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REVIEW

L-Cysteine metabolism and its nutritional implications

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L-Cysteine is a nutritionally semiessential amino acid and is present mainly in the form of L-cystine in the extracellular space. With the help of a transport system, extracellular L-cystine crosses the plasma membrane and is reduced to L-cysteine within cells by thioredoxin and reduced glutathione (GSH). Intracellular L-cysteine plays an important role in cellular homeostasis as a precursor for protein synthesis, and for production of GSH, hydrogen sulfide (H₂S), and taurine. L-Cysteine-dependent synthesis of GSH has been investigated in many pathological conditions, while the pathway for L-cysteine metabolism to form H₂S has received little attention with regard to prevention and treatment of disease in humans. The main objective of this review is to highlight the metabolic pathways of L-cysteine catabolism to GSH, H₂S, and taurine, with special emphasis on therapeutic and nutritional use of L-cysteine to improve the health and well-being of animals and humans.

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1 Introduction

L-Cysteine is a nutritionally semiessential amino acid. Three sources contribute to L-cysteine in the body: absorption from diets, the transsulfuration pathway from L-methionine degradation, and breakdown of endogenous proteins. In food and tissue proteins and in the blood, L-cysteine exists mainly in the form of L-cystine because L-cysteine is rapidly oxidized to L-cystine in normoxic conditions. Inside cells, L-cysteine is

the prevailing form due to the highly reducing conditions [1]. Imbalance of extracellular L-cysteine/L-cystine is associated with oxidative stress and other pathological disorders and has been reviewed by other researchers [2–4]. Although L-cysteine and L-cystine metabolism via multiple ways have not been fully explored in all tissues, results of previous studies indicate that the balance between extracellular and intracellular L-cysteine/L-cystine is largely regulated by transportation. Currently, L-cysteine and L-cystine transport have been shown to be associated with systems A, ASC, L, X_c⁻, B^{0,+}, and X_{AG}⁻ (Fig. 1) [1, 5, 6]. For more specific details of the contribution of these transport systems, readers are referred to the reviews by Conrad and Sato [1], Aoyama et al. [7], and Kilberg et al. [8].

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Abbreviations: **3MP**, 3-mercaptopyruvate; **γ-Glu-cys**, γ-glutamylcysteine; **CBS**, cystathionine β-synthase; **CDO**, cysteine dioxygenase; **CSD**, cysteinesulfinate decarboxylase; **CSE**, cystathionine γ-lyase; **GCL**, glutamate cysteine ligase; **GS**, GSH synthase; **GSH**, glutathione; **H₂S**, hydrogen sulfide; **PLP**, pyridoxal 5'-phosphate; **Trx**, thioredoxin

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Colour online: See the article online to view Fig. 1 in colour.

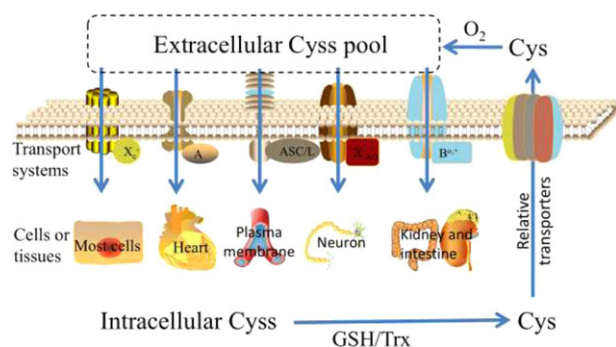


Figure 1. Extracellular and intracellular L-cysteine/L-cystine balance and L-cysteine/L-cystine transport systems. Glu, L-glutamate; Cyss, L-cystine; Cys, L-cysteine; GSH, glutathione; Trx, thioredoxin.

Efflux of L-cysteine from cells and uptake of L-cysteine by cells improve the intracellular ratio of L-cysteine to L-cystine. In contrast, uptake of L-cysteine by cells and its oxidation to L-cystine, and the efflux of L-cysteine by cells increase the extracellular ratio of L-cysteine to L-cystine (Fig. 1). Meanwhile, in order to satisfy cellular requirements, L-cysteine is widely transported into cells. Intracellular conversion of L-cysteine into L-cystine has been considered to be a key process to mediate extracellular L-cysteine/L-cystine redox, as well as the synthesis of protein and glutathione (GSH) [9]. However, specific redox systems or enzymes responsible for this reduction have not been fully identified. Based on the current literature, at least two related systems are known to catalyze the reduction of L-cysteine into L-cystine: thioredoxin-1/thioredoxin reductase 1 (Trx1/TR1) and glutaredoxin-1/GSH/GSH disulfide reductase (Grx1/GSH/GR) [10, 11]. Jones et al. [4] have modeled reduced (Trx or GSH) or oxidized (reactive oxygen species, O_2 or Cyss) redox-related reactions: $PrSH + Cys \rightarrow PrSS-cysteine + L-cysteine$ (activity “on” or “off”) and $Pr-SS-cysteine + Trx/GSH \rightarrow PrSH + CySSG$ (the opposite to the reaction above). Such network suggests that Trx and GSH contribute to intracellular conversion of L-cysteine to L-cystine and the intracellular reducing status, which has been further confirmed by other lines of evidence. The redox states of both Trx1 and GSH/oxidized GSH are more reducing than intracellular L-cysteine/L-cystine redox (–160 to –125 mV), with Trx1 being maintained in the range of –280 to –270 mV and GSH/oxidized GSH being fluctuated from –250 mV in rapidly proliferating cells to –200 mV in differentiated cells [3, 12, 13]. These data reveal the high capacity of Trx and GSH for L-cysteine reduction. The rate of intracellular conversion of L-cystine to L-cysteine has been estimated to be approximately 2 $\mu M/min$ in cells with 3 mM GSH and 30 μM L-cystine, while the value may be catalytically reached to about 7 $\mu M/min$ with the help of Grx or Trx [4, 14].

