

Deciphering cytochrome P450 reductase role in MASLD: molecular mechanisms and pathophysiological implications

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a prevalent and rapidly growing global health concern characterized by hepatic steatosis, oxidative stress and inflammation that can progress to metabolic dysfunction-associated steatohepatitis (MASH), fibrosis and cirrhosis. NADPH:cytochrome P450 oxidoreductase (CPR, encoded by *POR*), the essential electron donor for microsomal cytochrome P450 enzymes and other redox partners, orchestrates xenobiotic detoxification, lipid metabolism, steroid and bile acid biosynthesis, and redox balance – processes disrupted in MASLD. This Perspective examines emerging evidence from genetic, biochemical, transcriptomic and clinical studies that implicate CPR in the pathophysiology of MASLD, including its roles in lipid accumulation, oxidative stress, mitochondrial dysfunction, iron homeostasis, ferroptosis and inflammatory signalling. We discuss how CPR dysfunction, driven by genetic polymorphisms or metabolic stress, contributes to disease heterogeneity and progression. These insights highlight CPR as a relevant and potentially actionable regulator of hepatic metabolism. The current FDA approval of resmetirom, a thyroid hormone receptor- β agonist, which increases *POR* transcription levels, provides translational proof that restoring CPR-dependent pathways can ameliorate MASH with fibrosis. A deeper understanding of the multifaceted role of CPR could inform precision medicine strategies for this complex and widespread liver disease.

Sections

Introduction

Central role of CPR in physiological pathways

MASLD

Molecular mechanisms of CPR in MASLD pathogenesis

Conclusion and outlook

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease, is a leading cause of chronic liver disease, affecting 2 billion people worldwide^{1–3}. It encompasses a spectrum of hepatic histological disorders, ranging from steatosis to its progressive form, metabolic dysfunction-associated steatohepatitis (MASH, formerly known as non-alcoholic steatohepatitis), which involves inflammation, fibrosis and an elevated risk of cirrhosis and hepatocellular carcinoma (HCC)^{4–7}. The development of MASLD and MASH is multifactorial, involving the complex interplay of genetic predisposition, dietary factors, metabolic alterations, oxidative stress and inflammation^{8,9}.

NADPH:cytochrome P450 oxidoreductase (CPR), encoded by *POR* (chromosome 7q11.2), is a diflavin enzyme containing both flavin adenine dinucleotide and flavin mononucleotide cofactors within a single polypeptide chain (see the Supplementary Information and Supplementary Fig. 1 for structural details)^{10–13}. It is an essential electron donor for 48 human microsomal cytochrome P450 (CYP) enzymes located in the endoplasmic reticulum. CYPs are haem-containing monooxygenases involved in the metabolism of endogenous and exogenous compounds, including drugs, steroids, bile acids, fatty acids and retinoids. Although microsomal CYPs rely on CPR for electron transfer, mitochondrial CYPs utilize adrenodoxin (ADX, also known as FDX1) and adrenodoxin reductase (ADXR, also known as FDXR) for this function¹³. Beyond CYP-mediated metabolism, CPR also supports several non-CYP proteins such as cytochrome *b*₅ (CYB5), haem-oxygenase 1 (HO-1), HO-2 and squalene monooxygenase (SQLE)^{10,11,14,15}. These interactions position CPR as a nexus of lipid metabolism, oxidative stress regulation and steroid biosynthesis.

Given its pleiotropic functions, CPR dysregulation, whether from genetic polymorphisms or metabolic stressors, might implicate this master enzymatic regulator in MASLD and MASH pathophysiology. Emerging evidence suggests that CPR dysfunction influences lipid accumulation^{16–18}, oxidative stress and mitochondrial impairment^{19–21}, ferroptosis^{22,23} and inflammation^{24–26}, which are key drivers of MASLD progression²⁷. In addition, *POR* variants seem to modulate MASLD heterogeneity and individual susceptibility²⁸. Transcriptomic data from *SteatoSITE*, a large-scale multimodal MASLD database, further support the potential role of CPR in pathways associated with drug metabolism, steroid biosynthesis, xenobiotic metabolism, retinol metabolism and fatty acid metabolism²⁹.

Advances in multiomics, including spatial transcriptomics, proteomics and metabolomics, have begun to map the complex cellular and molecular heterogeneity of MASLD and MASH^{30,31}. However, current data present a nuanced picture: Govaere and colleagues found stable *POR* expression levels despite CYP downregulation³², whereas Zhang and colleagues showed a 12.8-fold interindividual CPR protein variation³³, probably reflecting diverse genetic, transcriptional and post-transcriptional regulation³⁴. *SteatoSITE*'s declining *POR* expression levels with fibrosis²⁹ could reflect that individuals with a decreased baseline CPR capacity experience disproportionate metabolic stress. Overall, these studies suggest that CPR functions as a variable 'metabolic buffer', in which individual regulatory capacity – rather than disease-specific downregulation of protein expression levels – determines CYP-dependent metabolic dysfunction and disease susceptibility.

In this Perspective, we examine the multifaceted role of CPR in MASLD, including its involvement in lipid metabolism, oxidative stress, mitochondrial dysfunction, iron homeostasis and inflammation.

By integrating genetic, biochemical and clinical evidence, we aim to highlight CPR as a potential determinant of MASLD heterogeneity and a promising therapeutic target^{35,36}.

Central role of CPR in physiological pathways

CPR has a pivotal role in numerous physiological processes by facilitating electron transfer to various CYP and non-CYP redox partners, enabling the metabolism of a wide array of endogenous and exogenous compounds³⁷ (Fig. 1). Its activity sustains critical pathways such as drug metabolism, cholesterol and steroid biosynthesis, bile acid and fatty acid metabolism, retinoid turnover and haem degradation. Studies using liver-specific *Por* knockout mouse models highlight the central role of CPR in maintaining hepatic metabolic homeostasis. These mice, although viable, show substantial loss of CYP activity, along with hepatic lipid accumulation, disrupted cholesterol and bile acid metabolism, and impaired redox balance^{18,38–40}. Collectively, these CPR-dependent processes illustrate their critical roles in metabolic pathways essential for maintaining hepatic health and homeostasis (Fig. 1). Beyond its role in metabolic oxidation, CPR contributes to the generation of reactive oxygen species (ROS), redox homeostasis and the modulation of cellular signalling networks, highlighting its important role in metabolic and cellular function^{19–21}.

In xenobiotic metabolism, CPR supports CYP isoforms from families 1–3, which catalyse most phase I drug reactions, enabling detoxification and subsequent conjugation by phase II enzymes. This makes CPR indispensable for maintaining hepatic clearance and systemic protection against toxic intermediates^{41–45}.

In endobiotic metabolism, CPR-dependent enzymes facilitate cholesterol (for example, CYP51A1 and SQLE)^{46–48} and steroid hormone biosynthesis (for example, CYP17A1 and 21A2)^{15,49,50}, fatty acid hydroxylation and epoxidation (CYP1–4 families)⁵¹ and bile acid production (CYP7A1)^{52–54}. In addition, CPR can reduce the haem iron of CYB5, which supports fatty acid desaturation and elongation^{55,56}. Retinoid homeostasis also relies on CPR, as CYP26A1, CYP26B1 and CYP26C1 enzymes degrade retinoic acid to prevent toxic accumulation^{57,58}. In addition, CPR donates electrons to HO-1 and HO-2, enabling haem degradation to biliverdin and bilirubin, producing metabolites with antioxidant and signalling properties^{59–63}.

By coordinating these diverse processes, CPR integrates xenobiotic detoxification with lipid, sterol and vitamin metabolism, supporting also redox signalling and cellular homeostasis. Its central role makes CPR a key regulator of health and disease susceptibility.

