Clinical and nutritional benefits of cysteine-enriched protein supplements

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Purpose of review
To review recently published research into the use of dietary cysteine and/or its derivatives as functional food supplements that will enhance antioxidant status and improve outcome in certain diseases.

Recent findings
L-cysteine is now widely recognized as a conditionally essential or (indispensible) sulphur amino acid. It plays a key role in the metabolic pathways involving methionine, taurine and glutathione (GSH), and may help fight chronic inflammation by boosting antioxidant status. In stressed and inflammatory states, sulphur amino acid metabolism adapts to meet the increased requirements for cysteine as a rate-limiting substrate for GSH. Critically ill patients receiving enteral or parenteral nutrition, enriched with cysteine, exhibit decreased cysteine catabolism and improved GSH synthesis. The naturally occurring cysteine-rich proteins, whey or keratin, have the potential to be manufactured into high quality, high cysteine-containing functional foods for clinical investigation.

Summary
Cysteine-rich proteins, such as keratin, may have advantages over the simple amino acid or its derivatives, as nutraceuticals, to safely and beneficially improve antioxidant status in health and disease.

Keywords
cysteine, glutathione, keratin, oxidative stress

Introduction

Cysteine, one of only two sulphur-containing amino acids making up the 22 proteinogenic amino acids, plays a critical role in cell metabolism. Its unique ability to form interchain and intrachain disulphide bonds, with other cysteine residues, nonenzymatically, also gives it an important role in protein structure and protein folding. A transulphurization pathway converts methionine, via homocysteine to cysteine, by enzymatic action, in the liver, kidney, intestine and pancreas. Many of the enzymes involved in methionine metabolism are increased in activity on ingestion of a high protein diet [1]. The conversion is an irreversible process (Fig. 1) [1], which explains why methionine is classified as an essential or indispensable amino acid. Cysteine, on the contrary, is dispensible – providing adequate methionine is available – but has been recently categorized as 'conditionally essential' in certain pathological conditions associated with inflammation. Cysteine has a sparing effect on methionine metabolism and indirectly increases methionine and its metabolites, markedly reducing the requirements for dietary methionine [2,3].

Cysteine contributes to many biological pathways, notably those involved in glutathione (GSH), taurine and methionine metabolism. Cysteine metabolites play a critical role in antioxidant defenses, which help ameliorate chronic inflammation. However, as we age, levels decrease dramatically [4]. Cysteine is lacking in many diets, and a dietary deficiency has been linked to ageing and various diseases. Cysteine can be generated from methionine via S-adenosylmethionine and homocysteine, but this pathway may be inactive in neonates, patients with liver disease, surgical stress and trauma.

GSH is a tripeptide of glutamate, cysteine and glycine and is one of the most abundant, ubiquitous, intracellular peptides, produced intracellularly in all organs. Quantitatively the most important and abundant antioxidant in humans, plentiful GSH is obtained in the diet from fruits and vegetables, but dietary GSH does not result in increased plasma GSH. The majority must be synthesized, primarily in the liver. Thence around 80% is exported to the plasma and the kidneys for detoxification. GSH synthesis is limited by cysteine availability and activity of the enzyme, glutamate cysteine ligase [5].

Peroxides increase the transulphurization flux that provides some cysteine for GSH synthesis, whereas antioxidants decrease transsulphurization. Boosting GSH synthesis may aid in ageing, seizure, Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anaemia, HIV/AIDS, cancer, stroke, and diabetes [6].

Taurine, the most abundant amino acid *in vivo*, has an intracellular concentration of 25 mmol/l and may also be 'conditionally essential' for human infants. It is synthesized from cysteine in the liver and brain and is required for energy and antioxidant metabolism [7]. A person on a meat-eating diet will ingest between 40 and 400 mg taurine daily but vegetarians receive negligible taurine [8•].

Supplementation with free cysteine and its analogues

Dietary supplementation with GSH or cysteine would be the ideal adjunct to many antioxidant therapies, but orally

Key points

- Cysteine and GSH metabolism is impaired in neonates and the critically ill.
- Enteral nutrition enriched with cysteine can decrease cysteine catabolism and improve GSH status.
- There is a positive association between plasma cysteine and reduced cysteine redox state after ingestion of a diet high in cysteine and methionine.
- Keratin has the highest cysteine content of all natural proteins, and can be processed into a high quality nutraceutical supplement for clinical investigation.

delivered GSH does not produce elevated plasma GSH, possibly due to digestive oxidation and degradation. Likewise, supplementation with taurine can be problematic, with issues of stability, accurate dosage and the important observation that dietary taurine has only a short

