Sulfide regulation of cardiovascular function in health and disease

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Abstract | Hydrogen sulfide (H_2S) has emerged as a gaseous signalling molecule with crucial implications for cardiovascular health. H_2S is involved in many biological functions, including interactions with nitric oxide, activation of molecular signalling cascades, post-translational modifications and redox regulation. Various preclinical and clinical studies have shown that H_2S and its synthesizing enzymes — cystathionine γ-lyase, cystathionine β-synthase and 3-mercaptosulfotransferase — can protect against cardiovascular pathologies, including arrhythmias, atherosclerosis, heart failure, myocardial infarction and ischaemia–reperfusion injury. The bioavailability of H_2S and its metabolites, such as hydropersulfides and polysulfides, is substantially reduced in cardiovascular disease and has been associated with single-nucleotide polymorphisms in H_2S synthesis enzymes. In this Review, we highlight the role of H_2S, its synthesizing enzymes and metabolites, their roles in the cardiovascular system, and their involvement in cardiovascular disease and associated pathologies. We also discuss the latest clinical findings from the field and outline areas for future study.

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https://doi.org/10.1038/s41569-022-00741-6

Hydrogen sulfide (H_2S) is a naturally occurring, colourless gas that is toxic, corrosive and flammable. H_2S is a major component of the sulfur cycle and is present in the environment (such as in decaying organic matter, groundwater and natural gases). With exposure to levels >100 ppm, H_2S typically causes asphyxiation, with shock and convulsions that can be fatal[1]. However, H_2S is also an important biological molecule that was crucial in the evolution of life[2,3] and is synthesized in nanomolar to micromolar concentrations in vivo. In the past few decades, the essential role of H_2S in cellular signalling and protection and in regulating numerous biological functions has been recognized[4].

H_2S is one of three known gaseous signalling molecules or 'gasotransmitters' with crucial pathophysiological roles in cardiovascular function[4–6]. Carbon monoxide (CO) and nitric oxide (NO) are the other two gaseous neurotransmitters in this class. Before the identification in the 1940s of the biological role of H_2S in vertebrates[4,7], NO had long been considered the major vascular gaseous signalling molecule[4]. The current literature clearly demonstrates that H_2S is an important independent effector[8–11], as well as an enhancer of NO-mediated signalling events affecting the cardiovascular system[12–14]. A cardioprotective role for H_2S has been suggested in cardiac arrhythmias, cardiac fibrosis, heart failure, cardiac hypertrophy, ischaemia–reperfusion injury (IRI) and myocardial infarction (MI)[10]. Although the role of H_2S and its metabolites as biomarkers of human cardiovascular disease (CVD) is not yet well established[15], improved detection techniques have identified novel sulfide metabolites, including hydropersulfides and polysulfides, and have begun to reveal previously unknown molecular mechanisms and their biological relevance in cardiovascular pathology. In this Review, we discuss the involvement of H_2S, hydropersulfides and polysulfides in cardiovascular function and CVD and provide timely insights into potential clinical applications and interventions.

Chemical biology of sulfides

The oxidation state of sulfur has a broad range, from −2 in H_2S, 0 in elemental sulfur (S_8), +2 in sulfur monoxide (SO), and a maximum oxidation state of +6 in sulfate (SO_4^{2-}). Owing to its lower oxidation state, H_2S acts as a reductant. Although H_2S does not react readily with oxygen in the air, it easily undergoes oxidation in aqueous solutions. Sulfide can be present as other oxidation products, including polythionates, thiosulfate, sulfite (SO_3^{2-}), sulfate and small oxoacids of sulfur (FIG. 1a). H_2S is just one form of the sulfur-containing molecules that contribute to other metabolites, such as acid-labile sulfide (such as iron–sulfur clusters) and bound sulfane sulfur[16–18] (such as hydropersulfides and polysulfides). H_2S predominantly exists (~80%) as the anionic form HS^- under physiological conditions (pH 7.4).

Key points

- Hydrogen sulfide (H_2S) has a crucial role in regulating cardiovascular function; reduced bioavailability is associated with cardiovascular pathologies, including arrhythmias, heart failure, ischaemic myocardial dysfunction and peripheral vascular disease.
- H_2S and its synthesizing enzymes, including cystathionine γ-lyase, can protect against atherosclerosis and cardiac ischaemia–reperfusion injury.
- H_2S regulates various pathophysiological functions via interaction with nitric oxide, activation of molecular signalling cascades, post-translational modification of proteins and control of redox-dependent responses.
- Findings from clinical studies demonstrate that H_2S and its metabolites, including hydropersulfides and polysulfides, have substantial therapeutic potential for various forms of cardiovascular disease.

H_2S is freely diffusible under acidic conditions, such as ischaemia, which has physiological relevance. However, the reactivity of this compound differs substantially depending on whether it is in gaseous (H_2S) or anionic (HS^-) form. H_2S does not react with reduced thiols, whereas HS^- reacts with oxidized thiol derivatives[19]. However, both HS^- and thiolate anions (RS^-) are nucleophiles and, therefore, do not react with each other[20,21]. The functions of H_2S metabolites, including polysulfides, have become an area of intense research interest in the past 5 years[15,22–24]. Hydropersulfides and polysulfides have been suggested to be stronger nucleophiles than cysteine, glutathione and even H_2S[19]. However, the formation, kinetics and biological relevance of these various sulfide compounds under physiological and pathological conditions in the cardiovascular system remain unclear.

Production of sulfides

Endogenous H_2S is produced in mammalian tissues by both enzymatic and non-enzymatic pathways[4,15,25]. The basal level of production is determined by the activity of three main enzymes — cystathionine γ-lyase (CTH), cystathionine β-synthase (CBS), 3-mercaptopyruvate sulfurtransferase (MPST) — as well as by cysteine aminotransferase[4,15] (FIG. 1b).

Homocysteine, L-cysteine and their derivatives are common substrates of these H_2S-generating enzymes. Cysteine can also produce H_2S in the blood, catalysed by iron and vitamin B_6 (REF.[25]). Additionally, D-cysteine can be metabolized by D-amino acid oxidase to 3-mercaptopyruvate, which is subsequently converted to H_2S via MPST in mammalian cells[26]. This pathway is functional only in the kidneys and the brain, particularly the cerebellum.

The synthesis of H_2S and its metabolites can be promiscuous with respect to substrate utilization and reactivity[27]. The transsulfuration pathway of H_2S production via CBS and CTH uses homocysteine and L-cysteine, but these enzymes can also produce other biochemical forms of sulfide[28,29]. CBS and CTH can use substrates such as cystine or glutathione disulfide, resulting in the formation of cysteine hydropersulfide or glutathione hydropersulfide as well as polysulfides that are biologically important forms of bound sulfane sulfur[30]. Hydropersulfides or polysulfides can be carried by proteins, such as plasma albumin, which can transport sulfane sulfur equivalents functioning as signalling mediators for various biological activities[15,31,32].

In addition to the four conventional H_2S-producing enzymes, studies have shown that cysteinyl–tRNA synthetases (CARSs; also known as cytoplasmic cysteine–tRNA ligase) are also a major source of endogenous protein hydropersulfide formation in mammalian cells[33,34] (FIG. 1b). CARS2 is a mitochondrial isoform that regulates mitochondrial bioenergetics and protein hydropersulfides, affecting cellular function[34]. These findings are important because they demonstrate that hydropersulfides and polysulfides can be synthesized independently of H_2S. However, further studies are required to understand how these various pathways participate in cardiovascular pathophysiological responses.

Localization of H_2S-producing enzymes

CBS and CTH are pyridoxal 5′-phosphate-dependent enzymes localized in the cytosol, with CBS being predominantly found in the brain and central nervous system and CTH primarily expressed in the cardiovascular system, although both enzymes are also found in the kidneys, liver, lymphocytes, placenta and pancreatic islets[6,35,36]. MPST is localized in mitochondria and has been found in the heart, kidneys, liver and retina[4,5,15]. Importantly, all three of these H_2S-synthesizing enzymes are expressed in cardiovascular cells[37]. Translocation of CTH to the mitochondria under hypoxic conditions has been reported, and this enzyme can metabolize cysteine to produce H_2S and increase ATP production in the mitochondria when MPST activity is concomitantly reduced[38]. Interestingly, this process has been attributed to CBS, which accumulates in mitochondria under hypoxic conditions because the degradation of CBS by Lon protease in the mitochondrial matrix is greatly reduced in the absence of oxygen[36]. However, H_2S production in the brain is possible via MPST as an alternative to CBS[39]. Likewise, upregulation of CBS can replenish H_2S levels in the cerebral cortex of CTH-deficient mice[40,41]. Together, these findings show that translocation or expression of any of these enzymes can change to maintain H_2S synthesis when one of the other enzymes is genetically removed[42,43]. Further studies are required to investigate the compensatory mechanisms of H_2S production under various pathophysiological conditions, including the tissue-specific roles of these enzymes.

Sulfide catabolism

The metabolic clearance of H_2S via detoxification pathways is crucial to maintaining an appropriate physiological balance of H_2S and its metabolites. The bioavailability of H_2S is influenced by both the direct catabolism and cysteine metabolism of endogenous H_2S in biological systems. Several enzymes catabolize H_2S — mitochondrial sulfide–quinone oxidoreductase (SQOR), which oxidizes H_2S to a hydropersulfide; mitochondrial persulfide dioxygenase ETHE1 (also known as ethylmalonic encephalopathy protein 1), which oxidizes the sulfide downstream of SQOR; and cysteine dioxygenase, which catabolizes cysteine to cysteine sulfinic acid[44,45]. Additionally, cytosolic methylation, glutathione disulfide, or other metallo-containing or disulfide-containing molecules can scavenge H_2S and regulate its levels[46,47]. Sulfates, such as thiosulfate, are major end products of

H_2S metabolism under physiological conditions[5] (FIG. 1a). Sulfates can be further converted into sulfite and sulfate by thiosulfate–cyanide sulfurtransferase and sulfite oxidase, respectively. Lastly, H_2S and methaemoglobin form sulfhaemoglobin, resulting in H_2S depletion[48].

Detection of sulfide metabolites

Improved technology and novel analytical methods to identify H_2S in its various chemical forms have allowed the intricacies of this molecule's bioavailability and biological function to be studied. However, the

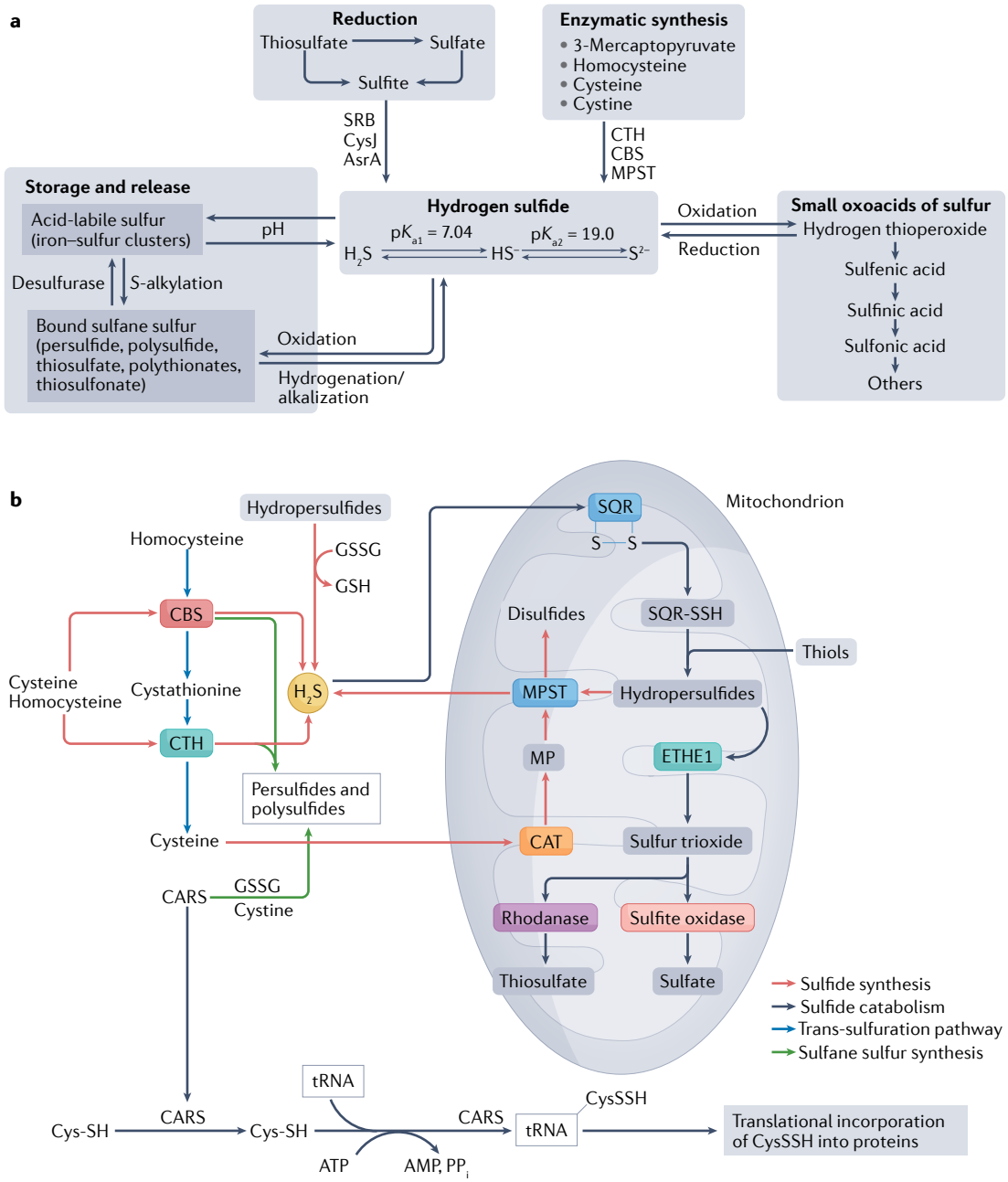


Fig. 1 | **Sulfide metabolite formation and fate. a** | Various chemical metabolite fate pathways for sulfide and its related species are shown. The basal level of production of hydrogen sulfide (H_2S) is determined by the activity of three main enzymes: cystathionine γ-lyase (CTH), cystathionine β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (MPST). In addition, bacterial enzymes (such as sulfate-reducing bacteria (SRB), sulfite reductase [NADPH] flavoprotein α-component (CysJ) and anaerobic sulfite reductase subunit A (AsrA)) can reduce terminal sulfide oxidation end products (such as thiosulfate, sulfate and sulfite) back to H_2S. H_2S can undergo a myriad of reactions leading to the formation of small oxoacids of sulfur, sulfane sulfur species and acid-labile sulfur species. **b** | Various enzymatic and non-enzymatic biochemical pathways are involved in sulfide metabolite formation. Sulfide catabolism through the mitochondrial H_2S oxidation pathway leads to the metabolic end products of sulfate and thiosulfate. CARS, cysteinyl–tRNA synthetase (also known as cytoplasmic cysteine–tRNA ligase); CAT, cysteine aminotransferase; CysSH, cysteine; CysSSH, cysteine hydropersulfide; ETHE1, persulfide dioxygenase; GSH, glutathione; GSSG, glutathione disulfide; MP, mercaptopyruvate; PP_i, inorganic pyrophosphate; SQR, sulfide–quinone oxidoreductase; SQR-SSH, sulfide–quinone oxidoreductase hydropersulfide.

