

ORIGINAL ARTICLE

Hydrogen sulphide in liver glucose/lipid metabolism and non-alcoholic fatty liver disease

Inês Mateus | Carina Prip-Buus 

Institut Cochin, INSERM, CNRS,
Université de Paris, Paris, France

Correspondence

Carina Prip-Buus, Institut Cochin,
INSERM U1016, CNRS UMR 8104;
Department of Endocrinology,
Metabolism and Diabetes, 24 Rue du
Faubourg Saint-Jacques, 75014, Paris,
France.
Email: carina.prip-buus@inserm.fr

Present address

Inês Mateus, Institut Cochin, INSERM
U1016, CNRS UMR8104, Université de
ParisParis, France

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Abstract

Background: For a long time, hydrogen sulphide (H₂S) was considered only as a toxic gas, inhibiting mitochondrial respiration at the level of cytochrome c oxidase, and an environmental pollutant. Nowadays, H₂S is recognized as the third mammalian gasotransmitter, playing an important role in inflammation, septic shock, ischaemia reperfusion events, cardiovascular disease and more recently in liver physiology and chronic liver diseases such as non-alcoholic fatty liver disease (NAFLD).

Methods: This narrative review is based on literature search using PubMed.

Results: From a bioenergetic perspective, H₂S is a very unique molecule, serving as a mitochondrial poison at high concentrations or as an inorganic mitochondrial substrate at low concentrations. By using transgenic animal models to specifically modulate liver H₂S biosynthesis or exogenous compounds that release H₂S, several studies demonstrated that H₂S is a key player in liver glucose and lipid metabolism. Liver H₂S content and biosynthesis were also altered in NAFLD animal models with the in vivo administration of H₂S-releasing molecules preventing the further escalation into non-alcoholic-steatohepatitis. Liver steady-state levels of H₂S, and hence its cell signalling properties, are controlled by a tight balance between its biosynthesis, mainly through the transsulphuration pathway, and its mitochondrial oxidation via the sulphide oxidizing unit. However, studies investigating mitochondrial H₂S oxidation in liver dysfunction still remain scarce.

Conclusions: Since H₂S emerges as a key regulator of liver metabolism and metabolic flexibility, further understanding the physiological relevance of mitochondrial H₂S oxidation in liver energy homeostasis and its potential implication in chronic liver diseases are of great interest.

KEYWORDS

hydrogen sulphide, liver, metabolism, mitochondria, non-alcoholic fatty liver disease

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1 | INTRODUCTION

Hydrogen sulphide (H_2S) is the third gasotransmitter discovered in mammals besides carbon monoxide (CO) and nitric oxide (NO).¹ Easily recognizable by its characteristic scent of rotten eggs, H_2S is considered an environmental pollutant, and its toxic effects in humans have been thoroughly described.² Nowadays, H_2S still constitutes the number one occupational hazard at oil and gas field well-heads, pipelines, processing plants and refineries.³ Much of its toxicity relies on the fact that, similarly to cyanide, NO and CO, H_2S inhibits mitochondrial cytochrome c oxidase (Complex IV), hence blocking mitochondrial respiration.⁴ Nonetheless, the public concern for its lethal effects was so great that the physiological importance of this gas was completely overlooked until the late 90s.⁵ It was not until 2001 that a physiological role for H_2S has been reported in cardio-vasorelaxation.⁶ Since then, thousands of studies were conducted to better understand and clarify the role of H_2S in a given system. The general assessment is that, as a gasotransmitter, H_2S acts as a pleiotropic agent, playing an active role in multiple physiological situations: vasorelaxation,^{7,8} angiogenesis,⁹ apoptosis,^{10,11} ageing¹² and metabolism.^{13,14} The biological importance of H_2S became more complex with the discovery that H_2S can also serve as an inorganic substrate for mammalian mitochondrial respiration through its oxidation by a mitochondrial sulphide quinone oxidoreductase (SQR).¹⁵ The presence of the SQR in many different cell types/organs of the mammalian organism suggests that tissue H_2S levels have to be tuned either to avoid toxic accumulation or to control H_2S signalling.

Over the past decades, the potential role of H_2S in liver pathophysiology has emerged.¹⁶ Liver metabolism is central to energy homeostasis, and liver metabolic inflexibility is known to be associated with several metabolic diseases, such as non-alcoholic fatty liver disease (NAFLD). The liver is uniquely positioned to be exposed to high levels of H_2S originating from the gut microbiota and from endogenous biosynthesis essentially via the transsulphuration pathway.¹⁷ In this review, we discuss the roles of H_2S in the regulation of liver metabolism and NAFLD development, with a particular focus on hepatic H_2S biosynthesis and mitochondrial oxidation and their potential exploitation as targeted therapeutic approaches.

2 | LIVER SOURCES OF H_2S

Liver H_2S levels were previously reported within the low nanomolar to middle micromolar range (17 nM–144 μ M).^{18–20} Although some of the H_2S found in the body comes from inhalation, the majority of it is produced

within the organism through the catabolism of dietary amino acids in tissues and through microbiota fermentation. Whether H_2S is of endogenous (hepatic non-enzymatic and enzymatic reactions) or exogenous (gut microbiota) sources, the liver is exceedingly likely to be exposed to high levels of H_2S (Figure 1).

