FORUM REVIEW: Diet and hydrogen sulfide production in mammals

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Abstract

In recent times it has emerged that some dietary sulfur compounds can act on mammalian cell signalling systems via their propensity to release hydrogen sulfide (H_2S). H_2S plays important biochemical and physiological roles in the heart, gastrointestinal tract, brain, kidney, and immune systems of mammals. Reduced levels of H_2S in cells and tissues correlate with a spectrum of pathophysiological conditions including heart disease, diabetes, obesity and altered immune function. In view of the important roles for this molecule, researchers have now begun to explore the mechanisms by which dietary derived sulfur compounds, in addition to cysteine, can act as sources of H_2S. Of the identified compounds, organic sulfides, isothiocyanates, and inorganic sulfur species including sulphate have received the most attention. Therefore, in the current review we will provide an overview of the literature focused on H_2S release by these molecules.

Innovation

Pathways for the production and catabolism of the gaseous signalling molecule H_2S have now been characterised in mammalian systems. Dysregulation in these pathways is seen in numerous diseases spanning various cancers through to neurological disorders. Less widely reported are the impacts of diet including lipids, caloric restriction, vitamins, and minerals, on H_2S producing systems in mammalian cells and tissues. Furthermore, recent research has shown that distinct pools of H_2S may also be produced in cells following the metabolism of various dietary derived sulfur-containing phytochemicals. Therefore, a better understanding of how diet impacts on H_2S, as described here, will enable a better understanding of gaseous signalling networks in mammalian cells and tissues.

1. Introduction

The human diet is composed of a diverse array of inorganic and organic dietary derived sulphur compounds (33). Inorganic sulfate (SO_4^{2-}) and sulfites (SO_3^{2-}), are common in water and foods and organic sulfur species like methionine, cysteine and its oxidised forms cystine, taurine, and the antioxidant glutathione present in meat products (15, 150). Many vitamins and co-factors also contain sulfur like biotin, and coenzyme A, and are common in many foods and food supplements (76, 106, 122 126). More widely, edible fungi and various dietary plants produce a whole spectrum of sulfur compounds including ergothionine (47, 83), and *S*-alkenyl-*L*-cysteine sulfoxides (ASCOs) and glucosinolates (GSLs); again all are common constituents in the human diet (9, 12, 41, 138, 164). Interestingly, some of these molecules are sources of H_2S, a recently characterised gaseous signalling molecule in mammals. Compounds like diallyl trisulfide (DATS), and isothiocyanates (ITCs) like sulforaphane are reported to have the capacity to release H_2S, these molecules occurring in edible cruciferous and allium vegetables (**Figure 1)**, (63, 138, 158). Interestingly, these plants have been widely investigated in human population studies and shown to be linked to reduction in the risk of some cancers, cardiovascular diseases, and type-2 diabetes, along with changes in the microbiome that may also be important to human health (4-5, 10-11, 29, 68, 115, 135, 173). Common to both plant groups is the production of a spectrum of reactive sulfur species produced during tissue damage [reviewed in (9, 12, 136). For example, GSLs are broken down by myrosinases (EC 3.2. 1.147) to form nitriles, ITCs, thiocyanates and oxazolidindione (9), while ASCOs are substrates for the enzyme allinase (EC 4.4.1.4). The catabolic action of alliinase towards ASCOs causes the production an array of thiosulfinates, sulfides and other sulfur compounds (**Figure 2**). Many of these sulfur species have a wide range of biologically activities including the induction of cell signalling cascades in mammalian cells (23), protein modification via S-thioallylation of cellular proteins (46), and

in the production of H_2S in mammalian cells (84), Other potential sources of H_2S occur in the gastrointestinal tract following the metabolism of sulfate, by sulfate reducing bacteria (133). However, whether H_2S at this site is important is hotly debated. Collectively, it is clear that several dietary sulfur compounds act as potential sources of H_2S in mammalian cells. Moreover, the production of this molecule could go some way in explaining the observed effects of these compounds on mammalian cells and tissues. Therefore, the current review will address how some sulfur species ubiquitous in the human diet, may contribute to the cellular pools of H_2S.

2. H_2S biosynthesis and catabolism

In mammals, H_2S functions as a gasotransmitter (176, 139), and plays roles in cellular proliferation and apoptosis (6, 65, 117, 187, 190), has pro- and anti-inflammatory effects in cells and tissues (16-17, 39, 78, 195), roles in the cardiovascular system (97, 149, 176, 191, 198), alters cellular metabolism and energy production (43, 82, 151, 154, 168, 178), effects neurological function (1, 93), and is an important component of the ageing processes (129-130). H_2S synthesis is controlled by the enzymes, cystathionine β synthase (CBS, EC 4.2.1.22), cystathionine-γ-lyase (CSE, EC 4.4.1.1), and 3-mercaptopyruvate sulfurtransferase (3-MST, EC 2.8.1.2); these enzymes utilise cysteine or 3-mercaptopyruvate as substrates. While much is known regarding the regulation and production of H_2S by CBS and CSE, only now are we beginning to understand the functional roles of 3-MST. Indeed, 3-MST produces H_2S in a coupled reaction with the enzyme cysteine aminotransferase (145) and appears to be important in cell proliferation (2), and in vascular tone (28). 3-MST expression is reduced during episodes of oxidative stress (107) and elevated by shear flow (58). Interestingly, 3-MST is also important in the production of endogenous polysulfides including H_2S_2, H_2S_3, cysteine persulfide (Cys-SSnH) and glutathione persulfide (GSSnH) species (n = 2–4), (69, 71, 110). Although the biological significance of these molecules is yet to be fully

