Published in final edited form as:

Exp Gerontol. 2015 August ; 68: 26–32. doi:10.1016/j.exger.2014.12.010.

Calorie restriction and methionine restriction in control of endogenous hydrogen sulfide production by the transsulfuration pathway

Christopher Hine and James R. Mitchell*

Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA 02115, USA

Abstract

H₂S is a gas easily identified by its distinctive odor. Although environmental exposure to H₂S has been viewed alternately as therapeutic or toxic through the centuries, H₂S has recently regained recognition for its numerous beneficial biological effects. Most experiments documenting such benefits, including improved glucose tolerance, increased stress resistance, and even lifespan extension, are based on exposure of experimental organisms to exogenous sources of H₂S. However, appreciation is growing for the importance of H₂S produced endogenously by the evolutionary conserved transsulfuration pathway (TSP) in health and longevity. Recent data implicate H₂S produced by the TSP in pleiotropic benefits of dietary restriction (DR), or reduced nutrient/energy intake without malnutrition. DR, best known as the most reliable way to extend lifespan in a wide range of experimental organisms, includes various regimens aimed at either reducing overall calorie intake (calorie restriction, intermittent/ every-other-day fasting) or reducing particular nutrients such as protein or the essential amino acid, methionine (methionine restriction), with overlapping functional benefits on stress resistance, metabolic fitness and lifespan. Here we will review the small but growing body of literature linking the TSP to the functional benefits of DR in part through the production of endogenous H₂S, with an emphasis on regulation of the TSP and H₂S production by diet and mechanisms of beneficial H₂S action.

Keywords

Dietary restriction; Methionine; Transsulfuration; Hydrogen sulfide; Aging; Stress; Metabolism

1. Introduction

1.1. Dietary restriction

Dietary restriction (DR) encompasses a variety of regimens characterized by nutrient and/or energy restriction leading to generally beneficial, but reversible, adaptive changes on the organismal level. Because DR-related nomenclature is poorly defined, we will refer to

regimens involving restriction of total food intake as calorie restriction (CR) in order to contrast them with regimens in which individual macronutrients such as protein, or specific amino acids such as methionine, are restricted (MetR) without enforced food restriction. CR and MetR result in overlapping phenotypes and associated benefits in multiple organisms, and as described below, may also share similar underlying mechanisms of benefits.

1.1.1. Calorie restriction—CR was originally identified as a lifespan extending regimen in rodents, and has been used as an experimental tool for nearly a century with which to study underlying mechanisms of the aging process. Functionally, CR regimens are diverse and organism specific; for example, in yeast reducing glucose in the media from 2% to 0.5% extends chronological and replicative lifespan, while in rodents daily restriction of food by 40% or alternating days of fasting and ad libitum feeding (every-other-day fasting) represent two extremes of CR regimens leading to longevity extension, improved metabolic fitness and multiple stress resistance (Anson et al., 2005). In recent decades, evolutionarily conserved pathways involved in nutrient and energy sensing, including insulin/IGF signaling, mTOR, AMPK, sirtuins, and GCN2 have been implicated in regulation of aging by CR, and associated benefits including stress resistance (Fontana et al., 2010). Because CR benefits are gained and lost rapidly upon the change from ad libitum to restricted feeding and vice versa, underlying mechanisms most likely involve adaptive changes linked to nutrient/energy restriction signal transduction pathways and downstream transcription factors including FOXO (Greer et al., 2007), NRF2 (Bishop and Guarente, 2007; Pearson et al., 2008), CREB (Mair et al., 2011) and ATF4 (Li et al., 2014). Nonetheless, evolutionarily conserved molecular requirements downstream of such transcriptional changes remain largely unresolved.

1.1.2. Methionine restriction—MetR also extends lifespan and stress resistance in yeast (Johnson and Johnson, 2014; Ruckenstuhl et al., 2014; Wu et al., 2013), flies (Troen et al., 2007), worms (Cabreiro et al., 2013) and rodents (Miller et al., 2005; Orentreich et al., 1993). In humans, it has been used to compliment cancer treatment (Thivat et al., 2007) and to improve metabolic fitness (Lees et al., 2014; Plaisance et al., 2011). Furthermore, MetR in yeast can be phenocopied by genetic manipulation of methionine bio-synthetic pathways Met15/17/25 or Met2 and shMTR in human and mouse cells (Fig. 1), imparting multiple stress resistance phenotypes (Johnson and Johnson, 2014). In mammals, MetR benefits actually require combined methionine and cysteine restriction (Elshorbagy et al., 2013), and thus could be more accurately referred to as sulfur amino acid (SAA) restriction. Because MetR regimens in rodents are given on an ad libitum basis without enforced restriction of calorie intake, it is currently unclear to what degree SAA restriction and CR share underlying molecular mechanisms of protection despite clear phenotypic overlap (Lopez-Torres and Barja, 2008). Evidence in favor of mechanistic overlap comes from flies, in which CR-mediated lifespan extension can be specifically abrogated by essential amino acids (EAA) including Met, but not EAA lacking Met (Grandison et al., 2009); and in mice, where SAA abrogate benefits of stress resistance (Hine et al., in press) as discussed further below.

1.2. Hydrogen sulfide

H₂S gas is released into the environment from inorganic sources or produced by sulfate-reducing bacteria, and is thus found in varying concentrations from different sources including well-water, thermal baths and volcanoes. When present in high concentrations, H₂S blocks respiration by inhibiting cytochrome c oxidase and interfering with iron-dependent biochemical reactions. However, as is typical of hormetic compounds that are toxic at high doses, at lower doses H₂S has a number of beneficial effects, probably via a variety of different mechanisms (discussed below). Interestingly, although H₂S was in vogue for centuries past as a cure-all (Forster, 1994), it is currently viewed by environmental/regulatory bodies as hazardous with little to no acceptable level of exposure (WHO, 2003). Nonetheless, in biology and medicine, interest in H₂S has entered a renaissance since the recognition that it acts as a vasodilator, similar to nitric oxide (NO) and carbon monoxide (CO), and has a number of other benefits in health and medicine (Zhang et al., 2013).