2.1 | Endogenous non-enzymatic H_2S production

In mammals, non-enzymatic H_2S can be produced in the presence of reducing agents such as glutathione (GSH), nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH).²¹ H_2S can also be released from bound sulphur as well as organic or inorganic polysulphides^{21,22} (Figure 1). An additional non-enzymatic way to produce H_2S is through thiosulphate.²¹ Koj et al.²³ reported that glutathione disulphide (GSSG), H_2S and labelled sulphite were produced when rat liver mitochondria were incubated with oxygen, GSH, and [³⁵S]thiosulphate. Other sources of non-enzymatic H_2S production can include thiocystine and cysteine.²¹ Indeed, the occurrence of coordinated catalysis of cysteine with ferric iron and vitamin B6 leads to dose-dependent intravascular release of abundant H_2S .²⁴ Such non-enzymatic H_2S generation is likely to occur in the liver, a tissue that usually holds iron storages. Presently, the existence and the physiological relevance of these non-enzymatic H_2S productions in the liver still remain to be fully addressed.

2.2 | Endogenous enzymatic H_2S biosynthesis

In the liver, sulphur-containing amino acids, such as homocysteine, cysteine and methionine, are metabolized during the transsulphuration pathway, in which H_2S is released as a by-product¹⁷ (Figure 1). The reactions constituting this pathway are essentially mediated by three enzymes: cystathionine β -synthase (CBS), cystathionine γ -lyase (CSE) and 3-mercaptopyruvate sulphurtransferase (MPST).

While CBS is mainly expressed in the central nervous system, CSE and MPST are mainly expressed in the peripheral tissues.^{5,25–27} In the liver, all three enzymes are highly detectable, although the CBS/CSE system is thought to be responsible for the majority of hepatic H_2S production while MPST plays a secondary role. Previous studies have shown that CSE is about 60-fold more expressed than CBS in the liver²⁸ and that mice with a global invalidation of the CSE gene presented 50% less serum H_2S levels.²⁶ Nevertheless, each enzyme uses a specific combination

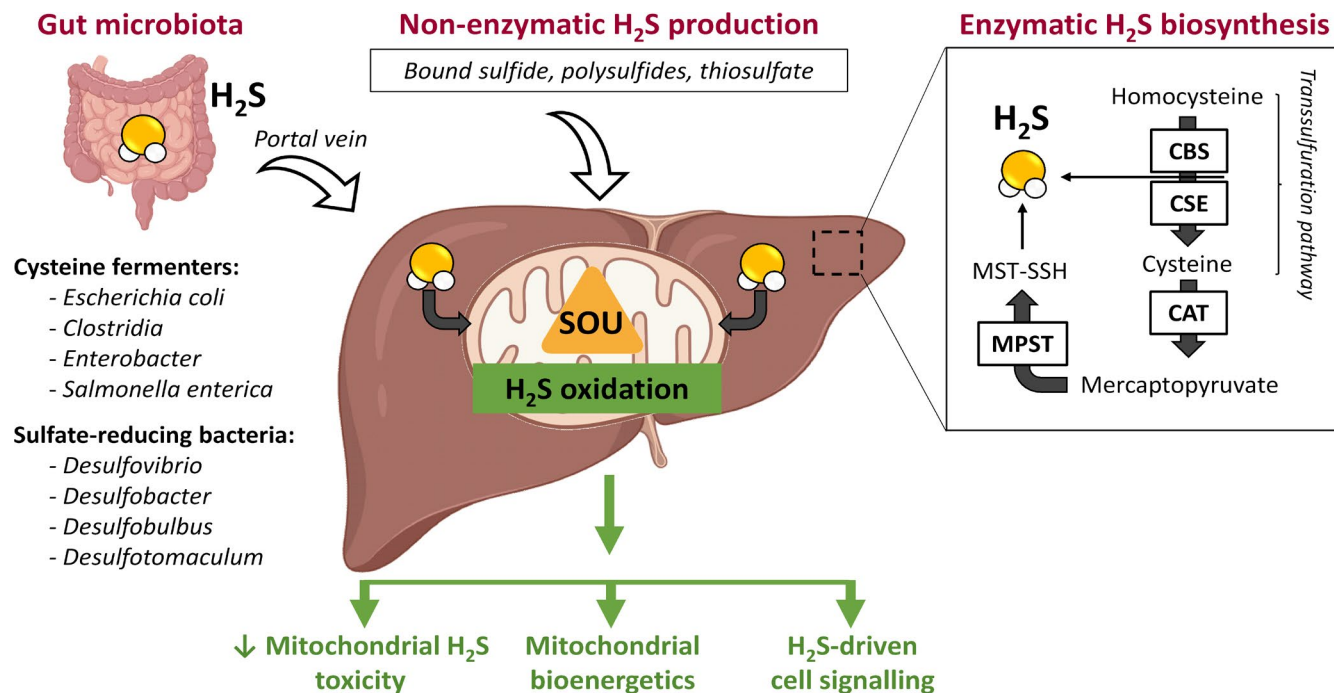


FIGURE 1 The sources of H₂S in the liver. The liver is exposed to H₂S from both exogenous and endogenous sources. Exogenously, gut microbiota represents a substantial source for the systemic bioavailability of H₂S, which is delivered to the liver via the portal vein. In the colonic lumen, certain bacteria, such as *Escherichia coli* and *Clostridia*, are capable to produce H₂S from the desulphydration of cysteine. Others, namely the sulphate-reducing bacteria, are able to reduce the sulphate present in epithelium-released sulphomucins to produce H₂S. Endogenously, in the liver, H₂S can be produced non-enzymatically from polysulphides and thiosulphate when reducing agents are present. However, H₂S is majorly synthesized as a by-product during the metabolism of sulphur-containing amino acids, such as homocysteine and cysteine, in the transsulfuration pathway, which involves cysteine β-synthase (CBS), cysteine γ-lyase (CSE), 3-mercaptopyruvate sulphurtransferase (MPST) and cysteine aminotransferase (CAT). H₂S is a strong mitochondrial poison when present at high concentrations, and cells have developed a system to maintain non-toxic levels of H₂S: the sulphide oxidizing unit (SOU). The mitochondrial oxidation of H₂S molecules conducted by the SOU generates electrons and protons that are injected into the mitochondrial electron transport chain, which makes H₂S the first inorganic energetic substrate used by mammalian cells

