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Hydrogen sulfide, microbiota, and sulfur amino acid restriction diet

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Abstract

Eukaryotes and microbiota produce H_2S, using the same substrates and enzymes which constitute the reverse-trans-sulfuration and transsulfuration pathways. The homeostasis of gut microbiota impacts on the structural and functional integrity of gut epithelial barrier. Microbiota also serve as signalling sources to inform the host of the metabolism and functional changes. Microbiota dysbiosis negatively affect human health, contributing to diseases like obesity, diabetes, inflammatory bowel diseases, and asthma. Not by coincidence, these pathological conditions are also closely related to the abnormal metabolism and function of H_2S signalling.H_2S serves as a bacterial signal to the host and the host-produced H_2S impacts on the population and size of microbiota. These bi-directional interactions become especially important for the digestion and utilization of sulfur amino acid in diet. Dietary restriction of sulfur amino acid increases the endogenous production of H_2S by the host and consequently offers many health benefits. It, on the other hand, decreases the nutritional supply to the microbiota, which could be remedied by the co-application of prebiotics and probiotics. It is strategically sound to target the expression of H_2S-producing enzymes in different organs to slow aging processes in our body and promote better health.

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Signalling molecules in eukaryote cells in the forms of gases or their ionic derivatives have taken the central court in cellular signaling arena. These molecules were categorized and conceptualized as “gasotransmitters” firstly in 2002[1-3] to reveal their common intrinsic attributes and differentiate them from other signaling molecules, such as neurotransmitters. Gasotransmitters, including nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H2S), are assessed by six classification criteria (Fig. 1).

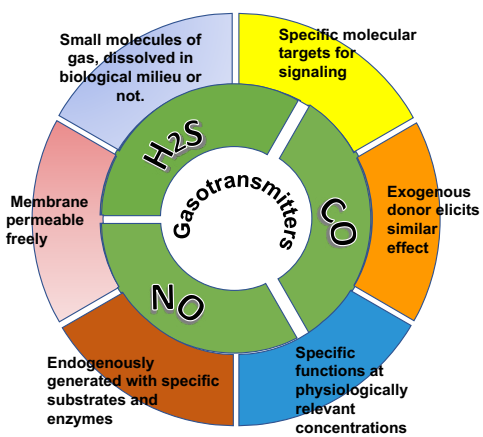

Fig. 1. Concept and membership of gasotransmitter family

1. H_2S is a gasotransmitter

H_2S was traditionally viewed as a toxic gas detected in contaminated environment[2,4]. Natural events, such as volcano eruptions or natural fermentation, and human activities in agriculture or industry generate H_2S. On the other hand, H_2S is a lifesaver. Its origination on earth has been linked to the survival of early life on this planet[4]. Three enzymes have been identified in eukaryotes for the catalyzation of H_2S production, i.e. cystathionine gamma-lyase (CSE), cystathionine beta-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (MST) which is also known as beta-mercaptopyruvate sulfurtransferase. The purification and detection of enzymatic activities of CSE, CBS, and MST were all accomplished about 60 years ago[5-7]. It took about 30 years to clone the genes of CSE[8], CBS[9], and MST[10] in eukaryotes. In mammalian cardiovascular system, the first H2S-generating enzyme (CSE) was cloned and sequenced in vascular smooth muscle cells in 2001[11].

Enzymatic production of H_2S in rodent liver and kidney was known long ago[12]. Human and microbiota produce H_2S through reverse trans-sulfuration and trans-sulfuration pathways, respectively, and use this gas molecule for their important physiological or biological functions. Basal levels of sulfide in rat and human brain tissues were detected about 30 years ago[13-14]. Clearly, thus detected sulfide was not the product of bacteria, neither the outcome of environmental intoxication.

The first identified molecular target of H_2S is ATP-sensitive K+ (KATP) channels in vascular smooth muscle cells[11]. Over the last 20 years, numerous other molecular targets of H_2S have been reported. Due to its reducing property, H2S functions as an antioxidant by directly scavenging reactive oxygen species[2]. In the mitochondria, H_2S stimulates mitochondrial bioenergetics by acting as an electron donor[15] and directly enhancing the activity of ATP synthase[16].