1.2.1. Exogenous hydrogen sulfide benefits—Exposure to exogenous H₂S can induce a state of suspended animation in rats, allowing them to survive hypoxia over six hours without irreversible effects (Blackstone et al., 2005; Blackstone and Roth, 2007). H₂S can also protect against global ischemia associated with severe blood loss (Morrison et al., 2008). In yeast and worms, H₂S significantly extends median lifespan (Hine et al., in press; Miller and Roth, 2007). Furthermore, H₂S is beneficial against metabolic syndrome (Xue et al., 2013), cancer (Lee et al., 2011, 2014), neurodegeneration (Kida et al., 2011), and multiple stress resistance, including heat shock in worms (Miller et al., 2011) and oxidative stress and hypoxia in mammalian cells (Hine et al., in press; Wen et al., 2013).

1.2.2. Endogenous hydrogen sulfide benefits—H₂S is also produced endogenously by organisms including mammals via the transsulfuration pathway (TSP) via the tandem enzymatic activity of CBS and CGL (Kimura, 2011). The importance of endogenous H₂S production was first demonstrated in mice lacking CGL, the final enzyme in the Cys biosynthesis pathway (Fig. 1). Under dietary conditions with adequate levels of Cys, KO mice still suffer from high blood pressure due to the lack of endogenous H₂S (Yang et al., 2008). H₂S production by CGL is also implicated in protection from a growing list of maladies including neurodegeneration associated with Huntington's disease (Paul et al., 2014), atherosclerosis (Mani et al., 2013) and type 1 diabetes (Manna et al., 2014). Because defects in TSP enzymes preventing H₂S production also affect other metabolites in the pathway, leading in some cases to cystathionuria or homocysteinuria (Zhu et al., 2008), or changes in downstream metabolites GSH and taurine, the causative role of H₂S depletion in these disorders is strongly suspected but remains to be proven.

1.3. Linking DR and H₂S

Recently, we reported a link between endogenous H₂S by the TSP and multiple DR benefits, including stress resistance in rodents and longevity extension in yeast, flies and worms (Hine et al., in press). While the requirement for a functional TSP in DR-mediated longevity was previously shown in flies (Kabil et al., 2011), this was the first attempt to elucidate the specific role of the TSP metabolite H₂S in this process. In this study, we made use of the fact that 50% CR offers robust protection against the multifactorial stress of hepatic

ischemia reperfusion injury to elucidate first dietary, and then molecular, mechanisms of protection. We found that protein restriction (PR) lent equal protection to 50% CR independent of calorie intake, and that adjusting the concentration of SAA, or just Cys, back to ad libitum levels significantly reduced CR- or PR-mediated protection. Together, these data implicate reduced Cys as the major trigger of protection in this model of DR-mediated stress resistance, prompting us to consider alterations in TSP activity and associated metabolites.

To uncover relevant molecular mechanisms underlying protection, we used a genetic model that fails to gain DR-mediated protection against hepatic ischemic injury due to hepatocyte-specific ablation of the mTORC1 repressor TSC1, and hence constitutively active mTORC1 in the liver (Harputlugil et al., 2014). Comparative analysis of TSP gene and protein expression and metabolomics profiling were strongly suggestive of increased H₂S production upon DR as the key diet/genotype interaction leading to stress resistance. To test this, we demonstrated the sufficiency of exogenous H₂S for protection, and that genetic or pharmacological inhibition of CGL abrogated DR-mediated protection, while CGL overexpression induced H₂S production capacity and stress resistance independent of diet (Hine et al., in press). We also found that species-specific DR regimens associated with extended longevity and stress resistance in mice (MetR, every-other-day fasting), flies (optimized protein/methionine levels), worms (eat-2 mutant) and yeast (glucose restriction) resulted in increased H₂S production capacity. Finally, overexpression of one particular TSP gene in worms, CBS-1, increased longevity independent of diet, while knocking down the same gene abrogated DR-mediated longevity extension.

Based on these data, we proposed that increased TSP activity is an evolutionarily conserved response to multiple DR regimens, including CR, PR and MetR, and that increased H₂S production under these conditions represents a common molecular mechanism underlying multiple DR benefits. In the following sections, we review recent findings regarding the regulation of TSP activity and H₂S production, and potential molecular mechanisms underlying H₂S benefits.

2. Regulation of transsulfuration pathway gene expression and activity

2.1. Dietary SAA requirements

Because most metazoans cannot reduce inorganic sulfur, sulfur-containing amino acids (SAA) methionine (Met) and cysteine (Cys) must be consumed in the diet. Met is of critical importance not only as the first amino acid incorporated into the growing polypeptide chain during protein synthesis, but with its X-CH₃ moiety is also the source of all one-carbon transfer reactions. In addition to its essential role in protein synthesis, Cys is also critical for glutathione production and thus redox homeostasis. Because Cys can be synthesized de novo from Met by the TSP (Fig. 1), Cys is nonessential unless Met is limiting. If dietary intake of both Met and Cys is low, then Cys also becomes an essential amino acid. Currently, the recommended daily intake of SAAs for humans independent of age or sex is approximately 14–20 mg/kg of body weight a day, with Met recommendations accounting for 10–19 mg/kg of body weight a day. These numbers are not universally agreed upon, especially in light of the lack of age and/or health dependent requirements (Fukagawa, 2013; Nimni et al., 2007).