of substrates and contributes differently to the total H₂S pool.^{28,29} At physiologically relevant concentrations of substrate, 70% of all H₂S is produced from CSE-mediated reactions.³⁰ However, it has been demonstrated that, at equimolar concentrations of CBS and CSE, H₂S production by CBS can account for approximately 25%–70% of the total H₂S pool.³¹ These seemingly contradictory studies could be explained by the observation that, at physiologically relevant concentrations of substrate, over 95% of all hepatic H₂S was produced via CSE-mediated reactions, while at high substrate concentrations CBS constituted the major contributor to hepatic H₂S source.²⁸

2.3 | Gut microbiota

Due to the portal circulation, the liver is the first destination for the different products originated in the gut, including H₂S molecules (Figure 1). By using a perfused rat liver system, a previous study demonstrated the high capacity of the liver for H₂S clearance from the circulation

and that H₂S infusion in the portal circulation had a direct impact on hepatic bioenergetics and oxygen availability.³² Thus, the gut-liver axis plays a key role not only in liver H₂S availability but also in H₂S clearance and liver physiology. Moreover, gut microbiota also represents a major regulator of the systemic bioavailability of H₂S since germ-free mice have up to 80% less plasma-free H₂S level than conventional mice.³³ Two classes of microorganisms produce considerable amounts of H₂S: the cysteine fermenters (e.g. *Escherichia coli*, *Salmonella enterica*, *Clostridia* and *Enterobacter*) and the sulphate-reducing bacteria (SRB) (e.g. *Desulfovibrio*, *Desulfobacter*, *Desulfobulbus* and *Desulfotomaculum*) (Figure 1). As their name suggests, cysteine fermenters produce H₂S, pyruvate and ammonia from the fermentation of cysteine by cysteine desulphydrase,³⁴ while SRB produce H₂S via the reduction of inorganic sulphate or via microbial catabolism of sulphomucins.³⁵

H₂S concentrations in the gut luminal content are usually found within the millimolar range (0.2–1.5 mM for rodents and up to 3.4 mM for humans).^{36–38} Since H₂S is

notably produced from sulphur-containing amino acids, high-protein diets (HPD) can modify gut microbiota composition towards the growth of H_2S -producing bacteria. Indeed, rats fed HPD presented a higher abundance of SRB and higher concentrations of colonic H_2S .³⁹ Similarly, individuals fed a high-meat diet for 10 days have 15 times more faecal sulphide than individuals fed a vegetarian diet for the same period of time.³⁸ H_2S production by intestinal bacteria also escalated during cases of inflammatory bowel disease.⁴⁰ Given that H_2S can be extremely toxic, colonocytes exposed to high levels of H_2S are able to develop adaptative metabolic response and increase their capacity for mitochondrial H_2S oxidation to limit the adverse effects of H_2S .³⁷

3 | H_2S AND MITOCHONDRIA: A DUAL FACED MOLECULE

At a molecular level, H_2S toxic properties are attributed to the inhibition of mitochondrial Complex IV. Complex IV catalyses the transfer of electrons from reduced cytochrome c to molecular oxygen (Figure 2). It has been established in several tissues including the liver that, at high concentrations (typically 10–100 μM), H_2S competitively binds to Complex IV, thereby inhibiting the binding of oxygen.^{41–43} While some studies suggested that this H_2S -induced inhibition led to increased mitochondrial levels

of reactive oxygen species (ROS),⁴⁴ other studies suggested that Complex IV inhibition by H_2S decreased ROS levels by increasing the activity of superoxide dismutases (SODs).⁴⁵

Nonetheless, H_2S actions on mitochondria are far more complex than those of a simple poison. Due to its highly toxic properties, microorganisms that dwell in sulfidic habitats must have efficient ways to survive in such abiotic conditions. Thorough investigations showed that H_2S was not only tolerated by these microorganisms, but rather exploited in an endosymbiotic way. In 1986, H_2S oxidation coupled to adenosine triphosphate (ATP) production was described in mitochondria isolated from the mussel *Solemya reidi*.⁴⁶ Subsequent studies conducted in the lugworm *Arenicola marina* showed that mitochondrial H_2S oxidation was linked to the electron transfer chain (ETC) at the level of ubiquinone via a sulphide quinone oxidoreductase (SQR).⁴⁷ Later, Hildebrandt and Grieshaber demonstrated that SQR did not act alone in this process, but was rather part of a larger pathway in which also participate ethylmalonic encephalopathy protein 1 (ETHE1), thiosulphate sulphurtransferase (TST) and sulphite oxidase (SUOX)⁴⁸ (Figure 2). This set of four enzymes, nowadays recognized as the sulphide oxidizing unit (SOU), conducts the transformation of H_2S into sulphite, thiosulphate and sulphate, respectively. In 2007, Goubert et al.¹⁵ demonstrated that not only bacteria and invertebrates but also mammalian cells used H_2S as an inorganic substrate via its oxidation in mitochondria.