Figure 1 Metabolic pathways for cysteine and other sulphur amino acids
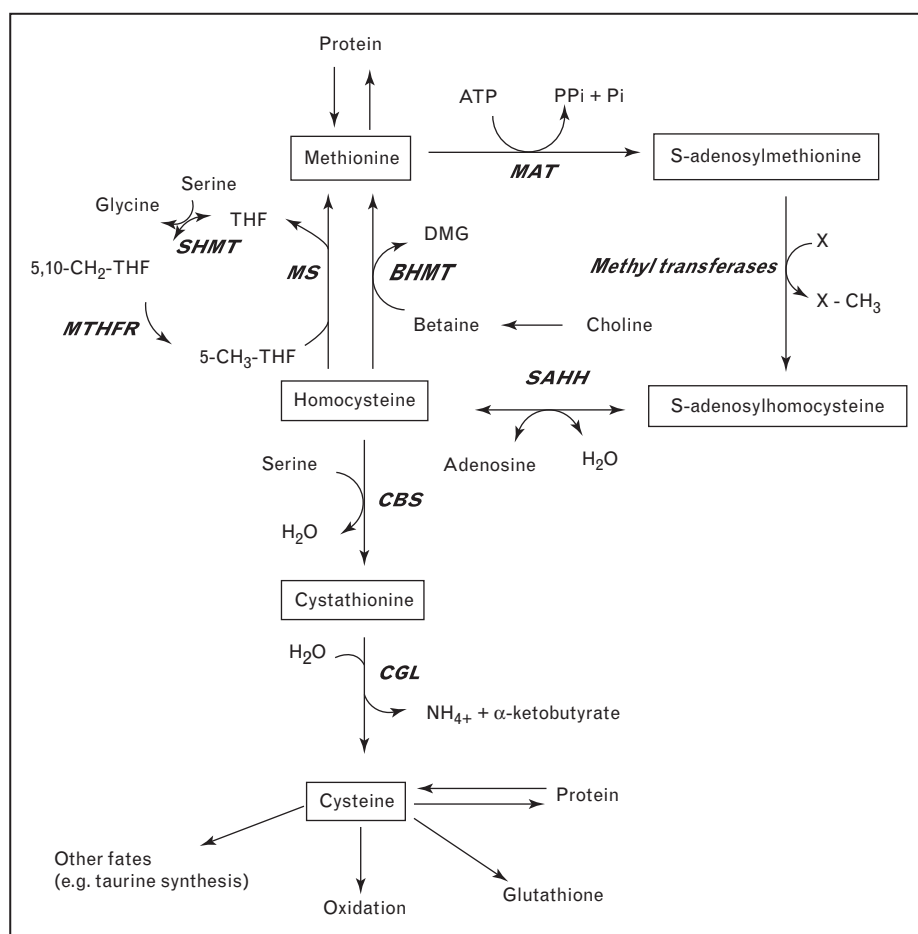
BHMT, betaine:homocysteine methyltransferase; CBS, cystathionine β-synthase; CGL, cystathionine γ-lyase; MAT, methionine adenosyltransferase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; SAHH, S-adenosylhomocysteine hydrolase; SHMT, serine hydroxymethyltransferase. Reproduced with permission from [1].

half life in plasma and may not raise plasma taurine significantly [7,9].

Cysteine itself readily oxidizes to the insoluble cystine dimer. Both free cysteine and cystine are toxic at high levels in the diet [10,11] but dietary cysteine in protein form as well as cysteine derivatives, largely lack this toxicity when included in animal diets [10,12] and can effectively substitute for free cysteine to boost anti-oxidant defences. The derivative N-acetyl-cysteine (NAC) has promising bioactivity *in vitro* and has been trialled with some successes, but thus far, NAC has not lived up to its promise in large-scale controlled clinical trials [13]. There is considerable debate on whether NAC is effective at all for some conditions as well as a growing recognition that there is a subset of individuals who might be at high risk from side-effects; including nausea, rash, wheezing, gastrointestinal problems and other adverse events.

In this review, we highlight current research on dietary cysteine and suggest that cysteine-rich proteins, rather than free cysteine compounds, may be worthy of further study as adjuncts to established therapies and as pre-ventatives against inflammatory diseases.

The redox mechanisms of cysteine and its metabolites

A high level of oxidative stress constitutes one of the main underlying mechanisms contributing to the patho-physiology and clinical features of many acute critical care situations as well as chronic diseases such as AIDS, cancer, inflammation, cardiovascular and neurological diseases. Critical illness increases production of reactive oxygen species (ROS) leading to oxidative stress through activation of the phagocytic cells of the immune system and vascular damage caused by ischaemic reperfusion.