Box 1 | **Detection and quantification of sulfide**

The methylene blue method is the easiest and most frequently used, but most controversial, method for the detection of sulfide[214]. First developed for quantification of hydrogen sulfide (H_2S) in non-biological samples, the assay is based on forming methylene blue in the presence of ferric iron under acidic conditions. Large background noise due to methylene blue aggregates and sulfide release from other biochemical forms due to acidic treatments contribute to the substantial limitations of the assay[54]. New analytical techniques have subsequently been developed to measure sulfide metabolites using the monobromobimane–high-performance liquid chromatography and liquid chromatography–mass spectrometry techniques, that enable highly accurate detection of sulfides[16,53]. The detection limit and the stability of the monobromobimane method allows batch storage and analysis and has been applied in both basic experimental and human research studies, validating the accuracy of this approach for detecting important metabolic responses[18,215–217]. Other analytical methods for detecting sulfide, hydropersulfides and polysulfides, such as those using β-(4-hydroxyphenyl)ethyl iodoacetamide (HPE-IAM) and *N*-iodoacetyl L-tyrosine methyl ester (TME-IAM), have also been reported[47,218]. Polarographic H_2S sensors can also detect H_2S levels in the nanomolar range and provide real-time measurement of H_2S from biological samples[218,219]. Although this method is reliable, some reports suggest that it might not detect sulfide[220]. A gas chromatography–chemiluminescence sulfur detection method using an alkylation technique to extract H_2S has also been reported to accurately measure H_2S in biological samples at the nanomolar level[221,222]. Numerous H_2S and sulfane sulfur-sensitive fluorescence probes (including Washington State probe-1, synchronous fluorescence-1/2, dansyl azide, sulfide-selective fluorescent probe-1/2,7-azido-4-methylcoumarin, sulfane sulfur probe 4 and PSP-3) have been identified, and their use has rapidly expanded in the field of H_2S pathobiology.

measurement of H_2S can still be challenging owing to its complex chemical signature and the various biological forms of sulfide. Detection methods for free and acid-labile H_2S and pools of sulfane sulfur — including hydropersulfides, polysulfides and oxoacids of sulfur — have been reviewed previously[16,22,49–53] (BOX 1).

In contrast to H_2S, the biological effects of sulfur metabolites, including hydropersulfides and polysulfides, are largely unknown. Also, the functions of the H_2S-producing enzymes in vascular disease remain unexplored and are a major knowledge gap. Fortunately, new analytical and biochemical methods have been developed to study hydropersulfide and polysulfide species[52]. The pitfalls associated with sulfide quantification analysis have been reviewed previously[22,54,55].

Sulfides in the cardiovascular system

The three gasotransmitters are involved in regulating an array of vital biological functions in the cardiovascular, neurological and immune systems at the cellular and organ levels[4,56]. NO and H_2S have similar and inter-relating physiological and pathological functions in the cardiovascular system[4], and the signalling pathways of these molecules often work in tangent[4,11]. H_2S was initially identified as an endogenous neuromodulator and vasorelaxant, with subsequent studies revealing broader functions[4,6,57,58]. The literature clearly demonstrates the protective effects and regulatory functions of H_2S in animal models of cardiovascular pathophysiology[59–61], but the role of H_2S and its metabolites in clinical CVD is less well studied[15].

Sulfide regulation and signalling in CVD

Evidence has increasingly demonstrated that disturbed H_2S production is relevant to cardiac pathologies. From a clinical perspective, H_2S has been posited to have a protective role against the onset and development of atherosclerosis[62–64]. Whereas defects in H_2S signalling, including its synthesizing enzymes, can lead to CVD and associated complications[15,65–68], H_2S-based interventions have proved to be beneficial in preventing adult-onset CVD in animal studies via the reversal of disease-programming processes[69]. Plasma H_2S levels have been shown to be significantly lower in patients with coronary heart disease than in angiographically normal control individuals[70]. Moreover, plasma H_2S levels are significantly lower in patients with unstable angina or acute MI than in those with stable angina[70]. In another study, patients with heart failure had marked reductions in circulating H_2S levels compared with healthy age-matched control individuals[71]. However, H_2S can be a 'double-edged sword' with beneficial effects at lower concentrations, but potentially harmful effects at higher concentrations. Balancing endogenous H_2S synthesis and the exogenous H_2S-releasing agents that can impinge on the delicate H_2S balance is important and requires scrutiny in the complex relationship between H_2S and CVD.

Initially, H_2S as a single entity was thought to mediate signalling events and biological functions. However, many other forms of sulfide (hydropersulfides and polysulfides) are also likely to have important signalling roles under physiological and pathophysiological conditions[15,72–74]. Whereas H_2S has emerged as an important molecule in various cardiovascular functions, less certainty exists about the synthesis and biological effects of other forms of sulfide in discrete cellular compartments. H_2S signals through distinct mechanisms to regulate various pathophysiological functions via interaction with other signalling molecules, including reactive sulfur species, NO, haem centres and antioxidant defence molecules, and post-translational modification of proteins via sulfhydration (also referred to as persulfuration). Sulfhydration alters protein function and has been shown to upregulate numerous protective signalling pathways[75–77]. However, the pathophysiological roles of hydropersulfides, polysulfides and small oxoacids of sulfide require further exploration.

H_2S-synthesizing enzyme polymorphisms. H_2S-synthesizing enzymes have a significant association with CVD[18,78,79]. A correlation was found between H_2S and NO bioavailability in patients with CVD, and H_2S metabolite levels were predictive of CVD in a sex-specific and ethnicity-specific manner[18]. Decreased levels of bound sulfane sulfur and total sulfide found in patients with coronary artery disease or peripheral artery disease were a statistically indicative biomarker for CVD[18]. Moreover, a specific single-nucleotide polymorphism (SNP) in *CTH* (1364G>T) was also identified as a potential risk factor in a substudy cohort, with a greater allelic mutation frequency across all forms of CVD than the previously identified 894G>T SNP in *NOS3* (encoding endothelial NO synthase (eNOS)), which was associated only with coronary artery disease[18]. Similarly, a *CTH* 1364G>T polymorphism was identified in 178 white Greek patients undergoing coronary artery bypass graft surgery[79]. Interestingly, the frequency

of the *CTH* 1364TT genotype was numerically higher (but not significantly different) in female patients than in healthy female control individuals, whereas there was no difference in the frequency of this SNP between male patients and controls. These studies suggest an association between CTH polymorphisms and CVD; however, molecular studies of these SNPs in other, larger populations is needed.

Cardioprotective effects in IRI. MI occurs when the heart muscle is deprived of blood carrying oxygen and nutrients, leading to acute tissue ischaemia and cell death[80]. Although reperfusion relieves ischaemia, it also results in complex reactions leading to inflammation and oxidative damage[81], which contribute to infarct development[82–84]. Growing evidence demonstrates that exogenous delivery of H_2S or modulation of endogenous H_2S improves cardiac function and reduces cardiac complications in IRI and various other cardiac conditions, including arrhythmias, heart failure, cardiac hypertrophy, myocardial fibrosis and MI[46,61,81–85]. Exogenous H_2S therapy was shown to be cardioprotective in a mouse model of IRI[68]. H_2S delivery reduced infarct size and preserved left ventricular function. Additionally, endogenous H_2S production by cardiac-specific *CTH* overexpression significantly limited myocardial injury. This study established that CTH–H_2S-mediated cryoprotection and inhibition of myocardial inflammation preserves myocardial and mitochondrial structure and function[68]. Subsequent research from the same group identified the underlying protective mechanisms of CTH–H_2S therapy in a mouse model of heart failure[85]. H_2S-induced cardiac protection was mediated via increased phosphorylation of RACα serine–threonine-protein kinase (AKT1; also known as protein kinase B), and nuclear localization of nuclear respiratory factor 1 and nuclear factor erythroid 2-related factor 2, which significantly increased antioxidative signalling, inhibited apoptosis and increased mitochondrial biogenesis[27]. Treatment with the H_2S donor diallyl trisulfide for 12 weeks preserved left ventricular function, reduced left ventricular remodelling and improved angiogenesis mediated via vascular endothelial growth factor (VEGF)–NO signalling in a mouse model of transverse aortic constriction[86]. These findings clearly imply the involvement of endogenous H_2S in maintaining basal physiological cardiac function.

H_2S therapy can protect against IRI via activation of the tyrosine–protein kinase JAK2–signal transducer and activator of transcription 3 (STAT3) signalling pathway. In a pig model of IRI, H_2S treatment markedly reduced MI-related damage, improving left ventricular function while concomitantly reducing apoptosis and increasing autophagy[87]. Sodium hydrosulfide pretreatment protected rat isolated hearts against IRI by inhibiting opening of the mitochondrial permeability transition pore[88]. Pharmacological inhibition of CTH increased infarct size in a rat model of IRI, which was rectified by H_2S therapy, leading to myocardial protection[89,90]. Additionally, cardiac-specific *CTH* overexpression in transgenic mice significantly reduced infarct size and improved cardiac function compared with wild-type mice after IRI[91]. These findings indicate that both exogenous H_2S donors and endogenously elevated H_2S levels protect the heart against IRI, revealing potentially important therapeutic targets.

Studies have suggested that the cardioprotective effects of H_2S are mediated through various pathways[71,92–94]. H_2S has an important role in promoting angiogenesis and ameliorating type 2 diabetes mellitus that also protect against IRI[8,95,96]. Endogenous H_2S production also augments antioxidant signalling via nuclear factor erythroid 2-related factor 2, reduces nuclear factor-κB (NF-κB)-mediated inflammatory signalling and facilitates NO signalling[60,97]. Studies in animal models of MI, IRI and heart failure have revealed significant reductions in endogenous H_2S production that contribute to disease progression[61]. H_2S also protects against MI and IRI by opening K^+_{ATP} channels[23,29,98–101]. Furthermore, H_2S interacts with NO in a $Cth^{-/-}$ mouse model of heart failure[61]. Cardiac remodelling and dysfunction were found to be worse in CTH-deficient mice than in wild-type mice. Reduced circulating H_2S levels in $Cth^{-/-}$ mice directly led to cardiac dilatation and dysfunction, whereas exogenous H_2S therapy had cardioprotective effects via upregulation of the VEGF–AKT1–eNOS–NO–cGMP pathway, resulting in preserved mitochondrial function and increased myocardial vascular density[61]. Therapy with the sulfur-donating drug SG1002 in $Cth^{-/-}$ mice increased myocardial vascular density and improved cardiac remodelling and function via the same pathway. In a later study, SG1002 was found to effectively increase circulating H_2S and circulating NO bioavailability, while attenuating B-type natriuretic peptide levels (a marker of cardiomyocyte stress and left ventricular dysfunction) in patients with heart failure with reduced ejection fraction (NYHA class II–III)[102].

Cardiac dysfunction and hypertrophy. Cardiac hypertrophy is a crucial compensatory mechanism in the failing heart. It increases cardiac output and can occur in response to chronic pressure or volume overload and after MI. However, persistent hypertrophy is deleterious, resulting in cardiac dilatation, loss of contractile function and decreased ejection fraction, subsequently leading to heart failure[103]. The protective role of H_2S in pathogenic cardiac hypertrophy is being increasingly demonstrated. In a model of cardiac hypertrophy, exogenous H_2S reduced the production of reactive oxygen species (ROS) in the mitochondria and preserved cardiac mitochondrial membrane potential, thereby inhibiting hypertrophy and cardiomyocyte apoptosis and improving cardiac function[104]. Furthermore, reduced levels of endogenous CTH and H_2S increased oxidative stress and induced cardiomyocyte apoptosis[104]. Hypertrophic signalling pathways activated in response to MI are defective in $Cth^{-/-}$ mice[105], but treatment with the exogenous H_2S donor GYY4137 from 2 h after the onset of MI reduced infarct size, cardiac hypertrophy and adverse remodelling and preserved cardiac function in both $Cth^{-/-}$ and wild-type mice[105]. An age-dependent association was found between MPST and cardiac hypertrophy in mice[106]. In young adult animals (aged 2–3 months), knocking out *Mpst* had a cardioprotective effect;

however, in older mice (aged >18 months), the *Mpst* knockout resulted in reduced antioxidant signalling and subsequent hypertension and cardiac hypertrophy[106].

Endothelial function and vasodilatation. The vascular endothelium is the active component lining the entire circulatory system and controls numerous responses, such as vascular tone, vessel remodelling, oxidative stress defences, thrombosis and inflammation[107,108]. Endothelial dysfunction is a crucial predictor of CVD[108–110]. NO is one of the most important substances produced by the vascular endothelium and, as discussed above, the association and interaction between the H_2S and NO signalling pathways have substantial implications for cardiovascular protection[107–109]. Our group and others have demonstrated that H_2S can preserve endothelial function through various mechanisms, including the post-translational stabilization of eNOS, leading to an increase in NO bioavailability, and the augmentation of nitrite–NO signalling[13,14,60,111,112].

H_2S can exert vasodilatory effects via regulation of the soluble guanylate cyclase (sGC)–phosphodiesterase–cGMP–protein kinase G (PKG; also known as cGMP-dependent protein kinase) vascular relaxation signalling pathway[113] or via K^+_{ATP}, L-type Ca^{2+} and other ion channels[114–116]. In a rat renal hypertension model, treatment with the fast-releasing H_2S donor sodium hydrosulfide (NaHS) dilated isolated aortic rings by relaxing vascular smooth muscle cells, mediated by increased cGMP–PKG activity, in a dose-dependent manner[113]. Similarly, the use of an H_2S and NO conjugated donor, ZYZ-803, induced time-dependent and dose-dependent vasodilatation of rat aortic rings by stimulating the cGMP pathway[117]. This vasorelaxant effect was suppressed with H_2S and NO inhibition. Inhibitors of PKG or the K^+_{ATP} channel had similar effects, demonstrating that the protective effects of H_2S and NO are mediated via K^+_{ATP} channel and cGMP pathways[117]. Another study, using human mesenteric arteries obtained from patients undergoing abdominal surgery, demonstrated NaHS-mediated K^+_{ATP} channel-dependent vasorelaxation[118]. This response was completely inhibited after endothelium denudation or inhibition of eNOS or cGMP, indicating a role for these signalling pathways in NaHS-mediated vasorelaxation[118]. Researchers demonstrated dose-dependent H_2S-induced vasoregulation in isolated blood vessels (including aortic, carotid, renal and iliac arteries) from rabbits[119]. As with NO donors, vasodilatation occurred with low doses of H_2S, but vasoconstriction occurred with high doses of H_2S[119]. These studies clearly indicate that H_2S has a prominent role in regulating endothelium-dependent signalling activities (FIG. 2a).

