

## Review Article

# Role of sulfur amino acids in controlling nutrient metabolism and cell functions: implications for nutrition

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Protein synthesis is affected when an insufficient level of sulfur amino acids is available. This defect may originate from dietary amino acid deficiency and/or excessive amino acid utilisation for other purposes such as the synthesis of glutathione and acute-phase proteins during catabolic stress. Sulfur amino acids are recognised to exert other significant functions since they are precursors of essential molecules, are involved in the methylation process, participate in the control of oxidative status, and may act as mediators affecting metabolism and cell functions. Despite this increased understanding of the role of sulfur amino acids, many questions still remain unanswered due to the complexity of the mechanisms involved. Moreover, surprising effects of dietary sulfur amino acids have been reported, with the development of disorders in cases of both deficiency and excess. These findings indicate the importance of defining adequate levels of intake and providing a rationale for nutritional advice. The aim of the present review is to provide an overview on the roles of sulfur amino acids as regulators of nutrient metabolism and cell functions, with emphasis placed on the implications for nutrition.

### Methionine: Cysteine: Nutrient metabolism: Oxidative stress: Methylation process: Gene expression

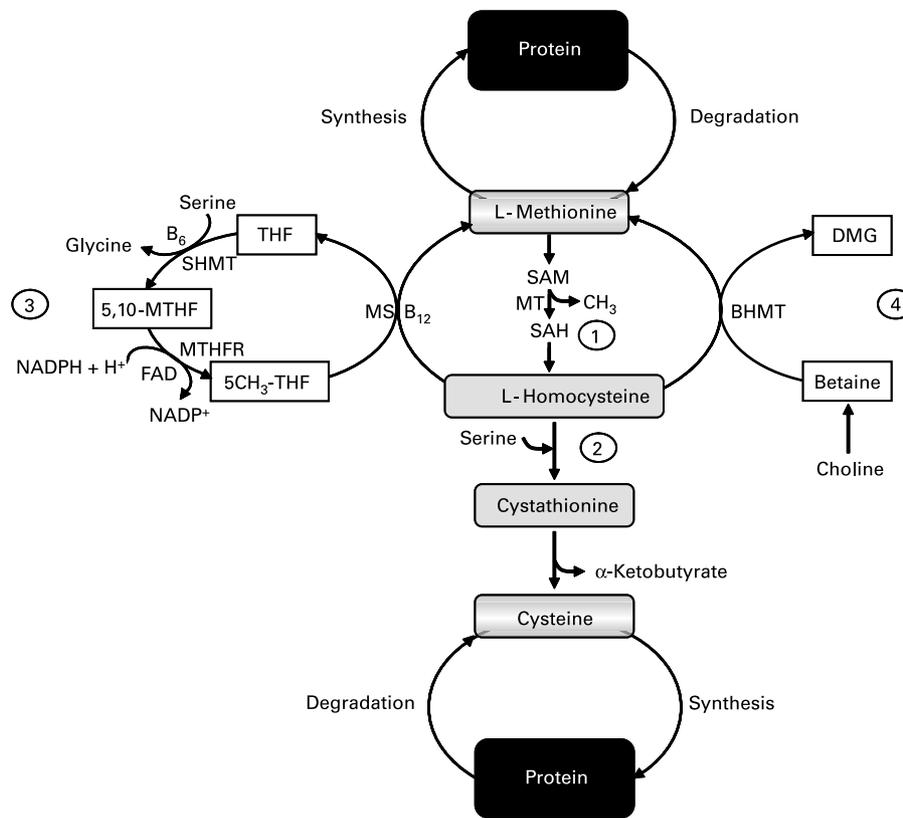
Like other amino acids, sulfur amino acids affect protein metabolism. They are the components of tissue proteins and, when provided at insufficient levels, they lead to reduced protein synthesis. Methionine is, for example, the first limiting factor in classical diets used for growing chickens because of the high requirement of sulfur amino acids for the synthesis of feathers, whereas poultry diets based on maize and soya-bean meal without supplementation are deficient in sulfur amino acids<sup>(1)</sup>. A positive effect of methionine supplementation on muscle growth has been reported in this species, since the addition of methionine to a methionine-deficient diet that is otherwise balanced in terms of other amino acids increases the accretion and synthesis of protein in skeletal muscles. Like methionine, cysteine is used for protein synthesis. Cysteine can be produced through the metabolism of methionine (Fig. 1) and can thus be considered as a non-essential amino acid. It can become conditionally indispensable in particular situations such as stress conditions or inflammatory states. In such situations, cysteine may be used for acute-phase proteins but above all for the synthesis of glutathione, i.e. the most important intracellular antioxidant of the body. This results in an increased cysteine requirement, with a demand for cysteine thus exceeding the body's capacity for cysteine production<sup>(2–4)</sup>. Although insufficient intake of sulfur amino

acids is clearly detrimental, negative effects of excess methionine or cysteine have also been reported<sup>(5–7)</sup>. It is therefore important to obtain a deeper understanding of the interaction between sulfur amino acid metabolism and nutritional demand, especially *in vivo*.