Nonetheless, long-term, severe deficiencies of SAAs can lead to infertility, embryonic developmental defects, reduced growth and bone strength, liver damage and death, while overconsumption of SAAs is conversely associated with metabolic syndrome.

2.2. Cys biosynthesis via the transsulfuration pathway

Cys can be produced from Met via a step-wise enzymatic process involving the transmethylation pathway (TMP) and TSP (Fig. 1) when dietary Cys intake is low. Two key enzymes of the TSP are cystathionine β -synthase (CBS) and cystathionine γ -lyase (CGL). Under normal physiological conditions, CBS converts serine and homocysteine, a product of Met methyl transfer, into cystathionine. Cystathionine is then converted into α -ketobutyrate and Cys by CGL. In mice lacking CGL, Cys is an essential amino acid, and mice die within 3 weeks of 50% cysteine restriction (Ishii et al., 2010). Mice lacking CBS, on the other hand, have a severe phenotype even when fed a normal diet (Gupta et al., 2009).

2.3. Dietary regulation of TSP expression

In mammals, TSP genes are upregulated on the transcriptional level in response to a lack of dietary Cys, consistent with a primary role in Cys biosynthesis (Lee et al., 2008).

Deprivation of even a single amino acid can activate the amino acid starvation response kinase GCN2, resulting in phosphorylation of its only known target, eIF2 α , and stabilization of transcription factors such as ATF4. The amino acid starvation response is activated in mouse liver upon PR and SAA restriction (Sikalidis and Stipanuk, 2010). ATF4 has been implicated in the regulation of CGL, as cells lacking this factor must be supplemented with Cys (Harding et al., 2003). Interestingly, ATF4 is increased in mouse liver upon CR and MetR, but also in other models of slow aging, including hypopituitary dwarfism, independent of diet (Li et al., 2014).

In addition to GCN2, eIF2 α can be phosphorylated on the same site by additional kinases such as PERK in response to oxidative or endoplasmic reticulum stress as part of the integrated stress response. Thus, whether ATF4 stabilization and CGL transcription upon Cys deprivation is due to the amino acid starvation response and GCN2, or redox stress and PERK, remains unclear.

The general transcription factor SP1 has also been implicated in promoting CGL expression (Paul et al., 2014; Yin et al., 2012). CGL is under negative regulation by glucocorticoids (Zhu et al., 2010) and anterior pituitary hormones lacking in long-lived Snell (Vitvitsky et al., 2013) and Ames (Uthus and Brown-Borg, 2006) dwarf mice. On the mRNA and protein level, CGL is increased upon 50% CR in mouse liver, and this increase in mRNA can be abrogated by specific add back of both SAAs or just Cys (Hine et al., in press). Additionally, the diet responsive non-coding micro RNA (miRNA) 21 and miRNA 30 family members epigenetically repress CGL expression and subsequent H₂S production (Khanna et al., 2011; Shen et al., 2014; Yang et al., 2012).

2.4. Regulation of H₂S production by TSP

The canonical product of the TSP is Cys, an amino acid used in protein synthesis. However, Cys is also integral for production of glutathione (GSH) (Lyons et al., 2000), taurine

(Stipanuk, 2004) and H₂S (Kimura, 2011). Of these, H₂S is the most challenging to measure accurately because of its relatively short biological half-life (Wang, 2009). While the capacity of CGL to produce H₂S can be ascertained fairly easily in organ extracts or live cells by providing them with an excess of substrate (Cys) and cofactor (vitamin B₆), the measurement of endogenously produced levels of this short-lived gas species is much more challenging. Using techniques such as gas chromatography, sulfide probes, and differential extraction methods, a number of issues arise due to sample preparation and oxidation, the release of endogenous and labile sulfides, sensitivity and specificity for H₂S, giving rise to differences of opinion on the actual levels in vivo (Olson, 2009).

Whatever the actual levels, the fact that H₂S is produced by the same enzymes that synthesize its substrate, Cys, presents an apparent paradox: how can H₂S levels be increased when its production depends on a substrate whose limitation increases the responsible enzymes in the first place? Although the answer isn't known, several non-mutually exclusive possibilities exist. First, Cys isn't the only substrate of H₂S production. TSP enzymes CBS and CGL are rather promiscuous, and can catabolize cysteine, cystathionine, and homocysteine to produce H₂S. A second possibility is based on localization of the enzyme CGL to the mitochondria, where local free Cys concentrations are high. A recent report indicates that CGL localizes to mitochondria upon conditions of ER stress via the translocase, Tom20 (Fu et al., 2012). A third possibility is that the source of free Cys for H₂S production comes not from de novo Cys via the TSP, but from autophagy. Autophagy is expected to be upregulated under conditions of nutrient/energy limitation as in DR and PR/ MetR. In support of this latter possibility, extended chronological longevity upon MetR in yeast requires autophagy (Ruckenstuhl et al., 2014). Interestingly, in long-lived Ames dwarfs with upregulated TMP and TSP enzyme activity, the flux of Met through these pathways is higher (Uthus and Brown-Borg, 2006; Vitvitsky et al., 2013). Inevitably, measurements of metabolite flux combined with accurate quantitation of H₂S will be required to determine the substrate of H₂S in vivo under conditions of CR or PR/MetR.