appreciated, current evidence shows that polysulfides are responsible for many of the signalling actions previously ascribed to H_2S. Indeed, polysulfides are cytoprotective (19-20), can activate the transient receptor potential ankyrin 1 (TRPA1) channel (48, 105, 111), and promote neuroblastoma cell differentiation (73). Other studies show polysulfides act as tumour suppressors (44), regulate the function of transcription factors like Nrf-2 (74), and various protein kinases (148), have antioxidant and pro-oxidant properties (103), and inhibit glucose-stimulated insulin secretion in mouse and rat pancreatic β-cell via the activation of ATP-sensitive potassium channels (146). The mechanisms of action for this class of sulfur compound are attributed to their ability to drive persulfidation of cysteine residues of target proteins (70).

The detoxification of H_2S is mediated by two enzymes, ethylmalonic encephalopathy protein 1 (ETHE1, EC: 1.13.11.18), and mitochondrial sulfide–quinone oxidoreductase (SQR, EC 1.8.5.4), respectively. Both enzymes prevent H_2S mediated cellular toxicity *viz.* the inhibition of cytochrome c oxidase (116, 120). Evidence for this comes from the observation that mice lacking ETHE1 show symptoms of H_2S toxicity, and typically have elevated levels of H_2S in tissues (157). Moreover, in humans the loss of a functional ETHE1 causes the accumulation of H_2S in tissues and promotes neurological impairment (67), and changes to the cellular proteome (141). H_2S is also a substrate of thiol-*S*-methyltransferase (EC 2.1.1.9) producing methanethiol (CH_3SH) and dimethylsulfide (CH_3SCH_3), and the enzyme rhodanese (thiosulfate:cyanide sulfurtransferase; EC 2.8.1.1), that produces thiocyanate (SCN^-) and SO_4^{2-}. Aside from enzymatic routes of detoxification, losses also occur via sulfide oxidation in tissues (13). For example, H_2S reacts with methemoglobin to form sulfhemoglobin, that can be rapidly oxidized to form thiosulfate ($S_2O_3^{2-}$), later forming sulfite (SO_3^{2-}) and sulfate (SO_4^{2-}) in mammalian tissues.

3.0 Known dietary factors that impact on H_2S biosynthesis

Studies describing the impact of diet on tissue levels of H_2S are limited (summarised in **Figure 3**). Of the available research, dietary restriction (DR), caloric restriction (CR) and methionine restrictive (MR) diets have received attention due to the known impacts of these dietary regimes on stress resistance and longevity in model organisms like yeast, drosophila and *Caenorhabditis elegans*. Work in this field shows that DR based diets promote H_2S biosynthesis via up-regulation of the trans-sulfuration pathway (51 - 52). Furthermore, in worms the overexpression of the H_2S generating enzyme CBS-1, increases longevity (51), while genetic deficiencies in mpst-1 (3-MST orthologue 1), but not cth-2 (CSE orthologue), reduces lifespan (129). Critically, these effects can be reversed using H_2S donor compounds like GYY4137 (130) and indicates a potential role of H_2S in aging. Other researchers have shown H_2S production slows aging and kidney senescence in mice (77, 177), and that increased H_2S production in the hippocampus of obese mice ameliorates impaired learning and memory function (186). MR causes reduced hepatic steatosis and oxidative stress via increasing hepatic H_2S production (193) and increases endogenous H_2S production via a miR-328-3p dependant mechanisms inducing protein metabolism in animals (184). Dysregulation in the trans-sulfuration pathway, due to altered availability of enzyme co-factors like pyridoxial-5-phosphate, or via the accumulation of metabolic intermediates like homocysteine (Hcy), can reduce the expression and activities of enzymes critical for H_2S production under certain conditions *viz*. CBS and CSE. Similarly, additional evidence points to associations with the levels of S-adenosylmethionine (SAM), an allosteric activator of CBS (38), folate (102), 1, 25 dihydroxyvitamin D1 (75), and betaine (189) with changes in the activities of H_2S biosynthetic enzymes like CSE and CBS. It should be stressed that in these studies no direct measures of H_2S levels were made however, it is reasonable to speculate that H_2S production rates may be impaired. Indeed, nutritional deficiencies in

