in primary hepatocytes and liver sinusoidal endothelial cells. Measure AMPK phosphorylation in response to SGLT2 inhibitors, GLP-1 receptor agonists, and statins to determine which kinase each class requires. To strengthen causal inference, favor genetic approaches (e.g., LKB1 or CaMKKβ silencing; AMPKα1/α2 knockdown) over broad small-molecule inhibitors with off-target effects; include receptor blockade for GLP-1 (e.g., peptide antagonism) and, for statins, GGPP/FPP add-back to separate prenylation-dependent from AMPK-mediated effects.

B. Downstream Profiling: Administer each drug class to validated MASH animal models as monotherapy and in combined regimens. Conduct integrated transcriptomic, proteomic, and metabolomic analyses to verify selective engagement of inflammatory and autophagic pathways versus lipid metabolic processes. Add phospho-proteomics (for AMPK/eNOS/ROCK nodes), spatial or single-cell transcriptomics (for cell-type specificity), and microvascular function readouts (e.g., eNOS phosphorylation, NO surrogates, sinusoidal perfusion).

C. Epigenetic Link (Statin): In activated HSCs, perform chromatin immunoprecipitation sequencing of BRG1 and assay for transposase-accessible chromatin sequencing. Compare BRG1 occupancy at promoters of fibrogenic genes with and without statin treatment to confirm a reduction that is independent of AMPK activity. Critically, test prenylation dependence by co-treating with geranylgeranyl precursors or GGTase modulators (GGPP add-back should reverse BRG1 occupancy changes if upstream RhoA/ROCK signaling is causal). Prefer genetic AMPK interventions to avoid confounding; profile YAP/TAZ and HIF-1α target accessibility as parallel readouts.

D. Synergy Confirmation: To formally confirm synergy, quantify key fibrosis, inflammation, and metabolic endpoints in animal models treated with individual drugs and with the triple combination. Use mathematical models of drug interaction to demonstrate that the combined effect exceeds the sum of each agent's individual effects. A response-surface framework (e.g., Bliss, Loewe/isobologram, HSA, or ZIP) and factorial designs will distinguish non-redundant from overlapping effects. Endpoints should include collagen proportionate area/hydroxyproline, α-SMA area, histologic stage, MRI-PDFF and elastography/MRE, portal-pressure surrogates, and microvascular measures, with prespecified safety monitoring (ketone dynamics/volume status for SGLT2 inhibitors; GI tolerance for GLP-1 RAs; myopathy/transaminases for statins). Finally, a translational "proof-of-mechanism" cohort can mirror these mechanistic biomarkers to bridge preclinical synergy into early clinical testing.

 doi:10.20944/preprints202508.0991.v3

6. Conclusion

In conclusion, we have developed a detailed and cohesive framework describing the mechanisms of action of leading metabolism-based therapies in metabolic dysfunction-associated steatohepatitis. We suggest that the pathological significance of SGLT2 derives from a two-phase sequence of adaptive upregulation followed by epigenetic lock-in. Adenosine monophosphate-activated protein kinase serves as the primary therapeutic nexus, being engaged by SGLT2 inhibitors, GLP-1 receptor agonists, and statins via three non-overlapping, context-linked upstream pathways and producing complementary downstream benefits. This hypothesis-generating yet experimentally tractable construct reconciles prior mechanistic inconsistencies, specifies testable predictions at the cell-type level, and supports rational, precision-guided combinations. As recent regulatory developments refocus attention on antifibrotic mechanisms in non-cirrhotic disease, the proposed program offers a clear path from bench to bedside while explicitly acknowledging remaining uncertainties in pathway directness and cell-specificity.

7. Statement on AI Collaboration

The initial conceptualization and core hypotheses presented in this manuscript were proposed by the human authors. The subsequent literature search and evaluation of these hypotheses were conducted using Liner (Liner, Republic of Korea) an AI-powered research platform. The final composition, drafting, and refinement of the manuscript were performed through an iterative, collaborative process between the human authors and a large language model (Gemini 2.5 Pro, Google). The human authors directed all stages of the project, critically reviewed all AI-generated contributions for scientific accuracy and integrity, and take full responsibility for the final content of this paper.