The mammalian relevance of H_2S metabolism to the physiological or biological functions was initially suggested from the study on the effects of exogenously applied H_2S salt and the pharmacological blockade of the known H2S-production enzymes on neuronal and vascular functions[11,17-18]. The first direct evidence for the physiological role of endogenous H_2S came from our 2008 study in which we reported the establishment of the first genetically engineered mouse strain with deficiency in cystathionine-γ-lyase (CSE) gene[19]. Global knock-out of CSE gene in mice leads to age-dependent development of hypertension as well as diminished endothelium-dependent relaxation of peripheral resistance arteries. We showed that H_2S suppressed early development of atherosclerosis[20] and the proliferation of vascular smooth muscle cells[2,21], but promoted angiogenesis and endothelial proliferation[22-23]. H_2S relaxes vascular tissues by opening KATP channels in vascular smooth muscle cells[11, 24-25] or by functioning as an endothelium-derived hyperpolarizing factor (EDHF) to cause endothelium-dependent vasorelaxation[26-27]. H_2S protects the heart from ischemia/reperfusion damage[28-30]. It also offers anti-inflammatory and antioxidant protections[2,11,19]. Increased endogenous production of H_2S beyond physiological range may also be detrimental. For example, high level of endogenous H_2S was shown to enhance hypoxic pulmonary vasoconstriction in rats[31]. Increased endogenous production of H_2S from pancreatic beta cells also contributes to the pathogenesis of diabetes[32].

Research progresses and discoveries over the last two decades have firmly established H_2S as one of the three gasotransmitters.

2. Production of H_2S by eukaryotes

CSE and CBS are pyridoxal 5'-phosphate (P5P)-dependent enzymes. They produce H_2S, pyruvate, and ammonium using homocysteine and/or L-cysteine as substrates and pyridoxal L-phosphate (PLP) as a cofactor[2]. Both CBS and CSE catalyze reactions in the reverse-trans-sulfuration pathway. The third enzyme for enzymatic H_2S production is a P5P-independent 3-mercaptopyruvate sulfurtransferase (MST). MST is widely distributed in prokaryotes and eukaryotes but not all eukaryotes express this enzyme. MST is localized in the cytoplasm and mitochondria. The subcellular distribution of MST and its correlation with H_2S production in mammalian liver, kidney, and adrenal cortex tissues were shown about 50 years ago[33-34].

The three canonical H_2S-producing enzymes are selectively expressed in different mammalian cells. CBS is the primary enzyme producing H_2S in the central nervous system. It is expressed in neurons and astrocytes, more in the hippocampus and cerebellum than in cerebral cortex and brain stem. In the cardiovascular system, respiratory system, testes, spleen and the pancreas, CBS expression is rare or absent, but CSE expression is abundant[1,2,19]. Both CSE and CBS are expressed in the liver and kidney but CSE appears to have more important physiological functions in these organs[2,35-36]. CSE is also expressed in different regions of the brain, such as cortex, striatum, cerebellum, brain stem, hippocampus and hypothalamus[37]. In the cardiovascular system, MST has been detected in the endothelium and other types of cells[38], but not in vascular smooth muscle cells or cardiomyocytes. MST is expressed in the liver and kidney. In the central nervous system, MST is localized in hippocampal pyramidal neurons, cerebellar Purkinje cells, and mitral cells in the olfactory bulb of the brain[38]. MST is also expressed in liver, kidney, and red blood cells[39-40] .

3. Production of H_2S by microbiota

Microbiota are the collection of bacteria, viruses, and fungi in a given environment. They reside in the gastrointestinal tract, the skin, mouth, and other organs with direct connection to the body surface under physiological conditions. More than 1014 cells from 500-1000 microbiota species, originating from endogenous and exogenous sources, habit in a mammalian body. For a better understanding of the bacterial production of H_2S in the gut, the process of diet digestion is critical[41].