In addition to sulfur-containing substrates, both CBS and CGL require the cofactor vitamin B₆ for transsulfuration activities including H₂S production. Vitamin B₆ from the diet is thus also relevant, as mutations in CGL that result in cystathioninuria affect binding of the cofactor to the enzyme and decrease its ability to synthesize H₂S (Zhu et al., 2008). Finally, in addition to causing relocation of TSP enzymes, oxidative stress stimulates CGL mRNA, protein and enzyme activity during the fetal to neonatal transition in rats. It remains to be seen if this results in increased H₂S production as well (Martin et al., 2007). H₂S may serve as a redox sensor in blood vessels, as hypoxia and H₂S have the same effects in multiple vessel types (Olson et al., 2006). In conclusion, H₂S production by the TSP can be influenced by the availability of substrate and enzyme cofactors, both of which are influenced by diet, as well as environmental conditions such as oxidative stress or endoplasmic reticulum stress that can influence subcellular localization and TSP enzyme activity levels.

2.5. Other pathways of H₂S production

In addition to the TSP, the enzyme 3MST in mammals can produce H₂S. However, the relevance of DR, PR or MetR to 3MST is unclear. In yeast, H₂S production is a major problem in wine-making, as it interferes with the smell and taste of the final product. Thus, there is considerable interest in strains that produce less H₂S. Interestingly, yeast are thought to produce most of their H₂S not by the TSP pathway, but by the assimilatory pathway that converts inorganic sulfate to sulfite, sulfide and then eventually into the organic homocysteine (Fig. 1). In the absence of this pathway, yeast cannot fix sulfur and are thus dependent on the addition of Met to the media for proper growth. They also produce considerably less H₂S under normal growth conditions. However, we found that glucose restriction, which extends chronological longevity, also increases H₂S production, and that this did not depend on the assimilatory pathway, strongly suggesting activity of the TSP as the source for H₂S. Interestingly, genetic mutants in the sulfate assimilatory pathway, including Met2 and/or Met15/17/25 (Fig. 1), produce an abundant amount of H₂S and have increased chronological lifespans (Johnson and Johnson, 2014).

3. Mechanisms of H₂S benefits

Like the other gasotransmitters NO and CO, H₂S is thought to act predominantly as a signaling molecule. H₂S can readily diffuse across membranes, and the production from one cell is thought to reach within a 200 cell radius of its origin (Cuevasanta et al., 2012). The major molecular mechanism of H₂S action is thought to be sulfhydrylation, or the formation of –SSH moieties, on surface-exposed Cys and thiol residues of proteins. This posttranslational modification can have a number of different effects on target protein function. Because exposed Cys residues are subject to nitrosylation, sulfhydrylation can compete for these moieties and affect protein function. For example, sulfhydrylation of Cys 38 of NFκB subunit p65 competes for nitrosylation and regulates the antiapoptotic activity of this transcription factor (Sen et al., 2012). Another target of H₂S with potential in health and longevity is the E3 ubiquitin ligase, parkin, whose dysfunction is implicated in aging-related neurodegeneration associated with Parkinson's disease. While nitrosylation of parkin inhibits protein activity, sulfhydrylation of specific Cys residues increases catalytic activity, thus potentially contributing to neuroprotection (Vandiver et al., 2013).

H₂S also has the ability to activate and/or open transmembrane receptors and channels via sulfhydrylation. As part of its ability to stimulate vascular angiogenesis, H₂S reduces a disulfide bond in the VEGFR2 cytoplasmic domain, changing the physical conformation of the receptor into the active kinase state (Tao et al., 2013). The role of H₂S as a vasodilator is based on its ability to sulfhydrylate the Kir6.2 regulatory subunit of the K_{ATP} channel in endothelial cells, leading to membrane hyperpolarization and vessel relaxation (Mustafa et al., 2011). Mutations in CGL or in K_{ATP} channel sulfhydrylation sites abolish the ability to relax, resulting in hypertension. Sulfhydrylation of K_{ATP} channels in insulin-secreting cells of the pancreas may also influence the ability of H₂S to regulate insulin secretion (Yang et al., 2005). Additionally, H₂S produced by the TSP in bone marrow mesenchymal stem cells (BMMSCs) regulates their self-renewal and osteogenic differentiation by maintaining proper calcium flux via sulfhydrylation of Cys residues on multiple calcium transient receptor

potential channels (TRP) channels (Liu et al., 2014). Thus, either by allosteric interaction, prevention of intra-molecular or intermolecular disulfide bond formation, structural changes, or competition for other potential modifications such as nitrosylation at the same residue, sulfhydrylation can serve to alter protein and cellular functions.

Sulfhydrylation can also serve a different purpose in energy metabolism. The mitochondrial protein SQR, an evolutionarily conserved component of the mitochondrial electron transport chain (ETC) functionally overlapping with Complex 2, has two exposed Cys residues that become sulfhydrylated sequentially by H₂S. Because SQR can donate electrons to Coenzyme Q, each sulfhydrylation results in the donation of electrons to the ETC with the potential to generate ATP. Reoxidation of the two Cys residues occurs with the concerted action of a number of mitochondrial proteins and results in the formation of thiosulfate, which can be exported and secreted in the urine, or under some condition such as hypoxia can itself be converted to H₂S (Olson et al., 2013). In this context, H₂S can thus be thought of as an inorganic energy source. While this may be extremely useful during periods of hypoxia, whether or not this can contribute significantly during periods of nutrient/energy deprivation, as in DR, remains to be determined. Furthermore, whether mitochondrial H₂S production requires CGL or the non-TSP component 3MST remains unclear (Modis et al., 2013). 3MST in the brain may be a major contributor to H₂S in that organ (Shibuya et al., 2009).

Finally, H₂S has the capacity to work directly as an antioxidant, protecting various cell types against hydrogen peroxide induced oxidative stress in HUVECs (Wen et al., 2013) and primary mouse endothelial cells (Hine et al., in press).