Systemic inflammatory response syndrome (SIRS) is a significant contributor to morbidity and mortality in ICU patients and it is now recognized that oxidative stress, leading to a strong and persistent inflammatory response, constitutes a serious factor for development of multiple organ failure (MOF) [14]. However, there is an elaborate defence system, involving antioxidants such as GSH, operating to protect cells from oxidative stress. These antioxidants quench ROS, delay oxidation of substrates, and can have beneficial effects on infectious complication rates and incidence of MOF.

Cysteine and glutathione in disease states

Intracellular GSH can be influenced by the availability of exogenous GSH precursors. The body has the capacity to synthesize GSH from cysteine, methionine, glutamate and glycine from foods. However, in the early stages of fasting and in metabolic stress, supplies of glutamine, cysteine and methionine are interrupted or reduced [15]. Consequently, GSH depletion is associated with severity of disease, increased morbidity and mortality. Critically ill patients with MOF and/or chronic obstruc-tive pulmonary disease have depleted GSH, with higher plasma cysteine levels than in whole blood, indicating a low intracellular concentration. During injury and trauma, ICU patients exhibit low GSH status and decreased muscle protein synthesis, suggesting that there might be an increased requirement for substrates such as cysteine.

In stress, muscle is known to serve as an amino acid reservoir, delivering substrates for anabolic reactions. Consequently, patients receiving enteral nutrition enriched with cysteine, appear to exhibit decreased cysteine catabolism and increased cysteine utilization, reflected by an improved sulphur balance due to increased GSH synthesis. Oral cysteine supplementation at 11 g/kg in septic rats maintains blood GSH status, improves fractional muscle protein synthesis rates and improves recovery [16]. This contrasts with NAC supple-mentation in HIV/AIDS, in which cysteine and GSH synthesis rates in erythrocytes are normalized, suggesting a different pathogenesis.

Bowel disease

Sulphur amino acid supplementation reduces the ileal and jejunal, but not the colonic GSH/the oxidized dimeric form of GSH redox state in resected (mid-jejuno-ileal) but not control transected (small bowel) rats [17••]. There was a reduction in cysteine redox state in resected, but not control rats, which was accompanied by an increase in growth rate in ileal and partially in jejunal, but not in colonic crypt, indicating that different parts of the intestine respond differently to dietary sulphur amino acids.

There is a distinct tissue-specific pattern of cysteine metabolic enzymes, recently reviewed [18••,19]. The presence or absence of cysteine metabolic enzymes in different tissues plays a major part in determining tissue and subsequent plasma cysteine levels in response to dietary supplementation. For example, colon tissue has a low amount of cysteine dioxygenase (CDO) but relatively high amounts of desulfuration enzymes. Stipanuk and Ueki are currently engaged in further studies of CDO regulation, the results of which are eagerly awaited.

Cardiovascular disease

Oxidation of cysteine and GSH and the associated improvement of both the cysteine and GSH redox states are correlated with markers of cardiovascular disease [20••]. A diminished cysteine redox state is accompanied

by cellular signalling events that are anti-inflammatory; conversely, increasing the cysteine redox state increases proinflammatory pathways. For example, the external cysteine redox state can directly regulate monocyte adhesion to aortic endothelial cells and mitochondrial, but not nuclear or cytoplasmic oxidation. These changes are at least partially mediated by changes in plasma membrane thiols and mitochondrial thioredoxin [21••]. This implies that reducing the plasma cysteine redox state might be beneficial either as a preventive or treatment adjunct for cardiovascular disease. Some confirmation of this hypothesis is provided in an elegant study of human dietary supplementation and plasma redox states [22••]. When healthy humans ingested a diet relatively high in cysteine and methionine (up to 117 mg/kg/day), there was a positive association between plasma cysteine concentration and cysteine redox state. However, there was no effect on plasma GSH or GSH redox state during these studies.

In monocyte cultures, improving the cysteine redox state increases proinflammatory interleukin (IL)-1 β expression [23••]. In the same study, dietary cysteine and methionine decreased the plasma cysteine in mice, but not the GSH redox state, while reducing plasma and lung IL-1 β expression in response to a proinflammatory challenge. In human participants there were correlations between (i) plasma IL-1 β and increased cysteine redox state, (ii) TNFα and plasma redox state increase and (iii) plasma cysteine and IL-1 β decrease [23••]. These data further confirm the link between cysteine supplementation and the resultant cysteine redox state and anti-inflammatory activity.

