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Emerging Evidence for Association of Transsulfuration Pathway with Hypoxia Responses

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ABSTRACT

When people ascend to a high altitude (HA), the body's oxygen (O_2) sensing mechanisms can sense perturbation in partial pressure and trigger adaptive responses. Rapid ascending to HA without ample time for acclimatisation culminates in high-altitude illnesses, which can derail the body functioning of lowlanders moving to HA. High-altitude native populations have undergone positive natural selection to efficiently overcome the challenges of chronic hypobaric hypoxia (HH) and thus offer a unique model to understand physiological and genetic adaptations at high altitudes. In addition, evolutionary shreds of evidence propose that sulfur belonging to the same periodic table family can mimic oxygen to bypass its metabolic oxygen demand and modulate energy production. Intriguingly, our group has identified a strong association between diminished hydrogen sulfide (H_2S) levels and HH-induced pathological responses. We have recently presented experimental evidence of cysteine deficit, which functionally regulates both lowered levels of endogenous H_2S and HH-induced neuropathological responses. In this review, we sought to understand the role of H_2S and the transsulfuration pathway at HA.

Keywords: High-altitude, Hydrogen Sulfide, Hypoxia, Transsulfuration Pathway

1. INTRODUCTION

Life on Earth is 3.9-4 billion years old. Archaeans are believed to be the oldest life forms and are typically ascribed as the 'first domain of diversified life' on Earth. These microbes are anaerobic and transfer electrons to nitrate (forming N_2), sulfate (forming H_2S), or CO_2 (comprising CH_4) to obtain energy. As life evolved, oxidative phosphorylation occurred in cyanobacteria, and eventually, the atmosphere became oxygenated. Following this, molecular oxygen (O_2) was identified as a vital substrate for the growth and development of most life forms. O_2 is an efficient electron acceptor most living forms utilise for ATP generation and an integral component of various enzymatic pathways. Recently, it was also proposed that acquiring mitochondria during evolution anticipated the necessity of O_2 for survival[1]. Consequently, an adequate concentration of O_2 emerged as a physiological requisite for most organisms.

In humans, an inbuilt O_2 sensing mechanism and a well-knit physiological network help maintain optimal tissue oxygenation by rapidly sensing the change in partial pressure of O_2 in arterial blood (PaO_2). Some pathological or environmental conditions can lead to cellular hypoxia and result in the progression of diseases such as cardiovascular, pulmonary, cancer, sleep, and inflammation-associated disorders[2]. Ascent to high altitude (more than 2700 m above sea level) is accompanied by alterations in environmental conditions[3]. Although the percentage of O_2 in the atmosphere remains constant (20.94%), a fall in partial pressure of O_2 (PO_2) due to decreased barometric pressure creates a state of O_2 deficit. This shortfall further leads to a reduced fraction of inspired O_2 (FiO_2), alveolar PO_2, arterial blood O_2, and arterial O_2 saturation, culminating in Hypobaric Hypoxia (HH).

An increasingly large number of people, about 40 million, are traveling to HA each year for recreational (including mountaineers, skiers, trekkers, and climbers), religious, or occupational (like soldiers, miners) purposes[4]. These groups are inevitably exposed to HH and experience hypoxemia (low level of O_2 in the blood). Therefore, the ascent to HA should follow the approved acclimatisation protocol as in Table 1[5,6]. Rapid rise without sufficient time for acclimatisation may contribute to the progression of high-altitude-related illnesses such as Acute-Mountain Sickness (AMS), High-Altitude Cerebral Edema (HACE), and High-Altitude Pulmonary Edema (HAPE). The severity of illnesses concomitantly increases with a decrease in arterial oxygen saturation from high (2700-3600 m) to extreme (above 5500 m) altitudes. The detailed text about pathologies caused by acute exposure to high-altitude, such as AMS, HACE, and HAPE, has been well-documented[7-10].