4. Conclusions

DR regimens, whether through restriction of overall calories or limitation of essential nutrient such as protein or SAAs, clearly have numerous health benefits. Similarly, H₂S from either exogenous sources or produced endogenously imparts benefits that overlap with DR (Fig. 2). Recently, we proposed that upregulation of the TSP and increased H₂S are causative of at least some of these benefits, including stress resistance and extended longevity (Hine et al., in press). As the biological effects of both DR and H₂S are pleiotropic, much remains to be done in order to determine the extent to which DR benefits depend on H₂S, as well as the molecular mechanisms underlying these effects. For example, H₂S production is not mutually exclusive with other consequences of increased TSP activity, including biosynthesis of downstream metabolites GSH and taurine. As we learn more about the potential of nutrient/energy sensors and downstream transcription factors already implicated in evolutionarily conserved DR benefits to regulate the TSP and H₂S production, the specific contributions of this gas as a downstream mediator of DR benefits should come into focus.

How could sulfhydrylation of target proteins such as NFκB, K_{ATP} channels or VEGFR2, or electron donation to the mitochondrial ETC, confer benefits of DR? Obviously much remains to be learned about the protein targets of sulfhydrylation, particularly by endogenously produced H₂S, and how this affects individual protein function and

physiology on the organism level. Nonetheless, changes in energy metabolism, mitochondrial biogenesis, ROS generation, insulin sensitivity, cardiovascular health and inflammatory capacity have all been implicated in DR benefits and can also be modulated by H₂S.

Resolving the potential overlap between CR and PR/MetR to DR benefits, and the contribution of H₂S to each, is of great interest and importance in both basic and clinical scientific realms. Identification of convergent points will undoubtedly lead to a better molecular understanding of this fascinating phenomenon. For the purposes of clinical translation, however, each beneficial treatment has different advantages and disadvantages. Relative to CR and the associated difficulties of reduced overall food intake, temporary or long lasting restriction of SAA intake, common in vegan diets already adopted by millions of people throughout the world (McCarty et al., 2009), may be more appealing to people wishing to obtain said benefits for various clinical applications or life-long improved health. For other purposes, H₂S supplementation may be easier, but whether exogenously added H₂S can safely recapitulate benefits of endogenously produced H₂S remains to be fully explored. While improved overall health is a worthy long-term goal, immediate impact on planned stressful events such as elective surgery or chemotherapy requiring only a short intervention would appear to be the low-hanging fruit with the most immediate potential for clinical translation.

Acknowledgments

This work was supported by grants from NIH (DK090629, AG036712) and the Glenn Foundation for Medical Research to JRM; CH was supported by T32CA0093823.

References

- Anson RM, Jones B, de Cabod R. The diet restriction paradigm: a brief review of the effects of every-other-day feeding. *Age (Dordr.)*. 2005; 27:17–25. [PubMed: 23598600]
- Bishop NA, Guarente L. Two neurons mediate diet-restriction-induced longevity in *C. elegans*. *Nature*. 2007; 447:545–549. [PubMed: 17538612]
- Blackstone E, Roth MB. Suspended animation-like state protects mice from lethal hypoxia. *Shock*. 2007; 27:370–372. [PubMed: 17414418]
- Blackstone E, Morrison M, Roth MB. H₂S induces a suspended animation-like state in mice. *Science*. 2005; 308:518. [PubMed: 15845845]
- Cabreiro F, Au C, Leung KY, Vergara-Irigaray N, Cocheme HM, Noori T, Weinkove D, Schuster E, Greene ND, Gems D. Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. *Cell*. 2013; 153:228–239. [PubMed: 23540700]
- Cuevasanta E, Denicola A, Alvarez B, Möller MN. Solubility and permeation of hydrogen sulfide in lipid membranes. *PLoS One*. 2012; 7:e34562. [PubMed: 22509322]
- Elshorbagy AK, Valdivia-Garcia M, Mattocks DA, Plummer JD, Orentreich DS, Orentreich N, Refsum H, Perrone CE. Effect of taurine and N-acetylcysteine on methionine restriction-mediated adiposity resistance. *Metab. Clin. Exp.* 2013; 62:509–517. [PubMed: 23154184]
- Fontana L, Partridge L, Longo VD. Extending healthy life span—from yeast to humans. *Science*. 2010; 328:321–326. [PubMed: 20395504]
- Forster MM. Mineral springs and miracles. *Can. Fam. Physician*. 1994; 40:729–737. [PubMed: 8199525]