The metabolic pathways of intracellular L-cysteine include protein synthesis, as well as the generation of GSH (γ -glutamyl-cysteinyl-glycine), hydrogen sulfide (H_2S), cysteine-sulfinate, taurine, pyruvate, and inorganic sulfur (Fig. 2) [15].

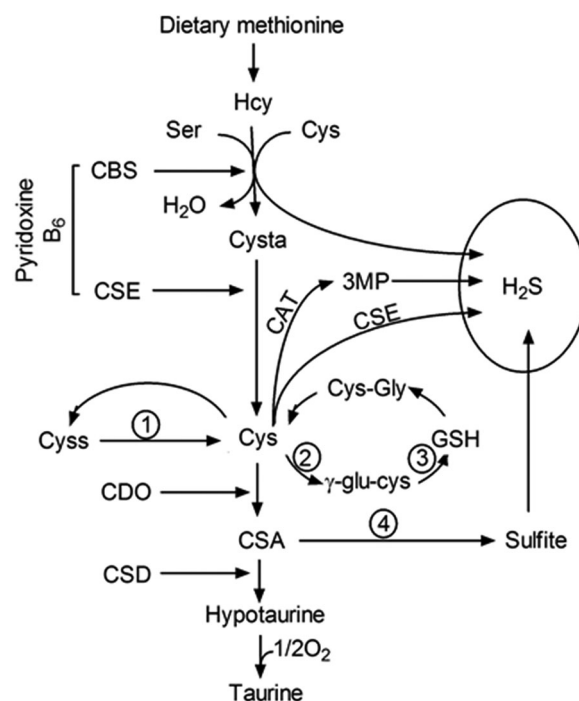


Figure 2. Intracellular cysteine metabolism. Hcy, homocysteine; Cysta, cystathionine; Cys, L-cysteine; L-Ser, serine; Cyss, L-cystine; γ -Glu-cys, γ -glutamylcysteine; GSH, glutathione; CSA, cysteine-sulfinate; CBS, cystathionine β -synthase; CSE, cystathionine γ -lyase; CDO, cysteine dioxygenase; CSD, cysteinesulfinate decarboxylase; 1, GSH/Trx systems; 2, GCL (glutamate cysteine ligase); 3, GS (GSH synthase); 4, aspartate (cysteinesulfinate) aminotransferase.

L-Cysteine can regulate nutrient metabolism, oxidative stress, physiologic signaling pathways, and associated diseases via the production of GSH, H_2S , and taurine. This review highlights the metabolic pathways of L-cysteine catabolism to GSH, H_2S , and taurine, with special emphasis on therapeutic and nutritional use of L-cysteine to improve the health and well-being of animals and humans.

2 L-Cysteine/GSH system

Apart from protein synthesis, L-cysteine mainly serves as a precursor for GSH along with L-glutamate and glycine. GSH is synthesized *de novo* in two successive enzymatic ATP-dependent reactions. First, L-cysteine and L-glutamate are coupled to form the dipeptide γ -glutamylcysteine (γ -Glu-cys), with the reaction being catalyzed by glutamate cysteine ligase (GCL). Then, GSH synthase (GS) converts γ -Glu-cys and glycine to GSH [16, 17]. However, the main sources of the GSH precursors have not been quantified. Thus, we have evaluated the combined coefficients using an orthogonal array design in the liver of mice receiving dietary supplementation with L-cysteine, glycine, and L-glutamate (Table 1A). The results showed that supplementation with L-cysteine

Table 1. Liver GSH concentrations in mice receiving dietary supplementation with L-cysteine, L-glutamate, and glycine for 7 days

A						
Groups	Dietary supplementation			GSH concentration (mmol/L) ^{a)}		
	L-Cysteine	L-Glutamate	Glycine			
1	0.0%	0.0%	0.0%	5.29 ± 1.02		
2	0.0%	0.5%	0.5%	3.58 ± 1.46		
3	0.0%	1.0%	1.0%	3.88 ± 1.70		
4	0.5%	0.0%	0.5%	5.90 ± 0.65		
5	0.5%	0.5%	1.0%	4.39 ± 1.09		
6	0.5%	1.0%	0.0%	6.39 ± 0.50		
7	1.0%	0.0%	1.0%	5.03 ± 1.79		
8	1.0%	0.5%	0.0%	4.86 ± 1.36		
9	1.0%	1.0%	0.5%	4.63 ± 1.40		
B						
Dietary supplementation	GSH concentration (mmol/L)			SS ^{c)}	MS ^{d)}	<i>p</i> -Value
	0.0% ^{b)}	0.5% ^{b)}	1.0% ^{b)}			
L-Cysteine	4.48 ^B	5.61 ^A	4.81 ^B	22.37	6.57	0.002
L-Glutamate	5.47 ^A	4.19 ^B	4.93 ^{AB}	23.48	6.90	0.002
Glycine	4.92	4.92	4.90	0.50	0.15	0.864