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Table 1. Acclimatisation protocol as per DGAFMS medical memorandum no.140 (issued in July 1997)

First Stage (2700m-3600m) Six Days	Days 1 and 2	Rest with short walks in the unit lines, not involving any climbs.
	Days 3 and 4	Stroll for 1.5-3 km and avoid steep climbs.
	Days 5 and 6	Walk up to 5km and climb up to 300m slowly.
Second Stage (Above 3600m up to 4500m) Four Days	Days 1 and 2	Stroll for 1.5-3 km and avoid steep climbs.
	Day 3	Slow walk and climb up to 300m.
	Day 4	Climb 300m without equipment.
Third Stage (Above 4500m) Four days	Days 1 and 2	Stroll for 1.5-3 km and avoid steep climbs.
	Day 3	Slow walk and climb up to 300m.
	Day 4	Climb 300m without equipment.

2. ROLE OF GASOTRANSMITTERS IN THE HYPOXIC ENVIRONMENT

With an ever-growing interest in the field of gasotransmitter biology, numerous studies have investigated the role of gaseous messengers (nitric oxide (NO), carbon monoxide (CO), and H_2S) in O_2 sensing and homeostasis[11-14]. As distinguished from CO and NO, there is a decrease in H_2S levels under normoxia, while its production increases under hypoxia[15,16]. An essential role of H_2S in mediating O_2 sensing by the carotid bodies and eliciting systemic responses to hypoxia has also been identified[17]. It is an excitatory gasotransmitter that regulates blood pressure and modulates inflammation. Interestingly, CO and NO have also been found to modulate H_2S production. A marked increase in basal H_2S levels has been found during normoxia in the presence of HO inhibitors. Conversely, a CO donor has been reported to inhibit H_2S generation during hypoxia. These effects were nullified in cystathionine γ-lyase (*Cth*) knockout mice[18]. Thus, it is plausible that CO does not elicit responses by itself; rather, it might suppress the generation of H_2S by inhibiting its enzyme cystathionine gamma-lyase, CSE.

However, most information on the contributions of gasotransmitters in O_2 sensing is limited to tissue/cellular hypoxia. Only a few reports have examined their importance under hypobaric hypoxia at altitude. The study involving highland Llama neonates has reported an enhanced pulmonary HO-1 protein expression and CO production by the pulmonary vessels compared to lowland Llama neonates[19]. This increase in the HO-CO system has been shown to protect against pulmonary vasoconstriction. In addition, there is increased production and expression of NO on exposure to HA for peripheral vasodilation at the systemic level and to counterbalance the tendency to develop pulmonary hypertension in the pulmonary system[20]. Furthermore, these increased NO levels in high-altitude populations have also been found to play a protective role[21]. It can result from increased eNOS (endothelial NOS) activity and/or an alternative pathway mediated by xanthine oxidoreductase and mitochondrial cytochrome c oxidase[22].

Further, in response to hypobaric hypoxia, our group has previously shown decreased levels of H_2S in multiple tissues, including the brain[23,24], lung[25], and heart[26]. This decrease in H_2S has been attributed to a marked limitation of cysteine (substrate for H_2S-producing enzyme cystathionine β-synthase (CBS)) during HH. However, these adverse effects were significantly prevented upon supplementation of H_2S (utilising an exogenous donor NaHS). Interestingly, H_2S levels were also normalised upon restoration of cysteine levels. In support of this, proteomics studies in alpine plants at HA also reported increased accumulation of H_2S and its synthesising enzymes[27]. They revealed the novel role of H_2S in facilitating long-term adaptations to environmental stress by enhancing antioxidant enzyme activity. Our understanding of the gasotransmitters is still evolving, and more work is required to establish a correlation between pathological conditions and long-term adaptations at altitude. This would further lead to a better understanding of addressing critical issues in the clinical management of hypoxemia.