Optimising sulfur amino acid nutrition involves considering the different roles of these amino acids, which are recognised to exert several significant influences. They are precursors of essential molecules and act as mediators affecting metabolism and cell functions. For example, cysteine is required for the synthesis of glutathione and taurine, which play a crucial role in oxidative stress conditions since they have the capacity to affect cellular redox status. Some mechanisms through which sulfur amino acids control oxidative status, and potentially amino acid signalling, have been detailed in a recent review<sup>(8)</sup> and they will be not presented here. In the present review, we first address the implications of the roles related to oxidative stress and cell function for nutrition recommendations. Since sulfur amino acids may also be involved in the regulation of gene transcription and participate in methyl group metabolism, we then provide an overview of the roles of sulfur amino acids in controlling gene expression and methylation processes, focusing particularly on the consequences for amino acid nutrition.

**Abbreviations:** ATF4, activating transcription factor 4; CHOP, CCAAT/enhancer binding protein homologous protein; CpG, cytosine-guanine dinucleotide.

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**Fig. 1.** Methionine–cysteine metabolic pathways. (1), Transmethylation; (2), trans-sulfuration; (3), folate-dependent remethylation; (4), folate-independent remethylation; B<sub>6</sub>, vitamin B<sub>6</sub>; B<sub>12</sub>, vitamin B<sub>12</sub>; BHMT, betaine homocysteine methyltransferase; 5-CH<sub>3</sub>THF, 5-methyltetrahydrofolate; DMG, dimethylglycine; MS, methionine synthase; MT, methyl transferase; 5,10-MTHF, 5,10-methyl tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

### Revisiting recommendations for methionine and cysteine in the light of their effects on oxidative stress and cell function

Sulfur amino acids participate in the control of oxidative status since they are involved in the synthesis of intracellular antioxidants, particularly glutathione<sup>(4,8,9)</sup>, and in the methionine sulfoxide reductase antioxidant system<sup>(8,10,11)</sup>. Using cysteine derivatives (i.e. *N*-acetylcysteine) or cysteine-rich dietary proteins has been shown to increase blood and/or intracellular glutathione, which may affect the thiol redox status<sup>(6,12–14)</sup>. Interestingly, dietary cysteine may also improve glucose control and alleviate sucrose-induced insulin resistance<sup>(15–17)</sup>. Moreover, several studies have reported a beneficial effect of cysteine supplementation at nutritional doses in the prevention of post-exercise oxidative stress in human subjects<sup>(18–21)</sup>. There is also evidence that the cysteine/cystine redox couple acts as an extracellular thiol redox buffer (i.e. it represents the major plasma redox pool) and a modulator of cell function. *In vitro* studies have indicated that the extracellular cysteine/cystine redox potential affects proliferation and the p44/p42 mitogen-activated protein kinase pathway via a transforming growth factor  $\alpha$ -dependent mechanism in enterocytes<sup>(22,23)</sup>, the production of reactive oxygen species and expression of cell–cell adhesion molecules in endothelial cells<sup>(24)</sup>, and modulates reactive oxygen species-induced apoptosis in retinal pigment epithelial cells<sup>(25)</sup> and Burkitt's lymphoma cells<sup>(26)</sup>. In these studies, the effects of the cysteine/

cystine redox potential as a thiol/disulfide redox couple were independent of intracellular glutathione status, suggesting a specific role of the extracellular redox environment in cell function<sup>(27)</sup>.