Approximately 50% of the dietary amino acids (AAs) are absorbed in the gut and recaptured in the liver via the portal drained viscera. The recycled AAs are partially metabolized by the liver or released into the peripheral circulatory system. About 30% of dietary AAs are used by a wide spectrum of bacteria in the small intestine for the metabolic need, survival and proliferation of the microbiota as well as the synthesis of bacterial constituents. The remaining dietary AAs are catabolized by bacteria in the large intestine via

two major mechanisms: deamination and decarboxylation.
In addition to dietary AAs, bacteria-produced AAs are also needed for gut AA homeostasis. Intestinal microbes synthesize some essential AAs de novo to meet host requirements.

Microbiota-derived H_2S constitutes an intertwined defense system against antibiotics and oxidative stress, serving a protective role for themselves and a detrimental role for the host. Some or all of the homologues of H_2S-producing enzymes (CSE, CBS, and MST) participate in bacterial production of H_2S. In gnotobiotic mice lacking a microbiome, H_2S levels are reduced in plasma, adipose, and lung tissues, and CSE activity is reduced in many organs consistent with bidirectional effects of microbiome and host on sulfide metabolism.[42] Bacteria also use sulfur amino acids (L-cysteine and methionine) as substrates for H_2S production. In most bacteria L-cysteine (Cys) is produced enzymatically via CysE, CysK and CysM from L-serine [43] .The key bacteria associated with the metabolism of methionine are mostly reside in large intestine and their metabolism generates various nitrogen- and sulfur-containing metabolites. These bacteria include Clostridium spp., Peptostreptococcus spp., Eubacterium, Salmonella enterica, Escherichia coli, Enterobacter aerogenes, and Klebsiella pneumoniae.

Non-enzymatic pathways also contribute to endogenous H_2S levels in bacteria. These include degradation of cysteine and other sulfur-containing amino acids/peptides, and dissimilatory reduction of inorganic sulfur compounds by sulfate-reducing bacteria (SRB). Being one of the oldest species of microbiota on earth[4], SRB represents a major class of normal gut microbiota. Human gut-inhabited SRB include Desulfovibrio, Desulfobacter, Desulfolobus, and Desulfotomaculum. Individuals with low bacterial richness and diversity have an increased potential for H_2S formation from SRB, more likely to develop obesity and insulin resistance by gaining more weight over time, and have increased inflammatory phenotypes[44].

Many antibiotics, such as spectinomycin, gentamycin, amikacin, and ampicillin, stimulate bacterial respiration and increase the production of hydroxyl radicals via Fe2+-catalysed Fenton reaction and oxidative damage to bacterial DNA. By producing endogenous H2S, Gram-negative and Gram-positive bacteria, including Bacillus anthracis, Pseudomonas aeruginosa, Staphylococcus aureus, and E. coli become resistant to antibiotics and oxidative stress. In comparison with wild-type bacterial strains, the strains with expressional deficiency of MST or CBS or CSE exhibits decreased endogenous H_2S production and higher susceptibility to a wide spectrum of antibiotics with different structures and functions. Overexpression of MST equips these bacteria with self-protection against spectinomycin. On the other hand, pharmacologically inhibiting the activities of MST, CBS, and CSE renders them

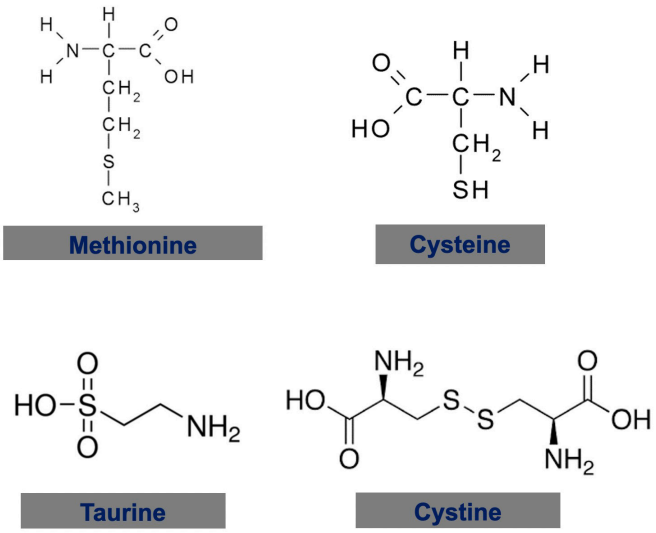

Fig. 2. Chemical formulas of sulfur amino acids

sensitive to a range of antibiotics. The application of exogenous H_2S salt and NaHS at low concentrations makes these pathogens resistant to antibiotics[45].