3. HYDROGEN SULFIDE

The prebiotic atmosphere comprised H_2S and NO rather than O_2 and was responsible for nearly 85% of the evolution of life. Considering this, H_2S may be a potential candidate for better adaptation to low O_2 conditions such as HA. The potential of H_2S as an essential biological messenger was realised in the landmark study of Abe and Kimura on the mammalian nervous system in 1996[28]. Szabo has comprehensively reviewed the chronology of H_2S research from a toxic substance to its recognition in the gasotransmitter family, along with NO and CO[29]. It is now unambiguously established as the third gaseous messenger and has been shown to regulate vital physiological processes such as vascular tone, inflammation, and redox homeostasis[30,31]. It is endogenously produced from L-cysteine via alternative desulfuration reactions catalysed by the CBS/CSE system or via a non-oxidative route. Cysteine Aminotransferase (CAT) produces 3-mercapto pyruvate, the substrate of 3-mercapto pyruvate sulfurtransferase (3-MST), to produce a protein-bound persulfide, that in turn can be reduced to form H_2S[32]. It exerts a biphasic effect to regulate mitochondrial function under physiological conditions—acts as an electron donor at low concentrations (100 nM to 1 μM) and inhibits mitochondrial complex IV at higher concentrations (10 μM and above)[33]. In the presence of O_2, it is oxidised to the level of elemental sulfur by Sulfide Quinone Oxidoreductase (SQOR).

3.1 H_2S AND HYPOXIA

H_2S holds evolutionary significance and is also known as "the sunlight of the deep ocean," according to the theory describing the evolution of first life near sulfide-emitting deep-sea vents with low O_2. There

H_2S proved to be a life supporter and the source of metabolic energy. Concurrently, accumulating literature in recent years has infallibly established the salubrious potential of H_2S, especially in the context of hypoxia and oxidative stress. It serves as an O_2 sensor[34, 35] and has been suggested as a downstream effector of CO-mediated O_2 sensing. Interestingly, CO suppresses the activity of H_2S-synthesising enzymes CSE and CBS[36]. The constitutive CO physiologically inhibits CSE by stimulating soluble guanylyl cyclase/protein kinase G (sGC/PKG) signaling, in contrast to the heme-dependent inhibition of CBS by CO (CO directly inhibits CBS by forming nitrosyl or ferrous carbonyl CBS). Thus, hypoxia-evoked decreased CO generation is responsible for increased H_2S generation, sensory nerve activity, and cerebral vasodilation.

H_2S is physiologically active at low endogenous concentrations and can regulate a variety of homeostatic functions (vasodilation, inflammation modulation) under hypoxic stress by three possible mechanisms-1) redox signaling to postulate antioxidant protection, 2) metabolism shift towards Pentose Phosphate Pathway (PPP) and 3) by regulating energy production in mitochondria[37].

3.1.1 Redox Signaling

Prolonged exposure to both normobaric and hypobaric hypoxia has been known to increase oxidative stress (ROS levels) and decrease antioxidant activity[38]. Thus, cellular redox balance is crucial in pathological conditions with limited O_2 availability. Intriguingly, a close link between ROS, redox-modulated signaling, and the action of H_2S has emerged in recent years. ROS-induced oxidation (most notably by NOx-generated oxidants, hydrogen peroxide (H_2O_2), and peroxide free radical ($O_2^{\bullet-}$)) can either directly affect the bioavailability and function or the endogenous enzymatic machinery responsible for the synthesis of H_2S[39]. The chemical nature of H_2S makes it highly vulnerable to oxidation. It reacts with H_2O_2 or O_2 to generate polysulfides (H_2S_n), further promoting disulfide bond formation within the PKG Iα subunit, necessary for its activation and role in lowering blood pressure. Besides chemical reaction, H_2S has also been demonstrated to impair mitochondrial ROS generation by S-sulfhydrating p66Shc at a site close to its phosphorylation site, thereby preventing activation of p66Shc from driving ROS production in mitochondria[40].

