Impact of Oral L-Glutamine on Glutathione, Glutamine, and Glutamate Blood Levels in Volunteers

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OBJECTIVE: We investigated the effect of glutamine supplementation on plasma glutamine (Gln), glutamate (Glu), and whole-blood glutathione (GSH) concentrations in human volunteers.

METHODS: Subjects first adapted to a standard diet with known intakes of protein, total GSH, cysteine, methionine, and total Glu (Glu values include Glu and Gln) for 3 d. Plasma Gln, Glu, and whole-blood GSH levels were then measured at 4-h intervals over 24 h. Supplemental oral Gln ($0.3\ g \cdot kg^{-1} \cdot d^{-1}$) was ingested for 10 d and then 24-h plasma levels of Gln, Glu, and whole-blood GSH were measured.

RESULTS: The plasma concentrations of Glu (116%; $P = 0.006$) and Gln (20%; $P = 0.046$) were significantly higher, whereas concentrations of GSH were significantly lower (37%; $P = 0.00091$) after oral Gln supplementation.

CONCLUSION: Oral Gln increases Glu and Gln levels in plasma of healthy subjects but does not increase GSH red cell (whole-blood) levels. Thus, GSH biosynthesis and preservation of GHS stores in red blood cells may involve rate-limiting substrates other than Gln. *Nutrition* 2002;18:367–370. ©Elsevier Science Inc. 2002

KEY WORDS: glutamine, glutamate, glutathione, antioxidant, amino acids

INTRODUCTION

Glutamine (Gln) is the most abundant free amino acid in the human body. It plays an important role in interorgan amino acid metabolism by shuttling nitrogen from skeletal muscle to the intestines (serving as energy substrate), to the liver (in case of starvation and injury), to the kidney (for aminogenesis and acid–base balance), and to proliferating cells of the immune system. Clinical interest in Gln metabolism is prompted by the fact that Gln deficiency occurs in various states of critical illness and that this deficiency might be a major determinant of outcome in these circumstances.[1–3] Gln is synthesized in skeletal muscle, from where it is released into the bloodstream and transported to a variety of tissues.[4–7] A high rate of Gln uptake, but only partial oxidation, is characteristic of rapidly dividing cells such as enterocytes, immunocytes, macrophages, and fibroblasts. This situation provides ideal conditions for the synthesis of key molecules, such as glutathione (GSH) and nucleotides.[8] Thus, Gln should be a GSH precursor and an important nutritional supplement to increase antioxidant stores in the body.

GSH (L-γ-glutamyl-L-cysteinyl-glycine) is the most abundant antioxidant inside cells. GSH performs a variety of physiologic and metabolic functions. These include thiol transfer reactions, which seem to protect cell membranes and proteins, metabolism of endogenous compounds, and transport of amino acids.[9] Lipid peroxidation is an oxidative process leading to the denaturation of biological membranes and it has been implicated in diverse pathophysiologic conditions including aging, atherosclerosis, rheumatic diseases, cancer, cardiac and cerebral ischemias, respiratory distress syndrome, various liver disorders, sepsis, trauma, and burns. It may also be involved in detoxification of certain metals, solvents, pesticides, and drugs.[10–21]

Whole-blood GSH metabolism has not been studied thoroughly. We hypothesized that red cells might, at least in part, use Gln for GSH production. To test this hypothesis, we measured plasma Gln, glutamate (Glu), and whole-blood GSH at 4-h intervals over a 24-h period in three healthy subjects eating a standard diet. We then examined the influence of supplemental oral Gln ($0.3\ g \cdot kg^{-1} \cdot d^{-1}$ taken with meals over 10 d) by repeating the assays at 4-h intervals over another 24-h period.

MATERIALS AND METHODS

Subjects

Three volunteers (two men and one woman) were classified as clinically healthy on the basis of complete medical check-ups. Mean age was 40 y, mean body weight was 64.6 kg, mean height was 166 cm, and mean body mass index was 23.2 kg/m^2. The purpose of the study was fully explained to each subject, and informed written consent was obtained from all subjects. All individuals had followed a regular diurnal rhythm for 3 wk before the experiments. During the 2-d study sampling began at 1 PM, and subjects continued their usual activities throughout the day, apart from the sampling periods. They went to sleep before 12 AM but were awakened for taking samples at 1 and 5 AM.

Diets

Subjects first adapted to a standard diet with known intakes of protein, total GSH, cysteine, methionine, and total Glu (Glu values included Glu and Gln) for 3 d. The meals were prepared individually to provide 25 $kcal \cdot kg^{-1} \cdot d^{-1}$, with 16.3% of the energy as protein ($1\ g \cdot kg^{-1} \cdot d^{-1}$), 60% as carbohydrate, and 23.7% as fat (polyunsaturated:saturated fatty acid ratio = 1). The calculated

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GSH content of each meal was 5.2 g for breakfast, 4.6 g for lunch and dinner, and 0.6 g for snacks; and the cysteine plus methionine content was 0.04 g for breakfast and snacks and 0.75 g for lunch and dinner. The Gln and Glu contents of each meal were 6.9 g for breakfast and midmorning and afternoon snacks and 4.98 g for lunch and dinner. Main meals were eaten at 8 AM, 12:30 PM, and 7 PM, and snacks were eaten at 10 AM and at 3 and 9:30 PM.

Subjects were then asked to adhere to the nutritional criteria of this diet by following a diet plan prepared by the dietitian (A.M.) with recommended food and drink for another 10 d, when they also ingested the Gln supplement (5- or 10-g sachets of GlutaminOx; Oxford Nutrition) as a slurry in water, after each meal.