4. Sulfur amino acid restriction diet

Sulfur amino acids (SAAs) include methionine, cysteine, cystine, and taurine (Fig. 2). Daily requirement of SAAs from diet is ~13–15 mg/kg for humans. Dietary SAA restriction (SAAR), restriction of methionine and cysteine contents in diet, has been shown to improve longevity, brain function, vascular endothelium integrity, and metabolism of experimental animals[46-48]. The emergence of SAAR as a potential approach in improving metabolic health is intriguing, especially when considering the paradoxical nature of the response since total diet restriction has been related to retarded growth and bone development[49]. We have previously reported that these beneficiary effects of total diet restriction and SAAR are mediated by increased endogenous H_2S production in different organs[46-47]. Methionine restriction is known to upregulate CSE and CBS genes in naked mole-rats. Furthermore, both CBS overexpression to produce more endogenous H_2S and application of exogenous H2S extend life span of worms.

Methionine is one of the nine essential amino acid which human body cannot make. The gastrointestinal tract has the capacity to metabolize approximately 20% of methionine from the digested food proteins[50]. The dietary methionine is absorbed into the host through intestinal epithelium, accounting for about 30% of the variance in serum levels of methionine. The symbiotic gastrointestinal microbes also synthesize methionine that is

available to the host. It may at least partially contribute to the host blood level of methionine[51].

Methionine is the first amino acid present in nuclear-encoded proteins, as the methionine codon signals the start of protein translation. Methionine is the precursor of cysteine. Homocysteine and cystathionine are the intermediates of this conversion. S-adenosyl methionine derived from methionine donates methyl group for methylation of DNA and proteins. Methionine is digested and absorbed through intestinal epithelium in small intestine via methionine transporters. Plasma methionine levels in mouse, rat, and human are in the range of 20–100 μM. Whether these levels of methionine are indicative of intracellular or organismal methionine status, however, has been unsettled[51]. In a rat experiment with methionine restriction diet, serum levels of methionine as well as cysteine were significantly lowered. Cysteine supplementation to methionine restriction diet elevated serum cysteine level, but not methionine level[52].

Dietary SAAR can be mimicked in a reduced system. In a cellular senescence study of culture duration >140 days, methionine concentration in the culture medium was purposely decreased from 30 mg/L to 1 mg/L. This methionine restriction increased the replication lifespan of primary human diploid fibroblasts, delaying cellular senescence. Suppressed mitochondrial protein synthesis and respiratory chain assembly as well as the activity of mitochondrial complex IV were believed underlying the effect of methionine restriction. Whether endogenous H2S metabolism had been changed by methionine restriction was not studied in this cellular experiment[53].

Cysteine is a semi-essential amino acid which is not exclusively obtained from diet since it can also be obtained via the breakdown of methionine. Cysteine is the precursor of glutathione (GSH). Serum levels of cysteine and GSH are 100–200 μM and 10–25 μM, respectively[50]. The relative cytosolic concentration of cysteine is 80–100 μM whereas in the mitochondrial matrix cysteine concentration is about 7-10 fold of that in the cytosol[50]. Cysteine is more reactive but less hydrophobic than methionine. The high concentration of cysteine in the mitochondrion is of great functional significance. It offers an essential adaptation mechanism for highly demanding redox balance in the mitochondrion. It enables cysteine transamination by cysteine/aspartate aminotransferase. It fuels the synthesis of other sulfur metabolites, such as GSH and taurine. It also provides the substrate for endogenous H_2S production inside the mitochondrion[15].

Oxidation of two monomers of cysteine forms cystine dimer. Cystine is required to establish and maintain the three-dimensional structures of certain proteins by constructing disulfide bond(s) between two cysteine molecules. In the oxidative microenvironments, such as ER and Golgi, and lysosomes), cystine residues exist to stabilize protein structures. Cysteine residues exist under reductive conditions, such as those found in the cytoplasm and nucleus. Dietary cystine is from meat, eggs, dairy products, and whole grains.