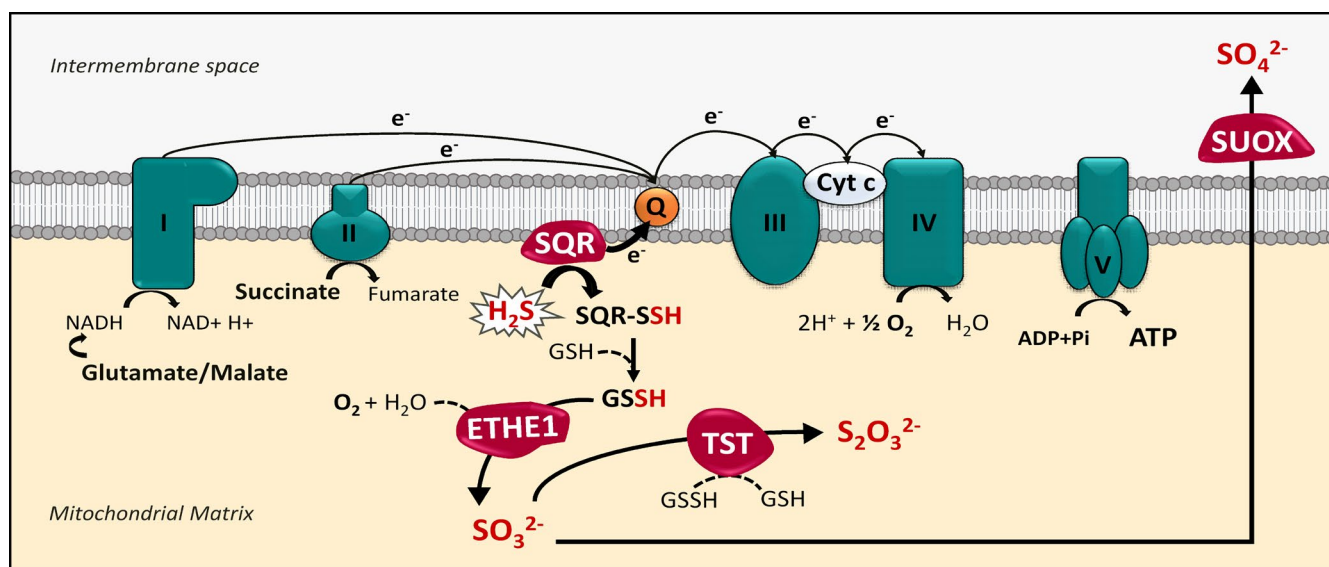


FIGURE 2 Mitochondrial H_2S oxidation in a mammalian cell. The sulphide oxidizing unit (SOU) allows H_2S to be used as an inorganic energetic substrate for mammalian mitochondria. H_2S is initially oxidized by sulphide quinone oxidoreductase (SQR), generating an SQR-bound persulphide (SQR-SSH) and releasing electrons (e^-) that are transferred to ubiquinone (Q) and carried down the electron transport chain. The SQR-SSH is transferred to a small molecule acceptor such as glutathione (GSH), forming a molecule of glutathione persulphide (GSSH) in the process. GSSH is then converted, with O_2 and H_2O as co-substrates, by ethylmalonic encephalopathy protein 1 (ETHE1, a sulphur dioxygenase) to sulphite (SO_3^{2-}). The sulphite formed can either be converted, with GSSH as co-substrate, into thiosulphate ($\text{S}_2\text{O}_3^{2-}$) by thiosulphate sulphurtransferase (TST, also called rhodanese) or be directly used by sulphite oxidase (SUOX) to produce sulphate (SO_4^{2-})

Almost all cells present the capacity to oxidize H₂S. In the central nervous system, only neurons, oligodendrocytes and endothelial cells present SQR protein expression.⁴⁹ In mammals, the main sites of sulphide oxidation are the liver, kidney and colon.^{50,51} The liver capacity to oxidize H₂S appears to be species-dependent, with human liver mitochondria showing the highest oxidation rates followed by pig, rat and finally mouse.^{52,53} From a mitochondrial bioenergetic point of view, H₂S is a very peculiar molecule, with a double-face effect on mitochondrial respiration: serving as an inorganic energetic substrate at low concentrations (nanomolar up to 10 μM) since H₂S oxidation exceeds its delivery rate and preserves mitochondrial respiration, or as an inhibitory molecule of mitochondrial Complex IV at higher concentrations (above 10 μM) when H₂S oxidation is lower than its delivery rate.⁵⁴⁻⁵⁶ Consequently, a marginal decrease in mitochondrial respiration may initiate a vicious cycle of H₂S accumulation leading to a block in respiration. The positive bioenergetic effects of H₂S required a basal activity of the Krebs cycle and is most pronounced at intermediate concentrations of succinate.⁵⁴ However, the concept of H₂S duality has recently been challenged by showing that high concentrations of sodium hydrosulphide (NaHS) (50–300 μM), an H₂S-releasing molecule, induced persulphidation of the α subunit of ATP synthase (ATP5A1) in HepG2 cells, rendering the ATP synthase enzyme more active.⁵⁷

4 | H₂S AS A REGULATOR OF LIVER METABOLISM

The liver is a key player in mammalian physiology with respect to energy homeostasis. It converts food nutrients absorbed by the intestine into substrates that the body can use (glucose, fatty acids [FA], amino acids...) and/or stores them (glycogen, triglycerides [TG]) before supplying them to cells when needed.⁵⁸ In addition, being the gateway to the body, the liver is also the main detoxification organ, able to metabolize and neutralize harmful substances, drugs, environmental toxins and endotoxins.^{59,60}

Although it is widely accepted that the liver is exceedingly well positioned to be exposed to elevated levels of H₂S, it still remains unknown how the liver copes with fluctuations in H₂S levels. Being a gas, H₂S can rapidly diffuse across membranes and react with different biological matrixes (e.g. proteins, haeme, GSH...), which makes this molecule incredibly hard to quantify. Therefore, up to now, this question has been indirectly addressed by using either transgenic knock-out (KO) CSE or CBS mouse models or exogenous H₂S

donors (molecules that release H₂S under in vivo or in vitro conditions) such as sulphide salts (NaHS and sodium sulphide [Na₂S]), natural donors (garlic-based compounds) or slow-release molecules (GYY4137) (Figure 3).