Liver disease

Men are more susceptible than women to liver damage. In a study of sex differences, female mice had slightly higher levels of GSH metabolism enzymes. The authors hypothesize that increased levels of GSH enzymes are at least partly responsible for increased resistance of female mice to liver injury [24•].

NAC improved markers of liver health during treatment of bile duct obstruction prior to endoscopic retrograde cholangiopancreatography [25]. The authors attributed this effect to the mucolytic action of NAC, which might reduce the viscosity of bile. In a mouse model of liver cirrhosis, oral NAC increased survival and restored cytosolic and mitochondrial GSH [26]. This recovery was accompanied by improvements in several markers of liver health. The mouse model was a previously developed liver-specific knockout of a GSH synthesis enzyme [27] so GSH must have either been synthesized outside of the liver tissue in response to NAC, or synthesized locally through an alternate pathway.

Progression of nonalcoholic fatty liver disease is worsened by antioxidant depletion. In a GSH-deficient knockout mouse model, in which liver GSH is 15% of normal, the mice appeared to adapt to decreased GSH [28•]. A consequence of this adaption was that knockout mice, fed a methionine and choline-deficient diet (MCD) that would induce liver disease in genetically normal animals, were protected against liver disease by the MCD diet. Adaptation to low GSH in the knockout mice was indicated by substantial changes in gene expression of metabolic pathway enzymes.

Paracetamol (acetaminophen), one of the most frequently used drugs, is detoxified by cysteine and its metabolites. Paracetamol intake causes oxidation of cysteine, but has no effect on the GSH redox state, regardless of dietary sulphur amino acid intake levels [29]. Pujos-Guillot and colleagues reasoned that long-term paracetamol usage, particularly in the elderly for arthritic pain, might increase the requirement for cysteine [30]. They found that older persons responded to long-term paracetamol by increasing their dietary protein intake substantially and as a consequence there was no depletion of cysteine and its metabolites.

S-allyl-cysteine, a component of garlic extract, is capable of reducing diabetic-induced glycoproteins in rat liver and kidney [31]. At the same time, blood and urine sugars are partially controlled. In this context it is possible that high cysteine protein is just as effective as NAC in negating the effects of a high sugar diet [32].

Cancer

The General Population Nutrition Trial, conducted in Linxian, China, has confirmed the association between serum cysteine and risk of some cancers. In that study, a higher serum cysteine quartile was associated with reduced risk of both gastric and oesophageal cancers [33••]. This relationship was even stronger in people aged over 60 years. It is worth noting this trial finished around 1991, but thanks to wise planning, the stored samples were analysed for cysteine using 'modern' methods.

Supplementation with cysteine and its analogues

A detailed study of the fate of dietary and arterial cysteine in minipigs showed, for the first time quantitatively, that net cysteine flux accounts for only 60% of dietary cysteine, suggesting further sequestration of 40% of cysteine in the intestine [34••]. Importantly, the portal drained viscera (PDV) released an additional 15–25% of nondietary cysteine, originating either from tissue breakdown, methionine metabolism or from reabsorption of

cysteine from endogenous secretions of biliary GSH. Thus, in a stress and inflammation model, sulphur amino acid metabolism adapts to cover the increased requirements of cysteine, demanded by the need for increased GSH synthesis. These data may partly explain the reason for inefficient oral cysteine availability, suggesting that, during supplementation, the colon, stomach, pancreas and spleen (PDV) could preferentially use circulating cysteine and methionine-containing peptides over dietary cysteine to synthesize more GSH. This raises the possibility that dietary cysteine contained within peptides and proteins will be more effective in boosting cysteine metabolism in SIRS and other inflammatory states than free dietary cysteine.

Clinical nutrition

The use of cysteine in parenteral nutrition has been recently reviewed by Yarandi et al. [35[•]] who noted the absence of convincing clinical evidence for benefits of sulphur amino acids in parenteral nutrition. In neonates the benefits of sulphur amino acids in parenteral nutrition mixtures is even less clear, especially given the well known low solubility and instability of free cysteine in aqueous solution. Early studies showed that neonates under stress lack enough cysteine to synthesize sufficient GSH [36]. In a small study of five sick infants, Courtney-Martin et al. [37[•]] studied whether added methionine could replace cysteine and boost GSH synthesis. They observed GSH was synthesized in the presence of parenteral methionine only. When cysteine was added as well as methionine, there was no increase in erythrocyte GSH and there was no significant difference in plasma cysteine. Clearly, there is a need to boost cysteine metabolism in parenteral nutrition patients using new strategies, but studies in neonates are challenging and more research is needed in this important area.

