

## Glutathione Metabolism and Its Implications for Health<sup>1</sup>

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**ABSTRACT** Glutathione ( $\gamma$ -glutamyl-cysteinyl-glycine; GSH) is the most abundant low-molecular-weight thiol, and GSH/glutathione disulfide is the major redox couple in animal cells. The synthesis of GSH from glutamate, cysteine, and glycine is catalyzed sequentially by two cytosolic enzymes,  $\gamma$ -glutamylcysteine synthetase and GSH synthetase. Compelling evidence shows that GSH synthesis is regulated primarily by  $\gamma$ -glutamylcysteine synthetase activity, cysteine availability, and GSH feedback inhibition. Animal and human studies demonstrate that adequate protein nutrition is crucial for the maintenance of GSH homeostasis. In addition, enteral or parenteral cystine, methionine, *N*-acetylcysteine, and *L*-2-oxothiazolidine-4-carboxylate are effective precursors of cysteine for tissue GSH synthesis. Glutathione plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation). Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases (including kwashiorkor, seizure, Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, HIV, AIDS, cancer, heart attack, stroke, and diabetes). New knowledge of the nutritional regulation of GSH metabolism is critical for the development of effective strategies to improve health and to treat these diseases. *J. Nutr.* 134: 489–492, 2004.

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The work with glutathione ( $\gamma$ -glutamyl-cysteinyl-glycine; GSH)<sup>3</sup> has greatly advanced biochemical and nutritional sciences over the past 125 y (1,2). Specifically, these studies have led to the free radical theory of human diseases and to the

advancement of nutritional therapies to improve GSH status under various pathological conditions (2,3). Remarkably, the past decade witnessed the discovery of novel roles for GSH in signal transduction, gene expression, apoptosis, protein glutathionylation, and nitric oxide (NO) metabolism (2,4). Most recently, studies of *in vivo* GSH turnover in humans were initiated to provide much-needed information about quantitative aspects of GSH synthesis and catabolism in the whole body and specific cell types (e.g., erythrocytes) (3,5–7). This article reviews the recent developments in GSH metabolism and its implications for health and disease.

**Abundance of GSH in Cells and Plasma.** Glutathione is the predominant low-molecular-weight thiol (0.5–10 mmol/L) in animal cells. Most of the cellular GSH (85–90%) is present in the cytosol, with the remainder in many organelles (including the mitochondria, nuclear matrix, and peroxisomes) (8). With the exception of bile acid, which may contain up to 10 mmol/L GSH, extracellular concentrations of GSH are relatively low (e.g., 2–20  $\mu$ mol/L in plasma) (4,9). Because of the cysteine residue, GSH is readily oxidized nonenzymatically to glutathione disulfide (GSSG) by electrophilic substances (e.g., free radicals and reactive oxygen/nitrogen species). The GSSG efflux from cells contributes to a net loss of intracellular GSH. Cellular GSH concentrations are reduced markedly in response to protein malnutrition, oxidative stress, and many pathological conditions (8,9). The GSH + 2GSSG concentration is usually denoted as total glutathione in cells, a significant amount of which (up to 15%) may be bound to protein (1). The [GSH]:[GSSG] ratio, which is often used as an indicator of the cellular redox state, is >10 under normal physiological conditions (9). GSH/GSSG is the major redox couple that determines the antioxidative capacity of cells, but its value can be affected by other redox couples, including NADPH/NADP<sup>+</sup> and thioredoxin<sub>red</sub>/thioredoxin<sub>ox</sub> (4).