Values are means ± SD, *n* = 10. Ninety male ICR mice (with an average body weight of 26 g) were randomly divided into one of nine groups (*n* = 10/group). Mice received dietary supplementation with L-cysteine, L-glutamate, or glycine or their combinations. The composition of the basal diet was the same as previously reported [108]. At the end of the 7-day supplementation period, liver samples were harvested and homogenized (1 g tissue in 9 mL saline) for GSH determination using an ELISA kit (Nanjing Jiancheng Bio. Institute, China).

a) Values are expressed as Mean ± SD.

b) Dietary dosage of amino acids.

c) Type II sum of squares.

d) Mean square; orthogonal analysis was subjected to general linear models. Multiple tests were performed using the Tukey's multiple comparisons test (IBM SPSS Statistic 20). Means in the same row with different superscripts are different (*p* < 0.05).

and L-glutamate increases hepatic GSH synthesis (Table 1B). Furthermore, we found that supplementation with an appropriate dose of L-cysteine improves GSH synthesis, while excessive dietary L-cysteine reduces liver GSH concentration (Table 1B).

2.1 GCL

Chen et al. have reported that several factors can affect GSH synthesis, including the amount of GCL, the availability of L-cysteine, and the extent of feedback inhibition of GCL by GSH [16]. Among these factors, GCL is a rate-controlling step and plays a critical role in L-cysteine metabolism and GSH synthesis. The eukaryotic GCL consists of a 73-kDa catalytic subunit (GCLC) and a 31-kDa modifier subunit (GCLM), each of which is encoded by separate genes and exhibits different function in γ -Glu-cys synthesis [18]. GCLC contains binding sites for L-glutamate, L-cysteine, and ATP and is responsible for all the catalytic activity of GCL [19]. In contrast, GCLM has a regulatory function affecting the affinity of the holoenzyme for glutamate and GSH [20]. Of note, feedback inhibition by GSH involves reduction of the enzyme and also competition between GSH and glutamate for the glutamate-binding site [19, 20]. Currently, two models of GCL activation are widely cited in the GCL-related literature. The first model holds that

the GCL holoenzyme is predominantly sequestered in the cytosol as an inactive heterodimer, which can be oxidized to its activated state by formation of a disulfide bridge between GCLC and GCLM [18]. The activated GCL holoenzyme substantially improves the efficiency of γ -Glu-cys synthesis. Another model indicates that the active status of GCL depends on a dynamic equilibrium between monomeric and holoenzyme forms of the enzyme [18]. The shift of GCL to the high activity pool involves a change in GCLC, such that an N-terminal GCLC epitope associated with enzyme activity is protected in extracts with high GCL activity. Likewise, increased formation of high activity heterodimeric complexes results in a shift to more efficient GSH production [18].

2.2 GS

In eukaryotes, GS is a homodimeric enzyme with two identical subunits to catalyze the condensation of γ -Glu-cys and glycine to form GSH [21]. Currently, GS has received relatively little attention in GSH biosynthesis, because GCL is considered to be the rate-limiting step and GS is not subject to feedback regulation by excessive GSH. However, we found that dietary supplementation with L-cysteine showed a dosage-dependent inhibitory effect on GS activity

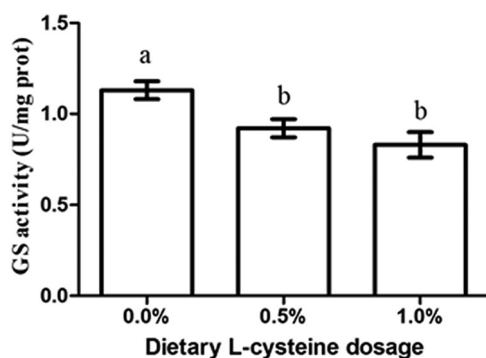


Figure 3. The response of liver GS activity to dietary L-cysteine in adult mice. The experimental detail is given in Table 1. Hepatic GS activity was measured using an ELISA kit (Nanjing Jiancheng Bio. Institute, China). Results were analyzed by one-way analysis of variance. Comparison of means was performed using the Tukey's multiple test (INM SPSS Statistic 2.0). Values are expressed as mean \pm SD. Means in the same row with different superscripts are different ($p < 0.05$).

in the liver of mice (Fig. 3), while L-glutamate and glycine failed to affect GS activity (unpublished data), indicating that supplementation with an appropriate dose of L-cysteine maintains a higher GS activity while excessive L-cysteine inhibits GS activity. Physiological abundance and activity of GS also play a key role in GSH synthesis and L-cysteine homeostasis. For example, decreased GS activity occurs in response to the depletion of the GSH pool under many pathological conditions, including surgical trauma [22]. GS deficiency can lead to the accumulation of γ -Glu-cys in cells, and this metabolite is further converted to 5-oxoproline. 5-Oxoproline is associated with severe metabolic acidosis, hemolytic anemia, and damage to the central nervous system [23, 24]. In support of this view, increased expression of GS by all-trans retinoic acid (which has no effect on GCL abundance) has been shown to enhance GSH synthesis in myeloid-derived suppressor cells [25].