References

1. Danpanichkul, P., et al., *Global Trend of MASH-associated Liver Cancer: A Systematic Analysis From the Global Burden of Disease 2021.* Clinical Gastroenterology and Hepatology, 2025. **23**(8): p. 1346–1355.
2. Younossi, Z.M., et al., *Addressing the High and Rising Global Burden of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and Metabolic Dysfunction-Associated Steatohepatitis (MASH): From the Growing Prevalence to Payors' Perspective.* Alimentary Pharmacology & Therapeutics, 2025. **61**(9): p. 1467–1478.
3. Younossi, Z.M. and L. Henry, *The Impact of Obesity and Type 2 Diabetes on Chronic Liver Disease.* Official journal of the American College of Gastroenterology | ACG, 2019. **114**(11): p. 1714–1715.
4. Day, C.P., *Natural History of NAFLD: Remarkably Benign in the Absence of Cirrhosis.* Gastroenterology, 2005. **129**(1): p. 375–378.
5. Falck-Ytter, Y., et al., *Clinical Features and Natural History of Nonalcoholic Steatosis Syndromes.* Semin Liver Dis, 2001. **21**(01): p. 017–026.
6. Browning, J.D. and J.D. Horton, *Molecular mediators of hepatic steatosis and liver injury.* The Journal of Clinical Investigation, 2004. **114**(2): p. 147–152.
7. Lackner, C., *Hepatocellular ballooning in nonalcoholic steatohepatitis: the pathologist's perspective.* Expert Review of Gastroenterology & Hepatology, 2011. **5**(2): p. 223–231.
8. Karsdal, M.A., et al., *Novel insights into the function and dynamics of extracellular matrix in liver fibrosis.* American Journal of Physiology-Gastrointestinal and Liver Physiology, 2015. **308**(10): p. G807–G830.
9. Arriazu, E., et al., *Extracellular Matrix and Liver Disease.* Antioxidants & Redox Signaling, 2013. **21**(7): p. 1078–1097.

 doi:10.20944/preprints202508.0991.v3

10. Pericàs, J.M., et al., *A roadmap for clinical trials in MASH-related compensated cirrhosis.* Nature Reviews Gastroenterology & Hepatology, 2024. **21**(11): p. 809–823.
11. Garcia-Tsao, G., et al., *Now there are many (stages) where before there was one: In search of a pathophysiological classification of cirrhosis†.* Hepatology, 2010. **51**(4): p. 1445–1449.
12. Xu, F., et al., *All-Cause Mortality and Progression Risks to Hepatic Decompensation and Hepatocellular Carcinoma in Patients Infected With Hepatitis C Virus.* Clinical Infectious Diseases, 2015. **62**(3): p. 289–297.
13. Tincopa, M.A., Q.M. Anstee, and R. Loomba, *New and emerging treatments for metabolic dysfunction-associated steatohepatitis.* Cell Metabolism, 2024. **36**(5): p. 912–926.
14. Allen, A.M., et al., *Envisioning how to advance the MASH field.* Nature Reviews Gastroenterology & Hepatology, 2024. **21**(10): p. 726–738.
15. Keam, S.J., *Resmetirom: First Approval.* Drugs, 2024. **84**(6): p. 729–735.
16. U.S. Food and Drug Administration. *FDA Approves Treatment for Serious Liver Disease Known as 'MASH'*. 2025 2025 Aug 15 [cited 2025 2025 Aug 18]; Available from: https://www.fda.gov/drugs/news-events-human-drugs/fda-approves-treatment-serious-liver-disease-known-mash.
17. Simon, T.G., *Semaglutide for Metabolic Dysfunction–Associated Steatohepatitis.* New England Journal of Medicine, 2025. **392**(21): p. 2160–2161.
18. Souza, M., et al., *Comparison of pharmacological therapies in metabolic dysfunction–associated steatohepatitis for fibrosis regression and MASH resolution: Systematic review and network meta-analysis.* Hepatology, 9900: p. 10.1097/HEP.0000000000001254.
19. Konings, L.A.M., et al., *Pharmacological treatment options for metabolic dysfunction-associated steatotic liver disease in patients with type 2 diabetes mellitus: A systematic review.* European Journal of Clinical Investigation, 2025. **55**(4): p. e70003.
20. Havranek, B., et al., *Glucagon-like peptide-1 receptor agonists improve metabolic dysfunction-associated steatotic liver disease outcomes.* Scientific Reports, 2025. **15**(1): p. 4947.
21. Liu, Y., M. Zhang, and Y. Wang, *Induction of Autophagy as a Therapeutic Breakthrough for NAFLD: Current Evidence and Perspectives.* Biology, 2025. **14**(8): p. 989.
22. Sourris, K.C., B.E. Harcourt, and J.M. Forbes, *A New Perspective on Therapeutic Inhibition of Advanced Glycation in Diabetic Microvascular Complications: Common Downstream Endpoints Achieved Through Disparate Therapeutic Approaches?* American Journal of Nephrology, 2009. **30**(4): p. 323–335.
23. Wang, X., et al., *Metabolomics Coupled with Proteomics Advancing Drug Discovery toward More Agile Development of Targeted Combination Therapies *.* Molecular & Cellular Proteomics, 2013. **12**(5): p. 1226–1238.
24. Hoong, C.W.S. and M.W.J. Chua, *SGLT2 Inhibitors as Calorie Restriction Mimetics: Insights on Longevity Pathways and Age-Related Diseases.* Endocrinology, 2021. **162**(8).
25. Rolski, F. and M. Mączewski, *Cardiac Fibrosis: Mechanistic Discoveries Linked to SGLT2 Inhibitors.* Pharmaceuticals, 2025. **18**(3): p. 313.
26. Rizzo, M., et al., *GLP-1 receptor agonists and reduction of cardiometabolic risk: Potential underlying mechanisms.* Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 2018. **1864**(9, Part B): p. 2814–2821.
27. Drucker, D.J., *Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1.* Cell Metabolism, 2018. **27**(4): p. 740–756.
28. Krasner, N.M., et al., *Glucagon-Like Peptide-1 (GLP-1) Analog Liraglutide Inhibits Endothelial Cell Inflammation through a Calcium and AMPK Dependent Mechanism.* PLOS ONE, 2014. **9**(5): p. e97554.
29. Xiao, H., et al., *Inhibition of Rho and Rac Geranylgeranylation by Atorvastatin Is Critical for Preservation of Endothelial Junction Integrity.* PLOS ONE, 2013. **8**(3): p. e59233.