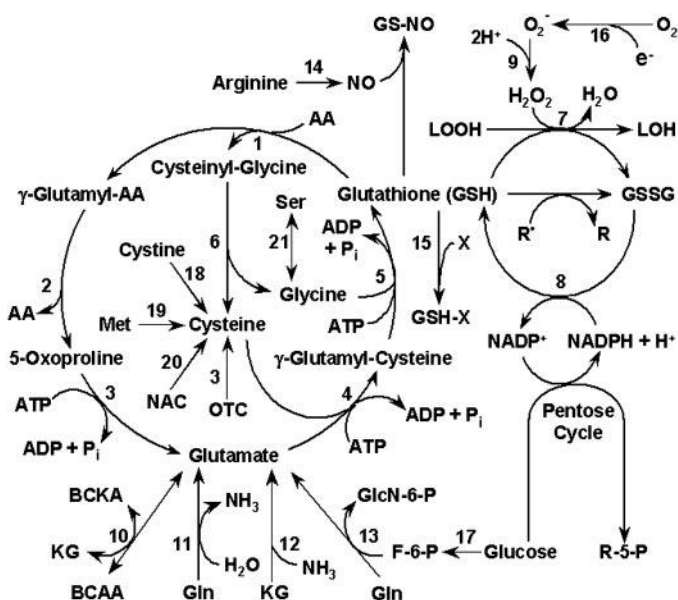
**GSH Synthesis.** The synthesis of GSH from glutamate, cysteine, and glycine is catalyzed sequentially by two cytosolic enzymes,  $\gamma$ -glutamylcysteine synthetase (GCS) and GSH synthetase (Fig. 1). This pathway occurs in virtually all cell types, with the liver being the major producer and exporter of GSH. In the GCS reaction, the  $\gamma$ -carboxyl group of glutamate reacts with the amino group of cysteine to form a peptidic  $\gamma$ -linkage, which protects GSH from hydrolysis by intracellular peptidases. Although  $\gamma$ -glutamyl-cysteine can be a substrate for  $\gamma$ -glutamylcyclotransferase, GSH synthesis is favored in animal cells because of the much higher affinity and activity of GSH synthetase (9).

Mammalian GCS is a heterodimer consisting of a catalytically active heavy subunit (73 kDa) and a light (regulatory) subunit (31 kDa) (8). The heavy subunit contains all substrate binding sites, whereas the light subunit modulates the affinity of the heavy subunit for substrates and inhibitors. The  $K_m$  values of mammalian GCS for glutamate and cysteine are 1.7 and 0.15 mmol/L, respectively, which are similar to the intracellular concentrations of glutamate (2–4 mmol/L) and cysteine (0.15–0.25 mmol/L) in rat liver (9). Mammalian GSH

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<sup>3</sup> Abbreviations used: GCS,  $\gamma$ -glutamylcysteine synthetase; GSH, glutathione; GSSG, glutathione disulfide.



**FIGURE 1** Glutathione synthesis and utilization in animals. Enzymes that catalyze the indicated reactions are: 1)  $\gamma$ -glutamyl transpeptidase, 2)  $\gamma$ -glutamyl cyclotransferase, 3) 5-oxoprolinase, 4)  $\gamma$ -glutamylcysteine synthetase, 5) glutathione synthetase, 6) dipeptidase, 7) glutathione peroxidase, 8) glutathione reductase, 9) superoxide dismutase, 10) BCAA transaminase (cytosolic and mitochondrial), 11) glutaminase, 12) glutamate dehydrogenase, 13) glutamine:fructose-6-phosphate transaminase (cytosolic), 14) nitric oxide synthase, 15) glutathione S-transferase, 16) NAD(P)H oxidase and mitochondrial respiratory complexes, 17) glycolysis, 18) glutathione-dependent thiol-disulfide or thioltransferase or nonenzymatic reaction, 19) transsulfuration pathway, 20) deacylase, and 21) serine hydroxymethyltransferase. Abbreviations: AA, amino acids; BCKA, branched-chain  $\alpha$ -ketoacids; GlcN-6-P, glucosamine-6-phosphate; GS-NO, glutathione-nitric oxide adduct; KG,  $\alpha$ -ketoglutarate; LOO $\cdot$ , lipid peroxyl radical; LOOH, lipid hydroperoxide; NAC, N-acetylcysteine; OTC, L-2-oxothiazolidine-4-carboxylate; R $\cdot$ , radicals; R, nonradicals; R-5-P, ribulose-5-phosphate; X, electrophilic xenobiotics.