3 L-Cysteine and H₂S synthesis

H₂S, nitric oxide, and carbon monoxide are the three gaseous signaling molecules that have received considerable attention from biological scientists in recent years. These three gasotransmitters perform a variety of homeostatic functions [26]. Endogenous H₂S is an anti-inflammatory, antioxidant, and neuroprotective agent. Many diseases, including neurological diseases, cardiovascular diseases, inflammation, and metabolic disorders, have been linked to metabolic disorders of endogenous H₂S [27–29]. The therapeutic administration of H₂S donors appears relevant in the treatment of various diseases. L-Cysteine is the preferred substrate for H₂S generation and accounts for 70% of the gas produced under normal conditions [30]. Meanwhile, a novel source of H₂S generation from D-cysteine has been observed in recent years,

and this metabolic pathway has been considered to be more effective than L-cysteine in neuroprotection against oxidative stress and ischemia-reperfusion injury [31].

3.1 H₂S production from L-cysteine

L-Cysteine-mediated generation of endogenous H₂S is catalyzed by two pyridoxal 5'-phosphate (PLP) dependent enzyme systems, including cystathionine β -synthase (CBS) and cystathionine γ -lyase (CSE) and PLP-independent mercaptopyruvate sulfurtransferase (MST) along with L-cysteine aminotransferase [32, 33]. CBS mainly catalyzes the β -replacement of the hydroxyl group of serine with homocysteine and then forms cystathionine with the release of H₂O [34]. L-Cysteine is structurally similar to serine with an OH group replaced by an SH. Thus, CBS can also use L-cysteine as a substrate to form cystathionine with the release of H₂S under pathological situations involving oxidative injury [34]. CBS has been demonstrated to be a major contributor for the production of H₂S. It is a highly regulated enzyme. S-adenosylmethionine serves as its allosteric activator and plays an important role in regulating its activity and concentration [35]. Stipanuk et al. [32] reported that an increase in CBS activity by supplementation with S-adenosylmethionine markedly promotes H₂S production by about 50% in both liver and kidney [32], while addition of amino-oxyacetate, a CBS inhibitor, blocks H₂S production and deteriorates oxidative injury [36]. Consequently, CBS knockout mice exhibit severe accumulation of homocysteine, as well as an inhibition of the β -replacement reactions involving both serine and L-cysteine [34].

However, Shibuya et al. [37] reported that brain homogenates of CBS knockout mice, even in the absence of PLP, produced H₂S at levels similar to those of wild-type mice, suggesting the presence of another H₂S-producing enzyme. Indeed, CSE has been reported to be the major alternative reaction for H₂S production. In addition to catalyzing the catabolism of cystathionine to form L-cysteine, CSE can directly facilitate the conversion of L-cysteine into L-serine and H₂S via α,β elimination [34, 38]. Furthermore, CSE participates in the disulfide elimination reaction to produce pyruvate, ammonia, and thiocysteine. Thiocysteine reacts with a thiol group in such substances as L-cysteine to generate H₂S [34]. Previous reports have estimated that CSE contributes to about 70% of the total H₂S generation under the normal conditions [38]. Treatment with propargylglycine, a CSE inhibitor, significantly suppresses sulfur anion production and L-cysteine metabolism by about 50% in rat renal cortical tubules [39]. The H₂S-producing activity of CSE is negatively regulated by cellular Ca²⁺ concentration. CSE efficiently produces H₂S at steady-state low Ca²⁺ concentrations, but this reaction is suppressed at high Ca²⁺ concentrations in the presence of PLP [40]. Thus, physiological calcium levels may control CSE-mediated H₂S formation. Emerging evidence has shown that a genetic deficiency of CSE results

in marked reductions in H₂S concentrations in the serum, heart, aorta, and other tissues in mice [41], leading to exacerbated myocardial ischemia/reperfusion injury, impaired cardiac mitochondrial function, and oxidative stress [42, 43]. Upregulation of the CSE/H₂S pathway provides various therapeutic avenues, including rescuing impaired arteriogenesis in mouse hind limb ischemia [44], improving TNF-induced insulin resistance associated with obesity and type 2 diabetes [45], and modulating central neurotransmitter input [46].

In the MST-mediated pathway for H₂S generation, L-cysteine firstly undergoes transamination with α -ketoglutarate to form 3-mercaptopyruvate (3MP). CAT (a PLP-dependent enzyme) is responsible for this reaction [47]. 3MP is then covalently linked via a disulfide bond to the active-site cysteine residue of MST to generate H₂S [37, 48]. MST also produces H₂S from thiosulfate, and several reducing substances, such as Trx and dihydrolipoic acid, are likely to be the major physiological persulfide acceptors and can facilitate H₂S release from MST [49, 50]. The MST/H₂S pathway is susceptible to oxidative stress, and treatment of H₂O₂ inhibits MST activity and interferes with the positive bioenergetic role of the 3MP/MST/H₂S pathway in vitro [51]. The MST/H₂S pathway may also be involved in the regulation of respiration and protection in cells [52].

