REVIEW ARTICLE

Hydrogen sulfide and hepatic lipid metabolism – a critical pairing for liver health

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Hydrogen sulfide (H₂S) is the most recently recognized gasotransmitter, influencing a wide range of physiological processes. As a critical regulator of metabolism, H₂S has been suggested to be involved in the pathology of many diseases, particularly obesity, diabetes and cardiovascular disorders. Its involvement in liver health has been brought to light more recently, particularly through knockout animal models, which show severe hepatic lipid accumulation upon ablation of H₂S metabolic pathways. A complex relationship between H₂S and lipid metabolism in the liver is emerging, which has significant implications for liver disease establishment and/or progression, regardless of the disease-causing agent. In this review, we discuss the critical importance of H₂S in hepatic lipid metabolism. We then describe the animal models so far related with H₂S and lipid-associated liver disease, as well as H₂S-based treatments available. Finally, we highlight important considerations for future studies and identify areas in which much still remains to be determined.

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Abbreviations

3-MST, 3-mercaptopyruvate sulfurtransferase; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; HFD, high-fat diet; KO, knockout; MCD, methionine–choline deficient; MDA, malondialdehyde; NAFLD, non-alcoholic fatty liver disease; WT, wild-type

Introduction

Hydrogen sulfide (H₂S) is a colourless gas originally recognized for its foul smell and toxic effects. The relatively recent discovery of endogenous H₂S synthesis, however, and its critical role in numerous biological functions, has now created more of an appreciation for this gas (Zhao *et al.*, 2001; Yang *et al.*, 2008a,b; Bibli *et al.*, 2018). In fact, H₂S is the most recently recognized gasotransmitter, joining **NO** and **carbon monoxide** as an established regulators of a multitude of physiological processes (Wang, 2002 and 2012). In line with this, scientific interest in H₂S is rising at a surprising rate, with over 400 articles published with H₂S included in the title yearly since 2015. This is twice as many that were published in 2010 and four times that of those published in 2007 (Yang and Wu, 2017).

The functioning of almost every organ is influenced, at least to some degree, by H₂S (Gadalla and Snyder, 2010; Wang, 2012). Some of the first links between H₂S and the liver related to endotoxaemia and hepatic ischaemia-reperfusion injury, which both altered expression of H₂S-producing enzymes and, thus ultimately, the subject's outcome (Li *et al.*, 2005; Jha *et al.*, 2008). Potentially, due to the identification of a relationship between H₂S and (i) glucose metabolism (Wu *et al.*, 2009; Suzuki *et al.*, 2011), (ii) atherosclerosis (Mani *et al.*, 2013; Xie *et al.*, 2016) and (iii) obesity (Candela *et al.*, 2017), studies have now also begun investigating how lipid metabolism is influenced by this gasotransmitter. With the liver being a very active site for lipid accumulation, storage and depletion (Figure 1), the regulation of hepatic lipid metabolism by H₂S is of particular interest. Non-alcoholic fatty liver disease (NAFLD) is one of the most frequent liver diseases worldwide, as defined by over 5% of hepatocytes presenting lipid accumulation (Loomba and Sanyal, 2013). Currently, no approved pharmacotherapy is available. The therapy can be directed at weight loss plus pharmacological intervention towards insulin resistance or dyslipidaemia, using pioglitazone and the statins. At the very late stage of NAFLD, liver transplant is required. The research on NAFLD is still in its infancy.

Recently, knockout (KO) animals and liver disease models have begun to demonstrate the critical importance of endogenous H₂S to normal liver function and how its dysregulation may contribute to the pathophysiology of NAFLD and also many other chronic liver diseases (Norris *et al.*, 2011; Mani *et al.*, 2014).

In this review, we first look at hepatic H₂S production and its importance in hepatic lipid metabolism. The mechanisms identified that link H₂S and lipid metabolism are reviewed, as well as therapeutic avenues involving H₂S signals and hepatic lipid-associated diseases. Finally, future perspectives for this growing field are discussed, as well as key considerations for further experimental work in order to ensure translation of findings from bench top to bedside.

Hepatic H₂S production

H₂S is a key gasotransmitter involved in regulating a wide variety of cellular processes, and it is synthesized *via* both non-enzymic and enzymic reactions. Non-enzymic synthesis of H₂S involves reduction of elemental sulfur and has been documented in erythrocytes (Searcy and Lee, 1998). This synthetic route accounts for only a small proportion of endogenous H₂S, with the rest resulting from enzymic pathways (Hine *et al.*, 2017). The three main enzymes responsible for H₂S synthesis in mammalian cells are **cystathionine γ -lyase (CSE)**, **cystathionine β -synthase (CBS)** and **3-mercaptopyruvate sulfurtransferase (3-MST)** (Shibuya *et al.*, 2009; Ahmad *et al.*, 2016; Yang *et al.*, 2018). The reactions through which these enzymes synthesize H₂S are summarized in Figure 2. Importantly, there are significant tissue-specific differences in the expression of H₂S-producing enzymes. This highlights the fact that different tissues or systems rely on different enzymes for the bulk of their H₂S production.

In the liver, although all three H₂S-producing enzymes are detectable, their contributions to endogenous H₂S production are different (Kabil *et al.*, 2011; Manna *et al.*, 2014; Ahmad *et al.*, 2016). CSE is approximately 60-fold more

Figure 1

Hepatic lipid metabolism. Simplified figure demonstrating key aspects of hepatic lipid metabolism. Solid purple line indicates hepatic lipid accumulation, blue dotted line indicates hepatic lipid storage and dashed green line indicates hepatic lipid depletion.

Figure 2

Enzymic synthesis of endogenous H₂S. Simplified diagram representing selected portions of the trans-sulfuration and desulfuration pathways, which contribute to enzymic synthesis of H₂S. Enzyme names are indicated on arrows. CAT: cysteine aminotransferase.

abundant than CBS, at least in murine liver tissues (Kabil *et al.*, 2011). Genetic deletion of CSE prevents more than 90% of H₂S production in liver, suggesting that CSE acts as the main H₂S-producing enzyme in hepatic tissues (Fiorucci *et al.*, 2005; Mustafa *et al.*, 2009; Mani *et al.*, 2015). Interestingly, inhibition of 3-MST significantly enhances rather than decreases H₂S production, pointing to the marginal role of 3-MST in endogenous H₂S production (Li *et al.*, 2017). Adding another level of complexity is the finding that expression of H₂S-producing enzymes shows important cell-type heterogeneity in liver tissues. For instance, hepatocytes, which constitute the parenchyma of the liver and approximately 80% of its volume, express both CBS and CSE (Ratnam *et al.*, 2002; Siebert *et al.*, 2008; Zhang *et al.*, 2013), with a particular subset expressing 3-MST, at least in rats (Nagahara *et al.*, 1998).

Table 1

Expression of H₂S-generating enzymes in hepatic non-parenchymal cells

Hepatic cell type	H ₂ S-producing enzyme expressed?		Organism and reference
Kupffer cells	N/A		–
Sinusoidal endothelial cells	CSE	No	Rat (Fiorucci <i>et al.</i> , 2005)
	CBS	No	Rat (Fiorucci <i>et al.</i> , 2005)
	3-MST	N/A	–
Hepatic stellate cells	CSE	Yes	Rat (Fiorucci <i>et al.</i> , 2005)
		Yes	Human cell line (Liu <i>et al.</i> , 2013)
	CBS	No	Rat (Fiorucci <i>et al.</i> , 2005)
	3-MST	N/A	–

N/A, not available.