MASLD

MASLD progression from 'simple steatosis' to inflammatory and fibrotic stages (MASH) involves complex metabolic and oxidative stress pathways in which CPR might have a regulatory role. Simple steatosis, histologically characterized as >5% of parenchyma fat without evidence of lobular inflammation and hepatocellular damage, is generally asymptomatic^{4–6}. Progression to the more aggressive MASH stage is characterized by inflammation and hepatocyte injury (including ballooning), which might lead to liver fibrosis^{4,7}. Chronic liver injury causes hepatic stellate cells to produce extracellular scar matrix (especially type I collagen), driving the progression of MASLD towards cirrhosis and, in some cases, HCC⁵. However, MASLD and MASH are potentially reversible and even established fibrosis might regress with lifestyle changes that lead to resolution of the inflammatory component, such as caloric restriction, weight loss and increased physical activity⁶⁴. MASLD results from the interplay of multiple risk

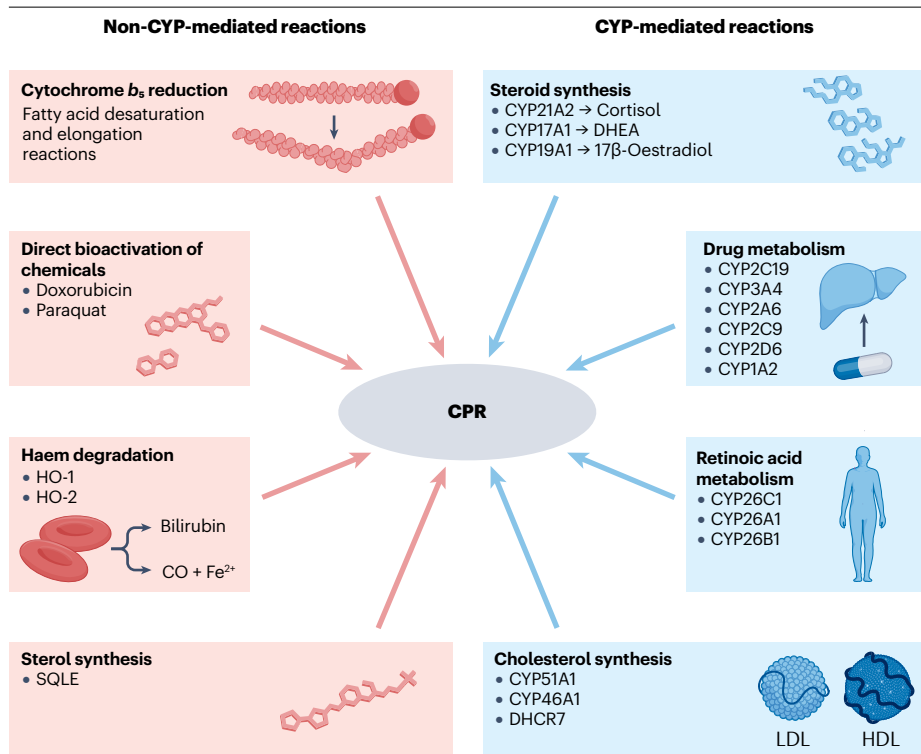


Fig. 1 | The central role of CPR in sustaining the activity of a panoply of redox partners and its physiological roles. Cytochrome P450 oxidoreductase (CPR) acts as the obligatory electron donor for microsomal cytochrome P450 (CYP) enzymes and several non-CYP redox partners. Through these interactions, CPR supports a wide range of physiological processes, including drug and xenobiotic metabolism, steroid and cholesterol biosynthesis, retinoic acid metabolism, and fatty acid modification via cytochrome b_5 -dependent reactions. CPR also contributes to sterol synthesis, haem degradation through haem oxygenases and the bioactivation of certain chemicals. Together, these pathways position CPR as a central regulator of hepatic metabolic homeostasis. CO, carbon monoxide; DHCR7, 7-dehydrocholesterol reductase; DHEA, dehydroepiandrosterone; Fe²⁺, ferrous iron; HO-1, haem-oxygenase 1; SQLE, squalene epoxidase.

factors spanning metabolic, dietary, genetic, environmental and demographic domains. Its complexity reflects the convergence of systemic metabolic dysfunction with lifestyle, environmental and host-specific susceptibilities.

Metabolic disorders

Obesity is a key factor in MASLD, promoting hepatic lipid accumulation, insulin resistance, and the release of pro-inflammatory cytokines and adipokines⁶⁵. Type 2 diabetes mellitus further increases susceptibility^{66,67}. By disrupting hepatic redox balance and insulin signalling, CPR dysfunction can contribute to metabolic syndrome and MASLD or MASH progression through impaired glucose utilization, oxidative stress and excessive lipid accumulation, as demonstrated in humanized liver mouse models and in the human HepG2 cell line in vitro^{34,40}. Dyslipidaemia, primarily presenting as hypertriglyceridaemia, is a hallmark of MASLD and is observed in 20–80% of patients across various global clinical settings⁶⁸. Similarly, hypertension is a major comorbid burden, affecting up to 50% of individuals with MASLD worldwide and increasing their cardiovascular risk two to three times compared with the general population⁶⁹. Indeed, bidirectional cross-talk between the liver and heart in cardiometabolic disease represents a crucial and evolving research area⁷⁰. Notably, *POR*-dependent CYP epoxygenases produce epoxyeicosatrienoic acids (EETs), which exert vasoprotective and anti-inflammatory effects. In mice, endothelial *Por* deletion reduced EET synthesis and enhanced angiotensin II-induced hypertension through prostanoid overproduction, effects reversed by cyclooxygenase inhibition⁷¹. Endothelial CYP overexpression, in mice, similarly protected against angiotensin II-induced hypertension and vascular dysfunction⁷². In humans with MASH, circulating EET levels decrease by 62% in advanced fibrosis and can accurately differentiate MASH from steatosis (AUROC 0.69–0.77)⁷³. By contrast,

CYP-dependent hydroxylation of arachidonic acid produces hydroxy-eicosatetraenoic acids (HETEs), lipid mediators that are more often linked with pro-inflammatory and vasoconstrictive signalling^{17,74}. A shift from vasoprotective EETs to HETE-dominated eicosanoids could occur when CPR–CYP electron transfer is disrupted. This imbalance might connect impaired liver redox and lipid metabolism to systemic vascular issues in MASLD and could also act as a dynamic marker of CPR–CYP network activity during disease progression.

Diet and gut microbiota

Diets high in saturated fats, fructose and omega-6 polyunsaturated fatty acids (PUFAs), combined with nutrient imbalances such as high iron and low copper, promote hepatic lipogenesis, inflammation and endoplasmic reticulum stress^{75,76}. Deficiencies in choline and methionine further impair lipid handling and increase oxidative stress, promoting hepatic steatosis^{77–79}. In parallel, gut dysbiosis enhances intestinal permeability or 'leaky gut', enabling microbial products such as lipopolysaccharides (LPS) to enter the liver, activating toll-like receptors and inflammasomes^{80,81}. Altered bile acid signalling via farnesoid X receptor, along with microbial metabolites such as trimethylamine *N*-oxide and ammonia, further exacerbates hepatic inflammation⁸⁰. The gut virome and mycobiome also seem altered in MASLD and are associated with disease severity⁸⁰, highlighting the complexity of host–microbiome interactions. Emerging evidence indicates that diet and microbiota-derived metabolites can modulate hepatic CYP activity and drug metabolism, thereby influencing CPR-dependent redox pathways and metabolic homeostasis⁸². Specifically, gut-derived inflammatory signals such as LPS have been shown to downregulate *POR* expression and CPR activity through the activation of nuclear receptors (for example, Pregnane X receptor and Constitutive androstane receptor) and pro-inflammatory cytokines, directly impairing the microsomal electron transport chain

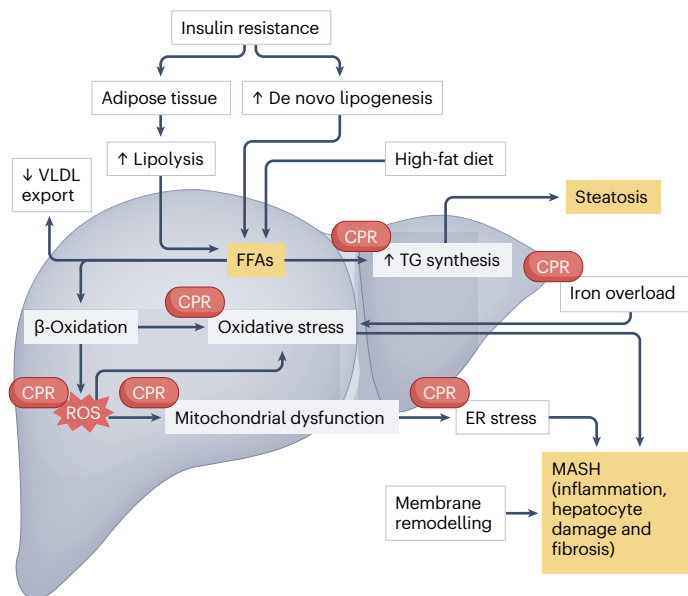


Fig. 2 | Molecular mechanisms in MASLD in which CPR might have a role. The pathways and processes that are increased are indicated by an upwards arrow (↑), and those that are decreased are indicated by a downwards arrow (↓). CPR, cytochrome P450 oxidoreductase; ER, endoplasmic reticulum; FFAs, free fatty acids; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; ROS, reactive oxygen species; TG, triglyceride.

and exacerbating oxidative stress⁸². These mechanisms suggest that diet–microbiome interactions might indirectly affect CPR–CYP network function in MASLD.