betaine decrease the activities of betaine-homocysteine S-methyltransferase (BHMT) and CBS in models of guanidinoacetic acid (GAA) and choline deprivation in rats causing hyperhomocysteinemia. Supplementation with betaine reverses these effects (88). In contrast, some studies report that Hcy can upregulate CSE but downregulates CBS in cardiomyocytes therefore impacting on the activities of each enzyme (113). Other nutritional related studies show that short-term protein restriction limits vein graft disease via up-regulation of CSE and increased endogenous production of H_2S in mice (165). Vitamin B6 deficiency in cultured HepG2 cells promotes reductions in H_2S synthesis and the rates of lanthionine and homolanthionine formation; products of H_2S metabolism (45). Moreover, reductions in the expression of CBS are diminished in animals maintained on high-salt diets (55). For example, high salt diminishes renal CBS expression and endogenous H_2S production in rats and the application of the H_2S donor sodium hydrosulfide (NaHS) reverses the impact of salt induced hypertension, oxidative stress, and inflammation (55-57). Some dietary compounds found in plants act as anti-nutrients like the non-protein amino acid 1-amino D-proline (1ADP) found in flax *Linum usitatissimum*. 1ADP reduce pyridoxal 5'-phosphate (PLP) levels in animals, and this in turn reduces the rates of sulfur amino acid metabolism. 1ADP is a vitamin B-6 antagonist and reduces plasma PLP concentrations and the enzymatic activities of CBS and CSE in animals (101). Other dietary components can act as inducers of H_2S production in animal tissues. These include sulfated polysaccharide from *Enteromorpha prolifera* that increase H_2S production in rat tissues via the upregulated hepatic mRNA and protein expression of CBS (134). Similarly, tyrosol, a major component of olive oil *Olea europaea,* increases the hepatic expression levels of CBS and CSE in the livers of mice fed a high fat diet. The associated increases in H_2S linked to reductions in hepatic lipid peroxidation and the restoration of the redox equilibrium of the antioxidant glutathione (142).

Another area attracting attention is the part played by H_2S and its effects on obesity and lipid metabolism. A potential role for H_2S in body composition comes from the observations that animals carrying gene knockouts of trans-sulfuration pathway enzymes are associated with decreased body weight and fat mass (34) and lipid deposition (192). In humans, adiposity levels are reported to correlate with diminished plasma levels of H_2S, an observation indicating that obesogenic diets negatively impact on the levels of H_2S in humans (182). Mice fed high fat diets have reduced capacity to synthesise H_2S. Indeed, CSE expression is reduced in liver, aortic endothelial cells and in lung, cystathionine β synthetase (CBS) elevated in liver and kidney, and 3-MST reduced in liver of animals fed high fat diets (125). In the murine fibroblast 3T3L1 cell line, H_2S-synthesising enzymes CBS, CSE and 3-MST are expressed during adipogenesis. It appears that both endogenous and exogenous H_2S is involved in adipogenesis and adipocyte maturation (166). In animal models, rats maintained on high fat diets have reduced expression of CBS and CSE in hepatic (14), and in aorta tissues (64). The inhibition of CSE using DL-propargylglycine (PAG) in rat adipocytes increases basal and isoproterenol stimulated lipolysis. In this instance, PAG was reported to cause phosphorylated of the protein kinase A substrate, perilipin 1 and hormone sensitive lipase. Interestingly replenishment of H_2S using either L-cysteine, or the prototypic donor molecule GYY4137, decreased lipolysis and reduces insulin resistance *in vivo* (42). Other studies report increased mRNA and protein expression levels of CBS and CSE in mice (C57BL/6) fed HFD (60% kcal fat) with this correlating with the development of fatty liver disease. Upregulation of CBS and CSE enzymes corresponding with increased rates of Hcy clearance; reducing the levels of this pro-atherogenic molecule in the general circulation of animals and correlating with an elevation in H_2S production in the liver (59). Similarly, 3-MST is upregulated in mice and humans with non-alcoholic fatty liver disease (NAFLD). This research suggesting roles of H_2S in the cellular regulator of lipid metabolism in NAFLD

(79), via the regulation sterol regulatory element-binding transcription factor 1 (SREBP-1), JNK and oxidative stress pathways impacting on lipid metabolism (79). Dysfunction of the 3-MST/H_2S pathway is also induced by hyperglycemia; a common metabolic change associated with diabetes and vascular complications. In rodents, treatment with 3-mercaptopyruvate (3-MP) facilitates wound healing, can promote relaxation of microvessels and enhances mitochondrial bioenergetic function via H_2S associated mechanisms. Interestingly, these effects were stimulated in the presence of the dietary antioxidant lipoic acid (28). Changes in the enzymatic routes of synthesis of H_2S are clearly important in many metabolic processes and more recent work has indicated that H_2S production in mammalian cells can also occur via the metabolism of dietary sulfur phytochemicals; a source of H_2S largely produced from plant-derived polysulfides (118, 140). Although detailed studies in humans have yet to be performed, it is compelling to predict that these reactions could be important routes of H_2S production in humans consuming diets rich in dietary sulfur phytochemicals. These areas will be reviewed in detail here.