Although the contribution of MST versus the other two H₂S generators, CBS and CSE, has been difficult to evaluate because of varied reaction conditions, we may make conclusions that the CBS-mediated H₂S production mainly occurs under pathological conditions involving oxidative stress, while the CSE-catalyzed H₂S formation largely contributes to normal H₂S metabolism. CBS and CSE are more likely to be potential therapeutic targets than MST for H₂S production as MST may not be responsible for the increased production of H₂S in various conditions [53].

3.2 H₂S production from D-cysteine

More recently, Shibuya et al. [31, 33] found an additional biosynthetic pathway for the production of H₂S from D-cysteine involving MST and D-amino acid oxidase (DAO). D-cysteine is derived from L-cysteine in food via racemization by heat and alkaline treatment during food processing [54]. The pathway for producing H₂S from D-cysteine is different from that from L-cysteine. The differences include the optimal pH, the dependency on PLP, and the stability against the freezing-thawing procedure [33]. Unlike the L-cysteine/H₂S pathway, in which the responsible enzymes are expressed in many tissues [32, 55, 56], D-cysteine-mediated H₂S generation occurs predominantly in the cerebellum and the kidney, as D-amino acid oxidase is mainly expressed in astrocytes, glia, and several types of neurons including the Golgi and Purkinje cells [31, 57]. Furthermore, there are no enzymes associated with D-cysteine metabolism, and D-cysteine has been widely hypothesized to produce H₂S directly via chemical degradation.

4 Hypotaurine and taurine

Several reports have indicated that an increase in L-cysteine availability as a result of the consumption of a sulfur amino acid rich diet can rapidly activates L-cysteine dioxygenase (CDO) [58], which catalyzes the oxidation of the L-cysteine thiol group to form cysteinesulfinic acid, which is also called cysteine sulfinic acid or 3-sulfinioalanine [34]. Cysteinesulfinic acid is a major precursor of taurine, and this metabolic pathway is involved in the decarboxylation and oxidation of cysteinesulfinic acid by cysteinesulfinic acid decarboxylase (CSD; Fig. 2).

4.1 CDO-mediated taurine formation

CDO is a highly regulated enzyme and widely expressed in hepatocytes, adipocytes, exocrine cells, goblet cells, and tubular epithelial [34]. Under conditions of a low intracellular concentration of L-cysteine, CDO activity is blocked via ubiquitination by 26S proteasome [59], while elevated levels of L-cysteine can rapidly increase hepatic or adipocyte CDO activity by up to 45- or tenfold, respectively [34]. For example, CDO activity increased with an increase in dietary protein levels, and the higher enzyme activity was paralleled by a greater rate of the production of taurine plus hypotaurine plus sulfate from L-cysteine [60]. Thus, CDO may serve as a major regulatory factor in intracellular L-cysteine levels and taurine formation. Previous studies with cell culture systems have shown that L-cysteine deprivation induces CDO ubiquitination, while addition of lactacystin or proteasome inhibitor 1 (PS1, N-carboxybenzyl-IleGlu[OtBu]AlaLeu-CHO), the 26S proteasome inhibitor, markedly blocks intracellular CDO degradation in L-cysteine-deficient cells [34, 61]. Studies in vivo have further indicated the switch of CDO activity in response to changes in intracellular L-cysteine levels. For example, feeding a L-cysteine-rich diet (100 g casein + 8.1 g L-cysteine/kg) or a high protein diet (400 g casein) resulted in a significant increase in hepatic CDO concentrations and a decrease in ubiquitinated forms of the CDO pool. Additionally, inhibition of 26S proteasome by PS1 stabilized hepatic CDO in rats fed a low protein diet [59, 62, 63]. Metabolic analysis has indicated that the increased CDO activity promotes L-cysteine metabolism toward hypotaurine and taurine production in that hepatic hypotaurine level was about 37 nmol/g in rats fed a low protein diet, but increased to 680 nmol/g at 3.5 h after the injection with PS1 [61]. Furthermore, primary hepatocytes from mice lacking CDO showed increases in L-cysteine concentrations and higher rates of metabolism of L-cysteine to H₂S and thiosulfate [64]. Thus, CDO sensitively responds to a high intracellular concentration of L-cysteine and plays an important role in the production of hypotaurine and taurine.