synthetase is a homodimer (52 kDa/subunit) and is an allosteric enzyme with cooperative binding for  $\gamma$ -glutamyl substrate (10). The  $K_m$  values of mammalian GSH synthetase for ATP and glycine are  $\sim 0.04$  and  $0.9$  mmol/L, respectively, which are lower than intracellular concentrations of ATP (2–4 mmol/L) and glycine (1.5–2 mmol/L) in rat liver. Both subunits of rat GCS and GSH synthetase have been cloned and sequenced (9), which facilitates the study of molecular regulation of GSH synthesis.  $\gamma$ -Glutamylcysteine synthetase is the rate-controlling enzyme in de novo synthesis of GSH (8).

Knowledge regarding in vivo GSH synthesis is limited, due in part to the complex compartmentalization of substrates and their metabolism at both the organ and subcellular levels. For example, the source of glutamate for GCS differs between the small intestine and kidney (e.g., diet vs. arterial blood). In addition, liver GSH synthesis occurs predominantly in perivenous hepatocytes and, to a lesser extent, in periportal cells (11). Thus, changes in plasma GSH levels may not necessarily reflect changes in GSH synthesis in specific cell types. However, recent studies involving stable isotopes (5–7) have expanded our understanding of GSH metabolism. In healthy adult humans, the endogenous disappearance rate (utilization rate) of GSH is  $25 \mu\text{mol}/(\text{kg} \cdot \text{h})$  (6), which accounts for 65% of whole body cysteine flux [ $38.3 \mu\text{mol}/(\text{kg} \cdot \text{h})$ ]. This finding supports the view that GSH acts as a major

transport form of cysteine in the body. On the basis of dietary cysteine intake [ $9 \mu\text{mol}/(\text{kg} \cdot \text{h})$ ] in healthy adult humans (6), it is estimated that most of the cysteine used for endogenous GSH synthesis is derived from intracellular protein degradation and/or endogenous synthesis. Interestingly, among extrahepatic cells, the erythrocyte has a relatively high turnover rate for GSH. For example, the whole-blood fractional synthesis rate of GSH in healthy adult subjects is 65%/d (6), which means that all the GSH is completely replaced in 1.5 d; this value is equivalent to  $3 \mu\text{mol}/(\text{kg} \cdot \text{h})$ . Thus, whole blood (mainly erythrocytes) may contribute up to 10% of whole-body GSH synthesis in humans (5,6).

**Regulation of GSH Synthesis by GCS.** Oxidant stress, nitrosative stress, inflammatory cytokines, cancer, cancer chemotherapy, ionizing radiation, heat shock, inhibition of GCS activity, GSH depletion, GSH conjugation, prostaglandin  $A_2$ , heavy metals, antioxidants, and insulin increase GCS transcription or activity in a variety of cells (2,8). In contrast, dietary protein deficiency, dexamethasone, erythropoietin, tumor growth factor  $\beta$ , hyperglycemia, and GCS phosphorylation decrease GCS transcription or activity. Nuclear factor  $\kappa\text{B}$  mediates the upregulation of GCS expression in response to oxidant stress, inflammatory cytokines, and buthionine sulfoximine-induced GSH depletion (2,8). S-nitrosation of GCS protein by NO donors (e.g., S-nitroso-L-cysteine and S-nitroso-L-cysteinylglycine) reduces enzyme activity (8), suggesting a link between NO (a metabolite of L-arginine) and GSH metabolism. Indeed, an increase in NO production by inducible NO synthase causes GCS inhibition and GSH depletion in cytokine-activated macrophages and neurons (12). In this regard, glucosamine, taurine, n-3 PUFAs, phytoestrogens, polyphenols, carotenoids, and zinc, which inhibit the expression of inducible NO synthase and NO production (13), may prevent or attenuate GSH depletion in cells. Conversely, high-fat diet, saturated long-chain fatty acids, low-density lipoproteins, linoleic acid, and iron, which enhance the expression of inducible NO synthase and NO production (13), may exacerbate the loss of GSH from cells.