 doi:10.20944/preprints202508.0991.v3

30. Jiang, X., et al., *CD47-mediated regulation of glucose and lipid metabolism: implications for the pathogenesis of MASLD.* Frontiers in Endocrinology, 2025. **Volume 16 - 2025**.
31. Klover, P.J. and R.A. Mooney, *Hepatocytes: critical for glucose homeostasis.* The International Journal of Biochemistry & Cell Biology, 2004. **36**(5): p. 753–758.
32. Ben-Moshe, S. and S. Itzkovitz, *Spatial heterogeneity in the mammalian liver.* Nature Reviews Gastroenterology & Hepatology, 2019. **16**(7): p. 395–410.
33. Ndibalema, A.R., et al., *Empagliflozin Protects Against Proximal Renal Tubular Cell Injury Induced by High Glucose via Regulation of Hypoxia-Inducible Factor 1-Alpha.* Diabetes, Metabolic Syndrome and Obesity, 2020. **13**(null): p. 1953–1967.
34. Tsuchida, T. and S.L. Friedman, *Mechanisms of hepatic stellate cell activation.* Nature Reviews Gastroenterology & Hepatology, 2017. **14**(7): p. 397–411.
35. Nakao, M., *Epigenetics: interaction of DNA methylation and chromatin.* Gene, 2001. **278**(1): p. 25–31.
36. Gibb, A.A., M.P. Lazaropoulos, and J.W. Elrod, *Myofibroblasts and Fibrosis.* Circulation Research, 2020. **127**(3): p. 427–447.
37. Kamm, D.R. and K.S. McCommis, *Hepatic stellate cells in physiology and pathology.* The Journal of Physiology, 2022. **600**(8): p. 1825–1837.
38. Hargreaves, D.C. and G.R. Crabtree, *ATP-dependent chromatin remodeling: genetics, genomics and mechanisms.* Cell Research, 2011. **21**(3): p. 396–420.
39. Ferrannini, E., et al., *Shift to Fatty Substrate Utilization in Response to Sodium–Glucose Cotransporter 2 Inhibition in Subjects Without Diabetes and Patients With Type 2 Diabetes.* Diabetes, 2016. **65**(5): p. 1190–1195.
40. Packer, M., *Critical Reanalysis of the Mechanisms Underlying the Cardiorenal Benefits of SGLT2 Inhibitors and Reaffirmation of the Nutrient Deprivation Signaling/Autophagy Hypothesis.* Circulation, 2022. **146**: p. 1383 – 1405.
41. Daniele, G., et al. *Dapagliflozin Enhances Fat Oxidation and Ketone Production in Patients With Type 2 Diabetes*. in *Diabetes Care*. 2016.
42. Roh, Y.J., H. Kim, and D.W. Choi, *Metabolic Sparks in the Liver: Metabolic and Epigenetic Reprogramming in Hepatic Stellate Cells Activation and Its Implications for Human Metabolic Diseases.* dmj, 2025. **49**(3): p. 368–385.
43. Gibert-Ramos, A., et al., *Sinusoidal communication in chronic liver disease.* Clin Mol Hepatol, 2025. **31**(1): p. 32–55.
44. Dimitriadis, K., et al., *The effect of SGLT2 inhibitors on the endothelium and the microcirculation: from bench to bedside and beyond.* European Heart Journal - Cardiovascular Pharmacotherapy, 2023. **9**(8): p. 741–757.
45. Xiong, Y., et al., *Mechanisms and therapeutic opportunities in metabolic aberrations of diabetic wounds: a narrative review.* Cell Death & Disease, 2025. **16**(1): p. 341.
46. Shaw, R.J., et al., *The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress.* Proceedings of the National Academy of Sciences, 2004. **101**(10): p. 3329–3335.
47. Bai, D., et al., *ALDOA maintains NLRP3 inflammasome activation by controlling AMPK activation.* Autophagy, 2022. **18**(7): p. 1673–1693.
48. Sun, X. and E.N. Harris, *New aspects of hepatic endothelial cells in physiology and nonalcoholic fatty liver disease.* American Journal of Physiology-Cell Physiology, 2020. **318**(6): p. C1200–C1213.
49. Dikalov, S., A. Panov, and A. Dikalova, *Critical Role of Mitochondrial Fatty Acid Metabolism in Normal Cell Function and Pathological Conditions.* International Journal of Molecular Sciences, 2024. **25**(12): p. 6498.
50. Ma, C., et al., *Metabolic reprogramming of immune cells in MASH.* Hepatology, 9900: p. 10.1097/HEP.0000000000001371.
51. Lunt, S.Y. and M.G. Vander Heiden, *Aerobic Glycolysis: Meeting the Metabolic Requirements of Cell Proliferation.* Annual Review of Cell and Developmental Biology, 2011. **27**(Volume 27, 2011): p. 441–464.