Interestingly, in addition to the effects of H_2S, prolonged NO and cGMP signalling might be sustained by sulfide metabolite modifications of eNOS, cGMP or PKG[120–122] (FIG. 2b). H_2S-mediated sulfhydration of eNOS Cys443 facilitates its catalytic activity, maximizing NO generation[120]. The HS^- anion can also mediate the electrophilic sulfhydration of 8-nitro-cGMP to form 8-SH-cGMP, which stabilizes cGMP release and modulates cellular redox signalling[122]. H_2S can also stabilize cGMP release by catalysing the formation of a protein disulfide within PKG1α, thereby stimulating the activity of PKG[121]. This modification has been shown to have substantial physiological effects that can reduce blood pressure. H_2S significantly lowers blood pressure in wild-type mice, but not in PKG1α Cys42Ser knock-in mice[123], revealing the functional implications of this modification.

H_2S can induce sGC activation and decrease cGMP degradation by blunting phosphodiesterase activity. The involvement of CTH and H_2S in mediating the vasodilatation of aortic rings via cGMP was demonstrated through inhibition of cGMP-specific 3′,5′-cyclic phosphodiesterase (PDE5; also known as phosphodiesterase type 5)[113]. H_2S can increase sGC levels via sulfhydration of sGCβ1 and reducing sGCαβ1 dimers in vascular tissues[124]. Furthermore, H_2S markedly decreased PDE5A homodimer formation via sulfhydration of PDE5, thereby reducing PDE5 activity, facilitating cGMP stabilization and significantly decreasing levels of 5′-GMP[124]. Other studies have also demonstrated endothelium-dependent vasodilatation in response to H_2S donors via a NO–cGMP-dependent pathway[113,125,126].

H_2S enzymatic pathways are important in the regulation of endothelial vascular function[127]. As discussed above, CTH-generated H_2S mediates smooth muscle relaxation and subsequent vasodilatation[113,124]. However, regulation of CTH in the vascular endothelium remains poorly characterized. Studies have shown that genetic deletion of H_2S-producing enzymes, and the subsequent reduction in H_2S levels, results in impaired vasodilatation[28,111]. In a global *Cth*$^{-/-}$ mouse model, reduced H_2S levels lead to hypertension[28]. Additionally, mesenteric arteries were markedly impaired in *Cth*$^{-/-}$ mice, and removal of the endothelium prevented methacholine-induced relaxation in both wild-type and mutant arteries[28]. Our group has extended this observation using a non-invasive, flow-mediated dilatation model in global *Cth*$^{-/-}$ mice[111]. Femoral artery dilatation was defective, and distal tissue blood flow was compromised. Both these effects were mediated by sulfide-dependent reduction of nitrite back to NO by xanthine oxidase and were reversed with diallyl trisulfide treatment[111] (FIG. 2c). Another study demonstrated that deletion of *Cth* decreased H_2S and cardiac NO production, impairing endothelial-dependent vasorelaxation. Transgenic overexpression of endothelial CTH restored H_2S and NO levels in the cardiovascular system and vasorelaxation in thoracic aorta[61,128]. These studies reveal interactions between H_2S and NO signalling in the regulation of vascular tone. However, further research is needed to understand the mechanisms mediated by cell-specific functions of CTH, H_2S and its metabolites.

Inflammation and atherosclerosis

Evidence suggests that H_2S protects against the development and progression of atherosclerosis[129,130], which involves endothelial dysfunction and vascular inflammation and is a major mediator of clinical CVD. Exogenous H_2S supplementation has salutary effects on atherogenesis, and the reduction in endogenous CTH or H_2S levels accelerates atherosclerosis[62,131,132]. Atherosclerotic

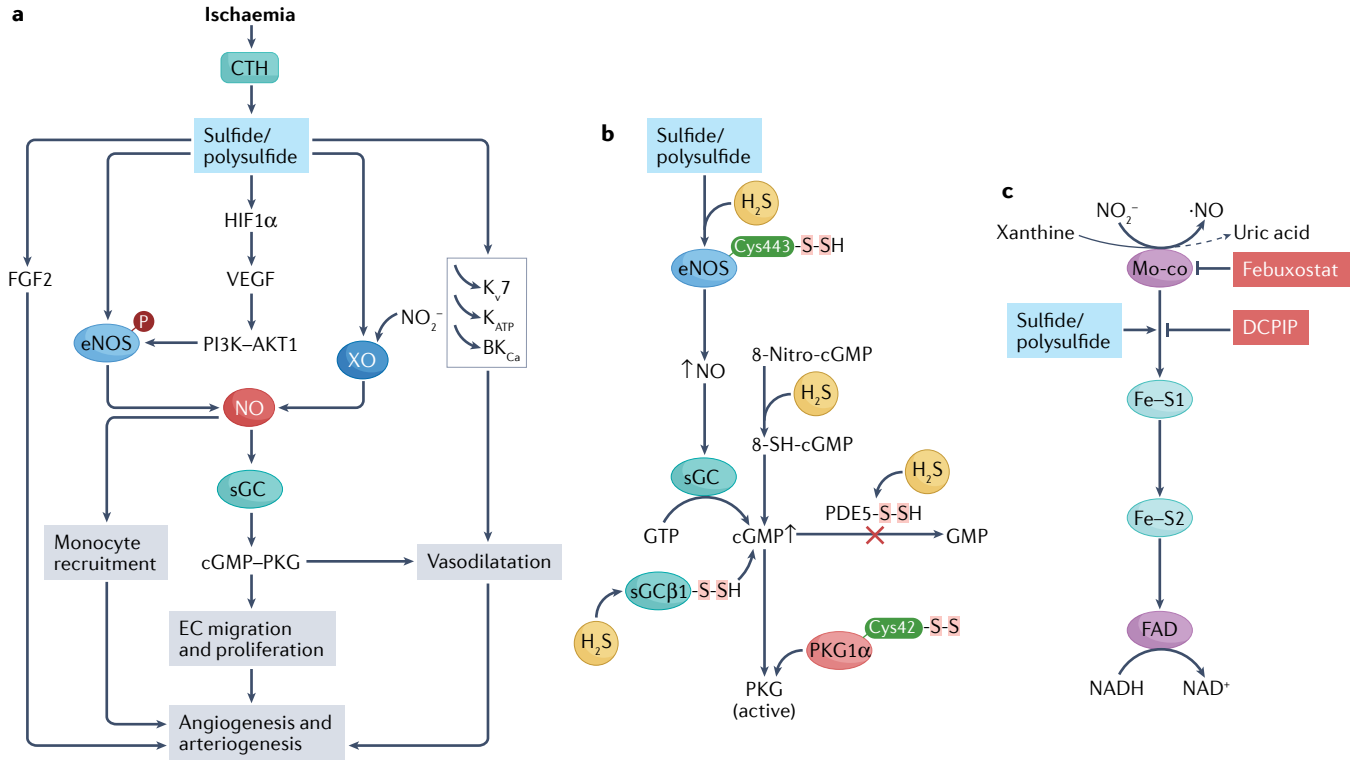
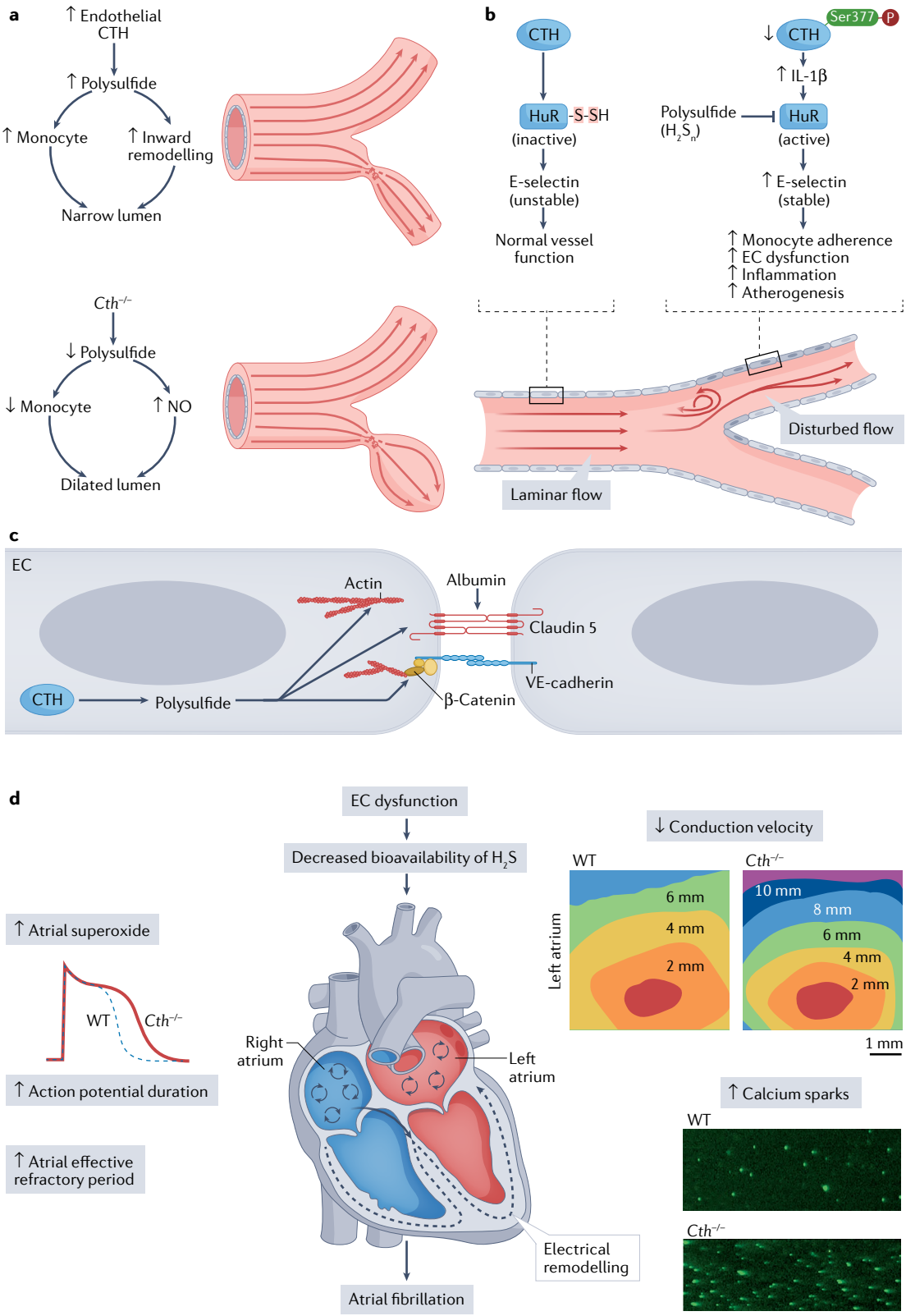


Fig. 2 | **Sulfide signalling and chemical reaction pathways. a** | An ischaemia-driven increase in the expression and function of cystathionine γ-lyase (CTH) leads to sulfide metabolite production, which affects both endothelial nitric oxide synthase (eNOS) phosphorylation and hypoxia-inducible factor 1α (HIF1α) activation. This cascade leads to vascular endothelial growth factor (VEGF) and nitric oxide (NO) production, stimulating the monocyte recruitment and endothelial cell (EC) proliferation necessary for angiogenesis and arteriogenesis. **b** | Sulfide post-translational modifications of eNOS and cGMP-dependent protein kinase 1α (PKG1α), together with electrophilic sulfhydration of 8-nitro-cGMP to 8-SH-cGMP, the soluble guanylate cyclase-β1 subunit (sGCβ1) to sGCβ1 persulfide (sGC-SSH) and phosphodiesterase type 5 (PDE5) to PDE5 persulfide (PDE5-SSH), contribute to increased cGMP levels and subsequent protein kinase G (PKG) activity. **c** | The effect of sulfide and polysulfide on xanthine oxidase (XO)-dependent nitrite (NO_2^-) reduction via interaction with either Fe–S clusters or a molybdenum cofactor (Mo-co) domain, which is inhibited by 2,6-dichlorophenolindophenol (DCPIP) or febuxostat, respectively. AKT1, RACα serine–threonine protein kinase; BK_{Ca}, large-conductance calcium-activated potassium channel; FGF2, fibroblast growth factor 2; H_2S, hydrogen sulfide; K_{ATP}, ATP-sensitive potassium channel; K_v7, voltage-gated potassium channels; PI3K, phosphatidylinositol 3-kinase.

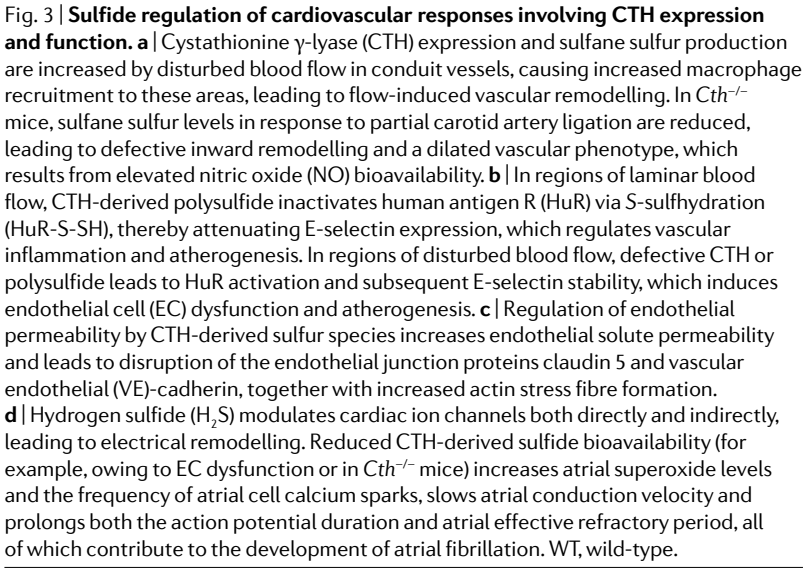
lesion formation was inhibited by NaHS in *Apoe*$^{-/-}$ mice, whereas the CTH inhibitor DL-propargylglycine significantly reduced H_2S levels and resulted in accelerated plaque formation[131]. Genetic CTH deficiency significantly increases atherosclerosis development in *Apoe*$^{-/-}$ mice[62]. Disruption of the vascular redox status was observed, as well as increased intimal proliferation and inflammatory adhesion molecule expression[62]. Exogenous H_2S treatment inhibits the expression of endothelial cell adhesion molecules, including intercellular adhesion molecule 1, vascular cell adhesion protein 1 and E-selectin, by suppressing NF-κB activity and attenuating atherosclerotic pathogenesis[131]. Exogenous H_2S therapy protects the endothelium, inhibits the development of vascular lesions and reduces blood pressure in *Apoe*$^{-/-}$ mice fed a high-fat diet[132]. In this study, H_2S donors such as diallyl disulfide and diallyl trisulfide protected against oxidized LDL-induced atherosclerotic plaque formation by inhibiting the activation of eNOS[132–134].