The cysteine/cystine redox potential in plasma has recently been shown to vary during the day, with a pattern influenced by meals which may reflect acute changes in plasma thiol redox state with dietary intake of sulfur amino acids<sup>(28)</sup>. Interestingly, it has been found in rats that the cysteine/cystine redox potential was affected by both decrease and increase in the sulfur amino acid (L-methionine + L-cystine) content of the diet, in contrast to glutathione redox potential<sup>(29)</sup>. Taken together, these studies provide new insights into the roles of cysteine and dietary sulfur amino acids in controlling cell function through modulation of the extracellular redox environment. These findings also highlight the complexity of the underlying mechanisms and do not support any systematic benefit from supplementing sulfur amino acids. In agreement with this assumption, decreasing methionine ingestion could be recommended in some situations. For example, due to the role of the methionine sulfoxide reductase antioxidant system, lowering dietary methionine levels appears to decrease the sensitivity of proteins to oxidative damage, oxidative stress and reactive oxygen species production<sup>(30,31)</sup>. Possible dietary implications for humans were discussed in a recent review<sup>(32)</sup>. Since the intake of proteins, and therefore methionine, is higher than required in humans in developed countries, the authors suggest that decreasing excessive

consumption could be an efficient way to reduce tissue oxidative stress and improve healthy life span.

Sulfur amino acids, particularly cysteine, may also act on cell function as precursors of hydrogen sulfide (H<sub>2</sub>S)<sup>(33)</sup>. First considered as a toxic gas, H<sub>2</sub>S is now recognised for its physiological function as the third (with NO and CO) gaseous transmitter in the body. In the brain, H<sub>2</sub>S may act as a neuromodulator and protect neurons from oxidative stress<sup>(34–38)</sup>. H<sub>2</sub>S has been shown to directly activate K<sub>ATP</sub> channels in vascular smooth muscle cells and insulin-secreting cells<sup>(39–41)</sup>, thus promoting vasorelaxation and inhibiting insulin secretion<sup>(39–45)</sup>. Although little is known regarding the production of endogenous H<sub>2</sub>S in response to changes in cysteine concentrations, it is important to consider the possibility that sulfur amino acids might affect cell function through modulation of H<sub>2</sub>S and not to ignore the possible consequences in terms of nutrition. Similarly, it is necessary to consider other products of sulfur amino acid metabolism such as sulfates. Sulfate is the predominant endproduct of sulfur amino acid catabolism, and a relationship has been reported between sulfate excretion and sulfur amino acid intake<sup>(46,47)</sup>. Sulfates contribute to glycosaminoglycan synthesis and detoxification processes through reactions depending on 3'-phosphoadenosine-5'-phosphosulfate, and these sulfation reactions account for 27% of inorganic sulfate turnover in humans<sup>(48)</sup>. In rats, ingesting a sulfur-deficient diet decreases plasma sulfate and elimination of the analgesic acetaminophen, with concomitant increase in its toxication<sup>(49)</sup>, highlighting the importance of adequate methionine and/or cysteine intake for detoxification processes. Nevertheless, sulfates have long been thought to increase urinary excretion of Ca<sup>(50,51)</sup>; a recent study suggests that sulfur amino acids counterbalance the positive association between protein intake and bone mineral density<sup>(52)</sup>. These positive and negative effects of sulfates should be taken into account when establishing recommendations for sulfur amino acid intake.

### Potential function of sulfur amino acids in controlling gene expression

Amino acids are recognised to act as modulators of signal transduction pathways that control gene transcription and translation<sup>(53–55)</sup>. Some *in vitro* experiments performed in mammals<sup>(56,57)</sup> and avian species<sup>(58)</sup> have suggested that methionine may have such a signal function by inducing the activation of an intracellular kinase involved in the control of mRNA translation, i.e. the p70 S6 kinase (p70S6K, also called S6K1). In addition, amino acid availability regulates the mammalian general control non-depressible 2/eukaryotic initiation factor 2 $\alpha$  (eIF2 $\alpha$ ) pathway that is thought to be affected by tRNA deacylation in the case of amino acid depletion<sup>(59–61)</sup>. By influencing the phosphorylation of eIF2 $\alpha$ , this pathway affects (1) the first step in the initiation of mRNA translation through the formation of eIF2-GTP, and (2) amino acid-controlled gene expression through the induction of activating transcription factor 4 (ATF4). Indeed, there is evidence that ATF4 can bind to the amino acid response element in the promoters of genes such as CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) and asparagine synthetase, thereby up-regulating their expression<sup>(54,62)</sup>. In relation to the present review, CHOP