4.1 | Studies targeting endogenous H₂S biosynthesis

Multiple studies reported that H₂S is an active modulator of different metabolic processes, such as glucose utilization,¹³ gluconeogenesis,^{13,14,61} lipolysis,^{62,63} adipogenesis,⁵⁵ insulin signalling⁶⁴ and insulin resistance (IR),^{65,66} with CSE and CBS being essential for liver lipid and glucose metabolism (Figure 3).

4.1.1 | Impact on hepatic glucose metabolism

Although hepatic H₂S can originate from CBS-mediated reactions, most studies assessing the role of H₂S in liver glucose metabolism focused on the CSE/H₂S system.

Liver glycogen content and glucose consumption were higher in CSE KO mice compared with wild-type mice.¹³ Adenovirus-mediated CSE overexpression increased endogenous H₂S production and decreased glycogen content in HepG2 cells whereas incubation of HepG2 cells with NaHS impaired glucose uptake and glycogen storage via decreasing glucokinase (GK) activity¹³ (Figure 3). Overnight-fasted CSE KO mice submitted to a pyruvate tolerance test had a lower rate of gluconeogenesis than wild-type mice, which was normalized by in vivo administration of NaHS through the upregulation of key gluconeogenic transcription factors such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and CCAAT/enhancer-binding protein beta.⁶¹ NaHS also upregulated the expression and the activity by persulphidation of glucose-6-phosphatase (G6Pase) and fructose-1,6-bisphosphatase (F1,6Pase). NaHS was further shown to stimulate pyruvate carboxylase (PC) activity and gluconeogenesis in HepG2 cells and mouse hepatocytes¹⁴ (Figure 3). CSE overexpression enhanced but CSE KO reduced PC activity and gluconeogenesis, and blockage of PC activity abolished H₂S-induced gluconeogenesis.¹⁴ In addition, insulin at the physiological range inhibited CSE expression, and H₂S decreased insulin-stimulated phosphorylation of the protein kinase B (Akt) in HepG2 cells. However, hepatic CSE expression was increased in an in vitro model of liver IR¹³ and in streptozotocin-induced diabetic rats.⁶⁷

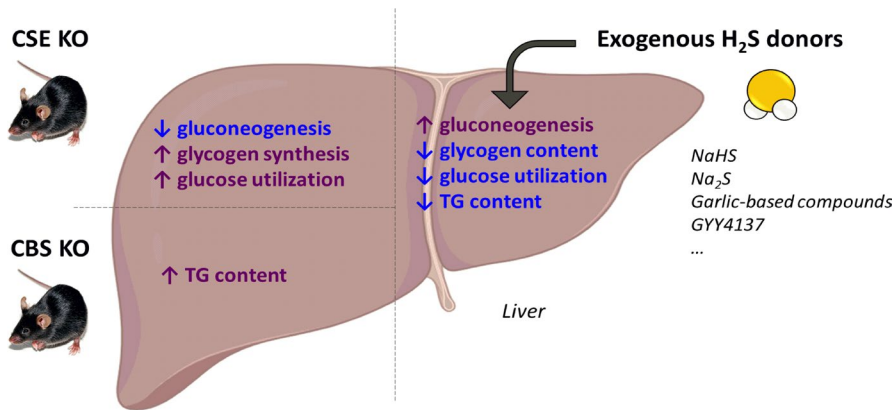


FIGURE 3 Regulation of liver metabolism by H₂S. Several studies using either transgenic knockout (KO) cysteine γ -lyase (CSE) or cysteine β -synthase (CBS) mouse models or exogenous H₂S donors demonstrated that H₂S regulates several hepatic metabolic pathways, including glucose utilization, gluconeogenesis, glycogen synthesis and triglyceride (TG) synthesis

4.1.2 | Impact on hepatic lipid metabolism

Humans carrying a CBS variant and CBS KO mice exhibited the same traits of hyperhomocysteinemia, oxidative stress, liver steatosis and fibrosis.^{68,69} CBS KO mice displayed altered mRNA levels of proteins required for cholesterol and FA biosynthesis and uptake, namely upregulation of liver X receptor (LXR), ATP binding cassette subfamily G member 1, 5 and 8 and ATP binding cassette subfamily A member 1, and downregulation of peroxisome proliferator receptor alpha (PPAR α)^{70,71} (Figure 3). These animals have also increased plasma levels of TG and non-esterified FA as well as lower hepatic activity of thiolase, a key enzyme for mitochondrial FA β -oxidation.⁷⁰ Recent evidence supported these findings by showing that CBS KO rabbits had higher plasma levels of TG, total cholesterol and low-density lipoprotein as well as hepatic microvesicular steatosis.⁷² CBS KO mice also showed significant alteration of a broad range of hepatic phospholipids and phosphatidylcholines.⁷³ Similarly, inhibition of hepatic CSE expression in a foetal hepatocyte line (L02) upregulates the sterol regulatory element-binding protein 1 (SREBP-1c) pathway, c-Jun N-terminal kinase (JNK) phosphorylation and hepatic oxidative stress.⁷⁴