Superoxide Dismutase (SOD), glutathione peroxidase (GPX), and catalase (Cat) are reported to increase markedly by lowered PO_2 levels during hypoxia and thus prevent H_2S oxidation[41]. In the absence of O_2, catalase acts as a selective sulfur reductase, thereby regenerating H_2S (reduced sulfur) from reduced thioredoxin, dithiothreitol, and NADPH. Interestingly, not only an excess of ROS but even an excess of reducing equivalents (reductive stress) is detrimental to the biological system, shifting the balance of redox couples (NAD^+/NADH, $NADP^+$/NADPH, GSSG/GSH) to a more reduced state. Their accumulation can modify transcriptional machinery, increase apoptosis, induce mitochondrial dysfunction, hinder protein disulfide bond formation, affect cell metabolism, and modulate cell signaling[42].

3.1.2 Metabolism Shift Towards Pentose Phosphate Pathway (PPP)

Metabolism shift towards anaerobic glycolysis for ATP production solves the energy deficit problem under hypoxia. Under normoxia, 92 % of glucose is catabolised through the classic Embden-Meyerhof glycolytic pathway. However, the absence of O_2 triggers the consumption of 90 % of glucose via the pentose phosphate pathway and maintains energetic homeostasis[43]. Routes are favored that maximise ATP production per mole of oxygen. PPP plays a significant role in supporting primary carbon homeostasis, nucleotide, and amino acid synthesis, generating reducing equivalents for anabolic reactions, and preventing oxidative stress. This pathway primarily provides cells with NADPH and ribose-5-phosphate through its oxidative and non-oxidative branches. NADPH is an integral component of redox-based primary biological functions–1) key molecule for the functioning of the cellular antioxidant system, 2) substrate for enzyme-like NADPH oxidase, NOS, cytochrome P450 oxidoreductase, 3) electron sources for anabolic reactions including fatty acid, steroid, and nucleic acid synthesis. Our group has established strong evidence for a direct link between intracellular H_2S levels and Glucose-6-phosphate Dehydrogenase (G6PD) activity–the rate-limiting enzyme of PPP[26,44].

3.1.3 Regulating Energy Production in Mitochondria

In the absence of molecular oxygen (highly oxidising agent and, therefore, terminal electron acceptor), there is decreased ATP production from mitochondria[45]. This energy deficit can be attenuated using other less-oxidising substances such as nitrate, sulfate, or carbon dioxide. Both oxygen and sulfur have six valence electrons, and much of their signaling is exerted by cysteine-sulfur interaction at regulatory sites. It is increasingly evident that H_2S is an ancient gaseous messenger system (well conserved across species) and has become an emergency or backup source of electrons under conditions where the Krebs cycle-derived electron donor supply is diminished. In this context, it is noteworthy that a low, steady-state concentration of sulfide (<5 μM) functions as an energy substrate and sustains ATP production under stress conditions[46, 47]. It reverses hypoxia-repressed mitochondrial ATP production and decreases the ADP/ATP ratio[46]. Thus, H_2S supplementation can increase ATP production under hypoxic conditions.

3.2 Post-translational Modifications Mediated by H_2S

Under hypoxia, mitochondrial ROS production increases at complex III, triggering transcriptional and post-transcriptional adaptive changes[48]. Post-translational oxidative modification of protein cysteine thiols (in the presence of O_2 or oxidants such as H_2O_2) leads to the generation of protein sulfenic acids. These volatile

derivatives can either be converted back to a reduced state by glutathione and thioredoxin systems or oxidised further to more stable forms, depending on the existing conditions[49]. The reversible sulfenic acid formation regulates the activity and function of many essential proteins in a redox-based manner. Extensive studies on H_2S suggest that it protects against hypoxia-induced cytotoxicity and inflammation through inhibition of ROS-activated ERK1/2 pathways or p38 MAPK pathways[50, 51]; suppresses both the elevation of intracellular ROS and the increase in cytosolic calcium in cultured neurons[52]; promotes proliferation and differentiation of neural stem cells, under hypoxic challenge[53]. Taken together, H_2S signaling serves as a guardian and reinforces stimulus-dependent endogenous regeneration pathways, rendering it more potent in curtailing the pathological effects of hypoxia.