Homocysteine metabolizes in the body to produce H_2S. However, increased homocysteine synthesis (hyperhomocysteinaemia) inactivates CTH[135]. Hyperhomocysteinaemia has a strong correlation with premature coronary artery disease[136,137] secondary to atherosclerosis via decreased H_2S production, which leads to sustained endothelial cell injury and the induction of vascular smooth muscle cell proliferation[138,139].

H_2S can induce anti-inflammatory signalling via peroxisome proliferator-activated receptor-δ (PPARδ) and suppressor of cytokine signalling 3 (SOCS3), which mediates vascular remodelling[140]. Therefore, endogenous H_2S deficiency could be a risk factor for vascular smooth muscle cell dysfunction. Endogenous H_2S deficiency generated vascular remodelling with aggravated active and passive contraction, thickened aortic walls, collagen deposition, increased STAT3 phosphorylation and decreased aortic production of PPARδ and SOCS3, which were all reversed by treatment with NaHS[140]. Importantly, SOCS3 mediates anti-inflammatory effects in hypertension and obesity via inhibition of tyrosine–protein kinase JAK1–STAT signalling[140], preserving endothelial function in experimental hypertension, suppressing inflammation in macrophages after treatment with lipopolysaccharides and inhibiting vascular smooth muscle cell proliferation[141,142]. These studies strongly establish anti-atherogenic and anti-inflammatory roles for CTH and H_2S in animal models of atherosclerosis.



Angiogenesis and vascular remodelling. Angiogenesis is a regulated process of microvascular growth that can revascularize ischaemic tissue. H_2S induces angiogenesis by increasing endothelial cell proliferation and migration[143]. Exogenous H_2S (NaHS) increases cell growth, migration and the formation of tube-like structures in cultured endothelial cells[143]. These effects were concentration-dependent and mediated via phosphatidylinositol 3-kinase (PI3K)–AKT1 signalling. The researchers confirmed their observations in vivo using a Matrigel plug assay to assess neovascularization in mice[143].



◂ Fig. 3 | **Sulfide regulation of cardiovascular responses involving CTH expression and function. a** | Cystathionine γ-lyase (CTH) expression and sulfane sulfur production are increased by disturbed blood flow in conduit vessels, causing increased macrophage recruitment to these areas, leading to flow-induced vascular remodelling. In *Cth*$^{-/-}$ mice, sulfane sulfur levels in response to partial carotid artery ligation are reduced, leading to defective inward remodelling and a dilated vascular phenotype, which results from elevated nitric oxide (NO) bioavailability. **b** | In regions of laminar blood flow, CTH-derived polysulfide inactivates human antigen R (HuR) via *S*-sulfhydration (HuR-S-SH), thereby attenuating E-selectin expression, which regulates vascular inflammation and atherogenesis. In regions of disturbed blood flow, defective CTH or polysulfide leads to HuR activation and subsequent E-selectin stability, which induces endothelial cell (EC) dysfunction and atherogenesis. **c** | Regulation of endothelial permeability by CTH-derived sulfur species increases endothelial solute permeability and leads to disruption of the endothelial junction proteins claudin 5 and vascular endothelial (VE)-cadherin, together with increased actin stress fibre formation. **d** | Hydrogen sulfide (H_2S) modulates cardiac ion channels both directly and indirectly, leading to electrical remodelling. Reduced CTH-derived sulfide bioavailability (for example, owing to EC dysfunction or in *Cth*$^{-/-}$ mice) increases atrial superoxide levels and the frequency of atrial cell calcium sparks, slows atrial conduction velocity and prolongs both the action potential duration and atrial effective refractory period, all of which contribute to the development of atrial fibrillation. WT, wild-type.

Studies from our group and others have established that H_2S promotes arteriogenesis and angiogenesis, and improves regional blood flow in ischaemic limbs, indicating prominent vascular growth and remodelling in ischaemic tissues[8,13,14,144]. Chronic ischaemia of the limb during peripheral vascular disease remains largely resistant to medical therapy[145], and translational approaches to restore perfusion to the distal limb and improve outcomes are limited[146]. Therefore, H_2S is an attractive therapeutic target for limb ischaemia. A study showed the pro-angiogenic effects of H_2S in a rat model of chronic limb ischaemia[144]. H_2S upregulated collateral vessel growth and capillary density mediated by upregulation of the VEGF–AKT1 pathway[144]. Similarly, an H_2S donor restored vascular density and remodelling and, subsequently, blood flow and tissue perfusion in mice with hindlimb ischaemia[14]. These effects were mediated via upregulation of the hypoxia-inducible factor 1α–VEGF–AKT1 pathway that induces the eNOS–sGC–cGMP–PKG system downstream[14,147]. Our group has demonstrated a unique interaction between H_2S and NO, in which H_2S significantly increases NO levels in plasma and ischaemic limb tissue, followed by downregulation of H_2S when NO levels are elevated, suggesting a hierarchical order of gasotransmitter production[13,14,111]. These beneficial effects of H_2S on NO levels in ischaemic tissue did not depend exclusively on NOS activity, because nitrite anion reduction back to NO was also involved and was blunted by febuxostat-dependent inhibition of xanthine oxidase[14,111].

In *Cth*$^{-/-}$ mice, chronic tissue ischaemia was associated with impaired ischaemic vascular remodelling and reductions in endogenous H_2S production, monocyte recruitment and expression of VEGF and fibroblast growth factor 2 (FGF2; also known as basic fibroblast growth factor)[13]. Exogenous treatment with diallyl trisulfide restored plasma and tissue NO levels, monocyte recruitment, arteriogenesis, ischaemic vascular remodelling and an angiogenic cytokine expression pattern[13]. VEGF receptor 2 (VEGFR2) can also act as a receptor target for H_2S during angiogenesis[148]. Downregulation of VEGFR2 during ischaemia can be reversed by H_2S via phosphorylation at Tyr996 of the receptor[144]. Exogenous H_2S can also increase AKT1 phosphorylation and upregulate angiogenic signalling including mitogen-activated protein kinase 1 (MAPK1), MAPK3 and MAPK11 (also known as ERK2, ERK1 and p38, respectively), which can be attenuated by MAPK inhibition, indicating a role for this pathway in H_2S-mediated angiogenesis[99].

Shear stress. Shear stress has major effects on vascular function and stimulates adaptive changes in blood vessel structure and size. Vascular endothelial cells are exposed to haemodynamic forces, which modulate their functions in health and disease. Low, or oscillatory, shear stress can promote vascular dysfunction and atherosclerosis, whereas physiological high shear stress is protective[149]. Changes in blood flow can trigger a cascade of biochemical signalling that mediates changes in biological events. Endothelial cells are crucial sensors of shear stress, but the mechanisms by which they decode complex shear stress environments to regulate physiological and pathophysiological responses are incompletely understood.

Shear stress-induced collateral vessel formation can be inhibited by blocking NO–VEGF–Rho GTPase signalling pathways and by upregulation of signalling mechanisms facilitating monocyte recruitment and attachment to the endothelium via adhesion molecules[150,151]. Our group has revealed the role of CTH and H_2S in shear stress[152]. In a *Cth*$^{-/-}$ mouse model of partial carotid ligation, reduced medial thickening and a dilated arterial phenotype was identified, indicating a defective inward vascular remodelling response (FIG. 3a). Oscillatory shear stress upregulated CTH expression and subsequent sulfane sulfur levels, which induced monocyte and macrophage recruitment into regions of disturbed flow. Importantly, a reduction in inward vascular remodelling in *Cth*$^{-/-}$ mice was associated with increased NO bioavailability that was reversed by the NO scavenger cPTIO[152]. These findings reveal that CTH expression is important in shear stress-dependent responses in atheroprone vascular regions and involves both endothelial activation and flow-dependent vascular remodelling through altered NO bioavailability. In accordance with our observations, other groups have demonstrated the role of CTH and sulfane sulfur in atherosclerosis under varied shear stress[153]. Endothelial-specific *Cth* deletion accelerated the development of endothelial dysfunction and atherosclerosis. CTH expression was upregulated in a mouse model of partial carotid artery ligation and in atheromas from human patients. However, circulating and intraplaque H_2S levels were reduced owing to Ser377 phosphorylation of CTH, which inhibits the enzyme[153] (FIG. 3b). Consistent with the loss of H_2S, human antigen R (HuR) sulfhydration was blunted in atherosclerosis, resulting in stabilization of the HuR target mRNAs encoding E-selectin and cathepsin S, both of which are linked to endothelial cell activation and atherosclerosis. CTH-derived H_2S can sulfhydrate HuR Cys13 and prevent its homodimerization and activity, thereby attenuating the expression of E-selectin and cathepsin S[153]. As such, increased E-selectin expression facilitates

monocyte adherence and recruitment under atherogenic conditions. The endothelial dysfunction and atherosclerosis associated with *Cth* deletion in endothelial cells were reversed with administration of the polysulfide donor SG1002, indicating its potential use in modulating inflammatory vascular responses[153].

Another study by the same group demonstrated the molecular mechanisms of shear stress-mediated reduction of CTH expression in human and mouse endothelial cells[154]. An inverse relationship was observed between CTH and Krüppel-like factor 2 (KLF2), which is involved in shear-stress mediated atheroprotective pathways[155]. CTH was identified as a direct target of the KLF2-regulated microRNA-27b[154]. Increased CTH expression in human plaque-derived endothelial cells also negatively correlated with KLF2 and microRNA-27b levels[154]. However, decreased CTH expression led to the loss of peroxiredoxin 6 (PRX6) Cys47 sulfhydration causing PRX6 hyperoxidation and inhibition, which subsequently increased endothelial ROS and lipid membrane peroxidation. These effects were reversed by polysulfide supplementation[154]. Additionally, statin therapy, which can activate KLF2, decreased CTH expression and increased CTH activity, thereby preventing phosphorylation of CTH at Ser377 and partially restoring PRX6 sulfhydration in plaque specimens from arteries of statin-treated patients[152].

In 2021, the same group of researchers reported mechanotransduction signalling changes via proteome *S*-sulfhydration in the setting of atherosclerotic vascular dysfunction[77]. In this study, 3,446 cysteine residues from 1,591 proteins in endothelial cells that can influence vascular reactivity were analysed. *S*-sulfhydration of β3 integrin was required for mechanotransduction in native endothelial cells isolated from mouse and human vessels. Exogenous sulfide treatment with SG1002 resulfhydrated endothelial cell proteins and β3 integrin, partially restoring endothelial cell function and vascular blood flow[77]. These observations indicate a potential role for polysulfide therapeutics in rectifying vascular function in human vascular disease.

Vascular barrier function. Vascular permeability and endothelial selective molecular sieving are crucial for several physiological functions, including tissue–fluid homeostasis, angiogenesis and vessel tone[156]. Regulated passage of macromolecules between the blood and interstitial space is important for physiological homeostasis. Vascular hyperpermeability is associated with numerous physiological and pathophysiological processes, such as inflammation, tumorigenesis, ischaemic injury, wound healing, and vascular growth and remodelling[157]. As discussed above, CTH and H_2S have important regulatory roles in vessel remodelling and maintenance of cellular homeostasis, and cytotoxic effects[147,158].

Vascular permeability can be increased via upregulation of VEGF and extracellular matrix signalling pathways, which causes endothelial contraction and junction protein disruption, resulting in intercellular gaps with greater permeability[159]. H_2S therapy inhibits vascular hyperpermeability and endothelial blood–brain barrier disruption in mice undergoing cardiac arrest. Treatment with exogenous H_2S was shown to decrease matrix metalloproteinase 9 (MMP9) activity and VEGF expression, and increase the expression of angiogenin I, preserving the normal function of the blood–brain barrier[160]. A study of ethanol-induced toxicity in mouse brain endothelial cells demonstrated the protective effects of H_2S on endothelial hyperpermeability[161]. In a subarachnoid haemorrhage model, NaHS therapy attenuated brain oedema, blood–brain barrier disruption and cerebral vasospasm[162]. In addition, exogenous H_2S was shown to reduce vascular protein leakage and leukocyte infiltration in a mouse model of particulate matter-induced lung inflammation[163].

Our group has shown that H_2S and polysulfides regulate permeability and barrier function in mouse aortic endothelial cells[164]. Reduction of CTH expression in either $Cth^{-/-}$ cells or via small interfering RNA inhibition resulted in tighter endothelial barrier function. Genetic loss of CTH expression and reduced bound sulfane sulfur levels prevented VEGF-mediated permeability in vivo. Importantly, the reduction in CTH and sulfide metabolite levels augmented claudin 5 expression and enhanced tight junction arrangement, contributing to improved endothelial barrier function (FIG. 3c). Although permeability is crucial in regulating both cardiovascular and cerebrovascular homeostasis, most of the literature is currently focused on the blood–brain barrier[165,166]. Further studies investigating CTH regulation of sulfide and its metabolites on changes in endothelial solute permeability are needed to increase our understanding of the endothelial barrier dysfunction during pathophysiological conditions.

Cardiac arrhythmias. H_2S is postulated to be antiarrhythmic but, although some molecular pathways have been explored, cell studies, animal models and translational research on this hypothesis are limited. The clearest evidence so far linking H_2S and arrhythmias is the capacity of this molecule to regulate the electrical properties of cardiac tissues. H_2S modulates ion channels both directly and indirectly, leading to electrical remodelling (FIG. 3d). Ca^{2+} and Ca^{2+}-binding proteins are intrinsically involved in cardiac arrhythmias. Variants in L-type Ca^{2+} channels are linked to a variety of arrhythmias, and sulfide donors are known to inhibit L-type Ca^{2+} currents and reduce intracellular Ca^{2+} concentrations[167,168]. A decrease in action potential duration (APD) was reported with sodium hydrosulfide, facilitated by the reduction in peak L-type Ca^{2+} current and Ca^{2+} transients[101]. Although sulfide donors are also known to modulate T-type Ca^{2+} channels in the nervous system and gastrointestinal tract, no studies have been reported on the effects of H_2S on T-type Ca^{2+} currents in cardiomyocytes[114,169].

In addition to regulating voltage-gated ion channels, H_2S also affects Ca^{2+}-binding proteins. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a ubiquitous and abundant serine–threonine kinase, has emerged as an important signalling molecule in cardiac arrhythmias. CaMKII has been implicated in the mechanisms of sinus node dysfunction, atrial tachyarrhythmias and ventricular arrhythmias[170–172]. H_2S inhibits

CaMKII, thereby potentially acting as an antiarrhythmic molecule. Sulfide donors, such as sodium NaHS, inhibit CaMKII phosphorylation through its sulfhydration. Moreover, reduced levels of H_2S in *Cth*$^{-/-}$ mice have been associated with increased CaMKII activity[173].

Treatment of rat atrial myocytes with NaHS has been shown to reduce APD and decelerate the sinus rhythm[98]. Decreases in APD at 50% and 90% repolarization by NaHS were blocked by the K^+_{ATP} channel blocker glibenclamide, suggesting that sulfide-induced APD shortening is mediated by K^+_{ATP} channels[98]. This effect of NaHS on APD shortening has been replicated in rat ventricular myocytes[174]. Although the mechanism behind the effects of sulfide donors in opening the K^+_{ATP} channels is not well understood, on the basis of findings in vascular smooth muscle cells, sulfide donors are thought to cause sulfhydration of the $K_{ir}6.1$ subunit of the K^+_{ATP} channel[175].