3.1 Allium sulfur compounds; polysulfides as H_2S donor compounds

Allium vegetables include species like garlic, *A. sativum*, onion, *A. cepa*, leek *A. ampeloprasum*, shallot, chive, *A. schoenoprasum,* and are representative of the main allium species consumed in the diets of humans. Current estimates of consumption for these vegetables is derived from studies from China, that report intake of allium vegetables of approximately 8.3 g/day, with garlic being the most commonly consumed allium vegetable (53). Most sulfur compounds in these plants are derived from the degradation of S-alkenyl-L-cysteine sulfoxides. (+)-*S*-allyl-*L*-cysteine sulfoxide (S-ACSO), commonly known as alliin, (+)-*S*-methyl-*L*-cysteine sulfoxide (methiin; MCSO), (+)-*S*-propyl-*L*-cysteine sulfoxide (propiin; PCSO), and (+)-*S*-trans-1-propenyl-*L*-cysteine sulfoxide or isoalliin (TPCSO) (12, 138) are the main sulfur storage compounds in allium vegetables. By far the most ubiquitous

is (+)-*S*-methyl-*L*-cysteine sulfoxide that is common in the intact tissue of *A. sativum, A. cepa, A. porrum*, and *A. ursinum* L., and some brassica vegetables including broccoli, *B. oleracea* (183). Generally, the occurrence of the sulfoxides is highly variable, as are the relative tissue levels (40). The enzyme alliinase (EC 4.4.1.4), breaks down ACSOs to produce pyruvate, ammonia, and sulfenic acids. The sulfenic acids produced are highly reactive and rapidly condense to form thiosulfinates. The thiosulfinates in turn produce additional sulfur compounds, ranging from compounds like ajoene, various sulfides including diallyl sulfide, and vinyl dithiins (**Figure 4**). These sulfur compounds are believed to be partly responsible for the reported health benefits attributed to the consumption of an allium- and possibly brassica-rich diet (4-5, 10-11, 29, 53).

In reference to H_2S, several edible plant species can generate this molecule including the stinky bean (*Parkia speciose*), durian (*Durio zibethinus*), yellow onion (*A. cepa*), leeks (*A. porrum*) and garlic (*A. sativum*); a property that is influenced by food preparative and cooking regimes (89, 159-161). Moreover, in culture cells exposed to oils derived from these plants intracellular H_2S can be detected (30, 80). Historically, the link between dietary derived sulfur compounds as a potential source of H_2S were first made following the observation that the compounds, DATs and S-allylcysteine (SAC) could be used to manipulated the levels of H_2S in blood and heart tissues of animals (8, 25, 127). Early work by Benavides *et al*. eloquently showed that human RBCs convert garlic-derived DATs into H_2S as measured in real time using a polarographic H_2S sensor (8). H_2S production was highest for the compound DATs followed by DADs and lowest for DPDs. H_2S from these sulfur compounds is liberated following their reaction with reduced thiols like glutathione. H_2S production occurs via two routes in these reactions, the first via nucleophilic substitution at the α carbon of the allyl moiety to form a hydropolysulfide (RSnH) and, second following the nucleophilic substitution at a sulfur atom

of the polysulfides (R-Sn-R′; n > 2) to yield RSnH and H_2S (81). Under normoxic conditions, the liberated H_2S causes vasorelaxation in phenylephrine pre-contracted rat aorta rings (8). In contrast, H_2S production by the cysteine derivative SAC is postulated to occur via the induction of H_2S biosynthetic enzymes, a mechanism affording cardioprotection in animal models (25). In rats pre-treated with SAC, significantly lower mortality rates and reduced infarct size were reported in animals. Importantly, SAC increases plasma H_2S levels and the activity of the H_2S biosynthetic enzyme, cystathionine-γ-lyase (CSE) in left ventricular tissues; an observation reversed in animals treated using the CSE inhibitor, propargylglycine (PAG). These observations provided a direct link between the cardioprotective effects of allium derived sulfur compounds with that of the production of H_2S. In addition, this research revealed two routes for the production of H_2S in mammalian systems *viz.* direct chemical production via reactions with cellular thiols or following the stimulation of known H_2S biosynthetic enzymes in tissues.

A focal point of much of the interest in this field of late has centred on the trisulfide, DATS. In mammalian cells and tissues, this molecule is cardioprotective (194), anti-inflammatory (60), induces antioxidant defences (22), prevents neurological dysfunction (87), and alters metabolic pathways linked to glucose (187) and lipid metabolism (86). H_2S produced from DATs via two processes, the first by increasing the protein expression of CSE and CBS protein in cells following exposure to DATs (61, 121, 166, 188) and the second following the reaction of DATs with cellular thiol compounds including cysteine (Cys) and glutathione (GSH) to liberate H_2S (157). Cai et al. recently showed using computational modelling that Cys and GSH react with DADs and DATs as thiol anions attacking the sulfur atoms present in DADs and DATs. This chemical reaction leads to the formation of an allyl perthiol anion (ASS-) that releases H_2S in the presence of other thiols via a proton-shuffle dependant process (18, 158). The production of H_2S from DATs likely contributes to some of the known biological effects attributed to this