**Regulation of GSH Synthesis by Amino Acids.** Cysteine is an essential amino acid in premature and newborn infants and in subjects stressed by disease (14). As noted above, the intracellular pool of cysteine is relatively small, compared with the much larger and often metabolically active pool of GSH in cells (15). Recent studies provide convincing data to support the view that cysteine is generally the limiting amino acid for GSH synthesis in humans, as in rats, pigs, and chickens (6,14,15). Thus, factors (e.g., insulin and growth factors) that stimulate cysteine (cystine) uptake by cells generally increase intracellular GSH concentrations (8). In addition, increasing the supply of cysteine or its precursors (e.g., cystine, N-acetylcysteine, and L-2-oxothiazolidine-4-carboxylate) via oral or intravenous administration enhances GSH synthesis and prevents GSH deficiency in humans and animals under various nutritional and pathological conditions (including protein malnutrition, adult respiratory distress syndrome, HIV, and AIDS) (2). Because cysteine generated from methionine catabolism via the transsulfuration pathway (primarily in hepatocytes) serves as a substrate for GCS, dietary methionine can replace cysteine to support GSH synthesis in vivo.

Cysteine is readily oxidized to cystine in oxygenated extracellular solutions. Thus, the plasma concentration of cysteine is low (10–25  $\mu\text{mol}/\text{L}$ ), compared with that of cystine (50–150  $\mu\text{mol}/\text{L}$ ). Cysteine and cystine are transported by distinct membrane carriers, and cells typically transport one more

efficiently than the other (8). It is interesting that some cell types (e.g., hepatocytes) have little or no capacity for direct transport of extracellular cystine. However, GSH that effluxes from the liver can reduce cystine to cysteine on the outer cell membrane, and the resulting cysteine is taken up by hepatocytes. Other cell types (e.g., endothelial cells) can take up cystine and reduce it intracellularly to cysteine (Fig. 1); cellular reducing conditions normally favor the presence of cysteine in animal cells.

Extracellular and intracellularly generated glutamate can be used for GSH synthesis (16). Because dietary glutamate is almost completely utilized by the small intestine (16), plasma glutamate is derived primarily from its de novo synthesis and protein degradation. Phosphate-dependent glutaminase, glutamate dehydrogenase, pyrroline-5-carboxylate dehydrogenase, BCAA transaminase, and glutamine:fructose-6-phosphate transaminase may catalyze glutamate formation (Fig. 1), but the relative importance of these enzymes likely varies among cells and tissues. Interestingly, rat erythrocytes do not take up or release glutamate (17), and glutamine and/or BCAAs may be the precursors of glutamate in these cells (Fig. 1). Indeed, glutamine is an effective precursor of the glutamate for GSH synthesis in many cell types, including enterocytes, neural cells, liver cells, and lymphocytes (18). Thus, glutamine supplementation to total parenteral nutrition maintains tissue GSH levels and improves survival after reperfusion injury, ischemia, acetaminophen toxicity, chemotherapy, inflammatory stress, and bone marrow transplantation (19).

Glutamate plays a regulatory role in GSH synthesis through two mechanisms: 1) the uptake of cystine, and 2) the prevention of GSH inhibition of GCS. Glutamate and cystine share the system X<sub>c</sub><sup>-</sup> amino acid transporter (8). When extracellular glutamate concentrations are high, as in patients with advanced cancer, HIV infection, and spinal cord or brain injury as well as in cell culture medium containing high levels of glutamate, cystine uptake is competitively inhibited by glutamate, resulting in reduced GSH synthesis (20). GSH is a nonallosteric feedback inhibitor of GCS, but the binding of GSH to the enzyme competes with glutamate (9). When intracellular glutamate concentrations are unusually high, as in canine erythrocytes, GSH synthesis is enhanced and its concentration is particularly high (9).

