Oxidative stress is related to several pathologies and it is also associated with surgical operations. Reactive oxygen species generated during oxidative stress can induce severe damage to biomolecules. To prevent this damage, cells are endowed with both enzymatic and nonenzymatic defenses. One of the most important antioxidant molecules is glutathione. Since glutamine is a precursor of glutathione, its supplementation in the clinical diet can be used to maintain high levels of glutathione and to avoid oxidative stress damage. Here, recent literature concerning this recurrent topic is critically reviewed.

**Key Words:** oxidative stress; glutamine; glutathione; total parenteral nutrition; enteral nutrition; ischemia/reperfusion.

The amino acid glutamine is usually described as a nonessential nutrient. However, the capacity to synthesize glutamine can be insufficient in some situations. In the last few years, interest in the possible benefits of glutamine supplementation in clinical nutrition is again increasing, mostly due to its currently recognized role in the prevention of oxidative stress. Up to now, one of the most important findings associated with the administration of glutamine-supplemented nutrition in the clinic has been the reduction in rates of infection. Here we review the recent scientific literature related to the role of glutamine, as a precursor of glutathione, in the prevention of medically or surgically relevant oxidative stress.

**MEDICAL INTEREST OF OXIDATIVE STRESS**

Human beings, as aerobic organisms, require oxygen for life. However, oxygen should be considered, at the same time, as a potentially toxic compound, due to its potential in rendering free radicals. In fact, oxidative stress is tightly related to aging. The term oxygen free radical (OFR) includes the superoxide anion free radical ($O_2^{-}$), the hydroxyl radical ($HO$), and lipid and other peroxyl radicals. OFR are part of the group of molecules called reactive oxygen species (ROS), all of them more strongly oxidizing than triplet molecular oxygen. Besides OFR, ROS include singlet oxygen, hydrogen peroxide, lipid peroxide, hypochlorous acid, and other N-chloramine compounds (1).

Although the mitochondrial electron transport chain has a high potential for the generation of ROS, this is not the only metabolic process which can contribute to generate them. In fact, ROS are generated in cells by both enzymatic and nonenzymatic reactions (2).

Oxidative stress has been related to several pathologies, including cancer, cardiovascular diseases, cataract, inflammation, and neurological disorders (3,4). Furthermore, in certain pathological situations, the triple cellular defense against oxidative stress is weakened. For instance, ROS seem to play a key role in carcinogenesis, in the phases of initia-
tion, promotion, or progression of the disease. In most of the cases, neoplastic cells have severe bad functioning of the three cellular defense lines against oxidative stress damage: (i) the levels of catalase, superoxide dismutase, and peroxidase activities are usually depressed; (ii) endogenous and circulating concentrations of antioxidant molecules are diminished; and (iii) repair machinery does not work conveniently and/or the induction of apoptosis is blocked or inhibited.

On the other hand, the phenomena of ischemia/reperfusion associated with surgical treatments generate ROS which can produce cell damage of diverse magnitude, all the more so when the increase in ROS levels is associated to a parallel reduction of the defenses against oxidative stress (4). The fact that both reduced and total glutathione levels are significantly diminished after surgical treatments can be especially relevant (5).

**GLUTAMINE, GLUTATHIONE, AND OXIDATIVE STRESS**

Both quantitatively and qualitatively, glutathione is one of the most important antioxidant small molecules in the human being, and it is essential for normal cell functioning and replication (6). Glutathione is the tripeptide \( \gamma \)-glutamyl-cysteinyl-glycine. Since cysteine contains a sulfhydryl residue, and it is easily oxidable, glutathione behaves as a very efficient sink of ROS. In a typical redox reaction, a ROS is reduced (and inactivated) through the generation of a disulfur bond between two glutathione molecules, yielding the oxidized glutathione pair. Once the ROS has been inactivated, two reduced glutathione molecules can be recovered through the enzyme reaction catalyzed by glutathione reductase.