compound (140). In a rodent model of Alzheimer's disease (AD), DATs enhances the performance of animals in maze tasks and reduces neuro-inflammation, oxidative stress, and cholinergic function via an H_2S associated mechanism (108). DATs treatment replenishes the levels of H_2S in animals replete of this molecule (24). A consequence of H_2S replenishment being the enhanced retention of injected bone marrow cells in ischemic tissues, improves blood perfusion, and cell survival in the ischemic hind limbs of diabetic mice (49). DAT_S can increase blood flow recovery, revascularization and increased capillary density in a model of hind-limb ischemia injury. These effects were only observed in wild type but not in eNOS knockout mice and provides evidence of an interaction between the H_2S derived from DATs with NO (72). Furthermore, DATs can reduce left ventricular dilatation and dysfunction, the severity of perivascular and intermuscular fibrosis, and cardiac hypertrophy (128). DATs also mitigates the production of intracellular reactive oxygen species (ROS), and inhibits hepatocyte apoptosis in an intragastric infusion model of alcohol fatty liver disease (AFL) by increasing expressions of CSE and CBS, and points to a potential therapeutic role of H_2S in AFL (21). More recently, nanoparticle-based deliver systems have been developed that deliver controlled amounts of DATs to tissues using mesoporous silica particles. This technology also overcoming the problem of poor solubility that is associated with this molecule. These novel formulations offer protection several models including ventilator-induced lung injury by inhibiting NF-kB signalling (174), the production of pro-inflammatory molecules, TNF-α, IL-1α/β and IL-2 in animals (152-153), ischemia/reperfusion (I/R) injury (175) and heart transplantation (152). Recently, a DATs based self-spray coating system for treating colitis has been described with foaming capabilities (CAP-w-FC). This system generates nano-scale micellar particles in the colorectal tract that are internalised by colon epithelial cells to yield H_2S (85). Additional delivery systems include a poly(D,L-lactic-co-glycolic acid) microparticle based DATs system (DATs@MPs). In vitro these carriers release DATs at slow

rates allowing for sustained intracellular production of H_2S. In turn, the liberated H_2S induces the cytoprotective transcription factor Nrf2 that upregulates gene expressions of antioxidant enzymes. In vivo, DATs@MPs induces angiogenesis and protects cells and tissues from damage (54). Finally, mesoporous iron oxide nanoparticles (MIONs) loaded with DATS have been generated that protect mouse myocardial tissues from ischemia-reperfusion injury by virtue of H_2S production. Collectively, the DATs/H_2S system has received the most attention but it is worth noting that other sulfur molecules including the compounds, 2-vinyl dithiin, 3-vinyl dithiin, and ajoene can also release H_2S (160). While information is sparse, H_2S production has been confirmed for these molecules using a cell-based (MCF-7 cells) H_2S-releasing capacity assay. This system uses a H_2S selective probe, based on a Cu^{II}-cyclen complex linked to a NIR-light-emitting BODIPY fluorophore that fluoresces in the presence of H_2S (185). At present, none of these molecules have been further developed as tools to aid H_2S research.

3.2 Brassica derived sulfur compounds

In recent times it has emerged that sulfur compounds found in brassica plants could also be important dietary components that produce H_2S. The Brassicaceae comprise a large group of agronomically important vegetables including familiar varieties of the *Brassica oleracea* complex like cabbage, cauliflower, Brussels sprouts, broccoli, and various salad species like watercress (*Nasturtium officinale*), and radish (*Raphanus sativus*). These plants contain glucosinolates (GSLs, **Figure 5**), sulfur phytochemicals composed of a beta-D-thioglucose moiety, sulfonated oxime residue and a variable side chain (9). The variable side chain consists of aliphatic, aromatic or indolyl moiety and is determined by the type of amino acid from which the GSL is synthesised (9, 164). Following damage to tissues, the stored GSLs are hydrolysed by plant myrosinase, or alternatively by intestinal bacteria following ingestion, to produce additional and often reactive sulfur molecules. At pH 5-7, hydrolysis

produces bioactive ITCs. This class of compound is biologically active in mammalian cells and tissues. Indeed, reports of the biological activities of isolated and synthetic ITCs are commonplace in the literature and focus mainly on 4-methylsulfinylbutyl ITC, β-phenylethyl ITC, benzyl ITC and allyl ITC [reviewed in 104]. Bioactivity is largely due to the presence of an electrophilic carbon atom positioned between the nitrogen and sulfur atom of the ITC group, R-N=C=S. These electrophilic compounds can participate in reactions by accepting electrons from nucleophilic donor molecules (112). In this regard, many naturally occurring ITCs and several synthetic analogues are known to react with nucleophilic centres of proteins (typically cysteine residues), or with cellular antioxidants like glutathione.

In recent times the possible role of ITCs as potential H_2S donor molecules has been postulated. Naturally occurring GSLs are typically catabolised by plant or bacterial myrosinase to liberate ITCs and nitriles, a property also common to many gut bacteria (90). The liberated ITCs can then be further metabolised to generate H_2S. In 1972, the first evidence that benzyl ITC can be catabolised by Enterobacteriaceae bacteria to liberate H_2S was published (155), (**Figure 6**). In this notable piece of work homogenates of mature papaya seeds, when kept overnight at room temperature developed an odour of H_2S. The authors postulated that the liberated H_2S was generated via the bacterial degradation of benzyl ITC to form benzylamine and H_2S (155). Only recently have ITCs been considered as possible sources of H_2S, and that this property may be important in explaining some of their known biological effects in mammalian cells and tissues. In this regard, sulforaphane (4-methylsulfinylbutyl ITC; SFN), commonly found in broccoli (*Brassica oleracea*), and is readily bioavailable in humans (164) has gained attention. SFN, like all ITCs, reacts with glutathione (GSH) priming the ITC for detoxification via the mercapturic acid pathway. Using the zinc trap assay, Pei et al. found that SFN releases H_2S in the presence of PC-3 cells or mouse liver homogenates. In this instance, H_2S concentrations in the cell culture medium