- Fu M, Zhang W, Wu L, Yang G, Li H, Wang R. Hydrogen sulfide (H₂S) metabolism in mitochondria and its regulatory role in energy production. *Proc. Natl. Acad. Sci. U. S. A.* 2012; 109:2943–2948. [PubMed: 22323590]
- Fukagawa NK. Protein and amino acid supplementation in older humans. *Amino Acids.* 2013; 44:1493–1509. [PubMed: 23563921]
- Grandison RC, Piper MD, Partridge L. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature.* 2009; 462:1061–1064. [PubMed: 19956092]
- Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, Blanchard D, Gygi SP, Brunet A. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. *Curr. Biol.* 2007; 17:1646–1656. [PubMed: 17900900]
- Gupta S, Kühnisch J, Mustafa A, Lhotak S, Schlachterman A, Slifker MJ, Klein-Szanto A, High KA, Austin RC, Kruger WD. Mouse models of cystathionine beta-synthase deficiency reveal significant threshold effects of hyperhomocysteinemia. *FASEB J.* 2009; 23:883–893. [PubMed: 18987302]
- Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calton M, Sadri N, Yun C, Popko B, Paules R, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol. Cell.* 2003; 11:619–633. [PubMed: 12667446]
- Harputiligil E, Hine C, Vargas D, Robertson L, Manning BD, Mitchell JR. The TSC complex is required for the benefits of dietary protein restriction on stress resistance in vivo. *Cell Rep.* 2014; 8:1160–1170. [PubMed: 25131199]
- Hine, C.; Harputlugil, E.; Zhang, Y.; Ruckenstein, C.; Lee, BC.; Brace, LE.; Longchamp, A.; Trevino-Villarreal, JH.; Mejia, P.; Ozaki, CK., et al. Endogenous Hydrogen Sulfide Production is Essential for Dietary Restriction Benefits.. *Cell.* 2015. <http://dx.doi.org/10.1016/j.cell.2014.11.048> (in press)
- Ishii I, Akahoshi N, Yamada H, Nakano S, Izumi T, Suematsu M. Cystathionine gamma-Lyase-deficient mice require dietary cysteine to protect against acute lethal myopathy and oxidative injury. *J. Biol. Chem.* 2010; 285:26358–26368. [PubMed: 20566639]
- Johnson JE, Johnson FB. Methionine restriction activates the retrograde response and confers both stress tolerance and lifespan extension to yeast, mouse and human cells. *PLoS One.* 2014; 9:e97729. [PubMed: 24830393]
- Kabil H, Kabil O, Banerjee R, Harshman LG, Pletcher SD. Increased transsulfuration mediates longevity and dietary restriction in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 2011; 108:16831–16836. [PubMed: 21930912]
- Khanna A, Muthusamy S, Liang R, Sarojini H, Wang E. Gain of survival signaling by down-regulation of three key miRNAs in brain of calorie-restricted mice. *Aging (Albany NY).* 2011; 3:223–236. [PubMed: 21415464]
- Kida K, Yamada M, Tokuda K, Marutani E, Kakinohana M, Kaneki M, Ichinose F. Inhaled hydrogen sulfide prevents neurodegeneration and movement disorder in a mouse model of Parkinson's disease. *Antioxid. Redox Signal.* 2011; 15:343–352. [PubMed: 21050138]
- Kimura H. Hydrogen sulfide: its production and functions. *Exp. Physiol.* 2011; 96:833–835. [PubMed: 21527544]
- Lee JI, Dominy JE Jr, Sikalidis AK, Hirschberger LL, Wang W, Stipanuk MH. HepG2/C3A cells respond to cysteine deprivation by induction of the amino acid deprivation/integrated stress response pathway. *Physiol. Genomics.* 2008; 33:218–229. [PubMed: 18285520]
- Lee ZW, Zhou J, Chen CS, Zhao Y, Tan CH, Li L, Moore PK, Deng LW. The slow-releasing hydrogen sulfide donor, GYY4137, exhibits novel anti-cancer effects in vitro and in vivo. *PLoS One.* 2011; 6:e21077. [PubMed: 21701688]
- Lee ZW, Teo XY, Tay EY, Tan CH, Hagen T, Moore PK, Deng LW. Utilizing hydrogen sulfide as a novel anti-cancer agent by targeting cancer glycolysis and pH imbalance. *Br. J. Pharmacol.* 2014; 171:4322–4336. [PubMed: 24827113]
- Lees EK, Krol E, Grant L, Shearer K, Wyse C, Moncur E, Bykowska AS, Mody N, Gettys TW, Delibegovic M. Methionine restriction restores a younger metabolic phenotype in adult mice with alterations in fibroblast growth factor 21. *Aging Cell.* 2014; 13(5):817–827. <http://dx.doi.org/10.1111/ace.12238>. [PubMed: 24935677]

