Restoring Mitochondrial Membrane Potential in Senescent Hepatocytes via Targeted NAD^+ Precursor Delivery Using Lipid Nanoparticle Vectors

Cameron Walker
Professor
Heidelberg University
Grabengasse 1, 69117 Heidelberg, Baden-Württemberg, Germany

Rowan Nelson
PhD
Seoul National University College of Medicine
103 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea

Rowan Mitchell
Associate Professor
University of Toronto
27 King's College Circle, Toronto, Ontario M5S 1A1, Canada

Abstract. Senescence-associated mitochondrial dysfunction in hepatocytes represents a critical driver of non-alcoholic fatty liver disease (NAFLD) progression and systemic metabolic dysregulation. This study investigates a targeted lipid nanoparticle (LNP) delivery system loaded with nicotinamide mononucleotide (NMN) to restore mitochondrial membrane potential ($\Delta\Psi m$) in replicatively senescent primary human hepatocytes. LNPs were surface-functionalized with asialoglycoprotein receptor (ASGPR)-binding galactose ligands to achieve hepatocyte-selective uptake. Cellular senescence was confirmed via p21/p53 immunostaining, β-galactosidase activity, and JC-1 mitochondrial staining. NMN-LNP treatment significantly restored $\Delta\Psi m$ ($p < 0.001$), reduced SASP cytokine secretion (IL-6, IL-8), and upregulated SIRT1/PGC-1α-mediated mitochondrial biogenesis markers. These findings identify hepatocyte-targeted NAD^+ repletion as a tractable therapeutic strategy for mitigating senescence-driven hepatic metabolic failure in aging populations.

Keywords: mitochondrial membrane potential, hepatocyte senescence, NAD^+ repletion, lipid nanoparticle delivery, nicotinamide mononucleotide, SASP attenuation, ASGPR-targeted therapy, SIRT1/PGC-1α axis, non-alcoholic fatty liver disease

Introduction

Cellular senescence, defined as an irreversible state of replicative arrest accompanied by a pro-inflammatory secretory phenotype (SASP), has emerged as a central mechanistic pillar in the pathogenesis of age-related metabolic liver disease. As global populations continue to age at unprecedented rates — with the World Health Organization projecting that individuals over 60 will constitute 22% of the world's population by 2050 — the hepatic consequences of organismal aging have become an urgent clinical priority. Non-alcoholic

fatty liver disease (NAFLD), now re-designated as metabolic dysfunction-associated steatotic liver disease (MASLD) under the 2023 Delphi consensus nomenclature, affects an estimated 38% of adults globally as of 2025, with senescent hepatocyte burden increasingly recognized as a non-trivial contributor to fibrotic progression and impaired lipid oxidation capacity. Central to the hepatocyte senescence phenotype is the progressive deterioration of mitochondrial integrity. Senescent hepatocytes exhibit characteristic reductions in mitochondrial membrane potential ($\Delta\Psi m$), impaired electron transport chain (ETC) complex I and III activity, elevated mitochondrial reactive oxygen species (mtROS) production, and a marked decline in intracellular NAD^+/NADH ratios. This bioenergetic collapse precipitates downstream dysfunction across fatty acid β-oxidation, tricarboxylic acid (TCA) cycle flux, and oxidative phosphorylation (OXPHOS) efficiency, collectively amplifying hepatic lipotoxicity and promoting SASP-driven paracrine senescence in adjacent stellate and Kupffer cell populations. Nicotinamide mononucleotide (NMN), a direct biosynthetic precursor to NAD^+ via the Preiss-Handler and salvage pathways, has attracted considerable translational interest as a mitochondria-restorative agent. However, poor hepatocyte-selective bioavailability and rapid systemic degradation of free NMN formulations have limited in vivo therapeutic efficacy in prior murine and early-phase human studies. To overcome these pharmacokinetic barriers, lipid nanoparticle (LNP) platforms — leveraging established ionizable lipid compositions proven in mRNA vaccine delivery — offer a compelling encapsulation strategy, particularly when surface-decorated with asialoglycoprotein receptor (ASGPR)-targeting galactose moieties that confer high hepatocyte selectivity. This article systematically addresses the challenge of achieving sufficient intracellular NAD^+ repletion within senescent hepatocytes to meaningfully restore $\Delta\Psi m$ and suppress SASP output. We first characterize the mitochondrial and metabolic phenotype of replicatively senescent primary human hepatocytes. We then detail the synthesis, physicochemical optimization, and ASGPR-targeting validation of the NMN-loaded LNP platform. Subsequently, we evaluate in vitro therapeutic efficacy across $\Delta\Psi m$ recovery, mtROS attenuation, SASP cytokine profiling, and SIRT1/PGC-1α pathway reactivation. Finally, we discuss the translational implications of these findings within the broader context of senolytic and senomorphic therapeutic strategies for MASLD and hepatic aging.

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