Taurine is not an essential amino acid, but the most abundant free amino acid. The plasma concentration of taurine is 50–90 μM in human and 200–400 μM in rodents[50]. The intracellular concentration of taurine, however, is enormously high, in a range of 10–50 mM[54]. Taurine is synthesized in the cysteine oxidation pathway and can be obtained from the diet as a non-protein amino acid. Unlike methionine and cysteine, taurine does not participate in the construction of proteins in our body. Its physiological functions include visual and neural development, detoxification, antioxidation, and anti-inflammatory. In addition to its stimulatory effect on hepatic bile acid synthesis, taurine conjugates with bile acids. Microbiota affects taurine absorption through the interaction of taurine and bile acids in the gut. As such, the ratio of conjugation of taurine with bile acids in the gut affects intestinal taurine absorption. Intestinal microbiota regulates the biotransformation, biosynthesis, and transportation of bile acids. Fiber in the diet may also increase taurine losses in the feces by influencing intestinal microorganism populations as well as through other effects on bile acid metabolism[55].

Although the mechanisms by which SAAR increases host H_2S level are not fully clear, an evolutionary conserved integrated stress response (ISR) has been proposed. Eukaryotic ISR, including nutritional stress, are sensed by integrated stress response regulators (ISRR) which are a variety of protein kinases. ISRR phosphorylate and activate eukaryotic initiation factor 2 (eIF2) during ISR, resulting in decreased protein synthesis. Deficiency in AAs supply can be sensed by general control non-depressible 2 (GCN2), one of ISRR, leading to decreased global protein synthesis. This would benefit the cells from disastrous depletion of the intracellular AAs pool. To date, a causative relationship between eIF2 phosphorylation and the activation of activating transcription factor 4 (ATF4) during SAAR has not been fully established. The regulation of ATF4 and its gene targets during SAAR remains unsettled. It has been reported that SAAR for one week resulted in increased rat hepatic eIF2 phosphorylation and ATF4 protein expression[56]. Feeding mice with a methionine-restricted diet for 5 weeks increased p-eIF2 and decreased cytosolic, but not mitochondrial, protein synthesis in both the liver and skeletal muscles. These ISR outcomes were not related to the status of GCN2[57]. When cytosolic levels of methionine and cysteine drop, the cognate tRNAs are less likely aminoacylated but more suitable for binding to GCN2. Dimerization and autophosphorylation of GCN2 lead to kinase activation. On the other hand, AA deprivation leads to targeted increases in the translation of selective mRNAs

with special sequence features in their 5′ leaders or untranslated regions (UTR). For example, ISR can enhance the translation of ATF4 which is the basic leucine zipper (bZIP) transcription factor. ATF4 promotes key processes affected by SAAR such as lipid metabolism, the trans-sulfuration pathway, and antioxidant defenses. The translation of ATF4 transcript is precisely controlled by two upstream open reading frames. With cytosolic levels of AAs at physiological range being sensed, ATF4 translation is repressed. Opposite situation occurs with ISR where ATF4 translation is increased and more ATF4 form homodimers or heterodimers with other bZIP transcription factors and bind promoter sequence motifs of its target molecules. ATF4 is known to induce transcription of CSE, stearoyl-Coenzyme A desaturase 1, and fibroblast growth factor 21. SAAR decreases total cysteine and cystathionine levels but increases total homocysteine levels. In response to low cellular cysteine levels, ATF expression is increased. The binding of ATF protein to sequences in the first intron of CSE activates the transcription of CSE[44]. Whether CBS or MST will be affected by ATF4 is unknown. We have found that SAAR triggered GCN2/ATF4-dependent signaling pathway in mice, leading to increased expression of vascular endothelial growth factor (VEGF) as well as H_2S production. Consequently, skeletal muscle angiogenesis is enhanced[46]. SAA deprivation of cultured human umbilical cord endothelial cells increased CSE-mediated H_2S production. The relationship between H_2S and eIF2 phosphorylation was demonstrated directly in cultured Hela and MEF cells. H_2S transiently increases the phosphorylation of eIF2α at least in part by inhibition of protein phosphatase-1 via S-sulfhydration at Cys-127[58].