- Li W, Li X, Miller RA. ATF4 activity: a common feature shared by many kinds of slow-aging mice. *Aging Cell*. 2014; 13(6):1012–1018. <http://dx.doi.org/10.1111/accel.12264>. [PubMed: 25156122]
- Liu Y, Yang R, Liu X, Zhou Y, Qu C, Kikuri T, Wang S, Zandi E, Du J, Ambudkar IS, et al. Hydrogen sulfide maintains mesenchymal stem cell function and bone homeostasis via regulation of Ca^{2+} channel sulphydration. *Cell Stem Cell*. 2014; 15:66–78. [PubMed: 24726192]
- Lopez-Torres M, Barja G. Lowered methionine ingestion as responsible for the decrease in rodent mitochondrial oxidative stress in protein and dietary restriction possible implications for humans. *Biochim. Biophys. Acta*. 2008; 1780:1337–1347. [PubMed: 18252204]
- Lyons J, Rauh-Pfeiffer A, Yu YM, Lu XM, Zurawski D, Tompkins RG, Ajami AM, Young VR, Castillo L. Blood glutathione synthesis rates in healthy adults receiving a sulfur amino acid-free diet. *Proc. Natl. Acad. Sci. U. S. A.* 2000; 97:5071–5076. [PubMed: 10792033]
- Mair W, Morante I, Rodrigues AP, Manning G, Montminy M, Shaw RJ, Dillin A. Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. *Nature*. 2011; 470:404–408. [PubMed: 21331044]
- Mani S, Li H, Untereiner A, Wu L, Yang G, Austin RC, Dickhout JG, Lhotak S, Meng QH, Wang R. Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. *Circulation*. 2013; 127:2523–2534. [PubMed: 23704252]
- Manna P, Gungor N, McVie R, Jain SK. Decreased cystathionine-gamma-lyase (CSE) activity in livers of type 1 diabetic rats and peripheral blood mononuclear cells (PBMC) of type 1 diabetic patients. *J. Biol. Chem*. 2014; 289:11767–11778. [PubMed: 24610811]
- Martin JA, Pereda J, Martinez-Lopez I, Escrig R, Miralles V, Pallardo FV, Vina JR, Vento M, Vina J, Sastre J. Oxidative stress as a signal to up-regulate gamma-cystathionase in the fetal-to-neonatal transition in rats. *Cell. Mol. Biol. (Noisy-le-grand)*. 2007; 53(Suppl.):OL1010–OL1017. [PubMed: 18184479]
- McCarty MF, Barroso-Aranda J, Contreras F. The low-methionine content of vegan diets may make methionine restriction feasible as a life extension strategy. *Med. Hypotheses*. 2009; 72:125–128. [PubMed: 18789600]
- Miller DL, Roth MB. Hydrogen sulfide increases thermotolerance and lifespan in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 2007; 104:20618–20622. [PubMed: 18077331]
- Miller RA, Buehner G, Chang Y, Harper JM, Sigler R, Smith-Wheelock M. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. *Aging Cell*. 2005; 4:119–125. [PubMed: 15924568]
- Miller DL, Budde MW, Roth MB. HIF-1 and SKN-1 coordinate the transcriptional response to hydrogen sulfide in *Caenorhabditis elegans*. *PLoS One*. 2011; 6:e25476. [PubMed: 21980473]
- Modis K, Coletta C, Erdelyi K, Papapetropoulos A, Szabo C. Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. *FASEB J*. 2013; 27:601–611. [PubMed: 23104984]
- Morrison ML, Blackwood JE, Lockett SL, Iwata A, Winn RK, Roth MB. Surviving blood loss using hydrogen sulfide. *J. Trauma*. 2008; 65:183–188. [PubMed: 18580516]
- Mustafa AK, Sikka G, Gazi SK, Stepan J, Jung SM, Bhunia AK, Barodka VM, Gazi FK, Barrow RK, Wang R, et al. Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulphydrates potassium channels. *Circ. Res*. 2011; 109:1259–1268. [PubMed: 21980127]
- Nimni ME, Han B, Cordoba F. Are we getting enough sulfur in our diet? *Nutr. Metab*. 2007; 4:24.
- Olson KR. Is hydrogen sulfide a circulating “gasotransmitter” in vertebrate blood? *Biochim. Biophys. Acta*. 2009; 1787:856–863. [PubMed: 19361483]
- Olson KR, Dombkowski RA, Russell MJ, Doellman MM, Head SK, Whitfield NL, Madden JA. Hydrogen sulfide as an oxygen sensor/transducer in vertebrate hypoxic vasoconstriction and hypoxic vasodilation. *J. Exp. Biol*. 2006; 209:4011–4023. [PubMed: 17023595]
- Olson KR, DeLeon ER, Gao Y, Hurley K, Sadauskas V, Batz C, Stoy GF. Thiosul-fate: a readily accessible source of hydrogen sulfide in oxygen sensing. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 2013; 305:R592–R603. [PubMed: 23804280]
- Orentreich N, Matias JR, DeFelice A, Zimmerman JA. Low methionine ingestion by rats extends life span. *J. Nutr*. 1993; 123:269–274. [PubMed: 8429371]

- Paul BD, Sbodio JI, Xu R, Vandiver MS, Cha JY, Snowman AM, Snyder SH. Cystathionine gamma-lyase deficiency mediates neurodegeneration in Huntington's disease. *Nature*. 2014; 509:96–100. [PubMed: 24670645]
- Pearson KJ, Lewis KN, Price NL, Chang JW, Perez E, Cascajo MV, Tamashiro KL, Poosala S, Csiszar A, Ungvari Z, et al. Nrf2 mediates cancer protection but not longevity induced by caloric restriction. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:2325–2330. [PubMed: 18287083]
- Plaisance EP, Greenway FL, Boudreau A, Hill KL, Johnson WD, Krajcik RA, Perrone CE, Orentreich N, Cefalu WT, Gettys TW. Dietary methionine restriction increases fat oxidation in obese adults with metabolic syndrome. *J. Clin. Endocrinol. Metab.* 2011; 96:E836–E840. [PubMed: 21346062]
- Ruckenstuhl C, Netzerberger C, Entfellner I, Carmona-Gutierrez D, Kickenweiz T, Stekovic S, Gleixner C, Schmid C, Klug L, Sorgo AG, et al. Lifespan extension by methionine restriction requires autophagy-dependent vacuolar acidification. *PLoS Genet.* 2014; 10:e1004347. [PubMed: 24785424]
- Sen N, Paul BD, Gadalla MM, Mustafa AK, Sen T, Xu R, Kim S, Snyder SH. Hydrogen sulfide-linked sulfhydration of NF-kappaB mediates its antiapoptotic actions. *Mol. Cell.* 2012; 45:13–24. [PubMed: 22244329]
- Shen, Y.; Shen, Z.; Miao, L.; Xin, X.; Lin, S.; Zhu, YC.; Guo, W.; Zhu, Y. MiRNA-30 family inhibition protects against cardiac ischemic injury by regulating cystathionine-γ-lyase expression. *Antioxid. Redox Signal.* 2014. <http://dx.doi.org/10.1089/ars.2014.5909>
- Shibuya N, Tanaka M, Yoshida M, Ogasawara Y, Togawa T, Ishii K, Kimura H. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid. Redox Signal.* 2009; 11:703–714. [PubMed: 18855522]
- Sikalidis AK, Stipanuk MH. Growing rats respond to a sulfur amino acid-deficient diet by phosphorylation of the alpha subunit of eukaryotic initiation factor 2 heterotrimeric complex and induction of adaptive components of the integrated stress response. *J. Nutr.* 2010; 140:1080–1085. [PubMed: 20357079]
- Stipanuk MH. Role of the liver in regulation of body cysteine and taurine levels: a brief review. *Neurochem. Res.* 2004; 29:105–110. [PubMed: 14992268]
- Tao BB, Liu SY, Zhang CC, Fu W, Cai WJ, Wang Y, Shen Q, Wang MJ, Chen Y, Zhang LJ, et al. VEGFR2 functions as an H2S-targeting receptor protein kinase with its novel Cys1045–Cys1024 disulfide bond serving as a specific molecular switch for hydrogen sulfide actions in vascular endothelial cells. *Antioxid. Redox Signal.* 2013; 19:448–464. [PubMed: 23199280]
- Thivat E, Durando X, Demidem A, Farges MC, Rapp M, Cellarier E, Guenin S, D'Incan M, Vasson MP, Chollet P. A methionine-free diet associated with nitrosourea treatment down-regulates methylguanine-DNA methyl transferase activity in patients with metastatic cancer. *Anticancer Res.* 2007; 27:2779–2783. [PubMed: 17695447]
- Troen AM, French EE, Roberts JF, Selhub J, Ordovas JM, Parnell LD, Lai CQ. Lifespan modification by glucose and methionine in *Drosophila melanogaster* fed a chemically defined diet. *Age (Dordr.)*. 2007; 29:29–39. [PubMed: 19424828]
- Uthus EO, Brown-Borg HM. Methionine flux to transsulfuration is enhanced in the long living Ames dwarf mouse. *Mech. Ageing Dev.* 2006; 127:444–450. [PubMed: 16519922]
- Vandiver MS, Paul BD, Xu R, Karuppagounder S, Rao F, Snowman AM, Ko HS, Lee YI, Dawson VL, Dawson TM, et al. Sulfhydration mediates neuroprotective actions of parkin. *Nat. Commun.* 2013; 4:1626. [PubMed: 23535647]
- Vitvitsky V, Martinov M, Ataullakhanov F, Miller RA, Banerjee R. Sulfur-based redox alterations in long-lived Snell dwarf mice. *Mech. Ageing Dev.* 2013; 134:321–330. [PubMed: 23707637]
- Wang R. Hydrogen sulfide: a new EDRF. *Kidney Int.* 2009; 76:700–704. [PubMed: 19536086]
- Wen YD, Wang H, Kho SH, Rinkiko S, Sheng X, Shen HM, Zhu YZ. Hydrogen sulfide protects HUVECs against hydrogen peroxide induced mitochondrial dysfunction and oxidative stress. *PLoS One.* 2013; 8:e53147. [PubMed: 23393548]
- WHO. Hydrogen Sulfide in the Drinking Water. 2003
- Wu Z, Song L, Liu SQ, Huang D. Independent and additive effects of glutamic acid and methionine on yeast longevity. *PLoS One.* 2013; 8:e79319. [PubMed: 24244480]