Further, H_2S also acts through S-sulfhydration, thus forming protein–SSH moieties. This S-sulfhydration reaction can modify many proteins, including GAPDH (at Cys150), albumin, actin, ADH1, AST, catalase, and thioredoxin-reductase. Indeed, there could well be crosstalk between S-nitrosylation and S-sulfhydration signaling. In many proteins, NO and H_2S have been found to target the same Cys residue, causing S-nitrosylation and S-sulfhydration, respectively. Based on the affinity for Cys residue, the post-translational modification that occurs governs the function of a target protein. For instance, S-sulfhydration of eNOS by H_2S at Cys 443 promotes eNOS dimerisation and phosphorylation (via Akt/p38 MAPK pathway), thereby increasing eNOS activity in a CSE-dependent manner[54, 55]. Whereas NO decreases eNOS activity by promoting the formation of eNOS monomers. In addition, S-sulfhydration of eNOS decreased its S-nitrosylation, but the vice-versa was invalid. The functional proof for the interaction of H_2S with eNOS-NO has also been generated using the ischemia/reperfusion (I/R) injury model. The anti-inflammatory role of H_2S in protecting organs against I/R injury was curtailed both in eNOS null mice and mice expressing trans-gene for non-phosphorylable eNOS, suggesting mediation of H_2S-based effects by NO- and p38 MAPK-dependent mechanism[56, 57]. Inferences from the literature discussed regarding the role of H_2S under hypoxia and what is well-established otherwise suggest its possible connection to adaptation to high altitude.

3.3 Role of H_2S in Glutathione Synthesis

Glutathione (GSH), synthesised in the liver, acts as an antioxidant and free radical scavenger. During detoxification (scavenging ROS and RNS), GSH is converted to GSSG (oxidised form). Glutathione reductase (GR) then converts GSSG back to GSH using NADPH. This GSH/GSSG ratio is critical in maintaining redox homeostasis inside the cell. H_2S has been reported to be involved in increasing intracellular GSH synthesis via different mechanisms, including–1) up-regulation of enzymes involved in GSH production[58], 2) reduction of cystine to cysteine by H_2S followed by its import through cysteine transporter for GSH synthesis inside the cells[59], 3) NaHS (H_2S)-enhanced transport of cystine inside cells via cystine/glutamate antiporter, its reduction to cysteine followed by GSH synthesis[60]. Further, biosynthesis of H_2S is also known to be linked to the metabolism of sulfur-containing amino acids, specifically cysteine and homocysteine (hCys), essential metabolites in the homocysteine transsulfuration pathway (TS).

4. TRANSSULFURATION PATHWAY

Considering that the limited oxygen availability at HA is inescapable, an alternative route for survival was necessary. The evolutionary evidence has advanced the role of sulfur in the prebiotic environment. The desulfuration pathway (also known as the transsulfuration (TS) pathway) redirects sulfur to increase the generation of antioxidants and H_2S. The forward (bacterial process) and reverse (mammalian process) transsulfuration pathways were identified by Vigneaud[61, 62]. The reverse transsulfuration pathway (often called the 'transsulfuration pathway' for simplicity) accepts hCys for regenerating cysteine via cystathionine. It serves two important purposes–1) it consumes hCys, which can modify proteins post-translationally, and 2) it regenerates cysteine, an essential precursor for several processes, including protein, glutathione, and taurine synthesis. This pathway has limited tissue distribution; it is restricted to the liver, kidney, intestine, and pancreas. Since they lack enzymes in transsulfuration, hCys detoxification occurs via the remethylation pathway in vascular tissues and the skin. Further, this pathway is sensitive to the balance between pro-oxidants and antioxidants; peroxide increases transsulfuration, whereas antioxidants decrease it[63].