4.2 | Studies targeting mitochondrial H₂S oxidation

Although most studies published thus far largely concern the regulation of H₂S levels through the modulation of its biosynthesis, hepatic mitochondrial H₂S oxidation may also regulate the levels of this gasotransmitter in the liver and hence its actions (Figure 2). Unfortunately, not many studies have been conducted to enlighten the physiological relevance of this pathway in liver energy homeostasis. SQR KO models of *Caenorhabditis elegans* exposed to H₂S exhibited increased phosphorylation of eukaryotic translation initiation factor 2- α with subsequent inhibition of protein synthesis.⁷⁵ The organism also presented

activation of endoplasmic reticulum and mitochondrial stress responses.⁷⁵ In humans, it has been recently reported the existence of two homozygous pathogenic variants of SQR, responsible for lack of protein and enzyme activity. The first variant (c.637G > A) was associated with decreased Complex IV activity in liver and muscle, lactic acidosis, hypotonicity, brain lesions and multiorgan failure.⁷⁶ The second variant (c.446delT) was associated with lactic acidosis, ketosis, dicarboxylic aciduria, Complex IV inhibition, encephalopathy and basal ganglia lesions. In all patients, the symptoms were triggered by infections or fasting, episodes associated with protein catabolism.⁷⁶ SQR has also been shown to be required for H₂S-mediated protection during nutrient/oxygen deprivation in Hepa1-6 cells.⁷⁷ In this study, addition of exogenous NaHS reduced lactate dehydrogenase (LDH) release (a marker of cell damage) in control Hepa1-6 cells during the ischaemic phase and upon reperfusion whereas LDH release was upregulated in cells partially depleted of SQR.⁷⁷ This study revealed that the resistance to ischaemia/reperfusion was linked to SQR capacity in donating electrons to the ETC via ubiquinone.⁷⁷

A complex interplay and feedback mechanism(s) may exist between the pathways of H₂S production and oxidation and their regulation. Dietary restriction (DR) was shown to enhance hepatic H₂S production by the upregulation of liver CSE via the activation of mechanistic target of rapamycin (mTOR).⁷⁷ This increased CSE expression presumes higher cysteine degradation into H₂S while cysteine concentrations are low in the context of DR, which appears to be counterintuitive.⁷⁷ However, the in vivo sources and substrates for CSE-derived H₂S production in cases of DR are not known, and free cysteine residues released upon autophagy could be a major fuel source for H₂S production. Additionally, Shirozu et al.⁷⁸ showed that hepatic H₂S levels in CSE KO mice were not significantly different from those found in control mice and that liver SQR mRNA and protein levels were increased, which is unexpected in a model of H₂S insufficiency. Therefore, further investigations are essential to better explore the

importance of SQR in liver metabolism and its interplay with the CSE/H₂S pathway.

5 | H₂S IN NON-ALCOHOLIC FATTY LIVER AND NON-ALCOHOLIC STEATOHEPATITIS

Non-alcoholic fatty liver disease (NAFLD) has been increasingly recognized as a major health burden in developed countries. Recent studies emphasize the role of IR, oxidative stress, lipid peroxidation, pro-inflammatory cytokines, adipokines and mitochondrial dysfunction in the onset and progression of non-alcoholic fatty liver (NAFL) towards non-alcoholic steatohepatitis (NASH).^{79,80} Over the past few years, several studies have reported alterations of H₂S metabolism in both human and animal models of NAFLD (Table 1).¹⁶

5.1 | Changes in H₂S biosynthesis

Some studies reported significant lower hepatic H₂S levels or CBS and CSE activities in mice and rats fed high-fat diet (HFD) when compared to control animals.^{81,82} These results have been corroborated by human studies, given that overweight individuals present lower plasma H₂S levels when compared to lean controls.⁸³ Hepatic H₂S biosynthesis was also impaired in a diet-induced rat model

of NASH.⁸⁴ Nonetheless, other studies reported that H₂S biosynthesis was upregulated in HFD-fed mice⁸⁵ and obese diabetic rats.⁸⁶ The discrepancies between these studies may either arise from differences in the composition of the diets and/or the duration of feeding or reflect spectrum-specific responses of these enzymes during the evolution of NAFLD.

Recently, CSE KO mice were shown to be more prone to develop obesity and IR when fed HFD as CSE invalidation aggravated obesity-related IR by inhibiting hepatic Akt activity and repressing forkhead box protein O1 (FoxO1) phosphorylation and degradation.⁸⁷ This resulted in the accumulation of nuclear FoxO1 and upregulation of phosphoenolpyruvate carboxykinase (PEPCK) and G6Pase, leading to increased gluconeogenesis.⁸⁷ Additional evidence demonstrated that CSE KO mice fed HFD had increased plasma and liver cholesterol levels due to decreased mRNA levels of LXR and the LXR-target gene cytochrome P450 family 7 subfamily A member 1 (CYP7A1), which halted cholesterol catabolism favouring its accumulation.⁸⁸ According to this study, deficiencies in the CSE/H₂S pathway might result in high susceptibility to HFD-induced fatty liver. In fact, HepG2 cells incubated with a mixture of free FA or high glucose concentration exhibited lower levels of CSE expression and H₂S production, as well as enhanced intracellular levels of acetyl-CoA and lipids.⁸⁹ Experiments using CSE KO mice fed high-fat/choline-deficient diet corroborated these results by showing hepatic accumulation of acetyl-CoA and lipids.⁸⁹

TABLE 1 Studies reporting changes in H₂S biosynthesis and H₂S levels in cases of non-alcoholic fatty liver disease (NAFLD)