were significantly increased 30 min post- incubation and were stable for at least 4 h (124). Citi et al provided additional confirmation of the H_2S-releasing capacity of some important natural ITCs using amperometric detection. Allyl ITC, erucin, benzyl ITC, and sinigrin were found to exhibit H_2S releasing capacity in the presence of the cellular thiol cysteine (26, 27). In addition, H_2S generated from 4-hydroxybenzyl ITC (HBITC), an ITC found in white mustard seeds (*Sinapis alba*), reportedly inhibits cellular proliferation of human neuroblastoma (SH-SY5Y) and glioblastoma (U87MG) cells (66). Wang et al showed H_2S production in the tissue extracts of *Moringa oleifera* Lam; a species rich in GSLs and ITCs (179). Other studies indicate that subcutaneous and oral administrations of GRA or SFN reduced neuropathic pain in a dose-dependent manner in chemotherapy-induced neuropathy (91). The co-administration of GRA and SFN in mixture with the H_2S binding molecule, haemoglobin, abolished the pain-relieving effect. This finding leading the authors to speculate that H_2S produced from GRA and SFN reduce neuropathic pain by releasing H_2S and by modulating Kv7 channels. Other research indicates that SFN is vasoactive when administered at doses of 10 μM-1 mM. At these levels SFN caused immediate and sustained dilation of pial arterioles; this occurring concomitantly with an elevation in the levels of H_2S in cerebrospinal fluids (123). In this work, SFN was not acting as a H_2S donor molecule directly but rather by inducing the expression of CSE and CBS in animal tissues. Moreover, the inhibition of CSE/CBS using PAG, aminooxyacetic acid or the inhibition of K_{ATP} and BK channels using glibenclamide, paxilline, and iberiotoxin blocked the vasodilator effects of SFN in the brain. Similarly, erucin can be used to generate H_2S inside human aortic smooth muscle cells with this causing hyperpolarization of the cellular membrane. Moreover, in rat aortic rings, erucin was also found to promote vasodilation and to inhibit noradrenaline-induced vasoconstriction. In vivo, erucin decreased systolic blood pressure to values seen in normotensive rats (100). Much of the research on naturally occurring ITCs has inspired the

development of a range of synthetic ITC derivatives developed as potential H_2S donor molecules (143). These synthetic ITCs can liberate H_2S. The novel H_2S donor 4-carboxy phenyl-ITC (4CPI), has vasorelaxant effects in rat aorta and coronary arteries (100), and in models of myocardial I/R (156), is reported to reduce neuropathic pain in animal models caused by the chemotherapeutic drugs, paclitaxel or oxaliplatin in experimental models (32), and diminishes [Ca^{2+}] availability and thus mast cell degranulation and renin release (98). Phenyl ITC (PITC) and 3-carboxyphenyl ITC (3C-PITC) reduce spontaneous pain induced by nerve injury and osteoarthritis (92). The molecule, DM-22, a therapeutic compound comprising alendronate (AL) and the H_2S-releasing moiety of aryl-ITC, inhibit h-OCs function and stimulate osteogenic differentiation. Amperometric measurement revealed that DM-22 releases H_2S at a slow rate via a thiol-dependent mechanism (132), (**Figure 7**). Collectively, these studies supporting the notion that thiol compounds are critical for H_2S release from ITC derivatives.

3.3 Other potential sources of H_2S in mammals

In the gastrointestinal tract H_2S is produced by resident gut bacteria following the metabolism of sulfate, sulfite and various proteins (94, 131, 147-148). Production of H_2S is largely mediated by the action of resident sulfate reducing bacteria (SRB) including members of the genera, *Desulfovibrio*, *Desulfobacter*, and *Desulfobulbus* (147-148). Following the metabolism of sulfate by these species H_2S is produced (131, 163). H_2S is also generated from sulphite by a spectrum of different bacteria species including *Bacillus, Corynebacterium, Enterobacter, E. coli, Klebsiella, Rhodococcus, Staphylococcus, and Salmonella,* respectively. In this instance sulfite is catabolised by the enzyme sulphite reductase to produce H_2S (7). An addition source of this molecule is via the action of bacterial cysteine desulfhydrase, present in anaerobic species like *Salmonella enterica*, and *Enterobacter aerogenes,* that catabolise cysteine to produce H_2S, pyruvate and ammonia; a