Demographic and genetic factors

Although people of all ages, including children, can develop MASLD, the prevalence increases with age and is higher in men than in women, although risk rises in women post menopause due to oestrogen loss^{28,83–85}. Ethnic disparities have also been documented in Hispanic people showing a higher prevalence, and African American individuals the lowest⁸⁴, partly explained by variants such as Patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) I148M (rs738409 C>G)⁸⁶ and Transmembrane 6 superfamily member 2 (*TM6SF2*) E167K (rs58542926 C>T)⁸⁷, which impair lipid metabolism⁷. Conversely, protective variants in 17-β hydroxysteroid dehydrogenase 13 (*HSD17B13*) (rs72613567, rs143404524 and rs62305723) mitigate progression despite elevated gene expression in MASLD^{5,88}. Broader regulatory mechanisms are also likely to contribute to MASLD pathogenesis. Epigenetic mechanisms, including DNA methylation of genes involved in lipid metabolism and inflammation, further modify risk^{86,87}. In addition, post-transcriptional downregulation of miRNA-122, the most abundant hepatic microRNA (miRNA), enhances lipogenesis and might promote progression to MASH and HCC^{86,89–93}. The regulation of *POR* expression is discussed later.

Environmental factors and drug exposure

Several drugs, including amiodarone, aspirin^{94–96}, non-steroidal anti-inflammatory drugs^{94–96} and glucocorticoids⁹⁶ can induce hepatic

steatosis and inflammation by impairing mitochondrial β-oxidation or disrupting lipid homeostasis. Pollutants, such as bisphenol A⁹⁷, diesel exhaust particles and fine particulate matter⁵, have been linked to oxidative stress and immune activation. Lifestyle factors, including alcohol^{97–99}, even at moderate levels of intake, and smoking⁵ accelerate inflammation, fibrosis and lipid accumulation. Many of these xenobiotics are metabolized by microsomal CYP enzymes⁴⁵ that rely on CPR for essential electron transfer. In MASLD, the frequent induction of CYP2E1 creates a high demand for CPR-mediated electrons, often leading to ‘uncoupling’ of the catalytic cycle in which electrons are diverted to oxygen, generating high levels of superoxide and hydrogen peroxide^{20,82}. Furthermore, certain pollutants and drugs can directly disrupt the CPR–CYP interface, impairing electron flow and worsening oxidative stress in the endoplasmic reticulum⁸². As a result, environmental and pharmacological exposures could promote MASLD progression by specifically impairing the CPR–CYP metabolic network, a mechanism examined in more detail in subsequent sections.

Molecular mechanisms of CPR in MASLD pathogenesis

MASLD exhibits substantial heterogeneity in its pathophysiological features. Its development and progression are complex and multifaceted processes, currently under extensive investigation⁸. Early concepts, such as the ‘two-hit hypothesis’, proposed that hepatic lipid accumulation caused by factors including high-fat diets, sedentary lifestyles, obesity and insulin resistance serves as the ‘first hit’, making hepatocytes more vulnerable to subsequent ‘second hits’ from insults such as oxidative stress and lipid peroxidation¹⁰⁰. This can cause hepatocyte damage, inflammation and fibrosis. However, this paradigm does not account for the complex interactions among genetic, environmental and metabolic factors that collectively drive MASLD in predisposed individuals⁸⁷.

Therefore, the ‘multiple-hit hypothesis’ is now widely accepted, incorporating insulin resistance, oxidative stress, dietary influences, gut microbiota, and genetic and epigenetic factors⁸. CPR and its redox partners have crucial roles in metabolic processes central to these factors, including lipid metabolism, ROS regulation, and cholesterol and bile acid synthesis, in addition to xenobiotic metabolism¹⁰¹. This indicates a potential role for CPR involvement in modulating MASLD initiation and progression, at multiple levels (Fig. 2).

Lipotoxicity

Disruptions to CPR function, whether (epi)genetic or stress induced, might impair metabolic pathways involved in lipid homeostasis⁵⁴, contributing to the pathogenesis of MASLD. CPR is essential for electron transfer to various CYP enzymes directly or indirectly involved in multistep lipid metabolism. CYP4A and CYP4F, for example, mediate ω-hydroxylation of fatty acids, facilitating their further oxidation¹⁶. Other microsomal CYPs (for example, CYP2E1, CYP3A4 and CYP3A5) also contribute to fatty acid processing, influencing lipid balance through reactions such as epoxidation and hydroxylation^{16,17,102,103}. The interaction of CPR with these enzymes ensures efficient execution of these metabolic processes, preventing lipid imbalances. For instance, CYP2E1 metabolizes free fatty acids (FFAs), producing bioactive lipid mediators and ROS, which are closely linked to MASLD progression¹⁰³. CYP3A subfamily members are involved in the metabolism of sterols and fatty acid derivatives, contributing to lipid regulation and transport¹⁰⁴. CPR also supports bile acid synthesis via CYP7A1 and sterol 27-hydroxylase, influencing cholesterol and lipid homeostasis¹⁰⁵.

Steatosis occurs when glycerol and FFAs are esterified to form lipids, primarily triglycerides⁸⁷. FFAs can be derived from diet, de novo lipogenesis (DNL) or increased lipolysis in adipose tissue. Within hepatocytes, FFAs are converted by acyl-CoA synthase into fatty acyl-CoAs, which can then be directed towards esterification to form triglycerides or towards the β -oxidation pathway for energy production⁸⁷. Cholesterol, phospholipids, proteins and triglycerides assemble into chylomicrons for transport to muscles and adipose tissue. In a fed state, the liver's primary energy source is glucose, and excess fatty acids are incorporated with triglycerides into VLDLs and/or lipid droplets instead of being oxidized¹⁰⁶.

Activating transcription factors such as the carbohydrate response element-binding protein and sterol regulatory element-binding protein-1 promotes DNL in MASLD¹⁰⁶. Notably, patients with MASLD show increased plasma FFA levels at night and increased DNL compared with control individuals (this pattern is unaffected by fasting), implicating circadian disruption with MASLD. It is also noteworthy that increasing carbohydrate consumption and insulin resistance are associated with increased DNL^{107,108}.

Chronic exposure to high levels of FFAs, ceramide, lysophosphatidylcholine or cholesterol during the onset of MASH and progression of MASLD can lead to lipotoxicity^{109,110}. An excess of FFAs has been shown to induce endoplasmic reticulum stress, mitochondrial dysfunction and lysosomal permeabilization, ultimately leading to cell death¹⁰⁷. Notably, increased FFA plasma levels are associated with increased disease severity. Although unsaturated FFAs can protect the liver from toxicity, saturated FFAs are particularly harmful and can promote hepatocellular injury¹¹¹.

Dysregulated CPR activity reduces the efficiency of fatty acid oxidation and hydroxylation, leading to lipid accumulation and lipotoxicity. Hepatic *Por*-null mouse models and small interfering RNA-mediated CPR inhibition have demonstrated significant ($P < 0.05$) lipid accumulation due to reduced triglycerides catabolism and secretion rather than enhanced synthesis¹⁸. Impaired bile acid production further exacerbates triglycerides and fatty acid build-up, highlighting CPR's central role in lipid regulation¹⁸. Thus, dysfunction of microsomal CYPs in lipid metabolism causes accumulation of toxic lipid intermediates, further contributing to lipotoxicity and MASLD progression.

Oxidative stress

CPR has a dual role in ROS production through both CYP-dependent and CYP-independent pathways. It facilitates electron transfer to CYP enzymes for metabolic oxidation reactions, concurrently contributing to ROS formation^{19,112}. During CPR-mediated electron transfer, uncoupling can occur, releasing electrons directly to oxygen and producing reactive species such as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2)¹¹². CPR also contributes to ROS production by redox-cycling ferric iron (Fe^{3+}) and quinones, generating hydroxyl and semiquinone radicals¹⁹. These pathways emphasize the crucial role of CPR in redox balance and its potential to worsen oxidative stress when dysregulated. Oxidative stress is a substantial factor in MASLD development, resulting from an imbalance between ROS production and the liver's antioxidant system's capacity for neutralization¹¹³. Although oxidative stress is mainly linked with mitochondrial dysfunction, other contributors include increased endoplasmic reticulum stress, C/EBP homologous protein (also known as CHOP or GADD153) production, microsomal metabolism and CYP activity¹¹⁴. Liver cells produce ROS such as $O_2^{\cdot-}$, hydroxyl radicals ($\cdot OH$) and H_2O_2 as metabolic by-products. Superoxide dismutase converts $O_2^{\cdot-}$ into H_2O_2 , which then undergoes an

iron-catalysed Fenton reaction to produce $\cdot OH$ and H_2O . These ROS further propagate oxidative damage by generating pro-oxidants such as hypochlorous acid, peroxynitrite and peroxy radicals¹¹⁴.