Naturally occurring cysteine-rich proteins

The most natural, and therefore, one might argue the best source of cysteine is dietary protein, in which it is present as the dimer, cystine, including linked sulphur–sulphur bonds. These disulphide bonds can be readily cleaved *in vivo*, or by heat or mechanical stress in the laboratory to liberate the monomer, cysteine. However, there is a dearth of published data on the nutritional value of high cysteine proteins.

Defatted egg protein, a byproduct of lecithin production, is digested with an enzyme mixture and then solid matter is centrifuged out to make egg yolk peptides (EYP). The protein has antioxidant activity and reduces peroxide-induced secretion of IL-8, a proinflammatory cytokine [38]. But there was no EYP rescue effect on the reduction of cell proliferation induced by peroxide. In a pig model,

GSH was induced in response to both peroxide and EYP/peroxide compared with isotonic saline infusion. GSH was induced in both the duodenum and jejunem, but not the ileum or colon. Erythrocyte GSH was induced by both peroxide and EYP/peroxide infusion, with the effect being more marked in the EYP/peroxide group. EYP was capable of decreasing the degree of peroxide-induced oxidant markers in pigs. Taken collectively, the data suggest that rather than acting as a high cysteine protein, EYP functions as an antioxidant protein.

Milk proteins, in particular cysteine-rich whey protein, are known for their ability to raise GSH. Although there is a positive relationship between milk consumption and growth [39], there can be negative impacts [40]. Allergies to cow's milk do develop but frequently disappear by adulthood. Allergies to whey proteins occur in children but they can be mostly ameliorated by hydrolysation [41]. Lactose intolerance, real or perceived, affects a substantial segment of Western populations [42] and an even larger proportion of the rest of the world [43]. Consumption of whey protein may also contribute to teen acne as a consequence of its high insulinotropic activity [44].

SelenoCysteine and SelenoMethionine [45] are abundant in eggs, and various other natural protein fractions are rich in selenium and cysteine [46]. The many selenoproteins are capable of modulating redox signalling, including cysteine and GSH redox states [47[•]]. Dietary selenium can modulate selenoprotein redox activity, so it makes sense that dietary selenoproteins might also modulate general redox states. Selenoproteins are essential for keratinocyte function and skin resistance to oxidative damage [48[•]].

Plant proteins tend to be deficient in sulphur amino acids. With aims towards improving both animal feed and human dietary applications, sulphur proteins have been expressed in plants. A recent study in sulphur protein-expressing soybean raises some doubts about current approaches in this area, as the new bean varieties have allergic potential, and may limit animal growth [49]. Therefore, the sulphur-containing proteins and plants selected for insertion need to be reconsidered, or alternatives for cysteine supplementation must be found.

Keratins are cysteine-rich proteins abundant in feather, skin, horn, nail, hair and wool. Hydrolysation by enzymatic or chemical means is required to achieve digestibility. Feather keratins have been trialled extensively as animal feed supplements and have the highest cysteine of the major food proteins [50]. Occurence of keratin in the human diet is widespread. In the USA, keratin-containing nutritional supplements have been available for over 50 years and it is not considered a new dietary ingredient. Recorded use in Europe dates back to 1911, as

evidenced by a monograph for keratin in the British Pharmaceutical Codex for tablet coating. Acute and chronic toxicity studies in a range of animal species have demonstrated no effect on LD50 and the common biochemical markers of toxicity, suggesting keratin is safe, which is not surprising given the widespread distribution of keratin in the human diet [51–53]. Keratin is present in all animal cells, being a principal component of the cytoplasm. It can be isolated as a pure protein powder, soluble in water above pH 4, with a relatively small variation in amino acid content compared with proteins from other sources and may contain bioactive sequences that are yet to be discovered [54••]. However whether any new studies may demonstrate allergenicity in a subset of individuals remains to be seen. Its potential as a nutraceutical or functional food component is currently under extensive investigation.

Conclusion

The sulphur amino acid, L-cysteine has a critical role in methionine, taurine and GSH metabolism. Oral or enteral supplementation with diets enriched with cysteine can lead to increased cysteine utilization and improved antioxidant status in various inflammatory conditions, but the simple amino acid and its derivatives, such as N-acetylcysteine, have limited practical applications in clinical nutrition because of stability issues and potential adverse reactions.

Cysteine-rich proteins, such as keratin, are abundant, and if processed correctly should result in high quality and demonstrably well tolerated nutraceuticals for use in a variety of clinical applications. Further basic research and clinical studies to elucidate the posology, mechanisms of action and potential clinical benefits are now necessary.

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