and asparagine synthetase are over-expressed when cells are deprived of a particular amino acid, for instance leucine, lysine, phenylalanine and methionine<sup>(63,64)</sup>. Different mechanisms are probably involved in such regulation since CHOP expression is strongly induced in response to methionine deprivation but only slightly affected by cysteine, asparagine or histidine deprivation, whereas asparagine synthetase expression is induced equally whatever the amino acid depleted<sup>(54,63)</sup>. Cysteine restriction may induce the expression of several genes via the mammalian general control non-depressible 2 protein kinase/ATF4-dependent integrated stress response pathway to cope with oxidative and chemical stresses<sup>(65)</sup>. It has also been reported that homocysteine (a metabolite of methionine) increases the expression of vascular endothelial growth factor by a mechanism involving ATF4<sup>(66)</sup>. It is of note that CHOP can interfere with C/EBP  $\alpha/\beta$  expression and function, thus regulating adipogenesis, which could provide a mechanism through which amino acids have an effect on the pathogenesis of insulin resistance, as proposed by Tremblay *et al.*<sup>(61)</sup>. Moreover, methionine and cysteine are potent inhibitors of insulin-stimulated glucose transport in muscle cells<sup>(67)</sup>. These results have been reported in non-physiological conditions, and the importance of a p70 S6 kinase-dependent regulatory loop in the development and/or the maintenance of insulin resistance remains to be determined<sup>(68)</sup>. More information is therefore needed on the potential adverse effects of sulfur amino acid supplementation before use for nutritional purposes.

Many other examples demonstrate that sulfur amino acids are able to regulate the expression of numerous genes, thereby controlling nutrient metabolism and cell functions. Methionine supply has been shown (1) to affect growth hormone-induced insulin-like growth factor I gene expression in ovine hepatocytes<sup>(57)</sup> and (2) to regulate the expression of an important gene controlling ubiquitin-proteasome-dependent proteolysis (i.e. E3 ubiquitin ligase atrogin-1) in avian fibroblasts<sup>(69)</sup>. Cysteine and cysteine derivatives (for example, *N*-acetylcysteine, or *N*-acetylcysteine amide) can modulate the activity of NF- $\kappa$ B<sup>(70–73)</sup>, which induces the expression of many genes that are involved in cell survival and proliferation, and in the regulation of immune and inflammatory responses (for reviews, see Barnes & Karin<sup>(74)</sup>, Chen *et al.*<sup>(75)</sup>, Grimbler<sup>(76)</sup> and Wek *et al.*<sup>(60)</sup>). The activation pathways of this transcription factor involve the thiol redox status that responds to antioxidants including cysteine (see the section 'Revisiting recommendations for methionine and cysteine in the light of their effects on oxidative stress and cell function' above). Despite the clear theoretical importance of sulfur amino acids in immune function, studies directly linking methionine and cysteine intake and immune function are still needed<sup>(76)</sup>. Sulfur amino acids are also recognised to control genes involved in lipid metabolism, including cholesterol 7 $\alpha$ -hydroxylase and apoA-I<sup>(77)</sup>, which could be in favour of sulfur amino acid supplementation in cases of abnormal lipid metabolism. Nevertheless, high methionine intake can promote the development of atheromatous disorder<sup>(78)</sup>, indicating that any such supplementation has to be undertaken with care to prevent atherosclerosis. The underlying mechanisms are still unclear. Methionine is the precursor of homocysteine (Fig. 1) and elevated levels of plasma homocysteine (hyperhomocysteinaemia) have been associated with occlusive

vascular disease<sup>(79)</sup>. However, Troen *et al.*<sup>(78)</sup> have shown in mice that the atherogenic effect of methionine exists even in the absence of hyperhomocysteinaemia. High plasma homocysteine levels appear not to be independently atherogenic as recently reported<sup>(80,81)</sup>. Vitamin B supplementation (its effect on methionine metabolism is discussed in the section 'Role of sulfur amino acids in the methylation processes' below) does not prevent CVD in humans despite lowering homocysteine<sup>(82,83)</sup>, similarly challenging the role of homocysteine as an independent cardiovascular risk factor.

### Role of sulfur amino acids in the methylation processes

In addition to the above roles, sulfur amino acids have another major function since methionine is a source of the methyl groups needed for all biological methylation reactions, including methylation of DNA and histones, a process that influences chromatin structure and gene expression<sup>(84–89)</sup>. DNA methylation generally occurs at cytosines within cytosine-guanine dinucleotides (CpG). The CpG sites are dispersed throughout the genome, with specific CpG-rich regions called CpG islands. The methylation status of cytosine residues within CpG dinucleotides and in the context of CpG islands determines which genes are active or not, and plays an important role in maintaining gene silencing that is needed for tissue- and development-specific gene expression, epigenetic mechanisms involved in the establishment and maintenance of gene expression patterns, and genomic imprinting.