The hepatic antioxidant system comprises enzymes such as glutathione peroxidase (GPX), catalase and superoxide dismutase, and non-enzymatic molecules such as glutathione (GSH), ascorbic acid, retinol and tocopherol (vitamins C, A and E, respectively), that collectively protect against ROS-induced damage¹¹⁴. In MASLD, hepatic lipid overload increases ROS production and depletes antioxidant defences¹¹³. This imbalance is exacerbated by oxidative inhibition of enzymatic activity and excessive consumption of antioxidants. In advanced stages such as MASH with established fibrosis, antioxidant capacity is further impaired, as seen in patients with cirrhosis¹¹⁵. In addition PUFAs undergo lipid peroxidation, producing reactive aldehydes such as malondialdehyde and 4-hydroxy-2-nonenal, which further contribute to MASLD progression¹¹⁶.

CPR is essential for metabolizing retinol and PUFAs through CYP-dependent pathways. CYP26 enzymes convert retinol into bioactive retinoic acids, crucial for cellular signalling and liver homeostasis¹¹⁷. Impaired CPR activity disrupts this process, resulting in retinol accumulation, dysregulation of retinoid signalling, and increased oxidative stress¹¹⁸. Similarly, PUFAs are oxidized into metabolites, such as HETEs and EETs, via lipoxygenase, cyclooxygenase and CYP pathways¹⁷. Indeed, in humans, EETs have anti-inflammatory properties, whereas HETEs promote inflammation and oxidative damage¹⁷. Altered EET metabolite levels in human liver are linked to the progression of MASLD and fibrosis^{73,119}. Thus, CPR dysfunction could shift this balance towards pro-inflammatory pathways.

CYP2E1, a key enzyme involved in ROS production in MASLD, depends on CPR in the endoplasmic reticulum to metabolize FFAs and other small polar xenobiotics²⁰. Its activity produces carbonyl radicals and increases ROS production through electron release^{20,120}. Elevated rates of $O_2^{\cdot-}$ and H_2O_2 production are thought to result primarily from poor coupling during electron transfer between CYP2E1 and CPR¹²¹. Furthermore, a mitochondrial form of CYP2E1, which depends on the ADX-ADX system for electron transfer, contributes to mitochondrial ROS generation and oxidative damage, potentially playing a part in the progression of steatosis to MASH^{120,121}. In hepatocytes, gene expression, protein levels and activity of CYP2E1 are elevated in MASLD. Its heightened protein activity diverts more electrons from CPR to oxygen, producing excess ROS that promote oxidative stress, mitochondrial dysfunction and inflammation¹²²⁻¹²⁴.

Key transcription factors, such as nuclear factor erythroid 2-related factor 2 (also known as NRF2) and nuclear factor- κB (NF- κB), have essential roles in regulating antioxidant responses and inflammation¹¹⁴. NRF2, a redox-sensitive transcription factor, is essential for cellular defence, activating the expression of antioxidant proteins, GSH synthesis-related proteins, and phase II detoxification enzymes by binding to antioxidant or stress-response elements. In addition, NRF2 is involved in the regulation of the expression of various CYP enzymes and HO-1 (ref. 21). Dysregulated CPR activity could disrupt this NRF2-dependent system, reducing antioxidant defences²¹. NF- κB , which is activated by ROS and endoplasmic reticulum stress, mediates predominantly pro-inflammatory and, to some extent, antioxidant responses¹¹⁴. Once activated, NF- κB regulates the production of inflammatory cytokines and CYP transcription and translation through binding to CYP promoter regions¹²⁵. In MASLD, NF- κB signalling links oxidative stress to inflammation and insulin resistance¹¹⁴, highlighting the integral role of CPR in these interconnected processes.

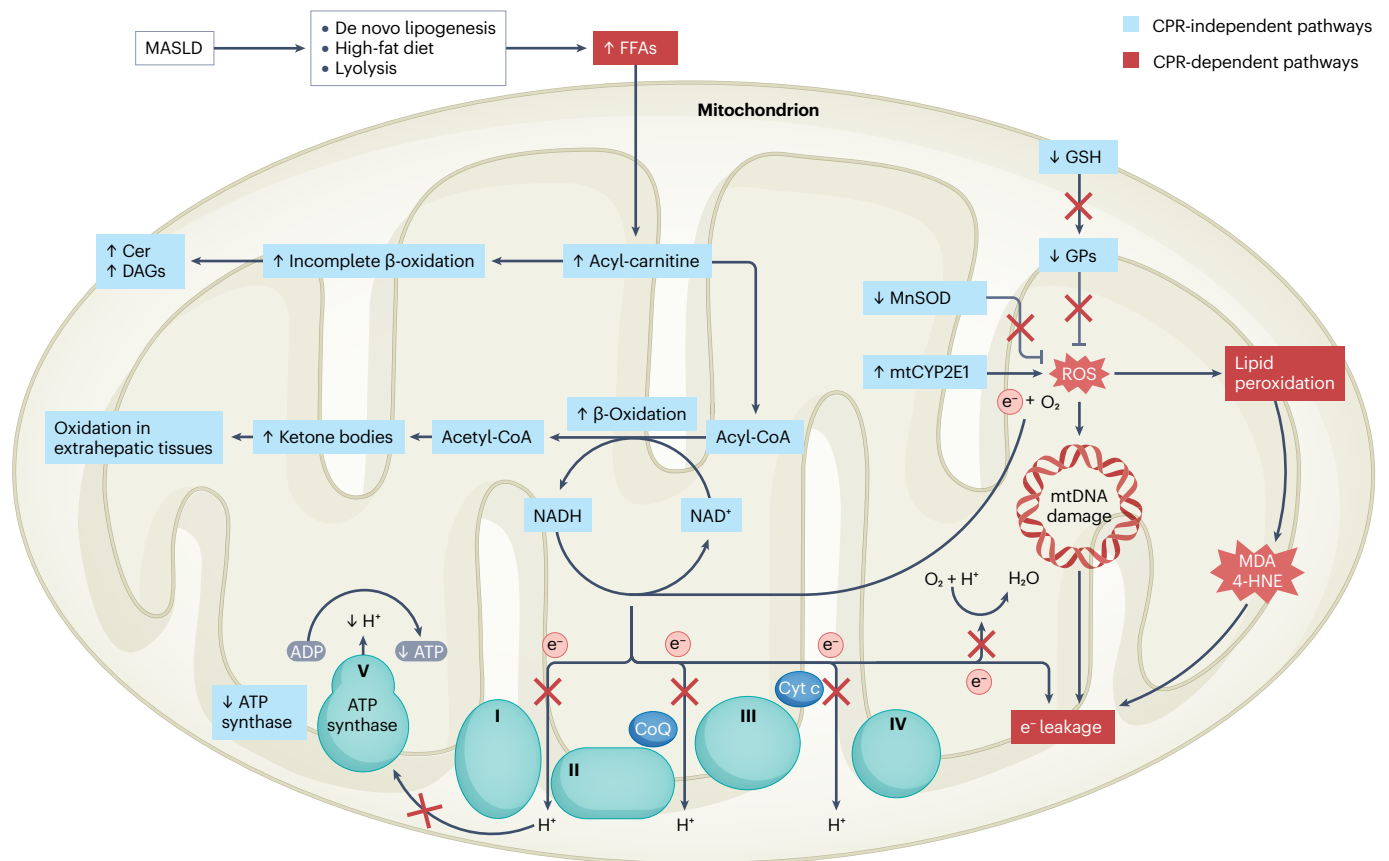


Fig. 3 | Potential roles of CPR in mitochondrial dysfunction in MASLD. Elevated free fatty acids (FFAs) lead to mitochondrial dysfunction, increased and incomplete β -oxidation, and accumulation of ceramide (Cer) and diacylglycerols (DAGs). Excess β -oxidation and electron leakage from the electron transport chain generate reactive oxygen species (ROS), exacerbated by increased mitochondrial CYP2E1 (mtCYP2E1) activity and diminished antioxidant defences, such as glutathione (GSH), glutathione peroxidases (GPs) and manganese superoxide dismutase (MnSOD). The resulting ROS triggers

lipid peroxidation, forming toxic by-products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), damaging mitochondrial DNA (mtDNA) and impairing ATP synthesis. Cytochrome P450 oxidoreductase (CPR)-influenced pathways are in dark red. CoQ, coenzyme Q; CYP, cytochrome P450; Cyt c, cytochrome c; e⁻, electron; H⁺, hydrogen ion; MASLD, metabolic dysfunction-associated steatotic liver disease; NAD⁺, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide.