metabolic step akin to the catabolic effects of mammalian CBS protein on cysteine (3). It is likely that these bacterial sources of H_2S bathe the surrounding tissues and will contribute to the total pool of H_2S in and around enteric cells (36). Attempts at measuring the levels of H_2S in the GI tract are sparse however, some researchers point to sulfide levels reaching concentrations of between 0.2–3.4 mmol/L (94). What is not currently know is how much of this free sulfide diffuses across epithelial cells and whether this contributes to circulatory pools in blood (31); current evidence suggests sulfide produced at this site does not enter the bloodstream. Interestingly, it has been reported that H_2S levels are reduced in germ-free animals (144), and in rodents that are vitamin B6 deficient (36). In addition, studies have shown diminished levels of bound sulfane sulfur; divalent sulfur atom bound to an adjacent sulfur atom which is highly reactive and labile (62, 162). Compounds containing sulfane sulfur are reported to act as a form of stored H_2S in mammalian tissues. At present, descriptions of any potential physiological roles for H_2S generated in the GI tract have yet to be fully described. Historically, H_2S production in the GI tract has been linked to colonic inflammation *viz.* Crohn's disease (109) and some cancers (180, 114), and has typically been viewed negatively by researchers. However, in the last decade, a number of studies point to potential beneficial effects of this molecule in the GI tract for example, as an energy substrate for colonic cells (43, 95, 170), to stimulate mucosal formation (172), and to act as a cytoprotective agent (37, 137, 170, 196). Studies in animals models consistently show H_2S donors to have therapeutic effects in the GI tract with reported anti-inflammatory effects and enhanced safety profiles (96, 169), chemopreventative effects in experimental models of colorectal cancer (35, 119), protection against stress induced gastric lesions (95-96), and reductions in colonic inflammation (37). Moreover, pioneering work by Wallace et al. has shown that ATB-346 a hydrogen sulfide (H_2S)-releasing anti-inflammatory and analgesic drug, has reduced toxicity in the GI tract of humans (171). These protective effects have been

attributed to the sustained and controlled release of H_2S coupled with the inhibition of cyclooxygenase. Indeed, participants receiving ATB-346 showed a significant (~50%) increases in plasma levels of H_2S over the course of the two-week intervention. Collectively, this body of work points to a beneficial effect of this molecule in the GI tract. Key questions still remain however and relate to the bioavailability of H_2S in the GI tract; i.e., whether or not the molecule reaches the systemic circulation in humans? How H_2S affects gut-inflammatory signalling networks? And whether in gastrointestinal diseases H_2S production is a by-product of bacterial infiltration, or a response of tissues to preserve cellular viability following damage? Hopefully, in the near future these questions will be answered.

4. Conclusion

The biological effects of dietary sulfur species are more complicated than first imagined. Many sulphur species derived from the diet are metabolised via a spectrum of separate metabolic pathways, leading to the products of sulfur molecules with differing chemical properties. Liberation of these sulfur species within cellular compartments induces a spectrum of separate signalling cascades (**Figure 8**). While research on the biological fate of these molecules and their role in health is still ongoing much still needs to be addressed. For example, does the consumption of allium or brassica plants produce H_2S in humans? The propensity to produce H_2S, and its wide range of biological effects in mammalian systems is intriguing, as such this area deserves further investigation. Critically, if H_2S is generated in vivo by people consuming diets rich in alliums and brassicas, then are the levels sufficient to mediate biological effects? Furthermore, several human intervention studies have shown that the consumption of allium vegetables can have modest effects on blood pressure in humans however, few studies have yet to consider potential links between brassica consumption and cardiovascular health. In addition, some restrictive diets alter H_2S production in the tissues of

animals however, the impact of this on health and healthy ageing processes needs additional scrutiny. Hopefully, these questions will be resolved in the near future.

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Abbreviations Used.

CBS = Cystathionine β synthase

CSE = Cystathionine-γ-lyase

3-MST = 3-Mercaptopyruvate sulfurtransferase

SQR-1 = Sulfide–quinone oxidoreductase

ETHE1 = Ethylmalonic encephalopathy protein 1

ASCOs = S-alkenyl-L-cysteine sulfoxides

GSLs = Glucosinolates

ITCs = Isothiocyanates

Nrf2 = Nuclear factor erythroid 2-related factor 2

NF-kB = Nuclear factor kappa-light-chain-enhancer of activated B cells

SREBP-1 = Sterol regulatory element-binding transcription factor 1

JNK = c-Jun N-terminal kinases

GAA = Guanidinoacetic acid

PAG = DL-propargylglycine

GYY4137 = p-Methoxyphenyl)morpholino-phosphinodithioic acid

ATB-346 = H2S-releasing naproxen derivative

DATs = Diallyl trisulfide

DPDs = Dipropyl disulfide

SAC = S-allylcysteine

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Figure 1. Small molecular weight sulfur compounds reported to release H_2S gas, (**A**) diallyl trisulfide, (**B**) S-allyl cysteine, (**C**) S-propargyl cysteine, (**D**) Ajoene, (**E**) 1,2 vinyldithiin, and (**F**) the isothiocyanate, sulforaphane.

Figure 2. Brassica and allium vegetables contain the sulfur storage compounds namely, glucosinolates and *S*-alk(en)yl-*L*-cysteine sulfoxides. Consumption of these vegetables is associated with reduced risk of developing several chronic diseases like cancer(s) and cardiovascular disease.

Figure 3. Summarised overview of the dietary factors influencing the expression of H_2S biosynthetic pathways. Arrows are used to highlight upregulation (↑), or down regulation (↓) of enzyme or protein levels.

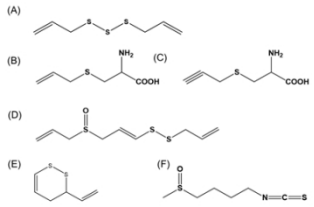
Figure 4. The catabolism of *S*-alk(en)yl-*L*-cysteine sulfoxides (ASCOs) by the enzyme alliinase produces a diverse array of reactive sulfur compounds. Some including diallyl trisulfide (DATs) can produce H_2S gas following reactions with cellular thiols.