	Diet/Genetic	Model	Observations	References
Animal models	HFD	NAFL	↓ liver CSE and MPST protein expression ↓ liver H ₂ S content	81
	HFD	NAFL	↑ liver MPST and ↓ liver CSE protein expression ↓ hepatic levels of H ₂ S	74
	HFD	NAFL	↑ liver CSE and CBS mRNA and protein ↑ hepatic H ₂ S levels	85
	Zucker diabetic fatty rats	NAFL	↑ liver CSE and CBS activity	86
	MCD	NASH	↓ liver CSE and MPST mRNA expression ↓ liver and plasma H ₂ S levels	84
	HFD	NAFL	↓ liver CSE activity and H ₂ S levels	62
	Humans		Overweight	↓ plasma H ₂ S levels
		Obese with T2DM	↓ plasma H ₂ S levels	83
		Overweight	↑ liver MPST and ↓ CSE protein expression	74
		Overweight with hypertriglyceridemia	↓ plasma H ₂ S levels	62

Abbreviations: CBS, cysteine β-synthase; CSE, cysteine γ-lyase; HFD, high-fat diet; MCD, methionine-choline-deficient diet; MPST, 3-mercaptopyruvate sulphurtransferase; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; T2DM, type 2 diabetes mellitus.

Similarly, HFD-fed CSE KO mice exhibited reduced plasmatic thiol levels, GSH, SOD activity and increased plasma malondialdehyde (MDA) levels.⁹⁰

Another study indicated that HFD-fed mice and NAFLD patients had an increased expression of hepatic MPST.⁷⁴ Both *in vivo* inhibition and *in vitro* inhibition of MPST significantly ameliorated intracellular fat accumulation. Interestingly though, H₂S production was enhanced by the inhibition of MPST and inhibited following MPST overexpression. Additional experiments showed that MPST negatively regulated CSE expression, which promoted steatosis by the upregulation of SREBP-1c, JNK phosphorylation and hepatic oxidative stress.⁷⁴ This study highlights the complexity of liver H₂S metabolism and the elaborate regulation to which the enzymes of the transsulfuration pathway are subjected.

5.2 | H₂S supplementation as a course of treatment for NAFLD

Recently, a growing number of evidence has shown that H₂S could be used as a drug for the treatment of NAFL/NASH (Table 2). Rats fed a methionine and choline-deficient (MCD) diet for 8 weeks had liver steatosis, inflammation and fibrosis as well as impaired hepatic H₂S production.⁸⁴ However, daily supplementation with NaHS prevented this MCD diet-induced NASH phenotype by reducing the cytochrome P450 family 2 subfamily E member 1 (Cyp2E1) expression, enhancing haeme oxygenase-1 (HO-1) expression and suppressing mitochondrial ROS production.⁸⁴ Similarly, treatment of MCD mice with S-propargyl-cysteine (SPRC), an H₂S donor, for 4 weeks significantly reduced hepatic ROS and MDA levels and increased SOD activity.⁴⁵ In HepG2 cells, the CSE inhibitor DL-propargylglycine completely abolished the anti-oxidant effects of SPRC through the downregulation of Akt phosphorylation, HO-1 and CSE expression, and by inhibiting the translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) to the nucleus.⁴⁵ HFD-fed mice daily injected with NaHS for 4 weeks displayed a decrease in hepatic lipid content, lower fatty acid synthase expression, increased carnitine palmitoyltransferase 1A (CPT1A) expression, reduced MDA levels and increased activity of both SOD and glutathione peroxidase.⁹¹ Liver proteomics showed that 58 proteins whose expression had changed upon HFD-feeding were normalized after NaHS treatment.⁹² The biological processes in which these proteins appear to be involved are fat digestion and absorption, FA metabolism, glutathione metabolism, drug metabolism-cytochrome P450 and steroid hormone biosynthesis.⁹²

Aside H₂S donors, other strategies may be applied to modulate endogenous H₂S production. Wang et al.⁹³ have

shown that HFD-fed mice subjected to moderate-intensity exercise for 24 weeks presented enhanced plasma and hepatic H₂S levels as well as increased expression of CBS, CSE and MPST. These changes were accompanied by attenuated systemic IR and glucose intolerance, as well as mitigated hepatic steatosis and fibrosis.⁹³ Administration of diallyl disulphides (DADs) (garlic-derived organic polysulphide compounds acting as H₂S donors) to mice fed MCD diet or HFD for 4 or 20 weeks, respectively, ameliorated hepatic steatosis by downregulating mRNA levels of both SREBP-1c and apolipoprotein A1 and increasing mRNA levels of fibroblast growth factor 21.⁹⁴ In HFD-fed mice, DADs also prevented lipotoxicity by increasing PPAR α expression and inhibiting stearoyl-CoA desaturase-1 expression. As to the MCD-fed group, DADs markedly inhibited lipid peroxidation by decreasing MDA, tumour necrosis factor- α , interleukin-6 levels, suppressing nuclear factor kappa B activation and increasing SOD expression.⁹⁴