Mitochondrial dysfunction

Although primarily located in the endoplasmic reticulum, where it mediates electron transfer to CYPs and other redox partners¹²⁶, CPR is also implicated in mitochondrial dysfunction, a condition central to the pathogenesis of MASLD^{112,114} (Fig. 3). In the uncoupling of CPR's electron transfer, electrons are prematurely released into the surrounding environment of the endoplasmic reticulum, often reducing O₂ to form ROS, as described earlier¹¹². This source of ROS can cause mitochondrial damage by disrupting mitochondrial membranes and key proteins within the electron transport chain, as well as impairing mitochondrial DNA (mtDNA)^{124,127} (Fig. 3). This damages mitochondrial respiration and reduces ATP production, ultimately leading to cellular energy failure¹²⁴ (Fig. 3). Also, mitochondrial-generated ROS further contributes to this degradation, exacerbating mitochondrial dysfunction^{124,127}. Although mitochondrial dysfunction in MASLD is characterized by mtDNA damage due to oxidative stress and limited repair capacity^{124,127}, the specific role of CPR in this process requires further investigation.

One marked consequence of CPR-mediated ROS production is lipid peroxidation, particularly the oxidation of PUFAs, which also contributes to oxidative stress. In mitochondria, lipid peroxidation amplifies oxidative damage. It enhances ROS production, sustaining mitochondrial dysfunction^{20,127} (Fig. 3). Mitochondrial CYP2E1 probably contributes substantially to ROS production and mitochondrial dysfunction^{20,120,124} (Fig. 3). ROS generated by CYP2E1 causes lipid peroxidation, forming reactive aldehydes that further damage cellular membranes, proteins and DNA²⁰.

Elevated gene, protein and activity levels of CYP2E1 are observed in steatosis and MASH in humans, as well as in vivo models, and in vitro human cell models, with its activity contributing to insulin resistance, inflammation, and the development of MASLD and its progressive form, MASH²⁰.

MtDNA is particularly susceptible to oxidative damage owing to its proximity to the inner mitochondrial membrane, where ROS are produced, its lack of protective histones (unlike nuclear DNA), and its limited repair capacity and ultimately depletion¹²⁸. In advanced

MASLD, hallmark features include abnormal mitochondrial biogenesis, insufficient mitophagy, reduced mtDNA content and activated inflammasomes^{114,129}. In addition, hepatic ischaemia–reperfusion injury, linked to oxidative stress and excessive ROS production, underscores the multifaceted role of ROS in liver damage. Mitochondrial ROS, generated during ischaemia, might contribute to reoxygenation-induced damage, although the specific contribution of CPR-dependent ROS production to this process remains to be determined¹⁹.

Iron overload and ferroptosis

Iron is essential for cellular processes such as mitochondrial respiration, DNA synthesis and oxygen transport. However, its pro-oxidant nature requires strict regulation to prevent harmful effects^{78,130}. The liver, as the primary storage organ, regulates iron uptake through transferrin receptors (TRF1 in most cells, TRF2 mainly in hepatocytes), storing it as ferritin, or incorporating it into metabolic pathways^{75,79,130} (Fig. 4). CPR supports enzymatic systems that maintain iron homeostasis. Disruptions in CPR function have been proposed to contribute to iron overload, a condition observed in approximately one-third of patients with MASLD^{22,23,79,131}. Iron accumulation increases ROS production via Fenton reactions^{75,130}, inducing lipid peroxidation (Fig. 4), leading to the formation of toxic by-products, including lipid hydroperoxides, aldehydes,

and oxidized sterols that exacerbate inflammation, mitochondrial dysfunction and fibrosis in MASLD^{79,132}.

Ferroptosis is a non-apoptotic form of cell death caused by iron metabolism disruption and characterized by widespread lipid peroxidation^{133,134}. CPR is hypothesized to be central in this mechanism by facilitating the generation of ROS and driving lipid peroxidation through its electron transfer functions²³. In humans, ROS-induced damage worsens due to GSH depletion, decreased GPX4 activity and dysregulation of genes linked to iron metabolism^{79,130,132,134} (Fig. 4). These factors collectively hinder cellular processes that neutralize lipid peroxides, leading to membrane destabilization and the onset of ferroptosis^{79,130,132,134}.

Ferroptosis is characterized by the absence of nuclear chromatin condensation and abnormal mitochondrial morphology, including rupture of the outer mitochondrial membrane, with associated ROS accumulation, GSH depletion, GPX4 inhibition and the release of PUFAs^{22,130,132–135} (Fig. 4). Ferroptosis is induced by peroxidation of PUFA-containing phospholipids (PL-PUFAs), resulting in lipid hydroperoxides (PL-PUFA-OOH) that destabilize the cellular membrane and drive the cell into ferroptosis^{130,133} (Fig. 4). CPR seems to amplify this cascade by generating ROS through uncoupling of its electron transfer, increasing peroxidation levels of PL-PUFAs²².

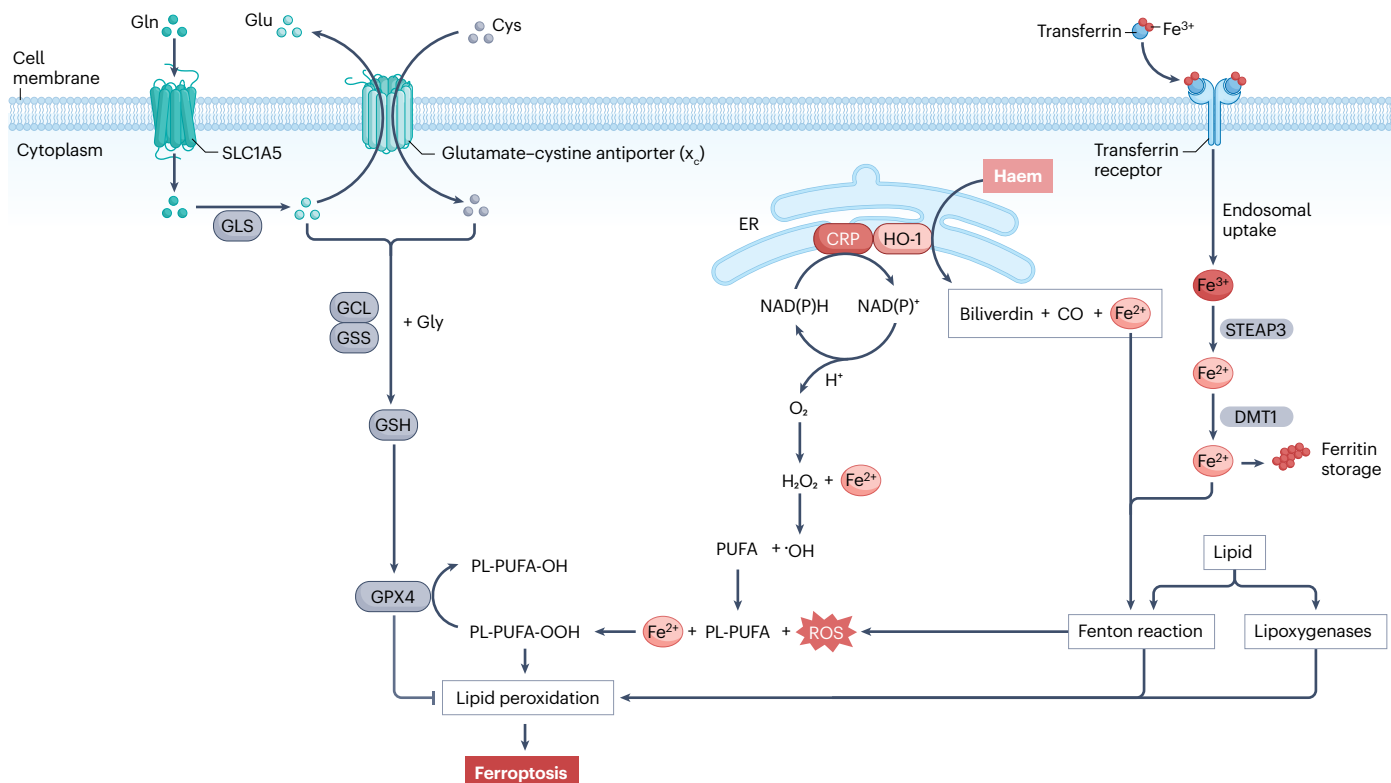


Fig. 4 | The suggested role of CPR in ferroptosis. Cytochrome P450 oxidoreductase (CPR) facilitates electron transfer from reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) to haem-oxygenase 1 (HO-1), but some react with oxygen, producing hydrogen peroxide (H_2O_2). The Fenton reaction (between H_2O_2 and ferrous iron (Fe^{2+})) generates a hydroxyl radical ($\cdot OH$), oxidizing polyunsaturated fatty acids (PUFAs) into phospholipid-bound PUFAs (PL-PUFAs). Reactive oxygen species (ROS) and Fe^{2+} convert PL-PUFAs into phospholipid-bound PUFA hydroperoxide (PL-PUFA-OOH). In metabolic dysfunction-associated steatotic liver disease (MASLD), glutathione peroxidase 4

(GPX4) fails to reduce PL-PUFA-OOH into phospholipid-bound PUFA alcohol (PL-PUFA-OH) using glutathione (GSH), leading to ferroptosis. Cys, cysteine; DMT1, divalent metal transporter 1; ER, endoplasmic reticulum; Fe^{3+} , ferric iron; GCL, glutamate cysteine ligase; Gln, glutamine; GLS, SLC1A5 glutaminase; Glu, glutamate; Gly, glycine; H^+ , hydrogen ion; NAD(P)⁺, nicotinamide adenine dinucleotide (phosphate); SLC1A5, solute carrier family 1A member 5; GSS, glutathione synthetase; STEAP3, six-transmembrane epithelial antigen of prostate 3.