The three main non-parenchymal liver cell types, however, show variation in which enzymes they express. Table 1 highlights that much remains unknown regarding hepatic cell-type specific regulation of CSE, CBS and 3-MST expression. As different cell types are responsible for different hepatic functions, determining how H₂S production by each cell type, rather than only the organ as whole, influences liver function may provide more accurate data regarding the relationship between H₂S and liver function.

The studies cited above have demonstrated that the liver abundantly expresses H₂S-producing enzymes. For this reason, additional studies have aimed to determine how modulating hepatic H₂S levels influences liver function. Because of the increase in liver steatosis, including more advanced stages of this disease, a particular focus on H₂S and the regulation of hepatic lipid metabolism has emerged (Abd El-Kader and El-Den Ashmawy, 2015). Multiple *in vivo* and *in vitro* models of liver disease are currently available and suitable to study hepatic lipid metabolism. As such, the mechanisms that link H₂S, hepatic lipid metabolism and liver disease are slowly beginning to be elucidated.

Regulation of hepatic lipid metabolism by H₂S – *in vivo* models

The liver is a key metabolic organ, coordinating the metabolism of carbohydrates, proteins and fats. Although multiple other tissues also contribute to various aspects of fat metabolism, the liver is responsible for the bulk of this activity in an organism, including the accumulation, storage and depletion of lipid (Figure 1). Many *in vivo* models are applicable to the study of the regulation of hepatic lipid metabolism by H₂S. Most of these models involve inducing liver stress, through altered dietary content or exposure to specific chemicals, for example. The resulting shift in H₂S-producing enzymes and lipid metabolism, as well as the phenotypic rescue upon restoration of H₂S levels, provide important evidence of the clinical relevance of this gasotransmitter to liver lipid homeostasis. In mammals, other organs apart from the liver are involved in lipid metabolism, including adipose tissue, muscles and components of the digestive tract. In addition, H₂S is produced from almost all tissues and can act locally,

or throughout the body (Ahmad *et al.*, 2016; Yang *et al.*, 2018). As such, *in vivo* models provide important considerations for H₂S and lipid metabolism studies that *in vitro* systems can simply not mimic and will thus be the focus here. One of the most powerful demonstrations of the importance of H₂S-producing enzymes to hepatic lipid metabolism, and even whole-body homeostasis, is provided by the knock-out models, CSE-KO, CBS-KO and 3-MST-KO, in mice, as discussed below (Table 2).

The KO models of H₂S-producing enzymes

Even before the development of KO animal models of H₂S-producing enzymes, pathogenic mutations in CBS and CSE in humans revealed how critical the expression of these enzymes is to hepatic lipid metabolism and even whole-body health. This is most clearly shown by pathogenic CBS mutations, which leads to homocystinuria in the affected individual and severe downstream consequences, including mental retardation and tissue disorders (Mudd *et al.*, 1964). In animal models of CBS-KO, the effects are also striking, with the genotype being semi-lethal and animals developing liver steatosis, significant oxidative damage and fibrosis (Watanabe *et al.*, 1995; Robert *et al.*, 2005). As CBS-KO mice present with such severely compromised liver function, additional experimental manipulations proved difficult. To overcome this obstacle, a specific mouse model was developed, in which a copy of the human CBS gene, with its endogenous human promoter, was inserted into the genome of mice lacking functional CBS; this strain was designated HO. These HO mice consequently have very little CBS expression but significantly higher **cystathionine** levels, approximately fourfold higher than CBS-KO mice (Maclean *et al.*, 2012). Interestingly, although the difference in CBS expression between CBS-KO and HO mice is quite minimal, HO mice have very little liver damage – without signs of lipid accumulation and oxidative damage (Maclean *et al.*, 2010, 2012).

CSE. In terms of CSE-KO, the phenotyping effects are much less obvious than with the CBS KO mice. Thus, CSE-KO mouse models fed with a normal diet show no noticeable differences compared with their wild-type (WT) counterparts, particularly in terms of liver health (Mani *et al.*, 2015). Altered gluconeogenesis, however, was observed (Untereiner *et al.*, 2016). In addition, although restriction of dietary **cysteine** leads to severe mortality in these CSE-KO mice, there were no identified hepatic changes, in terms of both liver structure and functionality (Mani *et al.*, 2011). Only when given a high-fat diet (HFD), hepatic lipid accumulation is exacerbated in CSE-KO mice (Mani *et al.*, 2015). Specifically, CSE-KO mice, compared with WT control, fed a HFD showed (i) a significant increase in hepatic total lipids, cholesterol and HDL cholesterol, (ii) double the normal liver-to-body weight ratios, (iii) lower hepatic apolipoproteins E and LDL-receptor levels and (iv) increased plasma aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (Mani *et al.*, 2015). In addition, increased levels of cholesterol and LDL were observed in the plasma of CSE-KO mice fed a HFD, compared with WT mice (Mani *et al.*, 2015). Importantly, this effect was reversed by introduction of exogenous H₂S, confirming that plasma lipid-level alterations were caused

by changes in CSE-derived H₂S. Of note, young CSE-KO mice fed a normal diet showed no visible liver damage (Mani *et al.*, 2011 and 2015). Taken together, these results thus suggest that CSE expression is key for adapting hepatic lipid metabolism to alterations in dietary fat intake and retaining homeostasis.

3-MST. As with CSE-KO mice, 3-MST-deficient mice show no difference in growth or pathological features when fed a normal diet, although some changes in increased anxiety-like behaviours have been described (Nagahara *et al.*, 2013). However, a recent study by Li *et al.* (2017) demonstrated that heterozygous 3-MST-KO mice displayed improved hepatic steatosis in mice on a HFD. The partial deletion of 3-MST markedly ameliorated the fatty liver phenotype with reduced hepatic contents of free fatty acids, triglycerides and cholesterol as well as lower plasma alanine transaminase and aspartate transaminase levels. These results indicate that 3-MST is an important factor that facilitates the development of NAFLD. This difference between 3-MST and CSE or CBS, in terms of NAFLD protection may be due to the unique catalytic activity of 3-MST. 3-MST reacts with 3-mercaptopyruvate to form persulfide-containing intermediates, which are labile and can transfer to many other groups leading to liver damage (Nagahara *et al.*, 2018). Furthermore, hepatic 3-MST up-regulates CSE enzymic activity *via* direct interaction (Li *et al.*, 2017). Further studies aimed at determining the importance of 3-MST to liver function are clearly warranted.

Taken together, the KO animal models described demonstrate that H₂S metabolism is critical for balanced hepatic lipid metabolism, particularly the CBS system. Additional liver damage models have been used to complement these KO studies and determine how H₂S production is influenced by hepatic damage. As such, modifications to the H₂S system may suggest a role for it in the pathophysiology of the associated liver disease.

Liver damage models

Many *in vivo* models of liver damage exist. Although some of these models have been studied in terms of their influence on liver H₂S metabolism, how exactly H₂S metabolism contributes to hepatic lipid metabolism and these liver pathologies remains largely to be elucidated.