In addition to modulating K^+_{ATP} channels, blockade of H_2S biosynthesis with DL-propargylglycine has been shown to increase angiotensin II-induced K^+_{ATP} expression in cultured atrial myocytes from neonatal rats[176]. Moreover, in the same study, 24-h rapid atrial pacing in a beagle model of atrial fibrillation (AF) increased atrial angiotensin II and K^+_{ATP} expression, which was inhibited by NaHS supplementation[70]. Although the effects of H_2S on ion channels might be the primary antiarrhythmic mechanism, H_2S can also reduce adverse structural remodelling[177]. Electrical anisotropy increases with age-related fibrosis by aiding electrotonic coupling between cardiomyocytes and fibroblasts or myofibroblasts, and can lead to electrical dissociation in the atrium and AF[178]. In cell proliferation assays with human cultured fibroblasts, NaHS reduced atrial fibroblast proliferation induced by transforming growth factor-β1, mothers against decapentaplegic homologue 3 (SMAD3) and angiotensin II[177]. Furthermore, H_2S also inhibits the differentiation of fibroblasts into myofibroblasts[177].

Diabetes increases atrial fibrosis, decreases atrial expression of the PI3K–AKT1–eNOS pathway, and increases the inducibility and duration of AF in rats[179]. These effects were inhibited by intraperitoneal injection of NaHS[179]. Our group found that *Cth*$^{-/-}$ mice with reduced levels of endogenous H_2S had increased AF inducibility and duration compared with wild-type mice, which was reversed by extrinsic supplementation with the H_2S donor diallyl trisulfide[180]. Low sulfide levels in the atria of *Cth*$^{-/-}$ mice were related to increased superoxide levels, increased frequency of atrial cell Ca^{2+} sparks, prolonged APD and atrial effective refractory period, and slower atrial conduction velocity (FIG. 3d). In a case–control analysis performed in parallel to this study, we found that patients with AF had reduced levels of acid-labile sulfide (the storage form of H_2S) compared with control individuals who had other cardiovascular conditions. We also showed a novel association between endothelial dysfunction and atrial remodelling mediated by H_2S in the pathogenesis of AF[180]. Uniquely, H_2S can also act as a paracrine signalling molecule. In the global *Cth*$^{-/-}$ mouse model of AF discussed above, transgenic reconstitution of CTH in endothelial cells reduced the atrial effective refractory period and APD, normalized the frequency of Ca^{2+} sparks, and decreased the inducibility and duration of AF[180].

H_2S has been shown to be antiarrhythmic not only in the atria; emerging research indicates that sulfide donors might also have a role in preventing life-threatening ventricular arrhythmias. NaHS was first shown to reduce the arrhythmia burden in an ex vivo model of IRI[181]. In another rat model of IRI, α-lipoic acid increased H_2S and sulfane sulfur levels, thereby reducing ventricular ectopy and sustained ventricular arrhythmias[182–184]. CTH was reported to be upregulated in the heart of rats with IRI and, interestingly, plasma H_2S levels were inversely related to the arrhythmia scores[185]. In a later study, mitochondrial sulfide donor compounds, but not global sulfide donors, reduced the incidence of ventricular arrhythmias in a rat in vivo model of ischaemia–reperfusion[186]. These studies show that intracellular and paracrine H_2S signalling can regulate electrical and structural remodelling in the heart, reducing the risk of arrhythmias mediated by various risk factors.

Sulfide therapies for CVD

As discussed in this Review, many cardiovascular conditions — including hypertension, stroke, IRI, cardiac hypertrophy and fibrosis, atherosclerosis, arrhythmias and vascular pathologies related to diabetes — can potentially be treated with H_2S[96,187–189]. Clinical studies have shown that plasma H_2S levels inversely correlate with the severity of CVD, particularly hypertension and stroke, and children with hypertension have reduced plasma H_2S levels compared with healthy children[190,191]. TABLE 1 lists interventional and observational clinical trials related to sulfide treatment for CVD.

Administration of sulfides

Many natural products and drugs in current use carry sulfur-derived functional groups. Garlic has been used for centuries in traditional medicine and contains allicin that rapidly degrades into diallyl polysulfides, which can act as H_2S donors in the presence of thiols[192]. Preclinical and clinical trials have shown that garlic consumption reduces the risk of CVD[192]. Pharmacologically, H_2S can be administered in several ways, including by direct inhalation of the gas and orally or intravenously as inorganic sulfides or natural and synthetic H_2S donors[96]. Each method has advantages and disadvantages. Inhalation of H_2S can provide targeted treatment for conditions involving pulmonary defects, but carries a risk of toxicity and flammability. Oral or intravenous administration of inorganic sulfides can be site-directed, but these compounds have short half-lives and oxidize rapidly, which limits their use. Many natural and synthetic H_2S donors have poorly understood pharmacological mechanistic effects and possible toxicities[96].

Synthetic H_2S donors

Many currently available sulfide salts, natural H_2S compounds and synthetic H_2S donors have unsuitable pharmacokinetic profiles and undergo rapid hydrolysis, releasing H_2S in an uncontrollable manner that limits their clinical utility[193]. Therefore, various novel, chemically stable and efficacious H_2S donors are being

Table 1 | **Selected interventional trials and observational studies on sulfides and CVD**

Trial name	Study type	Number of patients	Status	Study population	Main findings	Intervention	Study period (year)	Ref.
Interventional trials using sulfide donors								
Assessing the safety and ability of SG1002 to overcome deficits in hydrogen sulfide in heart failure patients	Randomized controlled trial	16	Completed	Patients with heart failure and healthy individuals	SG1002 increases H_2S and NO bioavailability	SG1002 versus placebo	2014–2015	102
Assessing the safety and bioactivity of SG1002 in heart failure patients	Randomized, double-blind, placebo-controlled trial	50	NA	Patients with heart failure	NA	Sodium polysulthionate versus placebo	2016–2018	204
Sodium thiosulfate to preserve cardiac function in STEMI	Multicentre, double-blind, randomized controlled trial	38	Active, not recruiting	Patients with MI and/or heart failure	NA	Sodium thiosulfate versus placebo	2018–2021	205
Taurine supplementation on lower extremity vasculopathy in patients with diabetes	Randomized, double-blind, placebo-controlled trial	20	NA	Patients with diabetes mellitus and/or lower-extremity artery disease	NA	Taurine versus placebo	2017–2018	206
Effects and safety of taurine granule on blood pressure in prehypertensive (ESTAB)	Randomized, double-blind, placebo-controlled trial	12	NA	Patients with prehypertension	Taurine supplementation mediated H_2S levels that reduced hypertensive effect and improved vascular function	Taurine granules versus placebo	2012–2015	207
Short-term endogenous hydrogen sulfide upregulation	Randomized clinical trial	Planned 40; actual 9	Completed	Patients with carotid stenosis and undergoing carotid endarterectomy	Dietary intervention increased abundance of sulfide-producing bacteria and was protective in patients undergoing carotid endarterectomy	Protein calorie restriction versus controlled regular diet	2017–2018	208
Effect of garlic (*Allium sativum*) on blood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease	Placebo-controlled trial (randomization unclear)	60	Completed	CAD	Polysulfides (diallyl disulfide and diallyl trisulfide) in garlic oil showed antiplatelet activity	Garlic oil versus placebo	1997	209
A randomized trial of the effects of garlic oil upon coronary heart disease risk factors in trained male runners	Randomized, double-blind, placebo-controlled trial	27	Completed	Healthy male runners aged 17–45 years	Garlic oil supplementation reduced total cholesterol and triglyceride levels, thereby lowering the risk of chronic heart disease	Garlic oil versus placebo	2000 (publication date)	210
Clinical study on effect of garlicin in stabilizing the carotid artery atherosclerotic plaque in patients with primary hypertension and coronary artery disease	Randomized controlled trial	79	Completed	Patients with primary hypertension and CAD	Garlicin is vasoprotective in patients with primary hypertension and carotid artery atherosclerotic plaque	Garlicin and fosinopril versus fosinopril alone	2006 (publication date)	211
Effect of combined supplementation of fish oil with garlic pearls on the serum lipid profile in hypercholesterolemic subjects	Controlled clinical trial (no randomization, no placebo)	32	Completed	Patients with hypercholesterolaemia	Co-administration of garlic pearls with fish oil can be effective in managing dyslipidaemia	Fish oil with garlic versus placebo	2005 (publication date)	212

Table 1 (cont.) | **Selected interventional trials and observational studies on sulfides and CVD**

Trial name	Study type	Number of patients	Status	Study population	Main findings	Intervention	Study period (year)	Ref.
Observational studies measuring sulfide metabolites								
Plasma hydrogen sulfide, nitric oxide and stress hyperglycemia in acute myocardial infarction	Prospective cohort study	Estimated 50	NA	Patients with acute MI versus patients 12 h after MI	NA	NR	2019	213
Hydrogen sulfide and peripheral arterial disease	Cross-sectional cohort study	252	Completed	Patients aged >40 years undergoing catheterization for CAD or PAD; symptomatic PAD versus asymptomatic PAD versus no PAD	Plasma-free H_2S levels are significantly elevated in acute vascular disease	NR	2011–2012	110
Measurement of distinct biological pools of hydrogen sulfide in women with cardiovascular disease	Prospective case–control study	137	Completed	Women with or without PAD or CAD, with or without CVD risk factors	Plasma-bound and total sulfide levels were significantly reduced and indicative of CVD	NR	2013–2017	18
Hydrogen sulfide and atrial fibrillation	Prospective case–control study	116	Completed	Patients aged 18–89 years with atrial fibrillation versus patients without atrial fibrillation	CTH and H_2S bioavailability regulates electrical remodelling and susceptibility to atrial fibrillation	NR	2018–2019	180

CAD, coronary artery disease; CTH, cystathionine γ-lyase; CVD, cardiovascular disease; MI, myocardial infarction; NA, not available; NR, not relevant; PAD, peripheral artery disease; STEMI, ST-segment elevation myocardial infarction.

developed[61,102,194–198]. Sodium thiosulfate is stable relative to other H_2S donors and is used for the treatment of cyanide intoxication, calcific uraemic arteriolopathy and renal toxicity induced by chemotherapy[194,196–198]. This compound could also have value in treating CVD[185–188]. For example, in mice with arteriovenous fistula-induced heart failure, treatment with sodium thiosulfate-supplemented drinking water attenuated cardiac decline and reduced the expression of MMP1, MMP9 and adenylate cyclase type 6 (REF.[197]). Sodium thiosulfate also normalized ventricular H_2S levels, which were reduced by fistula-induced heart failure, suggesting that this H_2S donor restores cardiac function partly by increasing endogenous ventricular H_2S synthesis[197]. In rats with angiotensin II-induced hypertensive heart disease, intraperitoneal injection of sodium thiosulfate attenuated hypertension, increased mRNA expression of natriuretic peptides, and reduced cardiac hypertrophy, oxidative stress, fibrosis and fibrosis-associated gene expression[198]. Similarly, in rats with chronic deficiency of NO induced by the administration of N^{ω}-nitro-L-arginine, sodium thiosulfate-supplemented drinking water improved systolic function and reduced hypertension, left ventricular hypertrophy, cardiac fibrosis and oxidative stress[196]. Interestingly, sodium thiosulfate was also cardioprotective in a rat model of cardiac ischaemia–reperfusion[195]. SG1002 is novel, α-sulfur oral formulation H_2S prodrug discussed above in this Review. In a phase I clinical trial, SG1002 was safe and well-tolerated, increased plasma H_2S and nitrite levels, and reduced B-type natriuretic peptide levels in patients with heart failure[102].

In addition, a mitochondria-targeted H_2S donor (AP39) has been developed, which stimulates mitochondrial bioenergetic functions and reduces damage induced by oxidative stress, thereby preserving cell viability, mitochondrial bioenergetics and genomic stability in endothelial cells[199]. In a mouse model of heart transplantation, AP39 significantly increased cardiomyocyte viability and protected heart graft function following prolonged cold IRI[200]. These findings suggest that AP39 could have value in preventing IRI in human heart transplantation. The development of AP39 also indicates that H_2S donors that target specific subcellular locations could have important clinical benefits. Evidence also exists that many currently available drugs could be modified through the addition of sulfur-derived functional groups. For example, an H_2S-releasing diclofenac derivative markedly suppresses gastric prostaglandin synthesis without causing the gastric mucosal damage associated with chronic administration of non-steroidal anti-inflammatory drugs[201].

Novel targets

An exciting aspect of H_2S donors and CVD lies in the many novel targets yet to be examined. For example, the mitochondrial protein mitofusin 2 is regulated by H_2S, and its dysfunction contributes to several cardiovascular pathologies, including dilated cardiomyopathy, heart failure and IRI[202,203]. Currently, no data exist on the

use of H_2S donors in mitofusin 2-related CVD, which could be an important target for future research.

Crucially, several other areas require additional investigation before H_2S donors can be used clinically. Chemically stable H_2S donors must be developed to enable long-term therapy, optimal monitoring of H_2S levels in patients must be established, H_2S-induced toxicities need to be minimized and H_2S-dependent biomarkers should be identified.

Conclusions

Sulfides are crucially involved in cardiovascular health and disease. Although much has been learned about the various roles of sulfides, their synthesis and their catabolism, the field is still striving to understand specific mechanisms, mediators and conditions in which therapeutic sulfides could affect cardiovascular pathophysiology. Many important questions remain in the field of sulfide-based therapeutics for CVD. For example, how do sulfide metabolites affect cardiovascular cell function and disease? How do sulfide-synthesizing enzymes function in specific cardiovascular cell types and under various pathological conditions? What are the key molecular targets for sulfide-dependent cytoprotection against CVD? Are these molecules robust biomarkers for measuring the clinical efficacy of sulfide therapies? Which sulfide-based therapies are most effective in the treatment of CVD? We hope that future studies will help to provide the data needed to support the clinical use of sulfides in the treatment of CVD.