5.3 | Mitochondrial H₂S oxidation as a potential player in NAFLD development

The reported effects of H₂S greatly depend on its concentration, which is determined by the balance between the rates of H₂S biosynthesis and oxidation. Although the majority of studies focused on the regulation of H₂S levels through the modulation of its biosynthesis, mitochondrial H₂S oxidation is another pathway that regulates the levels of this gasotransmitter. Given that SQR is the key regulatory enzyme in the mitochondrial H₂S oxidation pathway,^{17,56} changes in SQR expression or activity may impact H₂S signalling properties. In HFD-fed rats, Baiges et al.⁹⁵ reported that mRNA levels of SQR and ETHE1 were decreased by more than 50% compared with rats fed chow diet. Recently, it has been reported that SQR inhibited butyrate oxidation in colonocytes.⁹⁶ With this ability, SQR can exert a direct impact on IR, given that butyrate and butyrate-based compounds are known to improve liver mitochondrial respiration, mitochondrial FA β -oxidation and activate the AMP-activated protein kinase/acetyl-CoA carboxylase pathway.⁹⁶ Interestingly, a link has also been established between TST and NAFL animal models. Mice injected with an adipocyte-specific TST adenovirus resisted to HFD-induced obesity and presented smaller fat cell size despite similar food intake as the controls. This TST overexpression in adipose tissue was associated with elevated hepatic CPT1A mRNA levels and increased mitochondrial FA β -oxidation.⁹⁷ However, whether or not TST could play a role in liver homeostasis still remains to be addressed. Regarding ETHE1 mutant patients and ETHE1 KO mice, abnormally high concentrations of H₂S were observed in the liver, which led to Complex IV inhibition

TABLE 2 Studies reporting the protective effects of H₂S in non-alcoholic fatty liver disease (NAFLD)

Species	Diet	Model	Treatment	Effect	Mechanisms	References
Mouse	HFD	NAFL	NaHS (56 µmol/kg/day)	Amelioration of HFD-induced NAFL	Activation of liver autophagy via the AMPK-mTOR pathway	92
Mouse	HFD	NAFL	NaHS (50 µmol/kg/day)	Amelioration of HFD-induced NAFL	Improvement of lipid metabolism and antioxidant potential	91
Mouse	HFD	NAFL	DADs (20, 50 and 100 mg/kg)	Amelioration of HFD-induced NAFL	Increased PPARα, inhibition of SCD1, SREBP-1c and ApoA-1	94
Mouse	HFD	NAFL	Physical exercise for 24 days	Amelioration of HFD-induced NAFL	Physical exercise upregulated CBS, CSE and MPST expression, which decreased MDA levels as well as TNF-α and IL-6 expression	93
Mouse	MCD	NASH	DADs (20, 50 and 100 mg/kg)	Amelioration of MCD-induced NASH	Inhibition of lipid peroxidation through the downregulation of MDA, TNF-α, IL-6 and NF-κB	94
Rat	MCD	NASH	NaHS (28 µmol/kg/day)	Amelioration of MCD-induced NASH	Possibly through abating oxidative stress and suppressing inflammation	84
Mouse	MCD	NASH	SPRC (40 mg/kg/day)	Amelioration of MCD-induced NASH	Antioxidative effect through the PI3K/Akt/Nrf2/HO-1 signalling pathway	45

Abbreviations: Akt, protein kinase B; AMPK, 5' AMP-activated protein kinase; ApoA-1, apolipoprotein A1; CBS, cysteine β-synthase; CSE, cysteine γ-lyase; DADs, diallyl disulphides; HFD, high-fat diet; HO-1, haeme oxygenase-1; IL-6, interleukin-6; MCD, methionine-choline-deficient diet; MDA, malonaldehyde; MPST, 3-mercaptopyruvate sulphurtransferase; mTOR, mechanistic target of rapamycin; NAFL, non-alcoholic fatty liver; NaHS, sodium hydrosulphide; NASH, non-alcoholic steatohepatitis; NF-κB, nuclear factor erythroid-derived 2-like 2; PI3K, phosphoinositide 3-kinase; PPARα, peroxisome proliferator-activated receptor alpha; SCD1, stearoyl-CoA desaturase-1; SPRC, s-propargyl-cysteine; SREBP-1c, sterol regulatory element-binding protein-1c; TNFα, tumour necrosis factor-alpha.

and disruption of mitochondrial respiration.⁹⁸ Additional studies have demonstrated that some of the metabolic inhibitory effects observed in ETHE1 KO mice could be linked to decreased mRNA levels of the E2 and E3 subunits of the branched-chain α -keto acid dehydrogenase complex (BCKDH). BCKDH is the flux-generating step in the oxidation of branched-chain amino acids (valine, leucine and isoleucine), which occurs in the mitochondria, and has some steps overlapping with mitochondrial FA β -oxidation.⁹⁹

Although H₂S production and oxidation are both important to maintain steady-state levels of H₂S, the way by which these pathways control H₂S actions in a cell may vary. H₂S production impacts on H₂S ability to modify cysteine residues and regulate protein expression/activity, whereas H₂S oxidation participates in mitochondrial respiration, controlling oxygen levels as well as the production of reactive sulphur species, which have lately gained recognition as powerful cell signalling mediators.¹⁰⁰ On the other hand, since mitochondrial H₂S oxidation relies on oxygen consumption, an upregulation of this pathway would significantly modulate oxygen tissue levels, which would impact oxygen sensing and ROS production.

6 | CONCLUDING REMARKS

In the literature, the evidence linking H₂S to the development of NAFLD has been piling up. Alterations in H₂S biosynthesis have been associated with the development of liver steatosis, fibrosis, cirrhosis, IR and diabetes. However, the discover that H₂S can also be used, especially in human liver mitochondria, as an inorganic energetic substrate through its oxidation by the mitochondrial SOU, unveils a specific and previously ignored bioenergetic mechanism with promising consequences on the signalling properties of this new gasotransmitter. Given that previous studies linking cell perturbations to dysregulations in H₂S metabolism were mainly focused on H₂S biosynthesis, it becomes now imperative to explore the physiological relevance of mitochondrial H₂S oxidation, especially in liver pathophysiology and metabolic diseases.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID

Carina Prip-Buus  <https://orcid.org/0000-0002-2153-7857>

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