Methionine–choline-deficient diet-induced non-alcoholic steatohepatitis. This model is a diet-induced model for non-alcoholic steatohepatitis. As indicated in its name, the **methionine–choline**-deficient (MCD) diet contains reduced amounts of methionine and choline. The diet of control animals contains approximately 3 g·kg^{−1} of DL-methionine and 2 g·kg^{−1} of choline bitartrate, whereas the levels of these compounds are significantly reduced in the diet of the MCD animals (Teramoto *et al.*, 1993; Luo *et al.*, 2014). Following several weeks on this diet, animals will begin to present with hepatic lipid accumulation. An important aspect of the pathophysiology of this model is that it hinders synthesis of VLDL, which in turn reduces triglyceride and fatty acid export from hepatocytes and contributes to the observed hepatic lipid accumulation and consequent steatohepatitis. Of note, additional

Table 2Molecular mechanisms identified to date through which H₂S pathways influence hepatic lipid metabolism

Gene or signalling molecule and expression	Additional experimental parameters	Mechanisms identified and reference	Phenotype
CBS	KD (HO) Normal diet, MCD	N/A	Attenuated liver lipid accumulation and steatosis
KO	Normal diet, HFD, MCD	N/A	Severe liver lipid accumulation and steatosis
WT	Homocysteine-induced ER stress	<p>Activation of NF-κB, SREBP2, CREB $\rightarrow$ $\uparrow$ HMG-CoA reductase expression $\rightarrow$ $\uparrow$ cholesterol biosynthesis (Woo <i>et al.</i> 2005)</p> <p>$\downarrow$ phosphorylation of HMG-CoA reductase (inactive form) $\rightarrow$ $\uparrow$ cholesterol biosynthesis (Wu <i>et al.</i> 2011)</p> <p>$\uparrow$ LDL-mediated cholesterol uptake $\rightarrow$ $\uparrow$ hepatic cholesterol (Werstuck <i>et al.</i> 2001)</p> <p>Uncoupled protein response activation $\rightarrow$ $\uparrow$ SREBP-dependent genes (Werstuck <i>et al.</i> 2001)</p> <p>$\rightarrow$ eIF2α-ATF4 $\rightarrow$ $\uparrow$ CSE $\rightarrow$ $\uparrow$ Glutathione (Dickout <i>et al.</i> 2012)</p>	Severe liver lipid accumulation and steatosis
CSE	KO Normal diet HFD	N/A $\downarrow$ ApoE, LDL-R, $\downarrow$ PPAR α , PPAR γ , LXRA, CYP7A1 $\uparrow$ ABCG8	Healthy liver Liver lipid accumulation (increased total lipids, cholesterol, HDL-cholesterol) and steatosis
3-MST	KD	$\uparrow$ CSE activity $\downarrow$ SREBP-1, FAS and ACC (Li <i>et al.</i> 2017)	Reduced liver lipid accumulation
H ₂ S	$\uparrow$ MCD	$\downarrow$ CD36, SREBP-1c, FAS, TLR2, TLR4 $\uparrow$ PPAR γ , L-FABP (Luo <i>et al.</i> 2014)	Decreased liver triglyceride and cholesterol levels, with smaller liver-to-body weight ratio

ER, endoplasmic reticulum; N/A, not available.

consequences of the MCD diet include overall weight loss and decreased serum cholesterol and triglycerides. Liver weight, however, as demonstrated by increased liver-to-body weight ratios, is increased, as well as hepatic cholesterol and triglyceride content (Luo *et al.*, 2014). In fact, six-fold higher hepatic triglyceride levels were observed in MCD diet-fed animals (Maclean *et al.*, 2012). Malondialdehyde (MDA) levels are also higher in the livers of animals on a MCD diet, indicating oxidative stress (Maclean *et al.*, 2012), and increased endoplasmic reticulum stress has also been demonstrated (Rahman *et al.*, 2007).

Interestingly, hepatic CBS and CSE expressions at both the mRNA and protein levels as well as H₂S levels in both plasma and liver, were reduced in MCD-fed rats (Luo *et al.*, 2014). **Sodium hydrosulfide (NaHS)** treatment of MCD-fed animals reversed the toxic hepatic outcomes of the MCD diet, including (i) a reduction of the ratio of liver-to-body weight ratio and (ii) a decrease in liver triglyceride and cholesterol levels, without influencing plasma levels of these factors. These results suggest that the CSE and CBS down-regulation following a MCD diet results in reduced H₂S production, and thus, restoration of the levels of this gasotransmitter reverses the damaging hepatic effects of the MCD diet. Of note, CSE expression was even further reduced following NaHS treatment, suggesting a potential negative feedback mechanism (Luo *et al.*, 2014).

It is important to note that the HO mice described above, in which only human CBS is expressed at low levels, showed less liver injury, lipid accumulation and cell death following a MCD diet, compared with their WT counterparts (Maclean *et al.*, 2012). The authors suggested that this is to do the accumulation of cystathione in the HO model, which would attenuate the effects of the MCD diet (Figure 2). Deficiencies in CBS and CSE may thus result in different outcomes for the liver due to the metabolic intermediates synthesized.

High-fat-diet-induced fatty liver. The liver is an important site for lipid metabolism and so it is particularly susceptible to the effects of a HFD. As such, when animals are fed with a diet high in fat (approximately 60% of their daily caloric intake deriving from fat), they will develop a fatty liver within as little as 5 weeks (Hwang *et al.*, 2013). The expressions of H₂S-generating enzymes have been widely found to be altered upon introduction of an HFD (Table 3). Bravo *et al.* (2011), using male Wistar rats and a 35% fat diet, found that following 18 weeks of this diet, rats had decreased hepatic CBS and CSE expressions and enzymic activity. In contrast, Hwang *et al.* (2013) using C57BL/6 mice and a 60% kcal fat diet showed that following 5 weeks of this HFD, CBS and CSE mRNA, protein and enzymic activity was increased. They also observed increased MDA, lipid peroxides and oxidative stress at this time point. Peh *et al.* (2014), using C57BL/6 mice and a 33.5% HFD (16% fat, 12.5% cholesterol and 5% sodium cholic acid), determined that following 16 weeks of this HFD, hepatic CSE and 3-MST expressions as well as H₂S production were reduced, while hepatic CBS expression was increased. By feeding C57BL/6 mice with a 60% kcal fat diet for 8 weeks, Li *et al.* (2017) showed that 3-MST expression was increased

in liver and inhibition of 3-MST *via* either administration of adenovirus-mediated shRNA or heterozygous deletion of the 3-MST gene markedly attenuated HFD-induced hepatic steatosis, indicating the detrimental role of 3-MST in hepatic lipid accumulation. Interestingly, 3-MST knock-down is associated with enhanced H₂S production *via* up-regulation of CSE, implying a negative feedback between 3-MST and CSE.

As each study used different HFDs, animal models and time points, comparison of results is difficult. Although, taken together, they show that CSE and CBS expression is altered in mice on a HFD, and correlate expression with liver damage, more studies are required to fully understand the relationship between these factors and if it is, indeed, a causal relationship. In addition, as CBS and CSE expression seems to change over time in the HFD model, further studies must consider this variation in their work and realize that administration of exogenous H₂S, or alterations in H₂S-producing enzyme expression levels, may have different effects depending on the timing of such experimental manipulations over the duration of exposure to a HFD. Finally, although often overlooked, most likely due to its relatively low contribution to endogenous H₂S generation in liver tissue, consideration of 3-MST expression in such studies has now also been shown to be a potentially important factor. Further work in this area could build upon recent findings showing that when C57BL/6 mice with HFD-induced liver steatosis were treated with exogenous NaHS, they recovered their liver structure and showed decreased hepatic lipid accumulation, as well as decreased **fatty acid synthase** (FAS) and MDA levels (Wu *et al.*, 2015).