Published online 5 August 2022

1. Industrial Safety and Hygiene News. Many oil & gas workers risk hydrogen sulfide overexposure. https://www.ishn.com/articles/109717-many-oil-gas-workers-risk-hydrogen-sulfide-overexposure (2018).
2. Neubeck, A. & Freund, F. Sulfur chemistry may have paved the way for evolution of antioxidants. *Astrobiology* **20**, 670–675 (2020).
3. Olson, K. R. & Straub, K. D. The role of hydrogen sulfide in evolution and the evolution of hydrogen sulfide in metabolism and signaling. *Physiology* **31**, 60–72 (2016).
4. Kolluru, G. K., Shen, X., Bir, S. C. & Kevil, C. G. Hydrogen sulfide chemical biology: pathophysiological roles and detection. *Nitric Oxide* **35**, 5–20 (2013).
5. Kolluru, G. K., Shen, X. & Kevil, C. G. A tale of two gases: NO and H2S, foes or friends for life? *Redox Biol.* **1**, 313–318 (2013).
6. Abe, K. & Kimura, H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* **16**, 1066–1071 (1996).
7. Szabo, C. A timeline of hydrogen sulfide (H(2)S) research: from environmental toxin to biological mediator. *Biochem. Pharmacol.* **149**, 5–19 (2018).
8. Wu, D. et al. Role of hydrogen sulfide in ischemia-reperfusion Injury. *Oxid. Med. Cell Longev.* **2015**, 186908 (2015).
9. Liu, Y. H. et al. Hydrogen sulfide in the mammalian cardiovascular system. *Antioxid. Redox Signal.* **17**, 141–185 (2012).
10. LaPenna, K. B. et al. Hydrogen sulfide as a potential therapy for heart failure–past, present, and future. *Antioxidants* **10**, 485 (2021).
11. Pan, L.-L., Qin, M., Liu, X.-H. & Zhu, Y.-Z. The role of hydrogen sulfide on cardiovascular homeostasis: an overview with update on immunomodulation. *Front. Pharmacol.* **8**, 686 (2017).
12. Szabo, C. Hydrogen sulfide, an enhancer of vascular nitric oxide signaling: mechanisms and implications. *Am. J. Physiol. Cell Physiol.* **312**, C3–C15 (2017).
13. Kolluru, G. K. et al. Cystathionine γ-lyase regulates arteriogenesis through NO-dependent monocyte recruitment. *Cardiovasc. Res.* **107**, 590–600 (2015).
14. Bir, S. C. et al. Hydrogen sulfide stimulates ischemic vascular remodeling through nitric oxide synthase and nitrite reduction activity regulating hypoxia-inducible factor-1α and vascular endothelial growth factor-dependent angiogenesis. *J. Am. Heart Assoc.* **1**, e004093 (2012).
15. Kolluru, G. K., Shen, X. & Kevil, C. G. Reactive sulfur species: a new redox player in cardiovascular pathophysiology. *Arterioscler. Thromb. Vasc. Biol.* **40**, 874–884 (2020).
16. Shen, X., Kolluru, G. K., Yuan, S. & Kevil, C. G. Measurement of H2S in vivo and in vitro by the monobromobimane method. *Methods Enzymol.* **554**, 31–45 (2015).
17. Shen, X., Peter, E. A., Bir, S., Wang, R. & Kevil, C. G. Analytical measurement of discrete hydrogen sulfide pools in biological specimens. *Free Radic. Biol. Med.* **52**, 2276–2283 (2012).
18. Rajpal, S. et al. Total sulfane sulfur bioavailability reflects ethnic and gender disparities in cardiovascular disease. *Redox Biol.* **15**, 480–489 (2018).
19. Cuevasanta, E. et al. Reaction of hydrogen sulfide with disulfide and sulfenic acid to form the strongly nucleophilic persulfide. *J. Biol. Chem.* **290**, 26866–26880 (2015).
20. Dittmer, D. C. Hydrogen sulfide. *Encyclopedia of Reagents for Organic Synthesis* (Wiley, 2001) https://doi.org/10.1002/047084289X.rh049.
21. Li, Q. & Lancaster, J. R. Jr. Chemical foundations of hydrogen sulfide biology. *Nitric Oxide* **35**, 21–34 (2013).
22. Fukuto, J. M. et al. Biological hydropersulfides and related polysulfides–a new concept and perspective in redox biology. *FEBS Lett.* **592**, 2140–2152 (2018).
23. Sawa, T., Motohashi, H., Ihara, H. & Akaike, T. Enzymatic regulation and biological functions of reactive cysteine persulfides and polysulfides. *Biomolecules* **10**, 1245 (2020).
24. Sun, H. J., Wu, Z. Y., Nie, X. W. & Bian, J. S. Role of hydrogen sulfide and polysulfides in neurological diseases: focus on protein S-persulfidation. *Curr. Neuropharmacol.* **19**, 868–884 (2021).
25. Yang, J. et al. Non-enzymatic hydrogen sulfide production from cysteine in blood is catalyzed by iron and vitamin B6. *Commun. Biol.* **2**, 194 (2019).
26. Shibuya, N. et al. A novel pathway for the production of hydrogen sulfide from D-cysteine in mammalian cells. *Nat. Commun.* **4**, 1366 (2013).
27. Banerjee, R. Catalytic promiscuity and heme-dependent redox regulation of H2S synthesis. *Curr. Opin. Chem. Biol.* **37**, 115–121 (2017).
28. Yang, G. et al. H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine γ-lyase. *Science* **322**, 587–590 (2008).
29. Zhao, W., Zhang, J., Lu, Y. & Wang, R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. *EMBO J.* **20**, 6008–6016 (2001).
30. Ida, T. et al. Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling. *Proc. Natl Acad. Sci. USA* **111**, 7606–7611 (2014).
31. Kimura, H. Physiological roles of hydrogen sulfide and polysulfides. *Handb. Exp. Pharmacol.* **230**, 61–81 (2015).
32. Toohey, J. I. Sulphane sulphur in biological systems: a possible regulatory role. *Biochem. J.* **264**, 625–632 (1989).
33. Akaike, T. et al. Cysteinyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics. *Nat. Commun.* **8**, 1177 (2017).
34. Fujii, S., Sawa, T., Motohashi, H. & Akaike, T. Persulfide synthases that are functionally coupled with translation mediate sulfur respiration in mammalian cells. *Br. J. Pharmacol.* **176**, 607–615 (2019).
35. Kaneko, Y., Kimura, Y., Kimura, H. & Niki, I. l-Cysteine inhibits insulin release from the pancreatic β-cell: possible involvement of metabolic production of hydrogen sulfide, a novel gasotransmitter. *Diabetes* **55**, 1391–1397 (2006).
36. Teng, H. et al. Oxygen-sensitive mitochondrial accumulation of cystathionine β-synthase mediated by Lon protease. *Proc. Natl Acad. Sci. USA* **110**, 12679–12684 (2013).
37. Yang, G. & Wang, R. H2S and blood vessels: an overview. *Handb. Exp. Pharmacol.* **230**, 85–110 (2015).
38. Fu, M. et al. Hydrogen sulfide (H2S) metabolism in mitochondria and its regulatory role in energy production. *Proc. Natl Acad. Sci. USA* **109**, 2943–2948 (2012).
39. Wróbel, M., Włodek, L. & Srebro, Z. Sulfurtransferases activity and the level of low-molecular-weight thiols and sulfane sulfur compounds in cortex and brain stem of mouse. *Neurobiology* **4**, 217–222 (1996).
40. Eto, K., Ogasawara, M., Umemura, K., Nagai, Y. & Kimura, H. Hydrogen sulfide is produced in response to neuronal excitation. *J. Neurosci.* **22**, 3386–3391 (2002).
41. Jiang, Z. et al. Role of hydrogen sulfide in early blood-brain barrier disruption following transient focal cerebral ischemia. *PLoS ONE* **10**, e0117982 (2015).
42. Cao, X. et al. A review of hydrogen sulfide synthesis, metabolism, and measurement: is modulation of hydrogen sulfide a novel therapeutic for cancer? *Antioxid. Redox Signal.* **31**, 1–38 (2019).
43. Huang, C. W. & Moore, P. K. H2S synthesizing enzymes: biochemistry and molecular aspects. *Handb. Exp. Pharmacol.* **230**, 3–25 (2015).
44. Roman, H. B. et al. The cysteine dioxgenase knockout mouse: altered cysteine metabolism in nonhepatic tissues leads to excess H2S/HS$^-$ production and evidence of pancreatic and lung toxicity. *Antioxid. Redox Signal.* **19**, 1321–1336 (2013).
45. Tiranti, V. et al. Loss of ETHE1, a mitochondrial dioxygenase, causes fatal sulfide toxicity in ethylmalonic encephalopathy. *Nat. Med.* **15**, 200–205 (2009).
46. Donnarumma, E., Trivedi, R. K. & Lefer, D. J. Protective actions of H2S in acute myocardial infarction and heart failure. *Compr. Physiol.* **7**, 583–602 (2017).
47. Rose, P., Moore, P. K. & Zhu, Y. Z. H2S biosynthesis and catabolism: new insights from molecular studies. *Cell Mol. Life Sci.* **74**, 1391–1412 (2017).
48. Gharahbaghian, L., Massoudian, B. & Dimassa, G. Methemoglobinemia and sulfhemoglobinemia in two pediatric patients after ingestion of hydroxylamine sulfate. *West. J. Emerg. Med.* **10**, 197–201 (2009).
49. Kouroussis, E., Adhikari, B., Zivanovic, J. & Filipovic, M. R. Measurement of protein persulfidation: improved tag-switch method. *Methods Mol. Biol.* **2007**, 37–50 (2019).
50. Li, B., Kim, Y. L. & Lippert, A. R. Chemiluminescence measurement of reactive sulfur and nitrogen species. *Antioxid. Redox Signal.* **36**, 337–353 (2022).
51. Nagy, P., Doka, E., Ida, T. & Akaike, T. Measuring reactive sulfur species and thiol oxidation states: challenges and cautions in relation to alkylation-based protocols. *Antioxid. Redox Signal.* **33**, 1174–1189 (2020).
52. Takata, T. et al. Methods in sulfide and persulfide research. *Nitric Oxide* **116**, 47–64 (2021).
53. Shen, X., Chakraborty, S., Dugas, T. R. & Kevil, C. G. Hydrogen sulfide measurement using sulfide dibimane: critical evaluation with electrospray ion trap mass spectrometry. *Nitric Oxide* **41**, 97–104 (2014).
54. Kolluru, G. K., Shen, X. & Kevil, C. G. Detection of hydrogen sulfide in biological samples: current and future. *Expert. Rev. Clin. Pharmacol.* **4**, 9–12 (2011).
55. Nagy, P. et al. Chemical aspects of hydrogen sulfide measurements in physiological samples. *Biochim. Biophys. Acta* **1840**, 876–891 (2014).