4.1 Regulators of Transsulfuration Pathway

The bioavailability of substrates and the activity of metabolic enzymes involved–govern the regulation of the TS pathway at various levels (summarised in Figure 1).

4.1.1 Metabolites of the Methionine Cycle

The upstream precursor of the TS pathway, methionine, is the source of the sulfur atom present in newly synthesised cysteine via homocysteine. Cysteine was, therefore, considered a non-essential amino acid. However, recent studies have contemplated it to be conditionally essential for various pathophysiological processes[64]. Our group has also provided experimental evidence in view of limiting levels of cysteine during hypobaric hypoxia[24]. The enhanced requirement is coped up from the extracellular milieu, where cysteine exists in an oxidised dipeptide–cystine. There is upregulation in the expression of transporters (xCT, EAAT 1, EAAT2) for cystine uptake, which is then rapidly reduced to cysteine intracellularly by NADPH[65].

Further, augmented uptake of cystine has been reported in diverse cancers, and the TS pathway plays a crucial role in retaining the cysteine pool when the extracellular supply of cysteine diminishes. About 50 % of cysteine molecules are required for the ubiquitous

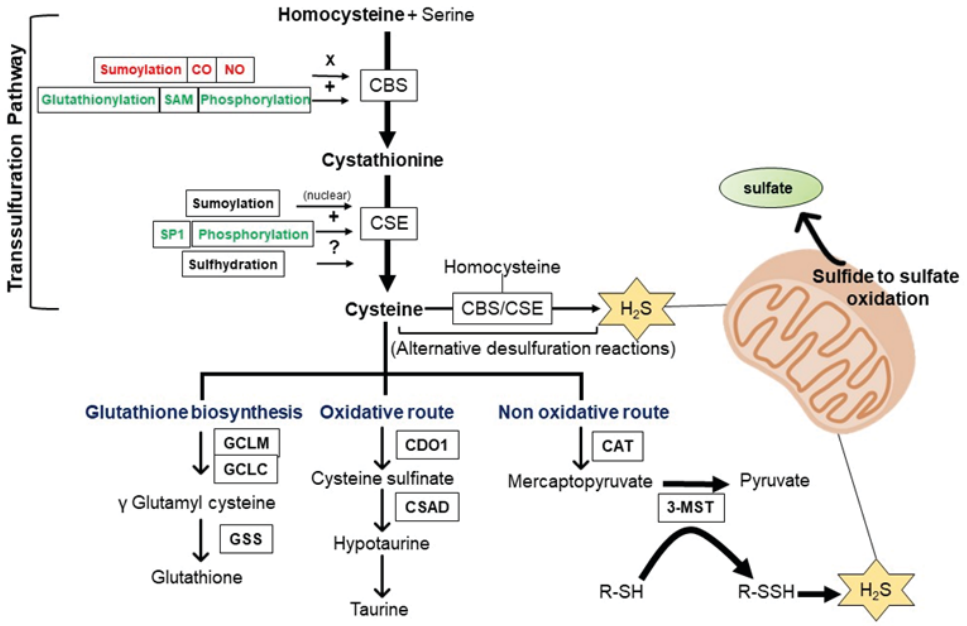

Figure 1. Overview of Transsulfuration Pathway and its regulators. The Transsulfuration pathway accepts homocysteine to generate cysteine via cystathionine. CBS condenses homocysteine with serine to generate cystathionine, the substrate for CSE, to generate cysteine. CSE can generate H2S from either cysteine or homocysteine. While CBS can utilise a combination of cysteine and homocysteine to generate H2S, CSE uses cysteine to generate H2S. MPST, in conjunction with CAT, utilises cysteine to generate H2S. Further, post-translational modifications (phosphorylation, sumoylation, and sulfhydration), gasotransmitters (CO and NO), and transcription factor (SP1) are known to regulate critical enzymes- CBS and CSE. Furthermore, there are three fates of cysteine produced by this pathway- oxidative route, non-oxidative route, and glutathione biosynthesis. Abbreviations – CBS: cystathionine β-synthase; CSE: cystathionine γ-lyase; CAT: cysteine aminotransferase; 3-MST: 3-mercaptopyruvate sulfurtransferase; CDO1: cysteine dioxygenase; CSAD: cysteine sulfinic acid decarboxylase; CO: carbon monoxide; NO: nitric oxide; SP1: specificity protein 1.