Previous studies have extensively addressed the role of CDO in L-cysteine metabolism and other neurological disorders [64–66], but the regulatory mechanism for the effect of

Table 2. Summary of effects of dietary cysteine or its precursors in different pathological conditions

Risk factor	Supplementary conditions	Response	Reference
HIV infection	L-Cysteine and L-glutamine or glycine	Taurine and GSH levels ↑, insulin sensitivity↑	[83, 109]
Aging	L-Cysteine and glycine; N-acetylcysteine	GSH levels ↑, oxidative stress ↓, proinflammatory state↓	[110, 111]
Type-2 diabetes	L-Cysteine or N-acetylcysteine	GSH levels ↑; the insulin-dependent signaling cascades of glucose metabolism↑; blood glucose, glycated hemoglobin, NF-kappaB activation↓	[112–115]
Cardiovascular disease	N-acetylcysteine	Proinflammatory cytokines↓, antioxidative capacity↑, energy metabolism↑	[116]
Inflammatory bowel disease	L-Cysteine	Proinflammatory cytokines↓, apoptosis↓	[117]
Nonalcoholic steatohepatitis patients	L-Cysteine-rich whey protein	GSH levels ↑, hepatic macrovesicular steatosis↓	[118]
Smoking	N-acetylcysteine	GSH levels ↑	[119]
Alzheimer's disease	N-acetylcysteine	Cognitive functioning↑, AD neuropathology↓	[120]
L-methionine deficiency	L-Cysteine	Plasma homocysteine concentration↓	[121]
Gastric cancer	S-propargyl-cysteine	H ₂ S production↑, tumor weights, and tumor volumes↓	[122]

↑, increase; ↓, decrease.

L-cysteine on CDO ubiquitination has not been fully explored. Stipanuk's group has investigated a substrate turnover dependent formation of a thioether cross-link between the sulfur of residue Cys93 and the aromatic side chain of residue Tyr157 in CDO [34]. Their results indicated that the immature CDO and inactive mutant forms of CDO fail to form any cross-link and exhibit low enzymatic activity and that a high catalytic efficiency can be achieved by the formation of Cys-Tyr cofactors. More recently, Goldberg's and Gao's group have reported structural and functional models for the active site of CDO [66, 67]. Formation of a CDO-Cys-Tyr cross-link requires a transition metal cofactor (ferrous iron [Fe²⁺] and oxygen [O₂]). It is speculated that the valence change of the Fe center makes the Cys-bound complexes effectively catalyze the oxidation of L-cysteine, as the ferric-superoxo species is an active oxidant and exhibits high reactivity in such a reaction.

4.2 CSD-mediated taurine formation

The cysteinesulfinate produced by CDO can be further metabolized by CSD to hypotaurine, which is subsequently oxidized to taurine. Transamination is another metabolic pathway for cysteinesulfinate to form pyruvate and sulfite by aspartate (cysteinesulfinate) aminotransferase. A previous report has estimated that 66 and 34% cysteinesulfinate participates in taurine and sulfite production, respectively [34]. The preferred metabolic pathway of cysteinesulfinate is likely dependent on the abundance of the enzymes and their affinities for their substrates. Although there is little information about intracellular concentrations of the enzymes, compelling evidence

has shown that high levels of CSD in liver and adipose tissue contributes to a higher capacity for taurine synthesis [68, 69]. Studies on kinetics of these enzymes have revealed that CSD has a higher affinity for cysteinesulfinate as the *K_m* in taurine synthesis for cysteinesulfinate is ~0.04–0.17 mM, while the value is ~3–25 mM for aspartate aminotransferase [34]. However, the L-cysteine/taurine pathway can be limited at high protein levels due to the decrease in CSD activity such that sulfate production from cysteinesulfinate is favored [60].

5 Nutritional use of L-cysteine

The balance between L-cysteine and L-cystine plays a vital role in controlling redox potential, synthesis of other active substrates (i.e., GSH, H₂S, and taurine), oxidative stress, and inflammatory response [3, 4, 70]. Dietary intake of sulfur amino acids affects cell signaling via modulating intracellular concentrations of L-cysteine and L-cystine, as well as L-cysteine/L-cystine redox state in the postprandial period [71]. Thus, recent years have witnessed growing interest in the use of L-cysteine for improving health in animals and humans (Table 2).

5.1 Oxidative stress

We found that various kinds of stress can lead to oxidative injury in animals [72–74]. Additionally, oxidative stress is thought to be involved in the development of many diseases or may exacerbate their symptoms [75]. GSH is the most abundant cellular thiol antioxidant and plays a

protective role against toxicity arising from excessive amounts of endogenous and exogenous electrophiles [16] via scavenging hydroxyl radical and superoxide directly, and serving as a cofactor for the enzyme GSH peroxidase (GPx) in metabolizing H_2O_2 and lipid peroxides. Thus, current reports mainly focused on the positive effects of L-cysteine in different pathological conditions via increasing GSH synthesis and suppressing oxidative stress or inflammatory response, while these reports failed to recognize other beneficial effects of dietary L-cysteine in the production of H_2S and taurine.