Glycine availability may be reduced in response to protein malnutrition, sepsis, and inflammatory stimuli (21,22). When hepatic glycine oxidation is enhanced in response to high levels of glucagon or diabetes (23), this amino acid may become a limiting factor for GSH synthesis. In vivo studies show that glycine availability limits erythrocyte GSH synthesis in burned patients (7) and in children recovering from severe malnutrition (21). It is important to note that dietary glycine supplementation enhances the hepatic GSH concentration in protein-deficient rats challenged with TNF- $\alpha$  (22).

The evidence indicates that the dietary amino acid balance has an important effect on protein nutrition and therefore on GSH homeostasis (8). In particular, the adequate provision of sulfur-containing amino acids as well as glutamate (glutamine or BCAAs) and glycine (or serine) is critical for the maximization of GSH synthesis. Thus, in the erythrocytes of children with edematous protein-energy malnutrition and piglets with protein deficiency, GSH synthesis is impaired, leading to GSH deficiency (3). An increase in urinary excretion of 5-oxoproline, an intermediate of the  $\gamma$ -glutamyl cycle (Fig. 1), is a useful indicator of reduced availability of cysteine and/or glycine for GSH synthesis in vivo (7,21)

**Interorgan GSH Transport.** Glutathione can be trans-

ported out of cells via a carrier-dependent facilitated mechanism (2). Plasma GSH originates primarily from the liver, but some of the dietary and intestinally derived GSH can enter the portal venous plasma (8). Glutathione molecules leave the liver either intact or as  $\gamma$ -Glu-(Cys)<sub>2</sub> owing to  $\gamma$ -glutamyl transpeptidase activity on the outer plasma membrane (Fig. 1). The extreme concentration gradient across the plasma membrane makes the transport of extracellular GSH or GSSG into cells thermodynamically unfavorable. However,  $\gamma$ -Glu-(Cys)<sub>2</sub> is readily taken up by extrahepatic cells for GSH synthesis. The kidney, lung, and intestine are major consumers of the liver-derived GSH (8). The interorgan metabolism of GSH functions to transport cysteine in a nontoxic form between tissues, and also helps to maintain intracellular GSH concentrations and redox state (8).

**Roles of GSH.** Glutathione participates in many cellular reactions. First, GSH effectively scavenges free radicals and other reactive oxygen species (e.g., hydroxyl radical, lipid peroxy radical, peroxynitrite, and H<sub>2</sub>O<sub>2</sub>) directly, and indirectly through enzymatic reactions (24). In such reactions, GSH is oxidized to form GSSG, which is then reduced to GSH by the NADPH-dependent glutathione reductase (Fig. 1). In addition, glutathione peroxidase (a selenium-containing enzyme) catalyzes the GSH-dependent reduction of H<sub>2</sub>O<sub>2</sub> and other peroxides (25).

Second, GSH reacts with various electrophiles, physiological metabolites (e.g., estrogen, melanins, prostaglandins, and leukotrienes), and xenobiotics (e.g., bromobenzene and acetaminophen) to form mercapturates (24). These reactions are initiated by glutathione-S-transferase (a family of Phase II detoxification enzymes).

Third, GSH conjugates with NO to form an S-nitroso-glutathione adduct, which is cleaved by the thioredoxin system to release GSH and NO (24). Recent evidence suggests that the targeting of endogenous NO is mediated by intracellular GSH (26). In addition, both NO and GSH are necessary for the hepatic action of insulin-sensitizing agents (27), indicating their critical role in regulating lipid, glucose, and amino acid utilization.