Hyperhomocysteinaemia diet. It is important to note that as CBS is the enzyme responsible for converting **homocysteine** to cystathionine, hyperhomocysteinaemia can be a clinical manifestation of CBS deficiency, and as such, this model is also considered to mimic certain aspects of CBS deficiency (Werstuck *et al.*, 2001). For instance, in rats, increased cholesterol biosynthesis and lipid accumulation was observed under a hyperhomocysteinaemia diet (Woo *et al.*, 2005). In addition, mice subjected to diet-induced hyperhomocysteinaemia demonstrated increased hepatic cholesterol and triglyceride levels (Werstuck *et al.*, 2001), which is consistent with the increased hepatic lipid in CBS-KO mice (Watanabe *et al.*, 1995; Robert *et al.*, 2005). It is important to note, however, that in this model, as CBS levels are not directly influenced, only its substrate levels are maximal, the authors can only conclude that homocysteine levels are responsible for the observed effect. Particularly in this study by Werstuck *et al.* and also in many other similar studies, H₂S levels were not assessed in any experimental conditions, and thus, the influence of this variable on liver health cannot be measured. In future studies examining the effects of CSE, CBS and 3-MST activity, the levels of their various metabolites, including H₂S, should be included. Likewise, it would be useful to include restoration of endogenous H₂S (through supplementation with an exogenous H₂S donor), to ensure any observed effects are indeed due the production of this gasotransmitter. This is particularly important since, as described above, other metabolites in the transsulfuration

pathway can have important effects (Figure 2). By including an examination of all these aspects of this particular pathway, we will be able to better delineate the importance of each enzyme, substrate or product and thus devise more effectively targeted treatment to prevent liver lipid accumulation and prevent liver damage.

In summary, *in vivo* models of hepatic disease, whether the disease is initiated by dietary, genetic or chemical methods, all demonstrate the possible interplay between altered H₂S signals and hepatic lipid metabolism. Additional models of liver disease have also been associated with altered expression of H₂S-generating enzymes. However, a potential link with altered hepatic lipid metabolism remains to be fully investigated. Such models include (i) chemical liver damage by CCl₄ and **LPS**, which increased liver damage in CSE-KO mice and decreased hepatic CSE expression (Ci *et al.*, 2017); (ii) liver cirrhosis induced by chronic CCl₄ or bile duct ligation, which decreased CSE and H₂S levels (Fiorucci *et al.*, 2005; Distrutti *et al.*, 2007); (iii) concanavalin A-induced acute hepatitis, where administration of H₂S influenced cell survival (Cheng *et al.*, 2014); and (iv) sepsis and ischaemic-reperfusion models, where, as described above, the H₂S pathway has been demonstrated as an important mediator of disease progression (Elrod *et al.*, 2007; Ahmad *et al.*, 2016). Much thus remains to be determined in terms of how alterations in H₂S signals and consequently hepatic lipid metabolism may contribute to the development of liver diseases. In addition, through the use of these *in vivo* models, as well as the manipulations possible with *in vitro* liver models, the molecular mechanisms that link H₂S, lipid metabolism and liver health are starting to be elucidated. Below, we gather together what has been established in this regard, highlighting key areas on which future projects could focus.

The intracellular mechanisms underlying the interactions between H₂S and lipid metabolism

It is established that CSE-KO and CBS-KO result in severe liver lipid accumulation. Relatively few studies, however, have provided mechanistic evidence regarding the mechanisms through which these enzymes and one of their products, H₂S, may influence hepatic lipid metabolism and thus liver health. Together, both *in vivo* and *in vitro* studies have started to examine the series of molecular events, which coordinate the phenotypic response observed and are detailed below.

CBS-KO models demonstrate how critical CBS expression is to normal hepatic lipid metabolism as summarised in Table 2. Briefly, increased homocysteine due to CBS deficiency influences many cellular processes related to lipid metabolism, including (i) the unfolded protein response, (ii) increased expression and activity of HMG-CoA reductase and consequently increases cholesterol biosynthesis (Woo *et al.*, 2005) and (iii) increased cholesterol import *via* LDL receptors (Werstuck *et al.*, 2001). Although these studies have provided some mechanistic insight as to how CBS deficiencies influences cellular processes, as they do not include modification of endogenous CBS levels, or a

measure/modification of H₂S produced, it is difficult to assess how the various consequences of excess homocysteine accumulation each contribute to the dysregulated liver lipid metabolism observed.

In contrast, CSE appears to be particularly critical in regulating hepatic lipid metabolism when there are excess lipids consumed through diet. The exact mechanism involved here remains to be determined, but one study identified several gene expression differences between CSE-KO and WT mice given a HFD (Mani *et al.*, 2015), which provide some insight as to the pathways involved (Table 2). HFD-fed CSE-KO mice have lower expression of liver LDL-R, apoE and **CYP7A1**, which are responsible for the decreased clearance of LDL from circulation and increased plasma LDL levels as well as decreased bile acid synthesis in the liver. **LXR α** , a steroid responsive transcription factor for the regulation of LDL-R, apoE and CYP7A1, was also decreased in the liver from HFD-fed CSE-KO mice, suggesting a potential link between CSE and LXR α on the regulation of lipid metabolism.

Apart from the studies stated above in which the expression of H₂S-generating enzymes were modified, few studies have directly investigated how altered hepatic (or systemic) H₂S levels can influence liver lipid metabolism. Under a MCD diet, which usually results in decreased CSE and CBS expressions, administration of exogenous H₂S reduced hepatic lipid accumulation and liver damage (Luo *et al.*, 2014). Treatment of MCD-fed rats with H₂S increased hepatic mRNA levels of **PPAR α** and the liver-type fatty-acid-binding protein (L-FABP) and reduced hepatic mRNA levels of CD36, the sterol regulatory element-binding protein-1c (SREBP-1c), **Fas**, **TLR-2** and **TLR-4** when compared with MCD-fed rats (Table 2) (Luo *et al.*, 2014). PPAR α , L-FABP and SREBP-1c are critical components of the modulation of lipid transport and metabolism. CD36, Fas, TLR-2 and TLR-4 are key players in inflammation development. These data indicate that H₂S would prevent MCD-induced non-alcoholic steatohepatitis in rats, possibly through improved lipid metabolism and inflammation. In another study, Wu *et al.* (2015) demonstrated that H₂S donor NaHS lowered the HFD-induced content of triglycerides and cholesterol in hepatocytes from mice, possibly by enhancing the activities of antioxidant enzymes, suggesting H₂S could mitigate the fatty liver by improving antioxidant potential. Consistent with this study, Chen *et al.* (2016) found that H₂S mediates the protective role of diallyl trisulfide against ethanol-induced oxidative stress and fatty liver in rats. The direct regulatory roles of H₂S on lipid metabolism, including lipid accumulation, storage and depletion, are still to be defined. Further studies need to look at the effects of H₂S on expression and activity of proteins related with lipogenesis, lipolysis and lipoprotein secretion.