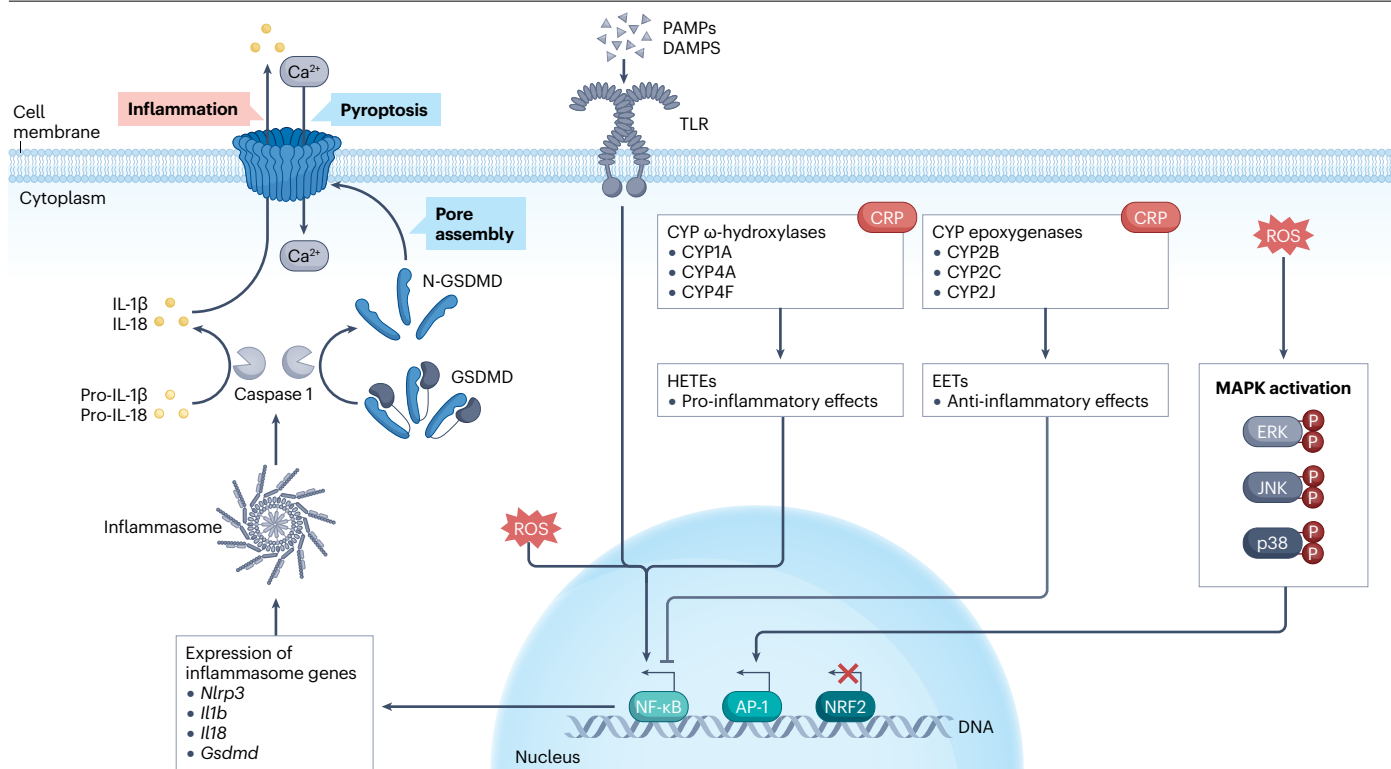


Fig. 5 | Suggested role of CPR in the initiation of the inflammatory process. Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) activate toll-like receptor (TLR) signalling and inflammasome pathways, leading to caspase 1 activation, gasdermin D (GSDMD) pore formation, pyroptosis, and the release of IL-1 β and IL-18. Cytochrome P450 oxidoreductase (CPR) supports cytochrome P450 (CYP) ω -hydroxylases and CYP epoxygenases that generate bioactive lipid mediators with opposing inflammatory functions: pro-inflammatory hydroxyeicosatetraenoic acids (HETEs) and anti-inflammatory epoxyeicosatrienoic acids (EETs), respectively.

CPR-dependent CYP activity also contributes to reactive oxygen species (ROS) generation, which activates mitogen-activated protein kinase (MAPK) signalling (extracellular-regulated kinase (ERK), JUN N-terminal kinase (JNK) and p38) and promotes nuclear factor- κ B (NF- κ B)-mediated and activator protein (AP-1)-mediated transcription while suppressing the nuclear factor erythroid 2-related factor 2 (NRF2) antioxidant response, collectively amplifying hepatic inflammation. Ca²⁺, calcium; NLRP3, leucine-rich repeat-containing receptor family pyrin domain-containing 3.

Haem-oxygenase, which exclusively relies on CPR, has a nuanced role in ferroptosis¹³³. Under normal conditions, the inducible HO-1 mediates haem degradation, which offers cytoprotection by preventing haem-induced ROS production. Yet excessive HO-1 activity in humans can paradoxically exacerbate iron release, promoting Fenton reactions and lipid peroxidation^{136,137} (Fig. 4). As a result, the level of HO-1 activity is critical in determining whether it acts as a protective or pathogenic factor in ferroptosis and consequently in MASLD progression. In MASLD livers, increased HO-1 and CPR levels can serve as an adaptive response to oxidative stress. However, their ability to promote ferroptosis suggests a complex role of CPR in iron overload and related pathologies^{133,136}.

Iron dysregulation, along with the roles of CPR in oxidative stress and ferroptosis, creates a vicious cycle in human MASLD, driven by iron overload. This dysregulation leads to increased hepatic ROS and lipid peroxidation, which causes hepatocyte injury and inflammation. Subsequently, it promotes further ROS production and ferroptotic signalling alongside CPR, thereby perpetuating liver damage^{79,132}. Clarifying the role of CPR in these processes will be important to establish whether it functions as a metabolic regulator and a potential therapeutic target (see the 'The potential role of CPR in MASLD' section).

Inflammation

Chronic inflammation is a hallmark of MASLD pathogenesis and a main driver of progression to MASH¹³⁰. CPR might influence inflammatory signalling both directly, through modulation of CYP enzyme activity and ROS generation, and indirectly, via the synthesis of lipid mediators that shape immune cell responses. Interestingly, in mouse atherogenic-diet MASLD models, CYP epoxygenase expression and EET biosynthesis are markedly suppressed with concomitant inflammation and hepatic injury^{73,119}. CPR supports the activity of CYP epoxygenases (for example, CYP2B, CYP2C and CYP2J subfamilies), which generate EETs, with strong anti-inflammatory properties. In humans, EETs inhibit leukocyte adhesion to vascular endothelial cells, suppress cytokine-induced NF- κ B activation and enhance endothelial nitric oxide production, collectively dampening hepatic and vascular inflammation^{24,138} (Fig. 5). Conversely, CPR facilitates the function of CYP ω -hydroxylases (for example, CYP1A, CYP4A and CYP4F subfamilies), producing HETEs such as 20-HETE, which activate NF- κ B signalling, promoting the expression of adhesion molecules and pro-inflammatory cytokines, thereby exacerbating inflammation, as shown *in vitro*^{24,74} (Fig. 5). The balance between these CPR-dependent lipid mediators seems to critically determine whether hepatic inflammation resolves or progresses.

The inflammatory mechanisms behind the pathological processes are not yet fully understood. Hepatocytes and non-parenchymal cells, such as Kupffer cells, have key roles in triggering inflammatory cascades¹³⁹. Hepatic lipotoxicity and the accumulation of FFAs lead to the activation of inflammasome complexes¹¹³. These complexes are triggered by pathogen-associated molecular patterns and damage-associated molecular patterns, including mtDNA, ROS, calcium, ATP and LPS¹¹³. Inflammasomes activate caspase-1, which processes pro-inflammatory cytokines, such as IL-1 β and IL-18, into their mature forms, perpetuating hepatic inflammation^{94,113} (Fig. 5).