Glutamine, with alanine, is the most abundant amino acid in the blood of mammals and it has been described as the main nitrogen vehicle among the tissues of the organism (7). Several transport systems, more or less specific, in both plasma membrane and inner mitochondrial membrane warrant the efficient uptake of glutamine into mitochondria, where it is metabolized (8,9). The first step in the metabolism of glutamine is its deamination by phosphate-activated glutaminase, yielding glutamate and ammonia (10). Glutamate is transported back to the cytosol and there it can be used in the synthesis of glutathione. In fact, glutamine supports the intracellular pool of glutamate, avoiding its depletion and the depletion of glutathione. On the other hand, excess glutamate can be exported via a counter-transport system which pumps cyst(e)ine within the cell (11). Thus, in the context in which we are interested here, glutamine addition can be a means of providing two of the three precursors necessary for glutathione synthesis; that is, glutamine itself can be considered a precursor molecule to glutathione (12). However, it must be stressed that this is not the only and most important role of glutamine in metabolism (13).

An ever-increasing amount of evidence is being published pointing to a protective role of externally added glutathione or glutamine (as its precursor) against oxidative stress. Looking back to the published literature on this issue in the nineties, a critical reading and contrast of available data permit some few and clear conclusions to be obtained:

1. **Exogenously added glutathione protects cultured cells from oxidative stress.** It has been shown that pretreatment with extracellular glutathione protects cultured rat gastric cells from hydrogen peroxide damage by accelerating intracellular glutathione synthesis (14). The concerted and sequential action of membrane-bound \( \gamma \)-glutamyl transpeptidase on extracellular glutathione and then of intracellular \( \gamma \)-glutamylcysteine synthetase would account for this increased intracellular glutathione synthesis as a response to the addition of glutathione to the culture medium. In fact, the protective role of extracellular glutathione is abolished when acivicin (an inhibitor of \( \gamma \)-glutamyl transpeptidase) or buthionine sulfoximine (an inhibitor of \( \gamma \)-glutamylcysteine synthetase) is also added to the culture medium (14).

2. **Glutathione precursors can rescue cells from glucose-deprivation-induced cytotoxicity.** It has been shown that glucose deprivation induces cell death in multidrug-resistant human breast carcinoma cells (15). Afterward, the same group has shown that glucose-deprivation-induced cytotoxicity alters mitogen-activated protein kinase activation and this effect is mediated by oxidative stress. Glutamate and N-acetyl-L-cysteine, precursors of glutathione, were able to rescue cells from glucose-deprivation-induced cytotoxicity and suppressed mitogen-activated protein kinase activation (16).

3. **Glutamine can be administered as an efficient precursor of glutathione.** Glutathione can be easily added to the extracellular medium in
cell cultures. However, in the treatment of animals and human beings, the simple administration of glutathione is not feasible. In such cases, some easily assimilable glutathione precursors should be used. Since glutamine is the main systemic vehicle of nitrogen in mammals and its carbon skeleton may be used in glutathione synthesis, it seems that glutamine could be the best choice as a precursor of glutathione with therapeutical value. For years, cysteine and other thiol compounds have been considered rate-limiting for glutathione synthesis, but recent studies have shown that glutamine is rate-limiting in the presence of cysteine when glutathione has been degraded in kidney, liver, or intestine during metabolic stress (6,12,17–19). As discussed above, systemic dissemination and efficient transport of glutamine into mitochondria are warranted; once inside the mitochondria, it is deaminated to glutamate and ammonia, glutamate is transported back to the cytosol, and here it is readily available for glutathione synthesis (12).

4. There is a wide consensus in recommending the use of a supplement of glutamine in regimes of postchirurgial total parenteral nutrition. Data coming from different laboratories show that a supplementation of glutamine in the diet enhances the antioxidant capacity of tissues and protects from surgical trauma-induced oxidative stress (6,12,19–21). It seems to be firmly demonstrated and contrasted that total parenteral nutrition enriched in glutamine enhances plasma glutathione in the resting state, while maintaining hepatic glutathione stores (6).