Taken together, these results from these studies demonstrate the involvement of CBS, CSE, 3-MST and H₂S in hepatic lipid metabolism. Many additional studies, however, are required to determine exactly how these factors influence liver lipid metabolism and how much of the effect of these enzymes are due to their changes in H₂S levels. In addition, the beneficial effects of H₂S on regulating hepatic metabolism have yet to be fully explored for their potential therapeutic effects.

Table 3Changes of hepatic H₂S-generating enzymes and the phenotype in HFD-fed animals

Animal species	Diet type	Feeding period	Altered H ₂ S signal	Phenotype	Reference
Male Wistar rats	35% kcal fat diet	18 weeks	Decreased hepatic CBS and CSE expression and enzymic activity	Fatty liver due to increased hepatic cholesterol and triglyceride levels	Bravo <i>et al.</i> (2011)
C57BL/6 mice	60% kcal fat diet	5 weeks	Increased mRNA and protein expression CBS and CSE as well as H ₂ S production in liver tissue	Fatty liver due to increased hepatic cholesterol and triglyceride levels	Hwang <i>et al.</i> (2013)
C57BL/6 mice	33.5% kcal fat diet	16 weeks	Decreased expression of CSE and 3-MST but increased CBS expression, no change plasma H ₂ S	N/A	Peh <i>et al.</i> (2014)
C57BL/6 mice	60% kcal fat diet	8 weeks	Decreased 3-MST expression	Fatty liver due to increased hepatic cholesterol and triglyceride levels	Li <i>et al.</i> (2017)

Targeting the H₂S pathway in the prevention and treatment of liver disease

The H₂S pathway influences numerous processes throughout the body, and H₂S has thus begun to be considered for the treatment of various disorders, particularly those affecting the cardiovascular, gastrointestinal and respiratory systems and CNS (Wei *et al.*, 2017; Das *et al.*, 2018). Traditionally, two different approaches for modulating H₂S levels have been used: enhancing H₂S levels, through increased production or bioavailability for instance, and decreasing H₂S levels, through inhibition of the enzymes responsible for H₂S synthesis, for example (Li *et al.*, 2008; Caliendo *et al.*, 2010). In addition, an ongoing clinical trial is determining if there are benefits to dietary-induced up-regulation of H₂S in patients before carotid endarterectomy (ClinicalTrials.gov Identifier: NCT03303534). Another study is examining the efficacy of SG1002, an H₂S prodrug, to reverse the decrease in patient H₂S levels following heart failure (Polhemus *et al.*, 2015) (ClinicalTrials.gov Identifier: NCT01989208). The H₂S pathway has also been targeted in an attempt to reduce the side effects of other drugs. For instance, H₂S donor groups have been added to the structure of established non-steroidal anti-inflammatory drugs in order to decrease their toxic effects, particularly in terms of gastrointestinal and cardiovascular side effects, as in ATB-337 (Caliendo *et al.*, 2010).

The link between H₂S and its regulation of hepatic lipid metabolism, however, is only beginning to be explored. The *in vitro* and *in vivo* works described above strongly suggest that H₂S pathway may be an effective target in the prevention and treatment of liver disease. There is, however, no clinical evidence to date regarding intentionally modulating H₂S pathways to treat liver disease in humans. Interestingly, some compounds used to treat various liver diseases have been shown to also modulate levels H₂S and/or expression of H₂S-producing enzymes, providing an important foundation

on which further work can build. One example of this is **metformin**. This insulin-sensitizing drug is used to treat Type 2 diabetes and is known to reverse fatty liver and steatosis in mice (Lin *et al.*, 2000). Interestingly, metformin treatment of mice also increased H₂S levels, with marked accumulation in the liver following specific dose regimens (Wiliński *et al.*, 2013). Likewise, **statins**, which work to lower lipid levels and prevent cardiovascular disease through inhibiting **HMG-CoA reductase**, have also been suggested to reduce steatosis and treat NAFLD (Ekstedt *et al.*, 2007; Pastori *et al.*, 2015). Like metformin, statins have also been shown to increase H₂S levels in the liver of rats (Wójcicka *et al.*, 2011). *n*-3 polyunsaturated fatty acid diets have demonstrated protective effects on the liver, particularly reducing hepatic lipid levels (Levy *et al.*, 2004). Interestingly, dietary inclusion of various types of polyunsaturated fats in rats, including **docosahexaenoic acid** and conjugated linoleic acid, has been shown to be associated with generally increased hepatic CBS expression, whereas that of CSE was not significantly modified (Huang *et al.*, 2013). In an *in vitro* model, however, involving human hepatoma HepG2 cells, treatment with docosahexaenoic acid and **eicosapentaenoic acid** correlated with a significant increase in CSE expression but unaltered levels of 3-MST and CBS (Huang *et al.*, 2012). These results suggest that the protective effects of *n*-3 polyunsaturated fatty acid diet against liver lipid accumulation are at least partially mediated by CBS and/or CSE activity.

In addition, compounds naturally found in various foods influence both liver lipid metabolism and H₂S production. Notably, garlic oil attenuated ethanol-induced hepatic steatosis in both mice and human cell line models (Zeng *et al.*, 2012) and increased intracellular H₂S levels in HEK cells (when cysteine or GSH was present) (DeLeon *et al.*, 2016). **Sulforaphane**, which is present in cruciferous vegetables, particularly young sprouts of broccoli, decreased hepatic lipid levels and accumulation in animal models of both NAFLD and alcohol-induced fatty liver (Zhou *et al.*, 2014; Yang *et al.*, 2016) and also increases H₂S levels (Pei *et al.*, 2011).

How critical the modulation of H₂S levels is to the therapeutic effects of these compounds, however, remains to be determined, in addition to any side effects that may be induced at the concentrations required to see their beneficial effects on human livers.

Additionally, a link between other treatments for liver disease and H₂S remains to be investigated. For instance, both aerobic and anaerobic exercise is a first-line therapy for NAFLD, but how this activity may affect endogenous H₂S production remains to be determined (Hashida *et al.*, 2017). It is known, however, that H₂S regulates mitochondrial metabolism, working to increase ATP production during periods of stress, providing some foundation for this suggested association (Fu *et al.*, 2012). Additionally, various diets have been suggested to modulate liver disease outcomes. Although a link between these diets in humans and endogenous H₂S levels remains to be shown, H₂S is known to be required to observe the beneficial effects of various diet restriction regimens (Hine *et al.*, 2015) and that its endogenous levels are modulated in many mouse models of diet-induced disease, as elaborated above. Taken together, the experimental findings described strongly support the pursuit of H₂S-based therapies for liver disease treatment. It is relevant to note that H₂S increases adipocyte numbers but attenuates insulin resistance in mice given a HFD, suggesting H₂S may play a dual effect in fat tissues and liver (Cai *et al.*, 2016; Yang *et al.*, 2018). On one hand, H₂S can stimulate more storage of free fatty acid in fat tissues and, on the other, H₂S induces more lipolysis in liver and attenuates liver lipid accumulation. A more thorough understanding of the molecular mechanisms through which the H₂S metabolic pathway regulates hepatic lipid levels, as well as the functioning of other organs, will be key for the development of effective therapeutic options. A full assessment of dose, mode of administration and off-target effects is also essential.