Figure 5. The glucosinolate–myrosinase system in brassica plants. Following tissue damage, glucosinolates are catabolised by myrosinases leading to the production of several sulfur containing products. At neutral pH, ITCs are produced, or in the presence of epithiospecifier-protein-like factor or alkali pH, nitriles. Glucosinolates hydroxylated at the third carbon atom of the side chain generate ITCs that can spontaneously cyclize to form oxazolidine-2-thione. Other compounds identified in brassica tissues include epithionitriles and thiocyanates. Abbreviation: R, variable side chain of aromatic, aliphatic or indole structures.

Figure 6. In homogenates of papaya seeds and benzyl isothiocyanate (BITC)-enriched papaya pulp the metabolism of BITC by *E. cloacae* generates benzylamine and H_2S.

Figure 7. Isothiocyanates (ITCs, R-NCS) rapidly form adducts with cysteine and rapidly cyclize to release amine ($R–NH_2$) and raphanusamic acid (RA) and minor amounts of 2-carbylamino-4,5-dihydrothiazole-4-carboxylic acids and H_2S. Adapter from (84).

Figure 8. Allium and brassica derived sulfur compounds *viz*. sulfides and isothiocyanates (ITCs) are hypothesised to generate H_2S in cells via two major routes. The first pathway includes the reaction of individual molecules with cellular thiols such as glutathione (GSH). In the second pathway, the up-regulation of biosynthetic enzymes involved in H_2S production is reported. The H_2S generated can then act on cell signalling processes to mediate effect or alternatively form various polysulfide species.



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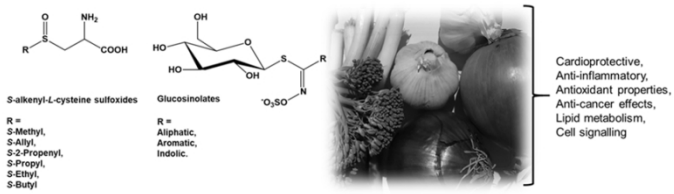

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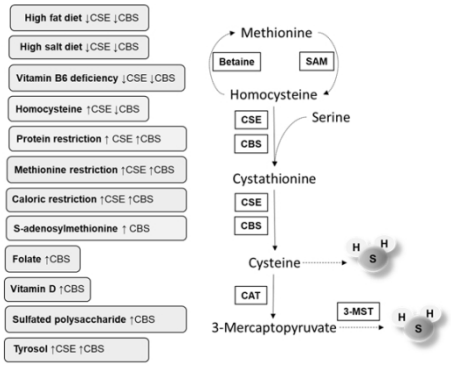

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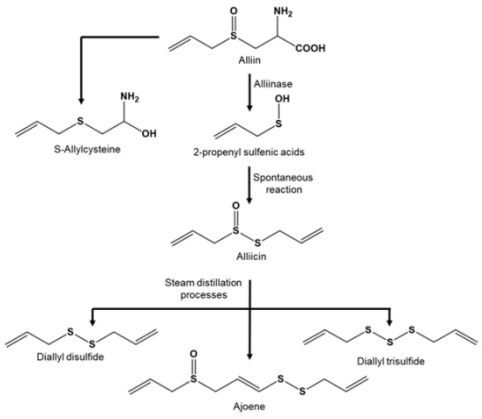

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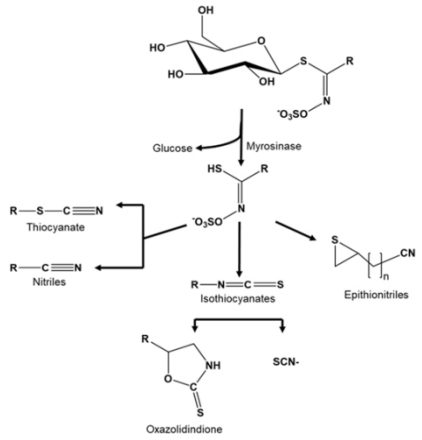

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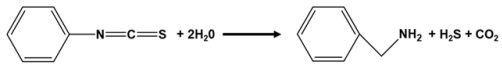

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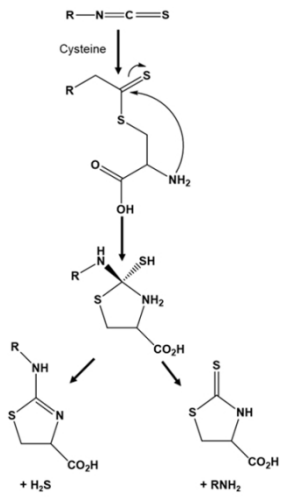

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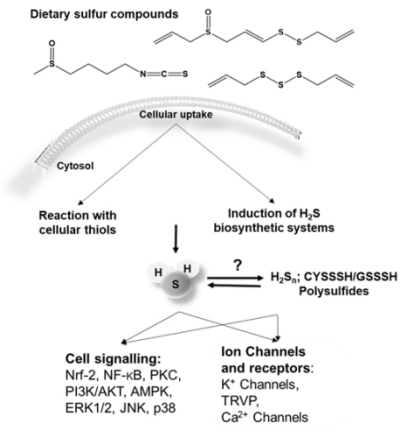

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