Fourth, GSH serves as a substrate for formaldehyde dehydrogenase, which converts formaldehyde and GSH to S-formyl-glutathione (2). The removal of formaldehyde (a carcinogen) is of physiological importance, because it is produced from the metabolism of methionine, choline, methanol (alcohol dehydrogenase), sarcosine (sarcosine oxidase), and xenobiotics (via the cytochrome P450-dependent monooxygenase system of the endoplasmic reticulum).

Fifth, GSH is required for the conversion of prostaglandin H<sub>2</sub> (a metabolite of arachidonic acid) into prostaglandins D<sub>2</sub> and E<sub>2</sub> by endoperoxide isomerase (8).

Sixth, GSH is involved in the glyoxalase system, which converts methylglyoxal to D-lactate, a pathway active in microorganisms. Finally, glutathionylation of proteins (e.g., thioredoxin, ubiquitin-conjugating enzyme, and cytochrome c oxidase) plays an important role in cell physiology (2).

Thus, GSH serves vital functions in animals (Table 1). Adequate GSH concentrations are necessary for the proliferation of cells, including lymphocytes and intestinal epithelial cells (28). Glutathione also plays an important role in spermatogenesis and sperm maturation (1). In addition, GSH is essential for the activation of T-lymphocytes and polymorphonuclear leukocytes as well as for cytokine production, and therefore for mounting successful immune responses when the host is immunologically challenged (2). Further, both in vitro and in vivo evidence show that GSH inhibits infection by the

TABLE 1

*Roles of glutathione in animals*

Antioxidant defense
Scavenging free radicals and other reactive species
Removing hydrogen and lipid peroxides
Preventing oxidation of biomolecules
Metabolism
Synthesis of leukotrienes and prostaglandins
Conversion of formaldehyde to formate
Production of D-lactate from methylglyoxal
Formation of mercapturates from electrophiles
Formation of glutathione-NO adduct
Storage and transport of cysteine
Regulation
Intracellular redox status
Signal transduction and gene expression
DNA and protein synthesis, and proteolysis
Cell proliferation and apoptosis
Cytokine production and immune response
Protein glutathionylation
Mitochondrial function and integrity

influenza virus (29). It is important to note that shifting the GSH/GSSG redox toward the oxidizing state activates several signaling pathways (including protein kinase B, protein phosphatases 1 and 2A, calcineurin, nuclear factor  $\kappa$ B, c-Jun N-terminal kinase, apoptosis signal-regulated kinase 1, and mitogen-activated protein kinase), thereby reducing cell proliferation and increasing apoptosis (30). Thus, oxidative stress (a deleterious imbalance between the production and removal of reactive oxygen/nitrogen species) plays a key role in the pathogenesis of many diseases, including cancer, inflammation, kwashiorkor (predominantly protein deficiency), seizure, Alzheimer's disease, Parkinson's disease, sickle cell anemia, liver disease, cystic fibrosis, HIV, AIDS, infection, heart attack, stroke, and diabetes (2,31).

**Concluding Remarks and Perspectives.** GSH displays remarkable metabolic and regulatory versatility. GSH/GSSG is the most important redox couple and plays crucial roles in antioxidant defense, nutrient metabolism, and the regulation of pathways essential for whole body homeostasis. Glutathione deficiency contributes to oxidative stress, and, therefore, may play a key role in aging and the pathogenesis of many diseases. This presents an emerging challenge to nutritional research. Protein (or amino acid) deficiency remains a significant nutritional problem in the world, owing to inadequate nutritional supply, nausea and vomiting, premature birth, HIV, AIDS, cancer, cancer chemotherapy, alcoholism, burns, and chronic digestive diseases. Thus, new knowledge regarding the efficient utilization of dietary protein or the precursors for GSH synthesis and its nutritional status is critical for the development of effective therapeutic strategies to prevent and treat a wide array of human diseases, including cardiovascular complications, cancer, and severe acute respiratory syndrome.

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