SAAs are required to maintain gut integrity and function, stimulating intestinal protein synthesis and cell growth. Gut microbiota need SAAs for their own metabolism and SAAR-induced disturbance of gut microbiome decreases intestinal epithelial barrier function and alters the composition and metabolism of gut microbiota. An increase in the dietary methionine intake would increase the amount of methionine in intestine lumen and provide an excellent source of fuel for rapid bacterial proliferation[59]. We are in a dilemma here. SAAR offers significant human health benefits. At the same time, SAAR diet would have an adverse effect on the homeostasis of gut microbiota. The pivotal factor links specific diet regimen to gut microflora and human health is H_2S.

At the practical level, we should restrict, not eliminate, high SAA-containing meat, regardless of red or white, in our daily diet. It would be beneficial by consuming more plant-based proteins, such as those from beans, lentils and legumes which are good sources of protein but low in SAA content. As an exception of plant-based proteins, soy protein is surprisingly rich in SAA content[49].

5. Probiotics, prebiotics, and sulfur metabolism in the gut

Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”[60]. So called “good bacteria”, probiotics provide health benefits when consumed, generally by assisting the maintenance of the natural balance of microorganisms (microflora) in the intestine, modulating immune response, and improving metabolism. Probiotics are considered generally safe to consume but may cause bacteria-host interactions and unwanted side effects in rare cases. Probiotics are usually supplemented by one or several commensal microbe species at one time, whereas prebiotics supplementation could stimulate a number of beneficial species simultaneously. It is interesting to note that some probiotic species can also metabolize methionine, such as Enterococcus faecalis, Enterococcus faecium, and Esherichia coli Nissle[61].

Prebiotics are compounds that are indigestible by the host gastrointestinal tract but can easily be fermented by gut microbiota[62]. The digestible parts of these foods are used by the human host whereas the indigestible elements are used by gut microbiota. In a more general term, prebiotics is the “food” for probiotics (Fig. 3) and, as such, many of plant-based human foods serve as prebiotics for microbiota. Included in this category of prebiotics are garlic, onion, leaks, chicory root, jicama, asparagus, bananas, whole grains/flower, leafy greens, broccoli, cabbage, cauliflower, kale, radish, to name a few. These plant-based prebiotic foods usually do not contain methionine/cysteine and as such do not contribute to gut bacterial production of H_2S in large

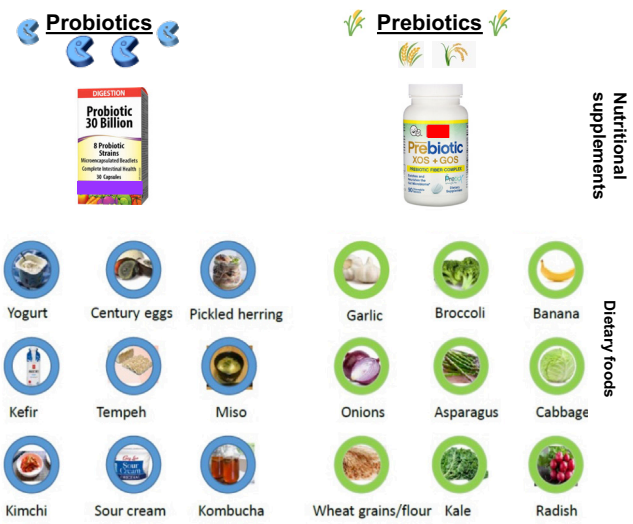

Fig. 3. Examples of probiotics and prebiotics from commercially available supplements and dietary foods

intestine (fermentation process). The majority of these foods, sch as garlic, onion, and broccoli, contain sulfur species and would lead to H_2S production in large intestine (sulfate reduction process) by feeding sulfate-reducing bacteria (SRB). Hence, the preferred food should have low SAA content, but high sulfur content. The former will boost endogenous H_2S production in the host and the latter offers the source of exogenous H_2S to the host. Consumers of vegan and lacto-ovo-vegetarian diets generally have lower intake of SAAs and this may constitute one plausible strategy of implementing SAAR in humans. These vegetarian diets often are good sources of prebiotics. Plant-based diets are typically lower in SAAs and higher in fiber.