On the other hand, total parenteral nutrition promotes intestinal atrophy, but glutamine might play a role in the maintenance of structural integrity of intestine (22,23). Furthermore, lipid emulsions are routinely administered to patients receiving nutritional support and impaired glucose regulation can develop associated with hyperlipidemia. Glutamine supplementation can prevent this effect (24).

Since glutamine itself is an unstable amino acid in solution, the use of stable glutamine dipeptides is highly recommended as a supplement in total parenteral nutrition (21).

5. The above-mentioned consensus is not absolute. There is at least one recent paper concluding that the addition of glutamine in the diet adds no benefit. In brief, the feeding of a protein hydrolysate-based elemental diet supplemented with added glutamine did not provide superior protection to the small intestine of dogs subjected to therapeutic pelvic irradiation. Diets both with and without the added glutamine significantly protected the intestine from radiation injury. Furthermore, after radiation, the activities of xanthine oxidase, superoxide dismutases, and glutathione peroxidases were significantly higher in the intestine of dogs fed without the added glutamine (25).

6. A supplement of glutamine in total parenteral nutrition contributes to a reduction in both morbidity and mortality associated with total parenteral nutrition. Recent studies have demonstrated that enteral feedings are associated with decreased morbidity and mortality when compared with parenteral feedings. It is speculated that alteration of microsomal cytochrome P450 and/or drug clearance may be related to the benefits of providing nutrients by the gastrointestinal route. At the same time the higher morbidity and mortality associated with total parenteral nutrition are related to the phenomenon of bacterial translocation. Glutamine has been reported to reduce bacterial translocation, thus attenuating the disadvantages associated with total parenteral nutrition (23,26).

7. Glutamine-enriched total parenteral nutrition decreases hospital stay and reduces mortality of long stayers in intensive care units. It is known that glutamine-enriched total parenteral nutrition decreases hospital stay after bone marrow transplantation (27,28). However, up to last year there was no reliable study pointing to an increase in intensive care unit-patient survival. Patients in the intensive care unit are one of the groups, together with gastrointestinal patients, short-bowel patients, and oncological patients, that are most likely to benefit from nutritional intervention (29). Malnutrition is common among patients admitted to the intensive care unit, and this is most often further accentuated among long-stay patients (30). Although less than 10% of the total number of patients admitted to the intensive care unit, the long stayers consume more than 50% of the resources and their mortality rate is very high, ranging from 30 to 80% (31,32). Obviously, this is a very important medical as well as economical problem.

We now have a clinical trial carried out by Griffith et al. in which mortality of long stayers in intensive care units is reduced when their total parenteral nutrition is supplemented with glutamine (33). The quality of this clinical trial is outstanding because it was carefully designed to identify and focus only on
those patients that were likely to benefit from the advantage of a supplement of glutamine in their diets, thus excluding a large number of patients who were not likely to benefit and who would just create a lot of noise in the statistics (29).

8. Glutamine supplementation in total parenteral nutrition has very positive effects on immune functioning. It seems that glutamine can exert its immunological effects through a combination of direct action on the cells of the immune system and indirect mechanisms, including the maintenance of gut barrier function, or the preservation of action of the antioxidant glutathione (21,34,35). Reduced natural killer activity has been associated with high levels of prostaglandin E2. The enhanced natural killer activity seen with glutamine supplementation in the diet can be explained, at least partly, by the inhibition of prostaglandin E2 synthesis mediated by glutathione (35). In any case, this is not a well-known aspect and should be further investigated.

9. A supplement of glutamine in diet contributes to diminish nitric oxide release. Nitric oxide is a lipophilic gas whose synthesis is stimulated under ischemic conditions. Nitric oxide is a highly reactive nitrogen species involved in several important functions for the cell (36).