Kupffer cells, the liver-resident macrophages, produce high levels of ROS, inflammatory cytokines (for example, tumour necrosis factor and IL-6) and growth factors such as transforming growth factor β in response to lipid accumulation and oxidative stress^{87,113}. CPR-dependent CYP enzymes, such as CYP2E1, further amplify these processes by generating ROS during the metabolism of excess FFAs, as described earlier. Elevated CYP2E1 expression, observed in human MASH, underscores its role in linking CPR activity to inflammation and oxidative stress in MASLD progression²⁰. Beyond Kupffer cells, hepatic stellate cells respond to CPR-dependent ROS and cytokine release by undergoing myofibroblastic activation, contributing to fibrogenesis, as shown in mice^{140,141}.

CPR might also affect the balance between pro-inflammatory and anti-inflammatory transcriptional programmes. Excessive CYP-dependent ROS can activate NF- κ B, perpetuating cytokine expression (Fig. 5), while simultaneously suppressing the NRF2 antioxidant response, shifting the balance towards a sustained pro-inflammatory state, a mechanism demonstrated primarily in human hepatoma cell lines^{25,26,142}. However, this specific redox-transcriptional crosstalk remains to be fully validated in MASLD preclinical models. This inflammatory cycle is further amplified by the shift in CPR-driven eicosanoid metabolism, moving towards pro-inflammatory HETEs and away from anti-inflammatory EETs, serving as a key lipidomic hallmark of MASH progression²⁴ (Fig. 5).

The role of CPR also extends to the gut–liver axis. By modulating CYP-mediated bile acid synthesis and detoxification, CPR indirectly influences the gut microbiota and intestinal barrier integrity. Altered bile acid profiles and increased LPS leakage stimulate toll-like receptor signalling in both hepatocytes and Kupffer cells, exacerbating NF- κ B and inflammasome activation and reinforcing chronic hepatic inflammation, as demonstrated in vitro^{143,144}.

Implications of genetic variations of *POR* in MASLD pathophysiology

Given the central role of CPR in supporting numerous CYP and non-CYP enzymes, we propose that genetic variation in the *POR* gene could contribute to MASLD susceptibility and phenotypic diversity. The *POR* locus contains many polymorphisms – listed in the databases ClinVar (around 760 variants) and gnomAD (around 3,700 variants; accessed 26 January 2025)¹⁴⁵ – some of which modify CPR activity and its interaction with specific CYP isoforms^{14,37,146,147}. In individuals with compound heterozygous mutations, the combined effects of both alleles probably produce a range of residual enzymatic activity, potentially affecting hepatic redox balance and lipid-metabolic outcomes^{148,149}.

Severe biallelic mutations, such as *A287P*, *Y181D* and *R457H*, are known to cause systemic endocrine and skeletal abnormalities associated with CPR deficiency^{150–153}. However, milder or more common polymorphisms^{147,153–155}, such as *A503V*, *Q153R* or *P284L*, can also influence CPR electron transfer efficiency and affect CYP-mediated drug and

lipid metabolism. These functional differences in CPR affect processes central to MASLD pathogenesis, including hepatic lipid accumulation, oxidative stress balance and hormone signalling^{147,153–155}. Thus, it is plausible that *POR* genetic diversity could have a mechanistic role in shaping disease severity, sex-based differences and therapeutic response.

Regulatory control of *POR* expression

Besides the effects of coding sequence polymorphisms, the *POR* gene is regulated by various factors that can influence CPR protein levels and activity. In MASH, impaired thyroid hormone receptor- β (THR β) function decreases mitochondrial activity, reduces fatty acid β -oxidation, and increases fibrosis¹⁵⁶. THR β directly targets the *POR* promoter, and the thyroid hormone binding to its response elements activates *POR* transcription, establishing a mechanistic connection between thyroid hormone signalling and the control of the microsomal CYP system¹⁵⁷.

Notably, in mice the hepatocyte circadian clock, through the transcription factor BMAL1, directly regulates *Por* expression, independent of feeding rhythms¹⁵⁸. Hepatocyte-specific *Bmal1* knockout mice showed reduced CPR protein and enzymatic activity, confirming that CPR is a rate-limiting step in microsomal electron transfer¹⁵⁸. Disruption of this circadian synchronization in MASLD could contribute to metabolic rigidity and excessive lipid accumulation.

At the post-transcriptional level, miRNA-214 binding to the 3'-untranslated region of *POR* downregulated its expression and increased oxidative stress in alcohol-induced liver injury both in vitro and in vivo (rats) could apply to MASLD⁹⁹, given their overlapping clinical and pathophysiological profiles. However, epigenetic regulation of *POR* has not yet been measured, despite the known roles of DNA methylation and histone modifications in MASLD gene expression^{159–161}. Indeed, if *POR* silencing in MASLD involves epigenetic repression, treatment with DNA methyltransferase, histone deacetylase inhibitors or Sirtuin activators could restore CPR and CYP network activity, particularly when combined with redox modulators or THR β agonists.

Reduced *Por* expression in preclinical in vivo models of MASLD probably reflects both quantitative decline in CPR protein and qualitative dysfunction¹⁶². In a mouse MASLD model, endoplasmic reticulum stress decreased CYP activity by reducing endoplasmic reticulum membrane fluidity and disrupting calcium homeostasis, effects that were reversible by the endoplasmic reticulum stress modulator silybin³⁴. Additional factors, such as protein misfolding, faulty flavin cofactor binding, oxidative modifications, altered membrane composition and NADPH depletion, could therefore further weaken CPR–CYP coupling^{14,37}. Differentiating between quantitative and qualitative CPR dysfunction is vital: transcriptional upregulation might suffice when expression is low, whereas broader therapeutic strategies targeting redox balance, protein stability and membrane integrity are needed when CPR function itself is compromised. Understanding these regulatory mechanisms is critical for interpreting CPR dysregulation in MASLD (discussed in the next section).

The potential role of CPR in MASLD

In MASLD, dysregulation of those mechanisms is likely to contribute to disease pathogenesis. Numerous studies have characterized the structural and functional diversity of CPR and the broad clinical spectrum of *POR* genetic variants^{18,19,39,40,60,71,146,147,155,158,159,162}. Despite this extensive knowledge, no direct mechanistic or clinical study has yet established a causal link between CPR dysfunction and MASLD.

Compared with well-established MASLD-associated genes such as *PNPLA3*, *TM6SF2* and *HSD17B13* (detailed earlier), which mainly affect

lipid metabolism or inflammatory regulation, CPR seems to act as a broader metabolic integrator. This broader control suggests that even subtle variations in *POR* expression or CPR activity could amplify metabolic downstream disturbances. Notably, *POR* has not been identified as a genome-wide significant locus in large-scale genome-wide association studies of MASLD, suggesting that CPR dysregulation in MASLD is probably acquired or regulatory (for example, transcriptional, epigenetic or metabolic), rather than genetically determined^{163,164}. Overall, the data presented earlier indicate a dual, context-dependent role of CPR in MASLD. This duality stems from the essential function of CPR in electron transfer to CYPs, which mediate diverse metabolic processes, spanning from protective metabolic functions to pathogenic mechanisms. On one hand, CPR supports metabolic homeostasis by facilitating CYP-dependent pathways critical for lipid metabolism, bile acid synthesis and detoxification that counteract lipotoxicity, a hallmark of MASLD^{51–54}. Its activity ensures the detoxification of potentially harmful metabolic intermediates and xenobiotics, safeguarding hepatocytes from damage^{41,42,44,45,165}. CPR-dependent enzymes also regulate retinoid and steroid metabolism, contributing to cellular function and overall metabolic equilibrium^{15,49,50,57,58}. Conversely, the role of CPR in ROS production could worsen oxidative stress, a major factor driving MASLD progression, causing mitochondrial dysfunction, lipid peroxidation and inflammation, key features of MASLD pathogenesis. Elevated ROS levels activate inflammatory signalling and fibrogenic pathways, promoting hepatocellular injury and fibrosis¹⁶⁶.

The dual nature of CPR's function could help explain the heterogeneous clinical course observed in MASLD, as CPR could exert both protective and pathogenic effects on metabolic homeostasis, depending on disease context. This hypothesis gains further support from transcriptomic data collated in SteatoSITE MASLD data commons, which combines histopathological assessments, hepatic bulk RNA sequencing, and electronic health record data from 940 patient-derived liver samples from Scotland²⁹. Using one-sided gene set enrichment

analysis, SteatoSITE identified multiple CPR-dependent pathways, including drug metabolism by CYPs as well as fatty acid, retinol and steroid metabolism, as significantly ($P < 0.05$) enriched in patients with MASLD-related fibrosis. Moreover, *POR* transcript levels progressively declined with increasing fibrosis stage (F0–1 to F4), indicating that low *POR* expression levels might contribute to metabolic collapse and fibrotic remodelling in late-stage disease²⁹. These findings align with the proposed duality of CPR: its enzymatic activity is indispensable for hepatic homeostasis, yet under chronic metabolic stress, reduced or dysregulated CPR function might exacerbate pathological processes.