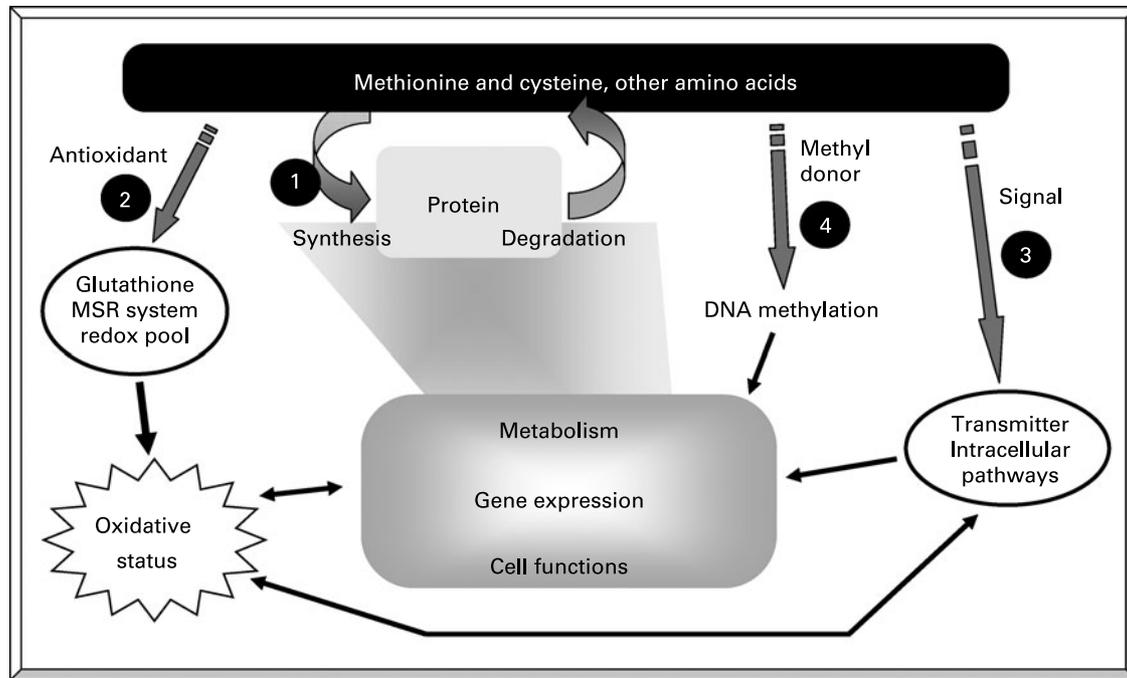
Being converted to *S*-adenosylmethionine, the major biological methylating agent, methionine provides the methyl groups that are needed for cellular methylation reactions. However, in order to adapt dietary methionine intake effectively, it is important to keep in mind the specific features of the methyl metabolism that is more generally involved in methylation regulation. *S*-adenosylmethionine is converted to *S*-adenosylhomocysteine following methyl donation, and to homocysteine (reactions of transmethylation; Fig. 1). Homocysteine can be remethylated into methionine through two separate pathways: (1) the folate-dependent remethylation pathway that involves 5,10-methyl tetrahydrofolate; (2) the folate-independent remethylation pathway in which the methyl group is provided by betaine. Disorders of methyl balance lead to several diseases (liver disease, CVD, closure of the neural tube, synthesis of creatine, cancer), illustrating the key role played by methylation reactions<sup>(90,91)</sup>. Whether DNA methylation is increased by high levels of methionine is still unclear, since, for example, excess methionine may impair DNA methylation by inhibiting remethylation of homocysteine<sup>(92)</sup>. These findings need further investigation to quantify their physiological relevance, particularly using more physiological amounts of methionine. In addition, based on the methionine cycle, nutritional factors affecting the supply of *S*-adenosylmethionine and/or removal of homocysteine (for example, dietary choline, betaine, folic acid and vitamin B<sub>12</sub>) potentially affect DNA methylation and must therefore be taken into account when assessing the supply of methionine. Mathematical models have been developed to consider the complexity of methionine metabolic pathways, and they will be useful to complement and help guide laboratory studies<sup>(93,94)</sup>.