Future perspectives

In this review, we have highlighted the importance of regulated hepatic lipid metabolism to liver health and the involvement of the H₂S pathway in the regulation of such processes and have described animal models in which these associations have been demonstrated. We have also highlighted key areas in which further studies are warranted. Overall, CBS has been shown to be a necessary protector of hepatic health, as down-regulation has particularly negative effects on liver lipid levels and liver disease development. Similarly, CSE knock-down results in hepatic lipid accumulation, but only under certain stressors, such as a HFD. The mechanisms through which this is regulated remained to be determined. 3-MST has largely been ignored in past studies, most likely due to its relatively low expression in liver. A recent finding has shown that 3-MST expression is in fact modified during a HFD and can regulate CSE activity (Li *et al.*, 2017), so the possible involvement of 3-MST in liver lipid metabolism needs to be thoroughly explored. In terms of H₂S, specifically, administration of this compound improves hepatic steatosis but more studies are required to determine how much of the effect of CSE and CBS are the result of

modulation of H₂S levels, and no other pathway metabolites, for example.

Another aspect that has received little consideration to date is in regard to specific ways in which lipids accumulate in the liver under various experimental conditions. To date, liver lipid accumulation, particularly in terms of regulation by the H₂S pathway, has been largely considered as a general phenomenon. Lipids can accumulate, however, in different ways in the liver. For instance, there are at least three types of lipid droplets that can form in the liver, with each having varied functions, downstream effects and thus potential clinical implications (Wang *et al.*, 2013; Gluchowski *et al.*, 2017). Evaluating this characteristic of hepatic lipid accumulation, as well as a more in-depth study of the molecular mechanisms that govern them, would undoubtedly advance our understanding of this process. In conclusion, a complex relationship between H₂S and hepatic lipid metabolism in relation to liver function is beginning to be unravelled. Although the molecular mechanisms that link these processes have yet to be fully determined, their critical importance to liver health suggests important clinical implications for future studies.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c,d).

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Conflict of interest

The authors declare no conflicts of interest.

References

- Ahmad A, Gerö D, Olah G, Szabo C (2016). Effect of endotoxemia in mice genetically deficient in cystathionine- γ -lyase, cystathionine- β -synthase or 3-mercaptopyruvate sulfurtransferase. *Int J Mol Med* 38: 1683–1692.
- Abd El-Kader SM, El-Den Ashmawy EM (2015). Non-alcoholic fatty liver disease: the diagnosis and management. *World J Hepatol* 7: 846–858.

Alexander SPH, Fabbro D, Kelly E, Marrion NV, Peters JA, Faccenda E *et al.* (2017a). The Concise Guide to PHARMACOLOGY 2017/18: Enzymes. *Br J Pharmacol* 174: S272–S359.

Alexander SPH, Kelly E, Marrion NV, Peters JA, Faccenda E, Harding SD *et al.* (2017b). The Concise Guide to PHARMACOLOGY 2017/18: Other proteins. *Br J Pharmacol* 174: S1–S16.

Alexander SPH, Cidowski JA, Kelly E, Marrion NV, Peters JA, Faccenda E *et al.* (2017c). The Concise Guide to PHARMACOLOGY 2017/18: Nuclear hormone receptors. *Br J Pharmacol* 174: S208–S224.

Alexander SPH, Fabbro D, Kelly E, Marrion NV, Peters JA, Faccenda E *et al.* (2017d). The Concise Guide to PHARMACOLOGY 2017/18: Catalytic receptors. *Br J Pharmacol* 174: S225–S271.

Bibli SI, Hu J, Sigala F, Wittig I, Heidler J, Zukunft S *et al.* (2018). Cystathionine γ lyase sulphydrates the RNA binding protein HuR to preserve endothelial cell function and delay atherogenesis. *Circulation* [Epub ahead of print].

Bravo E, Palleschi S, Aspichueta P, Buqué X, Rossi B, Cano A *et al.* (2011). High fat diet-induced non alcoholic fatty liver disease in rats is associated with hyperhomocysteinemia caused by down regulation of the transsulphuration pathway. *Lipids Health Dis* 10: 60.

Cai J, Shi X, Wang H, Fan J, Feng Y, Lin X *et al.* (2016). Cystathionine γ lyase–hydrogen sulfide increases peroxisome proliferator-activated receptor γ activity by sulphydration at C139 site thereby promoting glucose uptake and lipid storage in adipocytes. *Biochim Biophys Acta* 1861: 419–429.

Caliendo G, Cirino G, Santagada V, Wallace JL (2010). Synthesis and biological effects of hydrogen sulfide (H_2S): development of H_2S -releasing drugs as pharmaceuticals. *J Med Chem* 53: 6275–6286.

Candela J, Wang R, White C (2017). Microvascular endothelial dysfunction in obesity is driven by macrophage-dependent hydrogen sulfide depletion. *Arterioscler Thromb Vasc Biol* 37: 889–899.

Chen LY, Chen Q, Zhu XJ, Kong DS, Wu L, Shao JJ *et al.* (2016). Diallyl trisulfide protects against ethanol-induced oxidative stress and apoptosis via a hydrogen sulfide-mediated mechanism. *Int Immunopharmacol* 36: 23–30.

Cheng P, Chen K, Xia Y, Dai W, Wang F, Shen M *et al.* (2014). Hydrogen sulfide, a potential novel drug, attenuates concanavalin A-induced hepatitis. *Drug Des Devel Ther* 8: 1277–1286.

Ci L, Yang X, Gu X, Li Q, Guo Y, Zhou Z *et al.* (2017). Cystathionine γ -lyase deficiency exacerbates CCl_4 -induced acute hepatitis and fibrosis in the mouse liver. *Antioxid Redox Signal* 27: 133–149.

Das A, Huang GX, Bonkowski MS, Longchamp A, Li C, Schultz MB *et al.* (2018). Impairment of an endothelial NAD^+ - H_2S signaling network is a reversible cause of vascular aging. *Cell* 173: 74–89.

DeLeon ER, Gao Y, Huang E, Olson KR (2016). Garlic oil polysulfides: H_2S - and O_2 -independent prooxidants in buffer and antioxidants in cells. *Am J Physiol Regul Integr Comp Physiol* 310: R1212–R1225.

Distrutti E, Mencarelli A, Santucci L, Renga B, Orlandi S, Donini A *et al.* (2007). The methionine connection: homocysteine and hydrogen sulfide exert opposite effects on hepatic microcirculation in rats. *Hepatology* 47: 659–667.

Ekstedt M, Franzén LE, Mathiesen UL, Holmqvist M, Bodemar G, Kechagias S (2007). Statins in non-alcoholic fatty liver disease and chronically elevated liver enzymes: a histopathological follow-up study. *J Hepatol* 47: 135–141.

Elrod JW, Calvert JW, Morrison J, Doeller JE, Kraus DW, Tao L *et al.* (2007). Hydrogen sulfide attenuates myocardial

ischemia–reperfusion injury by preservation of mitochondrial function. *Proc Natl Acad Sci U S A* 104: 15560–15565.

Fiorucci S, Antonelli E, Mencarelli A, Orlandi S, Renga B, Rizzo G *et al.* (2005). The third gas: H_2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis. *Hepatology* 42: 539–548.

Fu M, Zhang W, Wu L, Yang G, Li H, Wang R (2012). Hydrogen sulfide (H_2S) metabolism in mitochondria and its regulatory role in energy production. *Proc Natl Acad Sci U S A* 109: 2943–2948.