SteatoSITE analysis also identified three master regulons, co-regulated gene networks, strongly linked to disease progression. One of these, the THR β regulon, was shown to be progressively downregulated with increasing severity of fibrosis and considered a pivotal modulator of pathways involved in lipid metabolism, inflammation and fibrosis^{29,36}. As the dominant THR isoform in hepatocytes, THR β has a critical role in lowering cholesterol and triglyceride levels, increasing bile acid synthesis and promoting fat oxidation, positioning it as a central player in MASLD pathogenesis^{35,36}. This regulon is of particular interest regarding CPR, as the expression of *POR* is primarily induced by THR β and thyroid hormone via specific sequences of its gene promoter, as shown in humans and rats^{157,167,168}. Downregulation of THR β will affect CPR-dependent pathways, as described earlier.

Interestingly, resmetirom, a THR β -specific agonist, has been approved by the FDA as the first oral treatment for patients with MASH and moderate to severe fibrosis^{35,36}. The therapeutic efficacy of resmetirom can be partly mediated through its direct transcriptional upregulation of *POR*, by selectively activating THR β ¹⁵⁷. It stimulates β -oxidation in liver mitochondria, which decreases the production and secretion of VLDLs, as well as free fatty acid uptake and synthesis, whereas it increases the expression of the low-density lipoprotein receptor in the liver¹⁶⁹. By increasing CPR expression, resmetirom could enhance the capacity of CPR-dependent metabolic pathways, including

Box 1 | Critical research questions

Establishing cytochrome P450 oxidoreductase (CPR) as a therapeutic target in metabolic dysfunction-associated steatotic liver disease (MASLD) requires addressing several key questions:

Causality and therapeutic proof-of-concept

Can restoring CPR activity — through strategies beyond thyroid hormone receptor- β agonism — reduce steatosis and inflammation in preclinical models? Are CPR-selective modulators feasible?

Which cytochrome P450 pathways matter most?

Not all CPR–cytochrome P450 interactions contribute equally to MASLD. Can we identify which specific pathways drive disease, and would selectively enhancing protective epoxygenases or inhibiting pro-oxidant ω -hydroxylases offer therapeutic benefit?

Non-invasive biomarkers for patient stratification

Could epoxyeicosatrienoic acid–hydroxyeicosatetraenoic acid ratios produced by circulating epoxygenases, *POR*-promoter methylation, or plasma miRNA-214 levels serve as accessible markers of hepatic CPR status? Such biomarkers could guide treatment decisions and monitor response.

What drives *POR* downregulation?

Understanding the molecular mechanisms — transcriptional, epigenetic or post-transcriptional — that silence *POR* in MASLD is essential. Are these changes reversible and druggable?

Precision-medicine potential

Does baseline CPR expression or activity predict who will respond to resmetirom or other MASLD therapies? Can CPR profiling identify patient subgroups for targeted intervention?

Genetic contributions and causal inference

How do *POR* variants influence MASLD susceptibility, progression or treatment response? Mendelian randomization and humanized knock-in mouse models could clarify whether genetic variation causally affects disease outcomes.

Answering these questions will require mechanistic studies in advanced human-relevant preclinical models, multiomics integration in well-characterized patient cohorts, and targeted clinical trials — ultimately translating CPR biology into therapeutic innovation for MASLD.

those involved in bile acid synthesis and cholesterol metabolism, therefore aiding in the resolution of hepatic steatosis and lipotoxicity. Indeed, these actions, many involving CPR, could improve lipid clearance, decrease hepatic steatosis, and lessen pro-inflammatory and pro-fibrotic signalling. Clinical trials demonstrated resmetirom's efficacy in substantially lowering lipotoxicity and improving fibrosis markers^{35,36,170}.

In addition, sex-specific hormonal regulation might further interact with CPR function to influence MASLD outcomes. This is probably due to the influence of sex hormones on CPR activity and the expression of related enzymes. For example, CPR-dependent steroidogenic enzymes affect the synthesis and balance of sex hormones (androstenedione, testosterone, estrone and oestradiol) that differ between men and women⁴⁸. Variations in CPR activity or *POR* genotype could therefore contribute to the observed sex dimorphism in MASLD, in which women in post menopause show increased susceptibility compared with age-matched men²⁸. Decreased oestrogen levels might heighten the effects of impaired CPR activity, whereas in men, androgen metabolism mediated by CPR-dependent CYPs could offer some protection in early-stage disease progression²⁸.

The clinical and molecular heterogeneity of MASLD probably reflects, in part, interindividual differences in CPR activity caused by *POR* genetic variability. Variants in *POR* can modify CYP electron transfer efficiency, either in a CYP isoform-specific manner or through cumulative reductions in compound heterozygotes, with different remaining enzymatic activity of the two *POR* alleles, thereby affecting multiple hepatic processes simultaneously¹⁵⁴. Although direct clinical associations between *POR* variants and MASLD have not yet been demonstrated, existing in vitro and heterologous expression studies consistently show that CPR variants exert isoform-specific effects on CYP enzymes, potentially influencing lipid metabolism, oxidative stress responses and inflammatory signalling pathways relevant to MASLD^{14,154,171–174}. Furthermore, this genetic variability could affect the metabolism of many clinically used drugs, including those prescribed for MASLD-associated comorbidities, such as those used for type 2 diabetes mellitus (pioglitazone), hypertension (beta blockers such as nifedipine and felodipine) and dyslipidemia (statins), leading to altered efficacy or increased risk of adverse reactions¹⁷⁵. Collectively, these findings support the view that *POR* genetic diversity represents a plausible yet underexplored determinant of MASLD susceptibility, progression and therapeutic response, underscoring the need for dedicated genotype–phenotype studies.

Conclusion and outlook

CPR is central in hepatic metabolic homeostasis as the essential electron donor to 48 microsomal CYP enzymes and numerous non-CYP redox partners. Through these interactions, it coordinates lipid, steroid and bile acid metabolism, xenobiotic detoxification, retinoid homeostasis and haem degradation. Several in vitro and mouse hepatic *POR* knockout models demonstrate that CPR deficiency directly induces steatosis and dysregulated cholesterol and bile acid metabolism, as well as oxidative stress, highlighting its essential role in liver metabolic integrity.

CPR dysregulation might be an underestimated factor in MASLD pathogenesis. Dysfunction arising from transcriptional repression, miRNA-mediated silencing, epigenetic modifications or metabolic stress could promote lipid accumulation, oxidative injury, ferroptosis and inflammation. The FDA approval of the THR β agonist resmetirom, which upregulates *POR* transcription, provides translational support

for the hypothesis that targeting CPR-dependent pathways could be beneficial in MASH with fibrosis.

However, CPR enzymatic activity shows a context-dependent duality: interactions can produce either protective or pro-oxidant effects. Although *POR* has not been identified as a genome-wide significant locus for MASLD, functional variants demonstrate modest, context-dependent effects, suggesting that acquired regulatory mechanisms, rather than genetic determinism, drive their contribution to MASLD risk. Integrative multiomics and longitudinal functional studies will be crucial for clarifying the role of CPR as a metabolic buffer and identifying patient subgroups most likely to benefit from CPR-targeted therapies.

CPR dysregulation might be an underestimated factor in MASLD development, and the ability of resmetirom to promote *POR* transcription highlights its importance. Further research into the molecular mechanisms of CPR and its spatial distribution in the liver is essential to deepen understanding of its metabolic buffering, support precision medicine, and inform future multiomics and longitudinal studies (Box 1).

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Author contributions

M.K. and L.J.N. conceived and developed the theme of this Perspective. M.K., L.J.N., and F.E. with C.B. developed the thematic structure. C.B. conducted research for the article and drafted the manuscript and figures under the supervision and support of M.K. and F.E. L.J.N., J.A.F. and T.J.K. contributed to critical discussions of the manuscript and the article’s content. All authors reviewed and edited the manuscript before submission.

Competing interests

T.J.K. serves as a consultant or advisory board member for Resolution Therapeutics, Clinovate Health, HistoIndex, Fibrofind, Kynos Therapeutics, Perspectrum, Concept Life Sciences, Servier Laboratories and Jazz Pharmaceuticals, and has received speakers’ fees from Servier Laboratories, Jazz Pharmaceuticals, AstraZeneca, HistoIndex and Incyte Corporation. J.A.F. serves as a consultant or advisory board member for Resolution Therapeutics, Kynos Therapeutics, Gyre Therapeutics, Ipsen, River 2 Renal Corp., Stimuliver, Guidepoint and ICON plc, and has received speaker fees from HistoIndex, International Society for Continuing Professional Development (SICLEO) and Resolution Therapeutics and has received a research grant from GlaxoSmithKline and Genentech. The other authors declare no competing interests.

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