- Xue R, Hao DD, Sun JP, Li WW, Zhao MM, Li XH, Chen Y, Zhu JH, Ding YJ, Liu J, et al. Hydrogen sulfide treatment promotes glucose uptake by increasing insulin receptor sensitivity and ameliorates kidney lesions in type 2 diabetes. *Antioxid. Redox Signal.* 2013; 19:5–23. [PubMed: 23293908]
- Yang W, Yang G, Jia X, Wu L, Wang R. Activation of K_{ATP} channels by H₂S in rat insulin-secreting cells and the underlying mechanisms. *J. Physiol.* 2005; 569:519–531. [PubMed: 16179362]
- Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, et al. H₂S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science.* 2008; 322:587–590. [PubMed: 18948540]
- Yang G, Pei Y, Cao Q, Wang R. MicroRNA-21 represses human cystathionine gamma-lyase expression by targeting at specificity protein-1 in smooth muscle cells. *J. Cell. Physiol.* 2012; 227:3192–3200. [PubMed: 22034194]
- Yin P, Zhao C, Li Z, Mei C, Yao W, Liu Y, Li N, Qi J, Wang L, Shi Y, et al. Sp1 is involved in regulation of cystathionine gamma-lyase gene expression and biological function by PI3K/Akt pathway in human hepatocellular carcinoma cell lines. *Cell. Signal.* 2012; 24:1229–1240. [PubMed: 22360859]
- Zhang Y, Tang ZH, Ren Z, Qu SL, Liu MH, Liu LS, Jiang ZS. Hydrogen sulfide, the next potent preventive and therapeutic agent in aging and age-associated diseases. *Mol. Cell. Biol.* 2013; 33:1104–1113. [PubMed: 23297346]
- Zhu W, Lin A, Banerjee R. Kinetic properties of polymorphic variants and pathogenic mutants in human cystathionine gamma-lyase. *Biochemistry.* 2008; 47:6226–6232. [PubMed: 18476726]
- Zhu XY, Liu SJ, Liu YJ, Wang S, Ni X. Glucocorticoids suppress cystathionine gamma-lyase expression and H₂S production in lipopolysaccharide-treated macrophages. *Cell. Mol. Life Sci.* 2010; 67:1119–1132. [PubMed: 20063035]

Fig. 1.

Pathways of H₂S production. Model of the transmethylation and transsulfuration pathway (TSP) in metazoans (left) and in yeast (right). Solid arrows trace canonical sulfur transfer from Met to Cys (in metazoans), or from inorganic sulfate and/or Met to Cys (in yeast), through various metabolites and downstream cellular processes via the enzymes Cystathionine Beta-Synthase (CBS;STR4) and Cystathionine Gamma-Lyase (CGL;STR1). Dotted arrows trace alternative pathways/usage of transmethylation products or TSP enzymes for production of H₂S. MAT: methionine adenosyl transferase, SAM: S-adenosylmethionine, SAH: S-adenosylhomocysteine, SAHH: S-adenosylhomocysteine hydrolase, and MS: methionine synthase.

Fig. 2. Overlapping benefits of DR and H₂S. Shared benefits of reduced intake of SAA common to various DR regimens including CR, PR and SAA restriction, and H₂S from endogenous or exogenous sources.