56. Li, L., Hsu, A. & Moore, P. K. Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation–a tale of three gases! *Pharmacol. Ther.* **123**, 386–400 (2009).
57. Li, L., Rose, P. & Moore, P. K. Hydrogen sulfide and cell signaling. *Annu. Rev. Pharmacol. Toxicol.* **51**, 169–187 (2011).
58. Szabo, C. Hydrogen sulphide and its therapeutic potential. *Nat. Rev. Drug Discov.* **6**, 917–935 (2007).
59. Shao, M. et al. Protective effect of hydrogen sulphide against myocardial hypertrophy in mice. *Oncotarget* **8**, 22344–22352 (2017).
60. King, A. L. et al. Hydrogen sulfide cytoprotective signaling is endothelial nitric oxide synthase-nitric oxide dependent. *Proc. Natl Acad. Sci. USA* **111**, 3182–3187 (2014).
61. Kondo, K. et al. H_2S protects against pressure overload-induced heart failure via upregulation of endothelial nitric oxide synthase. *Circulation* **127**, 1116–1127 (2013).
62. Mani, S., Untereiner, A., Wu, L. & Wang, R. Hydrogen sulfide and the pathogenesis of atherosclerosis. *Antioxid. Redox Signal.* **20**, 805–817 (2014).
63. Lin, Y. et al. Hydrogen sulfide attenuates atherosclerosis in a partially ligated carotid artery mouse model via regulating angiotensin converting enzyme 2 expression. *Front. Physiol.* **8**, 782 (2017).
64. Zhang, H. et al. Hydrogen sulfide inhibits the development of atherosclerosis with suppressing CX3CR1 and CX3CL1 expression. *PLoS ONE* **7**, e41147 (2012).
65. Casin, K. M. & Calvert, J. W. Harnessing the benefits of endogenous hydrogen sulfide to reduce cardiovascular disease. *Antioxidants* **10**, 383 (2021).
66. Corvino, A. et al. Trends in H2S-donors chemistry and their effects in cardiovascular diseases. *Antioxidants* **10**, 429 (2021).
67. Wilkie, S. E., Borland, G., Carter, R. N., Morton, N. M. & Selman, C. Hydrogen sulfide in ageing, longevity and disease. *Biochem. J.* **478**, 3485–3504 (2021).
68. Elrod, J. W. et al. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proc. Natl Acad. Sci. USA* **104**, 15560–15565 (2007).
69. Hsu, C.-N. & Tain, Y.-L. Preventing developmental origins of cardiovascular disease: hydrogen sulfide as a potential target? *Antioxidants* **10**, 247 (2021).
70. Jiang, H. L., Wu, H. C., Li, Z. L., Geng, B. & Tang, C. S. Changes of the new gaseous transmitter H2S in patients with coronary heart disease [Chinese]. *Di Yi Jun. Yi Da Xue Xue Bao* **25**, 951–954 (2005).
71. Polhemus, D. J., Calvert, J. W., Butler, J. & Lefer, D. J. The cardioprotective actions of hydrogen sulfide in acute myocardial infarction and heart failure. *Scientifica* **2014**, 768607 (2014).
72. Gorini, F., Bustaffa, E., Chatzianagnostou, K., Bianchi, F. & Vassalle, C. Hydrogen sulfide and cardiovascular disease: doubts, clues, and interpretation difficulties from studies in geothermal areas. *Sci. Total. Environ.* **743**, 140818 (2020).
73. Li, Z., Polhemus, D. J. & Lefer, D. J. Evolution of hydrogen sulfide therapeutics to treat cardiovascular disease. *Circ. Res.* **123**, 590–600 (2018).
74. Zhang, L. et al. Hydrogen sulfide (H2S)-releasing compounds: therapeutic potential in cardiovascular diseases. *Front. Pharmacol.* **9**, 1066 (2018).
75. Paul, B. D. & Snyder, S. H. H2S: a novel gasotransmitter that signals by sulfhydration. *Trends Biochem. Sci.* **40**, 687–700 (2015).
76. Paul, B. D., Snyder, S. H. & Kashfi, K. Effects of hydrogen sulfide on mitochondrial function and cellular bioenergetics. *Redox Biol.* **38**, 101772 (2021).
77. Bibli, S.-I. et al. Mapping the endothelial cell *S*-sulfhydrome highlights the crucial role of integrin sulfhydration in vascular function. *Circulation* **143**, 935–948 (2021).
78. Merz, T. et al. Cardiovascular disease and resuscitated septic shock lead to the downregulation of the H2S-producing enzyme cystathionine-γ-lyase in the porcine coronary artery. *Intensive Care Med. Exp.* **5**, 17 (2017).
79. Giannakopoulou, E. et al. Association study of the *CTH* 1364 G>T polymorphism with coronary artery disease in the Greek population. *Drug Metab. Pers. Ther.* https://doi.org/10.1515/dmpt-2018-0033 (2019).
80. Ghaderi, S. et al. Role of glycogen synthase kinase following myocardial infarction and ischemia–reperfusion. *Apoptosis* **22**, 887–897 (2017).
81. Dhalla, N. S., Elmoselhi, A. B., Hata, T. & Makino, N. Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc. Res.* **47**, 446–456 (2000).
82. Yellon, D. M. & Hausenloy, D. J. Myocardial reperfusion Injury. *N. Engl. J. Med.* **357**, 1121–1135 (2007).
83. Liu, J., Wang, H. & Li, J. Inflammation and inflammatory cells in myocardial infarction and reperfusion injury: a double-edged sword. *Clin. Med. Insights Cardiol.* **10**, 79–84 (2016).
84. Swirski, F. K. & Nahrendorf, M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. *Science* **339**, 161–166 (2013).
85. Calvert, J. W. et al. Genetic and pharmacologic hydrogen sulfide therapy attenuates ischemia-induced heart failure in mice. *Circulation* **122**, 11–19 (2010).
86. Polhemus, D. J. et al. Hydrogen sulfide attenuates cardiac dysfunction after heart failure via induction of angiogenesis. *Circulation Heart Fail.* **6**, 1077–1086 (2013).
87. Osipov, R. M. et al. Effect of hydrogen sulfide in a porcine model of myocardial ischemia-reperfusion: comparison of different administration regimens and characterization of the cellular mechanisms of protection. *J. Cardiovasc. Pharmacol.* **54**, 287–297 (2009).
88. Shymans'ka, T. V., Hoshovs'ka Iu, V., Semenikhina, O. M. & Sahach, V. F. Effect of hydrogen sulfide on isolated rat heart reaction under volume load and ischemia-reperfusion [Ukranian]. *Fiziol. Zh* . **58**, 57–66 (2012).
89. Ji, Y. et al. Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia-reperfusion injury. *Eur. J. Pharmacol.* **587**, 1–7 (2008).
90. Luan, H. F. et al. Hydrogen sulfide postconditioning protects isolated rat hearts against ischemia and reperfusion injury mediated by the JAK2/STAT3 survival pathway. *Braz. J. Med. Biol. Res.* **45**, 898–905 (2012).
91. Xia, H. et al. Endothelial cell cystathionine γ-lyase expression level modulates exercise capacity, vascular function, and myocardial ischemia reperfusion injury. *J. Am. Heart Assoc.* **9**, e017544 (2020).
92. Zhang, P. et al. Role of hydrogen sulfide in myocardial ischemia-reperfusion injury. *J. Cardiovasc. Pharmacol.* **77**, 130–141 (2021).
93. Huang, C. et al. Cardioprotective effects of a novel hydrogen sulfide agent-controlled release formulation of S-propargyl-cysteine on heart failure rats and molecular mechanisms. *PLoS ONE* **8**, e69205 (2013).
94. Shen, Y., Shen, Z., Luo, S., Guo, W. & Zhu, Y. Z. The cardioprotective effects of hydrogen sulfide in heart diseases: from molecular mechanisms to therapeutic potential. *Oxid. Med. Cell. Longev.* **2015**, 925167 (2015).
95. Cheng, Z. & Kishore, R. Potential role of hydrogen sulfide in diabetes-impaired angiogenesis and ischemic tissue repair. *Redox Biol.* **37**, 101704 (2020).
96. Wang, Y. Z. et al. The potential of hydrogen sulfide donors in treating cardiovascular diseases. *Int. J. Mol. Sci.* **22**, 2194 (2021).
97. Ling, K. et al. H(2)S attenuates oxidative stress via Nrf2/NF-κB signaling to regulate restenosis after percutaneous transluminal angioplasty. *Exp. Biol. Med.* **246**, 226–239 (2021).
98. Abramochkin, D. V., Moiseenko, L. S. & Kuzmin, V. S. The effect of hydrogen sulfide on electrical activity of rat atrial myocardium. *Bull. Exp. Biol. Med.* **147**, 683–686 (2009).
99. Papapetropoulos, A. et al. Hydrogen sulfide is an endogenous stimulator of angiogenesis. *Proc. Natl Acad. Sci. USA* **106**, 21972–21977 (2009).
100. Sivarajah, A., McDonald, M. C. & Thiemermann, C. The production of hydrogen sulfide limits myocardial ischemia and reperfusion injury and contributes to the cardioprotective effects of preconditioning with endotoxin, but not ischemia in the rat. *Shock* **26**, 154–161 (2006).
101. Sun, Y. G. et al. Hydrogen sulphide is an inhibitor of L-type calcium channels and mechanical contraction in rat cardiomyocytes. *Cardiovasc. Res.* **79**, 632–641 (2008).
102. Polhemus, D. J. et al. A novel hydrogen sulfide prodrug, SG1002, promotes hydrogen sulfide and nitric oxide bioavailability in heart failure patients. *Cardiovasc. Ther.* **33**, 216–226 (2015).
103. Drazner, M. H. The progression of hypertensive heart disease. *Circulation* **123**, 327–334 (2011).
104. Lu, F. et al. Exogenous hydrogen sulfide prevents cardiomyocyte apoptosis from cardiac hypertrophy induced by isoproterenol. *Mol. Cell. Biochem.* **381**, 41–50 (2013).
105. Ellmers, L. J. et al. Hydrogen sulfide treatment improves post-infarct remodeling and long-term cardiac function in CSE knockout and wild-type mice. *Int. J. Mol. Sci.* **21**, 4284 (2020).
106. Peleli, M. et al. Cardiovascular phenotype of mice lacking 3-mercaptopyruvate sulfurtransferase. *Biochem. Pharmacol.* **176**, 113833 (2020).
107. Deanfield, J. E., Halcox, J. P. & Rabelink, T. J. Endothelial function and dysfunction: testing and clinical relevance. *Circulation* **115**, 1285–1295 (2007).
108. Rajendran, P. et al. The vascular endothelium and human diseases. *Int. J. Biol. Sci.* **9**, 1057–1069 (2013).
109. Matsuzawa, Y. & Lerman, A. Endothelial dysfunction and coronary artery disease: assessment, prognosis, and treatment. *Coron. Artery Dis.* **25**, 713–724 (2014).
110. Peter, E. A. et al. Plasma free H2S levels are elevated in patients with cardiovascular disease. *J. Am. Heart Assoc.* **2**, e000387 (2013).
111. Pardue, S. et al. Hydrogen sulfide stimulates xanthine oxidoreductase conversion to nitrite reductase and formation of NO. *Redox Biol.* **34**, 101447 (2020).
112. Polhemus, D. J. & Lefer, D. J. Emergence of hydrogen sulfide as an endogenous gaseous signaling molecule in cardiovascular disease. *Circ. Res.* **114**, 730–737 (2014).
113. Bucci, M. et al. cGMP-dependent protein kinase contributes to hydrogen sulfide-stimulated vasorelaxation. *PLoS ONE* **7**, e53319 (2012).
114. Avanzato, D. et al. Role of calcium channels in the protective effect of hydrogen sulfide in rat cardiomyoblasts. *Cell Physiol. Biochem.* **33**, 1205–1214 (2014).
115. Elies, J. et al. Hydrogen sulfide inhibits $Ca_v3.2$ T-type Ca^{2+} channels. *FASEB J.* **28**, 5376–5387 (2014).
116. Sun, Y., Tang, C. S., Jin, H. F. & Du, J. B. The vasorelaxing effect of hydrogen sulfide on isolated rat aortic rings versus pulmonary artery rings. *Acta Pharmacol. Sin.* **32**, 456–464 (2011).
117. Xiong, Y. et al. ZYZ-803, a novel hydrogen sulfide-nitric oxide conjugated donor, promotes angiogenesis via cross-talk between STAT3 and CaMKII. *Acta Pharmacol. Sin.* **41**, 218–228 (2020).
118. Materazzi, S. et al. Vasodilator activity of hydrogen sulfide (H2S) in human mesenteric arteries. *Microvasc. Res.* **109**, 38–44 (2017).
119. Caprnda, M. et al. H2S causes contraction and relaxation of major arteries of the rabbit. *Biomed. Pharmacother.* **89**, 56–60 (2017).
120. Altaany, Z., Ju, Y., Yang, G. & Wang, R. The coordination of S-sulfhydration, S-nitrosylation, and phosphorylation of endothelial nitric oxide synthase by hydrogen sulfide. *Sci. Signal.* **7**, ra87 (2014).
121. Greiner, R. et al. Polysulfides link H2S to protein thiol oxidation. *Antioxid. Redox Signal.* **19**, 1749–1765 (2013).
122. Nishida, M. et al. Hydrogen sulfide anion regulates redox signaling via electrophile sulfhydration. *Nat. Chem. Biol.* **8**, 714–724 (2012).
123. Stubbert, D. et al. Protein kinase G Iα oxidation paradoxically underlies blood pressure lowering by the reductant hydrogen sulfide. *Hypertension* **64**, 1344–1351 (2014).
124. Sun, Y. et al. Sulfhydration-associated phosphodiesterase 5A dimerization mediates vasorelaxant effect of hydrogen sulfide. *Oncotarget* **8**, 31888–31900 (2017).
125. Coletta, C. et al. Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. *Proc. Natl Acad. Sci. USA* **109**, 9161–9166 (2012).
126. Citi, V. et al. Role of hydrogen sulfide in endothelial dysfunction: pathophysiology and therapeutic approaches. *J. Adv. Res.* **27**, 99–113 (2021).
127. Tang, G. et al. H_2S is an endothelium-derived hyperpolarizing factor. *Antioxid. Redox Signal.* **19**, 1634–1646 (2013).
128. Suzuki, K. et al. Hydrogen sulfide replacement therapy protects the vascular endothelium in hyperglycemia by preserving mitochondrial function. *Proc. Natl Acad. Sci. USA* **108**, 13829–13834 (2011).
129. Wang, Z.-J., Wu, J., Guo, W. & Zhu, Y.-Z. Atherosclerosis and the hydrogen sulfide signaling pathway–therapeutic approaches to disease prevention. *Cell. Physiol. Biochem.* **42**, 859–875 (2017).
130. Barton, M. & Meyer, M. R. HuR-ry up. *Circulation* **139**, 115–118 (2019).