Increases in endogenous H_2S generation by CBS and CSE contribute to some pathological conditions [76]. Lu et al. [36] also demonstrated that H_2S has potential therapeutic value for oxidative stress-induced brain damage via a mechanism involving enhanced L-glutamate uptake. Oxidative stress can impact the function of L-glutamate transporters [77] and result in L-glutamate accumulation in the synaptic cleft, which further leads to toxicity and neural injury via overactivation of related receptors. Thus, dysfunction of L-glutamate transporters is commonly associated with neurodegenerative diseases and some acute brain injuries [78]. In addition, L-glutamate is an inhibitor of the X_c^- system, which transports one molecule of L-cystine into cells and, therefore, releases one molecule of glutamate into the extracellular space [1]. The excessive extracellular glutamate plays a feedback inhibitory role in L-cystine influx and, therefore, L-glutamate neurotoxicity is primarily characterized by the depletion of cellular GSH [79]. In vitro studies have reported that addition of NaHS (an H_2S donor) reverses H_2O_2 -impaired L-glutamate transport and enhances GSH production [36]. This pathway may be another important factor contributing to the pathogenesis of brain and neural diseases.

Taurine is an organic osmolyte involved in modulation of intracellular free calcium concentration and has been considered as one of the most essential substances in the body due to: (i) its broad distribution, cytoprotective effects, antioxidant properties; (ii) its role in regulating intracellular Ca^{2+} concentration, movement of ions and neurotransmitters, proinflammatory response; and (iii) its functional significance in cell development, nutrition, and survival [80,81]. Recently, several reports have shown that taurine serves as a protective agent against several environmental toxins and drug-induced organ dysfunction and diabetes [82]. Thus, an increase in the conversion of L-cysteine to taurine provides a novel insight into L-cysteine nutrition and its therapeutic potential. For example, dietary N-acetylcysteine (a stable and water-soluble precursor of L-cysteine) significantly increased the plasma levels of taurine and GSH in patients with the human immunodeficiency virus (HIV) [83].

5.2 Gut function

Gut plays important roles in secretions, food digestion, nutrient absorption and metabolism, and cross-talk with the intestinal microbiota. Gut mucosal proteins and mucins, which

contribute to intestinal integrity, are rich in L-cysteine [84]. Compelling evidence from in vivo studies has shown that L-cysteine plays key roles in maintaining intestinal structure and function [84,85]. Bauchart-Thevret et al. [86] evaluated first-pass splanchnic metabolism of dietary L-cysteine in weanling pigs and found that gastrointestinal tract utilizes 25% of the dietary L-cysteine intake and that synthesis of mucosal epithelial proteins, such as GSH and mucins, is a major nonoxidative metabolic fate for L-cysteine. Thus, we can speculate that L-cysteine deficiency contributes significantly to the intestinal mucosal atrophy and reduced secretion of mucins [84]. Furthermore, Badaloo et al. [84] reported that children with malnutrition exhibited gut mucosal atrophy and depletion of mucins, produced less L-cysteine, and had a greater requirement for dietary L-cysteine during early and mid-nutritional rehabilitation. Thus, L-cysteine serves as an essential substrate for maintaining gut function.

5.3 Lipid metabolism

Dietary supplementation with L-cysteine can improve lipid metabolism. Elshorbagy et al. [87] reported that total L-cysteine concentration in serum was positively correlated with fat mass. Indeed, the correlation was stronger with total L-cysteine than with serum lipids such as triglycerides [87]. Triglycerides are formed by combining glycerol with three fatty acid molecules and play a critical role in lipid metabolic network as energy sources and transporters of dietary fat. In humans and animals, high plasma concentrations of triglycerides are associated with various diseases, including atherosclerosis, heart disease, and stroke [88]. Lee et al. [89] reported that L-cysteine effectively reduces triglyceride concentrations in serum and liver in a dose-dependent manner in rats fed a normal diet [89]. The derivatives of L-cysteine also regulate lipid metabolism. For example, S-methyl L-cysteine, a hydrophilic L-cysteine-containing compound, exhibits hypoglycemic and antihyperlipidemic properties through reduction in fasting plasma levels of glucose, total cholesterol, triglycerides, LDL cholesterol in fructose-induced diabetic rats [90]. N-acetylcysteine also has been demonstrated to improve lipid metabolism through affecting serum cholesterol, triglycerides, VLDL, and HDL levels [91]. Although little is known about the underlying mechanisms, some reports indicate (i) supplementation with L-cysteine targets at gene expression of the sterol response element-binding protein, fatty acid synthase, and stearoyl-coenzymeA desaturase-1 [92]; (ii) reduced oxidation of L-cysteine to form taurine leads to a deficiency of taurine [87,93] and abnormal lipid metabolism [94].

5.4 Growth

Previous reports have indicated that dietary supplementation with L-cysteine affects animal growth performance, including food intake, body weight gain, and feed efficiency. However, the effect of L-cysteine on growth performance is not always

Table 3. Effects of dietary L-cysteine on growth performance in rats

Health status	Dosage	Weight gain	Food intake	Reference
Healthy	1–2% diet	↓	↓	[89]
Healthy	1–4 mmol/kg	-	↓	[95]
Aging	6.8 g/kg	N	↑	[123]
Sucrose stress	5.5–16 g/kg	N	N	[115]
Diabetes	1 mg/kg	N	-	[114]
L-methionine-restricted diet	0.5%	↑	N	[124]

N, no effect; ↑, increase; ↓, decrease.

detected depending on experimental design (Table 3). McGavigan et al. [95] reported that a low dosage of L-cysteine (oral gavage: 4 mmol/kg) is more anorectic than L-arginine and L-lysine [95], high dosages of which can inhibit feed intake [96]. Lee et al. [89] further investigated the anorectic effect of L-cysteine and found that dietary supplementation with L-cysteine effectively reduces final body weight, body weight gain, food intake, and feed efficiency in rats [89]. We noted that animals in these studies were either aging ones or in a catabolic state, while an anorectic effect appeared in young and healthy animals (Table 3). Collectively, these studies suggest that the anorectic effect of L-cysteine depends on the health status, nutritional level, and age of the animals. Under normal conditions, supplementation with L-cysteine may reduce feed intake and weight gain in young animals.