Gadalla MM, Snyder SH (2010). Hydrogen sulfide as a gasotransmitter. *J Neurochem* 113: 14–26.

Gluchowski NL, Becuwe M, Walther TC, Farese RV Jr (2017). Lipid droplets and liver disease: from basic biology to clinical implications. *Nat Rev Gastroenterol Hepatol* 14: 343–355.

Harding S, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S *et al.* (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucl Acids Res* 46: D1091–D1106.

Hashida R, Kawaguchi T, Bekki M, Omoto M, Matsuse H, Nago T *et al.* (2017). Aerobic vs. resistance exercise in non-alcoholic fatty liver disease: a systematic review. *J Hepatol* 66: 142–152.

Hine C, Harputlugil E, Zhang Y, Ruckstuhl C, Lee BC, Brace L *et al.* (2015). Endogenous hydrogen sulfide production is essential for dietary restriction benefits. *Cell* 160: 132–144.

Hine C, Kim HJ, Zhu Y, Harputlugil E, Longchamp A, Matos MS *et al.* (2017). Hypothalamic–pituitary axis regulates hydrogen sulfide production. *Cell Metab* 25: 1320–1333.

Huang T, Hu X, Khan N, Yang J, Li D (2013). Effect of polyunsaturated fatty acids on homocysteine metabolism through regulating the gene expressions involved in methionine metabolism. *ScientificWorldJournal* 2013: 931626: 1–8.

Huang T, Wahlqvist ML, Li D (2012). Effect of *n*-3 polyunsaturated fatty acid on gene expression of the critical enzymes involved in homocysteine metabolism. *Nutr J* 11: 6.

Hwang SY, Sarna LK, Siow YL, O K (2013). High-fat diet stimulates hepatic cystathionine β -synthase and cystathionine γ -lyase expression. *Can J Physiol Pharmacol* 91: 913–919.

Jha S, Calvert JW, Duranski MR, Ramachandran A, Lefer DJ (2008). Hydrogen sulfide attenuates hepatic ischemia–reperfusion injury: role of antioxidant and antiapoptotic signaling. *Am J Physiol Heart Circ Physiol* 295: H801–H806.

Kabil O, Vitvitsky V, Xie P, Banerjee R (2011). The quantitative significance of the transsulfuration enzymes for H_2S production in murine tissues. *Antioxid Redox Signal* 15: 363–372.

Levy JR, Clore JN, Stevens W (2004). Dietary *n*-3 polyunsaturated fatty acids decrease hepatic triglycerides in Fischer 344 rats. *Hepatology* 39: 608–616.

Li L, Bhatia M, Zhu YZ, Zhu YC, Ramnath RD, Wang ZJ *et al.* (2005). Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. *FASEB J* 19: 1196–1198.

Li L, Whiteman M, Guan YY, Neo KL, Cheng Y, Lee SW *et al.* (2008). Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GY4137): new insights into the biology of hydrogen sulfide. *Circulation* 117: 2351–2360.

Li M, Xu C, Shi J, Ding J, Wan X, Chen D *et al.* (2017). Fatty acids promote fatty liver disease via the dysregulation of 3-mercaptopyruvate sulfurtransferase/hydrogen sulfide pathway. *Gut* [Epub ahead of print].

- Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM (2000). Metformin reverses fatty liver disease in obese, leptin-deficient mice. *Nat Med* 6: 998–1003.
- Liu Y, Li Y, Yang W, Cao G (2013). H₂S inhibits the activation of hepatic stellate cells and downregulates the expression of urotensin II. *Hepatology* 43: 670–678.
- Loomba R, Sanyal AJ (2013). The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 10: 686–690.
- Luo ZL, Tang LJ, Wang T, Dai RW, Ren JD, Cheng L *et al.* (2014). Effects of treatment with hydrogen sulfide on methionine–choline deficient diet-induced non-alcoholic steatohepatitis in rats. *J Gastroenterol Hepatol* 29: 215–222.
- Maclean KN, Sikora J, Kožich V, Jiang H, Greiner LS, Kraus E *et al.* (2010). A novel transgenic mouse model of CBS-deficient homocystinuria does not incur hepatic steatosis or fibrosis and exhibits a hypercoagulative phenotype that is ameliorated by betaine treatment. *Mol Genet Metab* 101: 153–162.
- Maclean KN, Greiner LS, Evans JR, Sood SK, Lhotak S, Markham NE *et al.* (2012). Cystathionine protects against endoplasmic reticulum stress-induced lipid accumulation, tissue injury, and apoptotic cell death. *J Biol Chem* 287: 31994–32005.
- Mani S, Li H, Untereiner A, Wu L, Yang G, Wang R *et al.* (2013). Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. *Circulation* 127: 2523–2534.
- Mani S, Cao W, Wu L, Wang R (2014). Hydrogen sulfide and the liver. *Nitric Oxide* 41: 62–71.
- Mani S, Li H, Wu L, Yang G, Wang R (2015). Deficiency of cystathionine gamma-lyase and hepatic cholesterol accumulation during mouse fatty liver development. *Science Bulletin* 60: 336–347.
- Mani S, Yang G, Wang R (2011). A critical life-supporting role for cystathionine γ -lyase in the absence of dietary cysteine supply. *Free Radic Biol Med* 50: 1280–1287.
- Manna P, Gungor N, McVie R, Jain SK (2014). Decreased cystathionine- γ -lyase (CSE) activity in livers of type 1 diabetic rats and peripheral blood mononuclear cells (PBMC) of type 1 diabetic patients. *J Biol Chem* 289: 11767–11778.
- Mudd SH, Finkelstein JD, Irreverre F, Laster L (1964). Homocystinuria: an enzymatic defect. *Science* 143: 1443–1445.
- Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK *et al.* (2009). H₂S signals through protein S-sulfhydration. *Sci Signal* 2: ra72.
- Nagahara N, Ito T, Kitamura H, Nishino T (1998). Tissue and subcellular distribution of mercaptopyruvate sulfurtransferase in the rat: confocal laser fluorescence and immunoelectron microscopic studies combined with biochemical analysis. *Histochem Cell Biol* 110: 243–250.
- Nagahara N, Nagano M, Ito T, Shimamura K, Akimoto T, Suzuki H (2013). Antioxidant enzyme, 3-mercaptopyruvate sulfurtransferase-knockout mice exhibit increased anxiety-like behaviors: a model for human mercaptolactate-cysteine disulfiduria. *Sci Rep* 3: 1986.
- Nagahara N, Koike S, Nirasawa T, Kimura H, Ogasawara Y (2018). Alternative pathway of H₂S and polysulfides production from sulfurated catalytic-cysteine of reaction intermediates of 3-mercaptopyruvate sulfurtransferase. *Biochem Biophys Res Commun* 496: 648–653.
- Norris EJ, Culbertson CR, Narasimhan S, Clemens MG (2011). The liver as a central regulator of hydrogen sulfide. *Shock* 6: 242–250.
- Pastori D, Polimeni L, Baratta F, Pani A, Del Ben M, Angelico F (2015). The efficacy and safety of statins for the treatment of non-alcoholic fatty liver disease. *Dig Liver Dis* 47: 4–11.
- Peh MT, Anwar AB, Ng DS, Atan MS, Kumar SD, Moore PK (2014). Effect of feeding a high fat diet on hydrogen sulfide (H₂S) metabolism in the mouse. *Nitric Oxide* 41: 138–145.
- Pei Y, Wu B, Cao Q, Wu L, Yang G (2011). Hydrogen sulfide mediates the anti-survival effect of sulforaphane on human prostate cancer cells. *Toxicol Appl Pharmacol* 257: 420–428.
- Polhemus DJ, Li Z, Pattillo CB, Gojon G Sr, Gojon G Jr, Giordano T *et al.* (2015). A novel hydrogen sulfide prodrug, SG1002, promotes hydrogen sulfide and nitric oxide bioavailability in heart failure patients. *Cardiovasc Ther* 33: 216–226.
- Rahman SM, Schroeder-Glockler JM, Janssen RC, Jiang H, Qadri I, Maclean KN *et al.* (2007). CCAAT/enhancing binding protein β deletion in mice attenuates inflammation, endoplasmic reticulum stress, and lipid accumulation in diet-induced nonalcoholic steatohepatitis. *Hepatology* 45: 1108–1117.
- Ratnam S, Maclean KN, Jacobs RL, Brosnan ME, Kraus JP, Brosnan JT (2002). Hormonal regulation of cystathionine β -synthase expression in liver. *J Biol Chem* 277: 42912–42918.
- Robert K, Nehmé J, Bourdon E, Pivert G, Friguet B, Delcayre C *et al.* (2005). Cystathionine β synthase deficiency promotes oxidative stress, fibrosis, and steatosis in mice liver. *Gastroenterology* 128: 1405–1415.
- Searcy DG, Lee SH (1998). Sulfur reduction by human erythrocytes. *J Exp Zool* 282: 310–322.
- Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K *et al.* (2009). 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid Redox Signal* 11: 703–714.
- Siebert N, Cantré D, Eipel C, Vollmar B (2008). H₂S contributes to the hepatic arterial buffer response and mediates vasorelaxation of the hepatic artery via activation of K_{ATP} channels. *Am J Physiol Gastrointest Liver Physiol* 295: G1266–G1273.
- Suzuki K, Olah G, Modis K, Coletta C, Kulp G, Gerö D *et al.* (2011). Hydrogen sulfide replacement therapy protects the vascular endothelium in hyperglycemia by preserving mitochondrial function. *Proc Natl Acad Sci U S A* 108: 13829–13834.
- Teramoto K, Bowers JL, Khettry U, Palombo JD, Clouse ME (1993). A rat fatty liver transplant model. *Transplantation* 55: 737–741.
- Untereiner AA, Wang R, Ju Y, Wu L (2016). Decreased gluconeogenesis in the absence of cystathionine gamma-lyase and the underlying mechanisms. *Antioxid Redox Signal* 24: 129–140.
- Wang H, Quiroga AD, Lehner R (2013). Analysis of lipid droplets in hepatocytes. *Methods Cell Biol* 116: 107–127.
- Wang R (2002). Two's company, three's a crowd: can H₂S be the third endogenous gaseous transmitter? *FASEB J* 16: 1792–1798.
- Wang R (2012). Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *Physiol Rev* 92: 791–896.
- Watanabe M, Osada J, Aratani Y, Kluckman K, Reddick R, Malinow MR *et al.* (1995). Mice deficient in cystathionine beta-synthase: animal models for mild and severe homocyst(e)inemia. *Proc Natl Acad Sci U S A* 92: 1585–1589.
- Wei W, Wang C, Li D (2017). The content of hydrogen sulfide in plasma of cirrhosis rats combined with portal hypertension and the correlation with indexes of liver function and liver fibrosis. *Exp Ther Med* 14: 5022–5026.