In studies in vitro, Arnal et al. have shown that nitric oxide release in response to bradykinin from cultured endothelial cells is inhibited by cultivating the cells in a medium containing glutamine (37). In this system, glutamine affected neither intracellular arginine levels nor nitric oxide synthase activity. Therefore, glutamine in concentrations similar to those found in blood in vivo may inhibit receptor-mediated nitric oxide release by interfering with signal transduction (37).

Recently, it has been shown that rabbits receiving glutamine have intestinal mucosal glutathione concentrations significantly higher than those rabbits which do not receive glutamine (20). Additionally, these authors have shown that nitric oxide concentrations increased in ischemia in both glutamine nonreceiving and receiving groups, while this increase was more prominent in the control group than in the glutamine-receiving group. From these data, the authors concluded that glutamine supplementation may protect the small intestine from ischemia/reperfusion injury and may play a regulatory role in the biosynthesis of nitric oxide. Another recent report shows that dietary glutamine supplementation reduces plasma nitrate levels, indicating that glutamine has an impact on arginine-nitric oxide metabolism (38). According to these data, it can be assumed that glutamine can act as an inhibitor of nitric oxide formation by nitric oxide synthase (39).

10. Glutamine supplementation in enteral nutrition contributes to gastrointestinal surface protection and mucosa reconditioning. There is increasing evidence that preservation of the ecology of the gastrointestinal tract and the surface protection system is important for the outcome in postoperative trauma patients, patients after bone marrow or liver transplantation, and patients with AIDS. Since the surface protection system consists of surfactants, mucus, and fiber, an enteral diet enriched in these components along with a resupply of species-specific lactobacilli and a supplement of glutamine is highly recommended. Bengmark and Jeppson have reviewed extensive experience in animal models and early experience in a patient population (40). Taking these data into account, they have designed a totally new enteral formula based on probiotic lactobacilli bacteria of the plantarum type (which have proven effective in colonizing the colonic mucosa, suppressing the potentially pathogenic flora) and oat as a substrate for fermentation because it contains surfactants (membrane lipids) a hundred times more than any other food, has a favorable amino acid pattern (particularly rich in glutamine), and is rich in water-soluble fermentable fiber β-glucans (40).

11. Glutamine and other glutathione precursors may play a protective role against acid-induced gastric damage. It has been shown that starvation aggravates acid-induced gastric damage and is associated with greater acid back-diffusion and oxygen radical generation, and lower mucosal glutathione and mucus production (41). Since decreased mucosal glutathione level is one of the factors associated with starvation-aggravated acid-induced gastric damage, the addition of glutathione precursors in the diet can prevent this risk.

12. A supplementation of glutamine in the diet may be beneficial in the treatment of cancer. Glutamine can play a double and antagonistic role with counterpoint effects. Tumor progression is associated with an avid consumption of host glutamine by tumor cells and with a depression in the activity of natural killer cells due to a decrease in the internal glutathione concentrations in these cells of the immune system. Therefore, a supplemen-
tation of glutamine in the diet could have the beneficial effect of restoring the levels of glutathione inside natural killer cells but, at the same time, it could have the pernicious effect of feeding the tumor. However, as glutamine consumption by tumors is almost absolutely dissipative, an increase in the growth rate of the tumor due to this cause should not be expected (42). In fact, there are experimental data which seem to indicate that a supplement of glutamine in the diet can, indeed, contribute to diminish tumor growth by restoring the function of natural killer cells and an improvement in the protein metabolism of the host or patient (43,44).

On the other hand, a supplement of glutamine via oral could increase the selectivity of antitumor drugs used in chemotherapy, protecting the patient from oxidative damage through an increase in glutathione contents (46). In contrast to the previously mentioned work on radiated dogs, several groups have shown that glutamine can also protect against oxidative damage induced by radiotherapy (34,47).

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**REFERENCES**