The representatives of commercially available prebiotics as nutritional supplements are inulin, fructooligosaccharides (FOS), and galactooligosaccharides (GOS)[63]. Prebiotics promote the growth and health of gut microbiota and significantly improved cognitive function and mood in healthy middle-aged adults[64]. FOS and GOS increase the expression of hippocampal neurotrophic factor and NMDA receptor subunits[65]. Prebiotics potentially minimize the adverse effect of SAAR diet on gut microbiota and at the same time maximize the utilization of SAAs by gut microbiota[66]. Prebiotics speed up the transit of diet through the small intestine so that SAAs would have less time to be absorbed by the host. Prebiotics also promote the integrity of intestine epithelia and enhance its barrier function[67]. Dietary inclusion of soluble non-starch polysaccharides can stimulate the growth of commensal microbes in the gut[68], thereby potentially minimizing the adverse effect of SAAR diet on gut microbiota and at the same time maximizing the utilization of SAAs by gut microbiota[66].

6. Challenges and Perspectives

1) Diet contents of SAAs vary with different cultures and populations. For example, traditional diet of far-northern indigenous people living in Nunavut, Canada, is composed of mainly animal meats from arctic char, seal, whale, caribou and ducks, etc. The extremely high-protein and high-fat composition of the so-called Inuit diet is supplemented with multi-vitamins and micronutrients. It contains limited vegetables and greens, such as crowberries and wild blueberries, as well as low carbohydrates[69-71]. The unusual makeup of the far-northern diet with highest content of proteins can result in higher dietary intake of SAAs. One might hypothesize that the longevity and ageing of people living on this diet would be adversely impacted. Dietary analysis of the SAA content of the far-northern diet and longitudinal studies of the aging process of regional indigenous people may provide the relevant clues.

2) The content of SAAs is not the only factor in determining dietary benefits to human health. High SAA ingestion from high-protein diet would lead to decreased endogenous H_2S level and the latter may result in high morbidity of the cardiovascular diseases. On the other hand, indigenous people in North America's frigid zones who lived on high-protein and high-fat traditional diet, before the introduction of westernized diets, had very low morbidity of cardiovascular diseases comparing to other populations, a phenomenon called "Inuit Paradox"[72]. Although this claim has not been substantiated fully by scientific evidence and morbidity statistics, it nevertheless emphasizes the importance of integrated outcome of all nutritional elements in the diet beyond SAAs and that of all signaling molecules involved beyond H2S. The key to the Inuit paradox is the meat-fat balance in which fats from wild animals provide more than 50 percent of the calories needed. The indigenous Inuit diet is particularly rich in polyunsaturated fats called omega-3 fatty acids, which contribute to the cardiovascular health. For example, the arctic char is the number one source of omega-3 fatty acids for the indigenous residents of Nunavut, Canada. Readers are referred to a massive collection of literatures for the cardiovascular benefits of polyunsaturated fats. What is intriguing is whether interaction of SAAs and fats in a given diet would affect the production and effects of endogenous H_2S on lipid metabolism in vivo. Moreover, it reminds us that what we eat not only affects longevity and ageing, but also impacts on the development of various diseases, such as those occurring in the cardiovascular system.

3) Would increasing dietary intake of H_2S and decreasing dietary intake of SAAs produce opposite health outcome to human body? Increased dietary intake of H_2S can be achieved by digesting selective groups of prebiotics. Gut microbiota-produced H_2S can also diffuse through the intestine. This exogenous source of H_2S produces transit surge of H_2S in gastrointestinal tissues and the circulation. In contrast, SAAR diet results in long-lasting increase in endogenous H_2S levels in various human organs/tissues. Once produced and regardless its origination, H_2S would have the same effects on the host cells. The only difference would be the concentration of H_2S in microenvironments. While endogenous H_2S produced by eukaryotes is at high nanomolar to low micromolar ranges, bacterial produced H_2S in the gut can reach high micromolar to low millimolar levels[73]. The combination of prebiotics and SAAR diet would provide complementary benefits to human health.

Conflict of Interest

Rui Wang is an Editorial Board Member. The article was subject to the journal's standard procedures, with peer review handled independently of this Member and his research groups.

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