L-Cysteine confers a bitter taste, which can contribute to its inhibitory effect on feed intake [89, 97]. This explanation is not convincing, as a latest report showed that intraperitoneal administration of 2 mmol/kg also reduces feed intake in rats [95]. McGavigan et al. [96] further investigated the mechanism for L-cysteine to reduce feed intake and found that L-cysteine activates promiscuous amino acid sensing receptors, such as T1R1/T1R3, CaSR, and GPRC6A [96]. However, these receptors may not mediate the effects of L-cysteine on appetite, as other amino acids (i.e., L-serine, L-threonine, and L-histidine) also induce a strong T1R1/T1R3-, CaSR-, and GPRC6A-mediated response [98] but do not inhibit food intake or growth performance of the animals [95]. More recently, acyl ghrelin has been suggested to play a decisive role in L-cysteine-mediated appetite stimulation, as a reduction in the circulating level of acyl ghrelin occurred in both rodents and humans receiving dietary supplementation with L-cysteine [95]. Meanwhile, the anorectic effect of L-cysteine is attenuated in transgenic mice overexpressing ghrelin [95].

5.5 Effects of supplemental L-methionine as a L-cysteine precursor

L-methionine is the physiological precursor of endogenous L-cysteine [99]. Thus, the metabolism and availability of

L-methionine can affect the nutritional efficacy of dietary L-cysteine in animals [100, 101]. L-methionine can replace L-cysteine in diets to maintain normal protein synthesis and normal growth in animals, but not vice versa [102]. However, L-cysteine can spare L-methionine in animals. Thus, supplementing L-cysteine to a L-methionine-restricted diet reverses the adverse effects of L-methionine deficiency [103, 104]. Several studies have concluded that when the diet contains both L-methionine and L-cysteine, the mean requirements of L-methionine and L-cysteine by infants are 38 and 91 mg kg⁻¹ day⁻¹ L-cysteine, respectively [106]. The values for adult men are 12.6 and 21 mg kg⁻¹ day⁻¹ for L-methionine and L-cysteine, respectively [102].

The ability of dietary L-methionine to supply endogenous L-cysteine has been studied in edematous severe acute malnutrition. In this case, L-methionine supplementation increases L-cysteine production but has no effect on GSH synthesis [105]. One explanation is that the conversion of L-methionine to L-cysteine in the liver is insufficient for sustaining GSH production. This necessitates dietary supplementation with L-cysteine to partially fulfill the demand for this amino acid in edematous severe acute malnutrition [105]. Effects of dietary supplementation with L-cysteine or its precursors on animals under different pathological conditions [106–124] are summarized in Tables 2 and 3. Taken together, these findings indicate that direct provision of L-cysteine in diets is required under conditions of impaired L-methionine catabolism so as to maintain whole-body protein synthesis and physiological homeostasis.

6 Conclusion and perspectives

L-Cysteine is not only a building block of protein, but is also a regulator of cell signaling pathways. Therefore, L-cysteine is now classified as a functional amino acid in nutrition [125]. There is a complex relationship between L-methionine and L-cysteine in their metabolism and nutrition such that dietary L-methionine is not always effective in supplying endogenous L-cysteine [126]. Under certain conditions when the absorption or catabolism of L-methionine is impaired, it is necessary to include L-cysteine in diets so as to maintain adequate protein synthesis in tissues and whole-body physiological homeostasis [127, 128]. Dietary L-cysteine supplementation can increase the synthesis of GSH, H₂S, and taurine in animals and humans [126–129]. However, the use of L-cysteine supplementation as a nutritional intervention of disease is limited. Emerging evidence shows a positive role of L-cysteine-rich meals in several pathological conditions, including oxidative stress, HIV infection, aging, type-2 diabetes, and neurodegenerative diseases. In addition, dietary supplementation with L-cysteine or its precursor N-acetyl-cysteine can improve gut function, growth, and health [128–132]. Future research should focus on: (i) optimal requirements of L-cysteine by animals and humans fed enteral or parenteral diets, (ii) nutritional regulation of GSH, H₂S, and taurine

synthesis in a cell- and tissue-specific manner, and (iii) roles of these metabolites to treat and prevent metabolic disorders. Additionally, caution must be exercised to avoid high dosages of L-cysteine supplementation in animals and humans, because L-cysteine exerts an N-methyl-D-aspartate receptor-mediated excitatory effect in the nervous system. A previous study indicated that a toxic dosage of intravenous administration of L-cysteine for 28 days was 1 g/kg body weight per day in adult male rats [107]. A toxic dosage of oral administration of L-cysteine remains to be determined for animals.

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