intracellular reducing agent glutathione. Thus, it is increasingly evident that cysteine and H_2S are central to maintaining redox homeostasis and integrating stress responses. Precisely, control of the transsulfuration pathway is critical for maintaining optimal cellular function under hypoxia.

Interestingly, supplementation of homocysteine to cysteine-depleted medium restored the proliferation of multiple cell lines[66], but neither serine nor methionine could combat cysteine depletion.

4.1.2 Activity of CBS

CBS lies at the branching point where the fate of homocysteine is decided. It redirects homocysteine flux from the methionine cycle to the transsulfuration pathway by catalysing the first step of converting homocysteine to cystathionine. CBS enzyme contains a heme moiety and can be modulated by different mechanisms- 1) increased production of NO and CO (during stress conditions), inhibits CBS due to formation of nitrosyl or ferrous carbonyl CBS. This results in the accumulation of hCys and production of H_2S by CSE[67]; 2) sumoylation by the E3 SUMO ligase decreases CBS activity[68]; 3) phosphorylation of Ser 227 occurs in a cGMP/protein kinase G-dependent manner to activate CBS and increase H_2S production; 4) allosteric activation of CBS by SAM promotes transsulfuration and 5) glutathionylation stimulates CBS activity to increase cystathionine production[69].

4.1.3 Activity of CSE

The enzyme CSE transforms methionine (derived from cystathionine) into cysteine by employing vitamin B6 as a cofactor. Post-translational modifications can also regulate CSE- 1) phosphorylated via the Akt pathway increases its catalytic activity[70]; 2) sumoylation is responsible for its nuclear localisation[68]; 3) sulfhydration has also been reported[71]. Besides, specificity protein 1 (SP1) can also regulate the expression of CSE.

Furthermore, inflammatory marker IL1RAP and components of the xCT transporter assemble to increase the expression of CSE, as seen in Ewing sarcoma, to promote the TS pathway[72].

4.2 Similar Pathological Conditions

The transsulfuration and its upstream and downstream pathways maintain homeostasis by regulating diverse metabolic functions. The deficiency of enzymes such as CBS, CSE, and MTHFR disrupts this pathway, leading to pathophysiological consequences, summarised in Table 2. Biochemically, they are characterised by increased levels of homocysteine and methionine in plasma, increased excretion of homocysteine in urine, and decreased cystathionine and cysteine in body fluids.

Table 2. Modulation in levels of homocysteine, cysteine, and methionine can lead to pathophysiological consequences

Complication	Associated diseases	References
Hyperhomocysteinemia	Neurodegeneration (Parkinson's, Schizophrenia, Dementia, Depression, Alzheimer's)	80-83
	Renal failure, chronic kidney disease (CKD)	84
	Cardiovascular diseases (venous thrombosis, carotid artery, atherosclerosis, vascular dementia, congenital heart defects, ischemic stroke)	85-91
	Dislocation of the optic lens	92
	Trauma, sepsis, and burn injury	93
	Obstructive sleep apnea	94
	Breast cancer	95
Hypercysteinemia	Obesity-related disorders such as cardiovascular disease (atherosclerosis) and metabolic syndrome	96, 97
Hypocysteinemia	AIDS (acquired immunodeficiency syndrome), Huntington's disease	98
Hypermethioninemia	Cirrhosis	99
	Islet cell hyperplasia, Renal tubular degeneration	100

Table 3. Polymorphism in the transsulfuration genes along with their clinical relevance. This data has been obtained from the ClinVar and SNP Database of the National Center for Biotechnology Information (NCBI).