131. Wang, Y. et al. Role of hydrogen sulfide in the development of atherosclerotic lesions in apolipoprotein E knockout mice. *Arterioscler. Thromb. Vasc. Biol.* **29**, 173–179 (2009).
132. Ford, A., Al-Magableh, M., Gaspari, T. A. & Hart, J. L. Chronic NaHS treatment is vasoprotective in high-fat-fed ApoE(-/-) mice. *Int. J. Vasc. Med.* **2013**, 915983 (2013).
133. Lei, Y. P., Chen, H. W., Sheen, L. Y. & Lii, C. K. Diallyl disulfide and diallyl trisulfide suppress oxidized LDL-induced vascular cell adhesion molecule and E-selectin expression through protein kinase A- and B-dependent signaling pathways. *J. Nutr.* **138**, 996–1003 (2008).
134. Lei, Y. P., Liu, C. T., Sheen, L. Y., Chen, H. W. & Lii, C. K. Diallyl disulfide and diallyl trisulfide protect endothelial nitric oxide synthase against damage by oxidized low-density lipoprotein. *Mol. Nutr. Food Res.* **54** (Suppl 1), S42–S52 (2010).
135. Yang, Q. & He, G.-W. Imbalance of homocysteine and H(2)S: significance, mechanisms, and therapeutic promise in vascular injury. *Oxid. Med. Cell. Longev.* **2019**, 7629673 (2019).
136. Ganguly, P. & Alam, S. F. Role of homocysteine in the development of cardiovascular disease. *Nutr. J.* **14**, 6 (2015).
137. Tinelli, C., Di Pino, A., Ficulle, E., Marcelli, S. & Feligioni, M. Hyperhomocysteinemia as a risk factor and potential nutraceutical target for certain pathologies. *Front. Nutr.* **6**, 49 (2019).
138. Sen, U., Mishra, P. K., Tyagi, N. & Tyagi, S. C. Homocysteine to hydrogen sulfide or hypertension. *Cell Biochem. Biophys.* **57**, 49–58 (2010).
139. Steed, M. M. & Tyagi, S. C. Mechanisms of cardiovascular remodeling in hyperhomocysteinemia. *Antioxid. Redox Signal.* **15**, 1927–1943 (2011).
140. Tian, D. et al. Endogenous hydrogen sulfide improves vascular remodeling through PPARδ/SOCS3 signaling. *J. Adv. Res.* **27**, 115–125 (2021).
141. Li, Y., Kinzenbaw, D. A., Modrick, M. L., Pewe, L. L. & Faraci, F. M. Context-dependent effects of SOCS3 in angiotensin II-induced vascular dysfunction and hypertension in mice: mechanisms and role of bone marrow-derived cells. *Am. J. Physiol. Heart Circ. Physiol.* **311**, H146–H156 (2016).
142. Wilson, H. M. SOCS proteins in macrophage polarization and function. *Front. Immunol.* **5**, 357 (2014).
143. Cai, W.-J. et al. The novel proangiogenic effect of hydrogen sulfide is dependent on Akt phosphorylation. *Cardiovasc. Res.* **76**, 29–40 (2007).
144. Wang, M. J. et al. The hydrogen sulfide donor NaHS promotes angiogenesis in a rat model of hind limb ischemia. *Antioxid. Redox Signal.* **12**, 1065–1077 (2010).
145. Ouma, G. O., Zafrir, B., Mohler, E. R. 3rd & Flugelman, M. Y. Therapeutic angiogenesis in critical limb ischemia. *Angiology* **64**, 466–480 (2013).
146. Annex, B. H. & Cooke, J. P. New directions in therapeutic angiogenesis and arteriogenesis in peripheral arterial disease. *Circulation Res.* **128**, 1944–1957 (2021).
147. Rajendran, S., Shen, X., Glawe, J., Kolluru, G. K. & Kevil, C. G. Nitric oxide and hydrogen sulfide regulation of ischemic vascular growth and remodeling. *Compr. Physiol.* **9**, 1213–1247 (2019).
148. Hoefer, I. E. Something is rotten in the state of angiogenesis–H2S as gaseous stimulator of angiogenesis. *Cardiovasc. Res.* **76**, 1–2 (2007).
149. Chiu, J. J. & Chien, S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. *Physiol. Rev.* **91**, 327–387 (2011).
150. Shyy, Y. J., Hsieh, H. J., Usami, S. & Chien, S. Fluid shear stress induces a biphasic response of human monocyte chemotactic protein 1 gene expression in vascular endothelium. *Proc. Natl Acad. Sci. USA* **91**, 4678–4682 (1994).
151. Rao, R. M., Yang, L., Garcia-Cardena, G. & Luscinskas, F. W. Endothelial-dependent mechanisms of leukocyte recruitment to the vascular wall. *Circ. Res.* **101**, 234–247 (2007).
152. Yuan, S. et al. Cystathionine γ-lyase modulates flow-dependent vascular remodeling. *Arterioscler. Thromb. Vasc. Biol.* **38**, 2126–2136 (2018).
153. Bibli, S. I. et al. Cystathionine γ-lyase sulfhydrates the RNA binding protein human antigen R to preserve endothelial cell function and delay atherogenesis. *Circulation* **139**, 101–114 (2019).
154. Bibli, S.-I. et al. Shear stress regulates cystathionine γ lyase expression to preserve endothelial redox balance and reduce membrane lipid peroxidation. *Redox Biol.* **28**, 101379 (2020).
155. Tsioufis C., Mantzouranis E., Kalos T., Konstantinidis D. & Tousoulis D. in *Coronary Artery Disease* Ch. 1.4 (ed. Tousoulis, D.) 43–66 (Academic Press, 2018).
156. Sukriti, S., Tauseef, M., Yazbeck, P. & Mehta, D. Mechanisms regulating endothelial permeability. *Pulm. Circ.* **4**, 535–551 (2014).
157. Yuan, S. & Kevil, C. G. Nitric oxide and hydrogen sulfide regulation of ischemic vascular remodeling. *Microcirculation* **23**, 134–145 (2016).
158. Lv, B. et al. Hydrogen sulfide and vascular regulation–an update. *J. Adv. Res.* **27**, 85–97 (2021).
159. Claesson-Welsh, L., Dejana, E. & McDonald, D. M. Permeability of the endothelial barrier: identifying and reconciling controversies. *Trends Mol. Med.* **27**, 314–331 (2021).
160. Geng, Y. et al. Hydrogen sulfide inhalation decreases early blood–brain barrier permeability and brain edema induced by cardiac arrest and resuscitation. *J. Cereb. Blood Flow. Metab.* **35**, 494–500 (2015).
161. Behera, J., Kelly, K. E. & Tyagi, N. Hydrogen sulfide prevents ethanol-induced ZO-1 CpG promoter hypermethylation-dependent vascular permeability via miR-218/DNMT3a axis. *J. Cell Physiol.* **236**, 6852–6867 (2021).
162. Cui, Y. et al. Hydrogen sulfide ameliorates early brain injury following subarachnoid hemorrhage in rats. *Mol. Neurobiol.* **53**, 3646–3657 (2016).
163. Wang, T. et al. Hydrogen sulfide attenuates particulate matter-induced human lung endothelial barrier disruption via combined reactive oxygen species scavenging and Akt activation. *Am. J. Respir. Cell Mol. Biol.* **47**, 491–496 (2012).
164. Yuan, S. et al. Hydrogen sulfide metabolism regulates endothelial solute barrier function. *Redox Biol.* **9**, 157–166 (2016).
165. Suzuki, Y., Nagai, N. & Umemura, K. A review of the mechanisms of blood–brain barrier permeability by tissue-type plasminogen activator treatment for cerebral ischemia. *Front. Cell. Neurosci.* **10**, 2 (2016).
166. Kadry, H., Noorani, B. & Cucullo, L. A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity. *Fluids Barriers CNS* **17**, 69 (2020).
167. Dai, L. et al. Hydrogen sulfide inhibited L-type calcium channels (CaV1.2) via up-regulation of the channel sulfhydration in vascular smooth muscle cells. *Eur. J. Pharmacol.* **858**, 172455 (2019).
168. Zhang, Q., Chen, J., Qin, Y., Wang, J. & Zhou, L. Mutations in voltage-gated L-type calcium channel: implications in cardiac arrhythmia. *Channels* **12**, 201–218 (2018).
169. Munaron, L., Avanzato, D., Moccia, F. & Mancardi, D. Hydrogen sulfide as a regulator of calcium channels. *Cell Calcium* **53**, 77–84 (2013).
170. Swaminathan, P. D. et al. Oxidized CaMKII causes cardiac sinus node dysfunction in mice. *J. Clin. Invest* **121**, 3277–3288 (2011).
171. Purohit, A. et al. Oxidized Ca(2+)/calmodulin-dependent protein kinase II triggers atrial fibrillation. *Circulation* **128**, 1748–1757 (2013).
172. Swaminathan, P. D. & Anderson, M. E. CaMKII inhibition: breaking the cycle of electrical storm? *Circulation* **123**, 2183–2186 (2011).
173. Wu, D. et al. Amelioration of mitochondrial dysfunction in heart failure through S-sulfhydration of Ca(2+)/calmodulin-dependent protein kinase II. *Redox Biol.* **19**, 250–262 (2018).
174. Zhong, G. Z. et al. Hydrogen sulfide opens the KATP channel on rat atrial and ventricular myocytes. *Cardiology* **115**, 120–126 (2010).
175. Kang, M., Hashimoto, A., Gade, A. & Akbarali, H. I. Interaction between hydrogen sulfide-induced sulfhydration and tyrosine nitration in the KATP channel complex. *Am. J. Physiol. Gastrointest. Liver Physiol.* **308**, G532–G539 (2015).
176. Lu, G. et al. H2S inhibits angiotensin II-induced atrial Kv1.5 upregulation by attenuating Nox4-mediated ROS generation during atrial fibrillation. *Biochem. Biophys. Res. Commun.* **483**, 534–540 (2017).
177. Zhang, Y. et al. Hydrogen sulfide suppresses transforming growth factor-β1-induced differentiation of human cardiac fibroblasts into myofibroblasts. *Sci. China Life Sci.* **58**, 1126–1134 (2015).
178. Krul, S. P. et al. Atrial fibrosis and conduction slowing in the left atrial appendage of patients undergoing thoracoscopic surgical pulmonary vein isolation for atrial fibrillation. *Circ. Arrhythm. Electrophysiol.* **8**, 288–295 (2015).
179. Xue, X. et al. Exogenous hydrogen sulfide reduces atrial remodeling and atrial fibrillation induced by diabetes mellitus via activation of the PI3K/Akt/eNOS pathway. *Mol. Med. Rep.* **22**, 1759–1766 (2020).
180. Watts, M. et al. Decreased bioavailability of hydrogen sulfide links vascular endothelium and atrial remodeling in atrial fibrillation. *Redox Biol.* **38**, 101817 (2021).
181. Sun, X. et al. A long-term and slow-releasing hydrogen sulfide donor protects against myocardial ischemia/reperfusion injury. *Sci. Rep.* **7**, 3541 (2017).
182. Bilska-Wilkosz, A. et al. Lipoic acid as a possible pharmacological source of hydrogen sulfide/sulfane sulfur. *Molecules* **22**, 388 (2017).
183. Dudek, M. & Meng, Q. J. Running on time: the role of circadian clocks in the musculoskeletal system. *Biochem. J.* **463**, 1–8 (2014).
184. Dudek, M. et al. Alpha lipoic acid protects the heart against myocardial post ischemia-reperfusion arrhythmias via KATP channel activation in isolated rat hearts. *Pharmacol. Rep.* **66**, 499–504 (2014).
185. Sun, Y. G., Wang, X. Y., Chen, X., Shen, C. X. & Li, Y. G. Hydrogen sulfide improves cardiomyocytes electrical remodeling post ischemia/reperfusion injury in rats. *Int. J. Clin. Exp. Pathol.* **8**, 474–481 (2015).
186. Whiteman, M., Karwi, Q. G., Wood, M. E. & Baxter, G. F. Mitochondria-targeted hydrogen sulfide (H2S), but not untargeted H2S, reverses ventricular arrhythmia at reperfusion [abstract 176]. *Free Radic. Biol. Med.* **112**, 124–125 (2017).
187. Ertugrul, I. A. et al. Donor heart preservation with hydrogen sulfide: a systematic review and meta-analysis. *Int. J. Mol. Sci.* **22**, 5737 (2021).
188. Myszkowska, J., Derevenkov, I., Makarov, S. V., Spiekerkoetter, U. & Hannibal, L. Biosynthesis, quantification and genetic diseases of the smallest signaling thiol metabolite: hydrogen sulfide. *Antioxidants* **10**, 1065 (2021).
189. Zaorska, E., Tomasova, L., Koszelewski, D., Ostaszewski, R. & Ufnal, M. Hydrogen sulfide in pharmacotherapy, beyond the hydrogen sulfide-donors. *Biomolecules* **10**, 323 (2020).
190. Chen, L. et al. Imbalance of endogenous homocysteine and hydrogen sulfide metabolic pathway in essential hypertensive children. *Chin. Med. J.* **120**, 389–393 (2007).
191. Sun, N. L., Xi, Y., Yang, S. N., Ma, Z. & Tang, C. S. Plasma hydrogen sulfide and homocysteine levels in hypertensive patients with different blood pressure levels and complications [Chinese]. *Zhonghua Xin Xue Guan Bing. Za Zhi* **35**, 1145–81148 (2007).
192. Bradley, J. M., Organ, C. L. & Lefer, D. J. Garlic-derived organic polysulfides and myocardial protection. *J. Nutr.* **146**, 403s–409s (2016).
193. Szabo, C. & Papapetropoulos, A. International union of basic and clinical pharmacology. CII: pharmacological modulation of H(2)S levels: H(2)S donors and H(2)S biosynthesis inhibitors. *Pharmacol. Rev.* **69**, 497–564 (2017).
194. D'Huart, E. et al. Physico-chemical stability of sodium thiosulfate infusion solutions in polyolefin bags at room temperature over a period of 24 hours. *Pharm. Technol. Hospital Pharm.* **3**, 135–142 (2018).
195. Kannan, S., Boovarahan, S. R., Rengaraju, J., Prem, P. & Kurian, G. A. Attenuation of cardiac ischemia-reperfusion injury by sodium thiosulfate is partially dependent on the effect of cystathione beta synthase in the myocardium. *Cell Biochem. Biophys.* **77**, 261–272 (2019).
196. Nguyen, I. T. N. et al. Cardiac protection by oral sodium thiosulfate in a rat model of L-NNA-induced heart disease. *Front. Pharmacol.* **12**, 650968 (2021).
197. Sen, U. et al. Cardioprotective role of sodium thiosulfate on chronic heart failure by modulating endogenous H2S generation. *Pharmacology* **82**, 201–213 (2008).
198. Snijder, P. M. et al. Exogenous administration of thiosulfate, a donor of hydrogen sulfide, attenuates angiotensin II-induced hypertensive heart disease in rats. *Br. J. Pharmacol.* **172**, 1494–1504 (2015).
199. Szczesny, B. et al. AP39, a novel mitochondria-targeted hydrogen sulfide donor, stimulates cellular bioenergetics, exerts cytoprotective effects and protects against the loss of mitochondrial DNA integrity in oxidatively stressed endothelial cells in vitro. *Nitric Oxide* **41**, 120–130 (2014).
200. Zhu, C. et al. Supplementing preservation solution with mitochondria-targeted H2S donor AP39 protects cardiac grafts from prolonged cold ischemia-reperfusion injury in heart transplantation. *Am. J. Transpl.* **19**, 3139–3148 (2019).

201. Wallace, J. L., Caliendo, G., Santagada, V., Cirino, G. & Fiorucci, S. Gastrointestinal safety and anti-inflammatory effects of a hydrogen sulfide-releasing diclofenac derivative in the rat. *Gastroenterology* **132**, 261–271 (2007).
202. Chakraborty, P. K. et al. Cystathionine β-synthase regulates mitochondrial morphogenesis in ovarian cancer. *FASEB J.* **32**, 4145–4157 (2018).
203. Chen, L. et al. Mitochondrial fusion protein Mfn2 and its role in heart failure. *Front. Mol. Biosci.* **8**, 681237 (2021).
204. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT02278276 (2015).
205. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT02899364 (2021).
206. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT03410537 (2018).
207. Sun, Q. et al. Taurine supplementation lowers blood pressure and improves vascular function in prehypertension: randomized, double-blind, placebo-controlled study. *Hypertension* **67**, 541–549 (2016).
208. Kip, P. et al. Insights from a short-term protein-calorie restriction exploratory trial in elective carotid endarterectomy patients. *Vasc. Endovasc. Surg.* **53**, 470–476 (2019).
209. Bordia, A., Verma, S. K. & Srivastava, K. C. Effect of garlic (*Allium sativum*) on blood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease. *Prostaglandins Leukot. Essent. Fat. Acids* **58**, 257–263 (1998).
210. Zhang, X. H. et al. A randomized trial of the effects of garlic oil upon coronary heart disease risk factors in trained male runners. *Blood Coagul. Fibrinolysis* **12**, 67–74 (2001).
211. Cheng, W. L. et al. Clinical study on effect of Garlicin in stabilizing the carotid artery atherosclerotic plaque in patients with primary hypertension and coronary artery disease. *Chin. J. Integr. Med.* **12**, 166–170 (2006).
212. Jeyaraj, S., Shivaji, G., Jeyaraj, S. D. & Vengatesan, A. Effect of combined supplementation of fish oil with garlic pearls on the serum lipid profile in hypercholesterolemic subjects. *Indian Heart J.* **57**, 327–331 (2005).
213. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT03829605 (2019).
214. Fischer E. in *Untersuchungen aus Verschiedenen Gebieten* (ed. Bergmann, M.) 117–119 (Springer, 1924).
215. D'Alessandro, A. et al. AltitudeOmics: red blood cell metabolic adaptation to high altitude hypoxia. *J. Proteome Res.* **15**, 3883–3895 (2016).
216. Revsbech, I. G. et al. Hydrogen sulfide and nitric oxide metabolites in the blood of free-ranging brown bears and their potential roles in hibernation. *Free Radic. Biol. Med.* **73**, 349–357 (2014).
217. Shen, X. et al. Microbial regulation of host hydrogen sulfide bioavailability and metabolism. *Free Radic. Biol. Med.* **60**, 195–200 (2013).
218. Doeller, J. E. et al. Polarographic measurement of hydrogen sulfide production and consumption by mammalian tissues. *Anal. Biochem.* **341**, 40–51 (2005).
219. Koenitzer, J. R. et al. Hydrogen sulfide mediates vasoactivity in an O2-dependent manner. *Am. J. Physiol. Heart Circ. Physiol.* **292**, H1953–H1960 (2007).
220. Whitfield, N. L., Kreimier, E. L., Verdial, F. C., Skovgaard, N. & Olson, K. R. Reappraisal of H2S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signaling. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R1930–R1937 (2008).
221. Furne, J., Saeed, A. & Levitt, M. D. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **295**, R1479–R1485 (2008).
222. Levitt, M. D., Abdel-Rehim, M. S. & Furne, J. Free and acid-labile hydrogen sulfide concentrations in mouse tissues: anomalously high free hydrogen sulfide in aortic tissue. *Antioxid. Redox Signal.* **15**, 373–378 (2011).

Acknowledgements
The authors are supported by an LSUHSC-S CCDS COVID-19 Research Award and CoBRE Pilot Grant Award to G.K.K; an LSUHSC-S CCDS COVID-19 Research Award and CoBRE Project Grant Award to P.D.; and an Institutional Development Award (IDeA) from the National Institutes of General Medical Sciences of the NIH under grant number GM121307 and HL149264 from the National Heart, Lung, and Blood Institute of the NIH to C.G.K.

Author contributions
All the authors researched data for the article. G.K.K., R.E.S., P.D. and C.G.K. wrote the manuscript. G.K.K., R.E.S., X.S. and C.G.K. reviewed and edited the article before submission.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Cardiology thanks the anonymous reviewer(s) for their contribution to the peer review of this work.

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