- Werstuck GH, Lentz SR, Dayal S, Hossain GS, Sood SK, Shi YY *et al.* (2001). Homocysteine-induced endoplasmic reticulum stress causes dysregulation of the cholesterol and triglyceride biosynthetic pathways. *J Clin Invest* 107: 1263–1273.
- Wiliński B, Wiliński J, Somogyi E, Piotrowska J, Opoka W (2013). Metformin raises hydrogen sulfide tissue concentrations in various mouse organs. *Pharmacol Rep* 65: 737–742.
- Wójcicka G, Jamroz-Wiśniewska A, Atanasova P, Chaldakov GN, Chylińska-Kula B, Bełtowski J (2011). Differential effects of statins on endogenous H₂S formation in perivascular adipose tissue. *Pharmacol Res* 63: 68–76.
- Woo CWH, Sow YL, Pierce GN, Choy PC, Minuk GY, Mymin D *et al.* (2005). Hyperhomocysteinemia induces hepatic cholesterol biosynthesis and lipid accumulation via activation of transcription factors. *Am J Physiol Endocrinol Metab* 288: E1002–E1010.
- Wu L, Yang W, Jia X, Yang G, Duridanova D, Cao K *et al.* (2009). Pancreatic islet overproduction of H₂S and suppressed insulin release in Zucker diabetic rats. *Lab Invest* 89: 59–67.
- Wu D, Zheng N, Qi K, Cheng H, Sun Z, Gao B *et al.* (2015). Exogenous hydrogen sulfide mitigates the fatty liver in obese mice through improving lipid metabolism and antioxidant potential. *Med Gas Res* 5.
- Xie L, Gu Y, Wen M, Zhao S, Wang W, Ma Y *et al.* (2016). Hydrogen sulfide induces Keap1 S-sulfhydration and suppresses diabetes-accelerated atherosclerosis via Nrf2 activation. *Diabetes* 65: 3171–3184.
- Yang F, Yan S, He Y, Wang F, Song H, Guo Y *et al.* (2008b). Expression of hepatitis B virus proteins in transgenic mice alters lipid metabolism and induces oxidative stress in the liver. *J Hepatol* 48: 12–19.
- Yang G, Ju Y, Fu M, Zhang Y, Pei Y, Racine M *et al.* (2018). Cystathionine gamma-lyase/hydrogen sulfide system is essential for adipogenesis and fat mass accumulation in mice. *Biochim Biophys Acta* 1863: 165–176.
- Yang G, Lee HE, Lee JY (2016). A pharmacological inhibitor of NLRP3 inflammasome prevents non-alcoholic fatty liver disease in a mouse model induced by high fat diet. *Sci Rep* 6: 24399.
- Yang G, Wu L (2017). Trend in H₂S biology and medicine research – a bibliometric analysis. *Molecules* 22: 2087.
- Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K *et al.* (2008a). H₂S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 322: 587–590.
- Zeng T, Zhang CL, Song FY, Zhao XL, Xie KQ (2012). Garlic oil alleviated ethanol-induced fat accumulation via modulation of SREBP-1, PPAR- α , and CYP2E1. *Food Chem Toxicol* 50: 485–491.
- Zhang L, Yang G, Untereiner A, Ju Y, Wu L, Wang R (2013). Hydrogen sulfide impairs glucose utilization and increases gluconeogenesis in hepatocytes. *Endocrinology* 154: 114–126.
- Zhao W, Zhang J, Lu Y, Wang R (2001). The vasorelaxant effect of H₂S as a novel endogenous gaseous K_{ATP} channel opener. *EMBO J* 20: 6008–6016.
- Zhou R, Lin J, Wu D (2014). Sulforaphane induces Nrf2 and protects against CYP2E1-dependent binge alcohol-induced liver steatosis. *Biochim Biophys Acta* 1840: 209–218.