 doi:10.20944/preprints202508.0991.v3

52. Devaraj, E., et al., *Liver fibrosis: Extracellular vesicles mediated intercellular communication in perisinusoidal space.* Hepatology, 2022. **76**(1): p. 275–285.
53. Chen, H., et al., *Adiponectin Stimulates Production of Nitric Oxide in Vascular Endothelial Cells *.* Journal of Biological Chemistry, 2003. **278**(45): p. 45021–45026.
54. Velliou, R.-I., et al., *Liver endothelial cells in NAFLD and transition to NASH and HCC.* Cellular and Molecular Life Sciences, 2023. **80**(11): p. 314.
55. Cunningham, R.P., et al., *Critical Role for Hepatocyte-Specific eNOS in NAFLD and NASH.* Diabetes, 2021. **70**(11): p. 2476–2491.
56. Faubert, B., et al., *AMPK Is a Negative Regulator of the Warburg Effect and Suppresses Tumor Growth In Vivo.* Cell Metabolism, 2013. **17**(1): p. 113–124.
57. Rowlands, J., et al., *Pleiotropic Effects of GLP-1 and Analogs on Cell Signaling, Metabolism, and Function.* Frontiers in Endocrinology, 2018. **Volume 9 - 2018**.
58. Harrison, S.A., et al., *Safety and Efficacy of Efruxifermin in Combination With a GLP-1 Receptor Agonist in Patients With NASH/MASH and Type 2 Diabetes in a Randomized Phase 2 Study.* Clinical Gastroenterology and Hepatology, 2025. **23**(1): p. 103–113.
59. Abdelmalek, M.F., S.A. Harrison, and A.J. Sanyal, *The role of glucagon-like peptide-1 receptor agonists in metabolic dysfunction-associated steatohepatitis.* Diabetes, Obesity and Metabolism, 2024. **26**(6): p. 2001–2016.
60. Aslam, M. and Y. Ladilov, *Emerging Role of cAMP/AMPK Signaling.* Cells, 2022. **11**(2): p. 308.
61. Petrovic, A., et al., *The Role of GLP1-RAs in Direct Modulation of Lipid Metabolism in Hepatic Tissue as Determined Using In Vitro Models of NAFLD.* Current Issues in Molecular Biology, 2023. **45**(6): p. 4544–4556.
62. Istvan, E.S. and J. Deisenhofer, *Structural Mechanism for Statin Inhibition of HMG-CoA Reductase.* Science, 2001. **292**(5519): p. 1160–1164.
63. Lee, M.H., Y.S. Cho, and Y.M. Han, *Simvastatin Suppresses Self-Renewal of Mouse Embryonic Stem Cells by Inhibiting RhoA Geranylgeranylation.* Stem Cells, 2007. **25**(7): p. 1654–1663.