Gene	SNP Id	Polymorphism	Clinical Relevance	References
Cystathionine Beta-Synthase *(Cbs)*	rs28934891	G>A	Hyperhomocysteinemia, thrombotic, homocystinuria, pyridoxine-responsive	101, 102
	rs121964969	-	Homocystinuria, pyridoxine-responsive, Cardiovascular phenotype	103
	rs12613	G>A	Homocystinuria	104, 105, 89
	rs706208	T>C		
	rs234706	G>A	Homocystinuria, Cardiovascular phenotype	106-108
	rs5742905	T>C>A	Homocystinuria, Hyperhomocysteinemia, Cardiovascular phenotype	109
Cystathionine Gamma-Lyase *(Cth)*	rs1021737	G>T	Cystathioninuria, elevated homocysteine	110
	rs28941785	C>T>A	Cystathioninuria	110,111
	rs28941786	-		
Methylene tetrahydrofolate reductase *(Mthfr)*	rs121434296	C>T	Homocysteinemia, Homocystinuria	112, 113
	rs121434297	T>C	Homocystinuria	114
	rs121434294	-		
	rs121434295	G>A		
	rs267606886	-		
	rs377443637	G>A		
	rs267606887	-		
	rs45590836	G>A		
	rs138189536	G>A		
	rs373398993	A>T	Homocysteinemia	115
	rs367585605	-		
	rs147257424	C>A>T		

4.3 Among the Different Eukaryotic Systems

Some animal and plant species have adapted to high-altitude and hypobaric hypoxia. Llama (*Lama glama*) have developed adaptation mechanisms to avoid hypoxia-induced pulmonary hypertension; these include- low concentrations of ADMA and homocysteine, low expression of arginase type II, DDAH-2, and CBS, as well as its insensitivity to activation by homocysteine[73]. In addition, the adaptation of Drosophila, Andean hummingbirds, and deer mice to experimental hypoxia has also been reported[74-77]. Furthermore, the utilisation of transsulfuration pathways among different organisms has also been suggested[78].

4.4 Genetic Perspective of the TS Pathway and Evidence for its Association with Hypoxia

Utilising candidate gene association is the most common approach to studying genes associated with HA illnesses. The genes usually code for proteins of a particular pathway likely to be involved in adaptation. In the current understanding of the high-altitude native's adaptation and the role of TS in modulating H_2S levels, there is a sweep in investigating the involvement of this pathway–both at the genetic and molecular level for the adaptation of high-altitude natives.

Various studies have shown that mutations and polymorphisms exist in genes involved in transsulfuration pathways (*Cbs, Cth,* and *Mthfr*). These polymorphic alleles of genes were found to be linked with hyperhomocysteinemia and hypermethioninemia (as outlined in Table 3). Further, hCys decreases NO secretion in the endothelial cells, leading to impaired endothelium-dependent vasodilation, increased platelet aggregation, and decreased antithrombotic activities[79]. At an elevated level, homocysteine inactivates proteins by homocysteinylation, including its endogenous metabolising enzyme, CSE. Thus, there is reduced production of H_2S during hyperhomocysteinemia. This review suggests a preeminent role for redox regulation in modulating transsulfuration genes that can be exerted through H_2S. H_2S and TS pathways work in coordination to elicit adaptive responses to hypoxia. However, most of these aspects need to be addressed by experimental evidence.

5. CONCLUSION

In this review, we sought to examine the association of the transsulfuration pathway during acclimatisation to high altitudes. The modulation in circulating levels of H_2S in low landers ascending to HA and HA natives through the regulation of genes in this pathway clearly merits attention. It is known that cysteine and homocysteine are substrates for H_2S synthesis, but their utilisation during specific conditions posed by high altitudes necessitates careful investigation. A comprehensive understanding of this aspect might lead to new prophylactic and interventional strategies culminating in regulating biological levels of H_2S and better acclimatisation. Further, under said conditions, the fundamental mechanisms underlying the biological effects of H_2S will also require future efforts.

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