Recent *in vivo* studies have demonstrated opposite diet-related changes in DNA methylation according to the tissue studied: long-term administration of a folate/methyl-deficient diet in rats results in progressive hypomethylation of DNA in the liver<sup>(95)</sup>, but an increase in DNA methylation in the brain<sup>(96)</sup>. Although the underlying mechanisms and significance of these findings are not understood, insufficient supply of the nutrients involved in methyl metabolism clearly affects methylation reactions and concomitantly alters patterns of gene expression<sup>(96)</sup>. This function of methyl group donors represents another mechanism through which sulfur amino acids can regulate gene expression (see section 'Potential function of sulfur amino acids in controlling gene expression' above) with potentially both negative and positive consequences, for example, either unsuitable dysregulation of gene expression or conversely restoration of appropriate locus-specific DNA methylation using therapeutic pro-methylation diets<sup>(89)</sup>. Epigenetic regulation thus plays an important role in the potential development (or in contrast prevention) of cancer, CVD, type 2 diabetes and obesity. For example, the availability of methyl group donors regulates the expression of genes involved in the development of the digestive tract through a mechanism dependent on DNA methylation, at least for the homeobox gene CDX1, which could explain the protective role attributed to folates in colon cancer<sup>(97)</sup>.

An increasing number of reviews have highlighted the importance of perinatal nutrition (i.e. nutrition in early life), since aberrant methyl metabolism *in utero* is linked with certain disorders (for reviews, see Ulrey *et al.*<sup>(85)</sup>, Waterland<sup>(89)</sup>, Rees *et al.*<sup>(98)</sup>, Waterland & Michels<sup>(87)</sup> and Nafee *et al.*<sup>(88)</sup>). For example, a murine metastable epiallele (axin fused, Axin<sup>Fu</sup>) exhibits epigenetic plasticity to the maternal diet, since supplementation with methyl group donors before and during pregnancy increases offspring DNA methylation at Axin<sup>Fu</sup><sup>(99)</sup>. As discussed by some authors<sup>(89,98,100)</sup>, manipulating the sulfur amino acid content of the early diet may induce chronic changes in cell functions that have implications for long-term health. More recent findings have provided evidence that changes in the supply of methionine and specific B vitamins such as B<sub>12</sub> during the periconception period can lead to widespread epigenetic changes in DNA methylation in offspring, and modify adult health-related phenotypes<sup>(101)</sup>. For instance, adult offspring exhibit changes in body composition (fatness), immune function and insulin response. Interestingly, these effects were obtained with modest early dietary intervention (changes within physiological ranges), indicating the importance of adequate dietary methyl group supply for metabolic programming and the need for further studies to define the level of intake. Such studies will certainly provide a rationale for nutritional advice in the future.

### Conclusion

Sulfur amino acids have a very significant place among amino acids due to their numerous roles. They are precursors of major components such as glutathione, taurine, H<sub>2</sub>S and sulfates, and thus act on oxidative status and various signalling pathways. In addition, methionine plays a unique role in epigenetic regulation by affecting DNA methylation. Sulfur amino acids consequently affect metabolism, gene expression



**Fig. 2.** Regulation of metabolism and cell functions, and possible roles of sulfur amino acids: (1) precursors of protein synthesis; (2) antioxidant function; (3) nutrient signal; (4) methyl group donor. The figure represents a summary of the findings discussed in the present review. MSR, methionine sulfoxide reductase.

and cell functions, as illustrated in Fig. 2, and a change in their dietary levels may have either beneficial or deleterious consequences. An increasing number of studies have been devoted to exploring the key roles of sulfur amino acids, but further experiments are still necessary to obtain direct evidence regarding some of their effects on nutrient metabolism, cell functions and more generally physiological responses in animals and humans. Understanding of the mechanisms of amino acid actions is essential to optimise dietary amino acid requirements whatever the conditions, whether physiological (pregnancy, early nutrition) or physiopathological (disease, ageing). In particular, the effects of sulfur amino acids should be analysed under circumstances affecting oxidative status and gene expression. Recommendations for methionine and cysteine intake have to be revisited in the light of their roles and the intricacies of sulfur amino acid metabolism. The availability of other dietary nutrients (for example, cysteine, vitamins) has of course to be taken into account when adjusting the supply of methionine, for instance. However, a major challenge for future experiments is to improve the integration of the various roles of sulfur amino acids at the whole-body level for rational use in nutrition.

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