Blood and Plasma Sample Collection for Gln, Glu, and GSH Assays

Plasma Gln, Glu, and whole-blood GSH were measured on two occasions, before and after ingestion of an oral Gln supplement. Glutaminase, Glu dihydrogenase, nicotinamide-adenine dinucleotide, adenosine 5′-diphosphate, *N*-ethylmaleimide, reduced GSH, glyoxalase-I, and methylglyoxal were purchased from Sigma Chemical Company (Poole, Dorset, UK). Approximately 5 mL of whole blood was taken from the forearm vein at 1, 5, and 9 AM and at 1, 5, and 9 PM. Blood samples were obtained by venipuncture and placed into a tube containing (25 mg) ethylene diaminetetraacetic acid for anticoagulation. All preparations were performed at room temperature.

GLN AND GLU SAMPLE PREPARATIONS. Within 30 min, the tube was centrifuged at 15 000 rpm for 2 min. The plasma sample (0.5 mL) was treated immediately with 0.2 mL of perchloric acid (2%) and centrifuged again at 15 000 rpm for 5 min. The acidic supernatant was stored at −20°C for 24 to 48 h before analysis. Gln and Glu determinations in plasma samples were performed with the glutaminase–Glu dehydrogenase assay.[22]

GSH SAMPLE PREPARATION. Whole blood (0.5 mL) was treated immediately with 0.5 mL of trichloroacetic acid (25%) containing 40 mM of *N*-ethylmaleimide. Within 30 min, the tube was centrifuged at 15 000 rpm for 10 min; the acid supernatant was then stored at −20°C for 24 to 48 h before analysis. GSH determination in whole-blood samples was performed according to the glyoxalase-I assay described by Akerboom et al.,[23] with minor modifications. Briefly, the reaction between methylglyoxal and GSH in 0.1 M of sodium phosphate buffer, pH 7.0, catalyzed by glyoxalase-I, was followed spectrophotometrically at 240 nm. This method is specific for GSH due to the specificity of glyoxalase-I measurement for GSH.

Statistical Analysis

Data were analyzed with Student's *t* test (paired, two-tailed) or the Wilcoxon test depending on how parametric the results were. Correlation coefficients were computed, and Pearson's or Spearman's test was done to determine whether the *r* value differed from zero. In addition, rhythmometric data were summarized by means. For all analytic procedures, $P < 0.05$ was considered statistically significant. All statistics were computed with SPSS 9 for Windows.

RESULTS

We found no correlation in any subject between content of Gln and Glu in plasma and the diet ($r = -0.27$, $P = 0.59$ and $r = 0.37$, $P = 0.47$, respectively) or protein in the diet ($r = 0.41$, $P = 0.41$ and $r = 0.23$, $P = 0.65$, respectively) before oral Gln supplementation. Further, we found no correlation between content of Gln and Glu in plasma and the diet ($r = 0.37$, $P = 0.47$ and $r = -0.24$, $P = 0.63$, respectively) or protein in diet ($r = 0.11$, $P = 0.82$ and $r = 0.63$, $P = 0.17$, respectively) after oral Gln ingestion (0.3 $g \cdot kg^{-1} \cdot d^{-1}$).

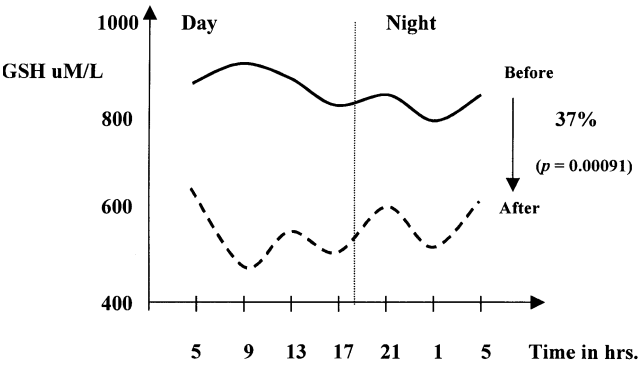

FIG. 1. Whole-blood GSH circadian variations changes before and after glutamine supplementation. GSH, glutathione.

Reduced GSH was measured in whole blood. The critical step in the determination of GSH is the time between blood sampling and analysis or freezing at −20°C or in liquid nitrogen. Analysis of whole blood instantly deproteinized and stored at −207°C for 48 h yields practically the same value as an immediate analysis of a fresh sample. We found no close correlation between plasma GSH content and GSH ($r = 0.61$, $P = 0.19$), methionine plus cysteine ($r = 0.09$, $P = 0.86$), or protein ($r = 0.50$, $P = 0.30$) in the diet before oral Gln ingestion. Further, we found no correlation between GSH content in whole blood and GSH ($r = -0.35$, $P = 0.49$), methionine plus cysteine ($r = 0.41$, $P = 0.41$), and protein ($r = 0.19$, $P = 0.70$) in the diet after oral Gln ingestion (Fig. 3).

Without correlations between dietary components and measured values of Gln, Glu and GSH, we were confident that any results obtained after Gln supplementation would be a direct effect of the substance under study.

Concentrations of Gln, Glu, and GSH from six consecutive blood aspirations sampled at 4-h intervals from the same individual during a 24-h period varied from 465 to 575 μM for Gln, from 47 to 93 μM for Glu, and from 803 to 929 μM for GSH before Gln supplementation. The levels after Gln supplementation ranged from 562 to 670 μM for Glu, from 109 to 229 μM for Glu, and from 483 to 632 μM for GSH. The mean ± standard deviation levels of Gln, Glu, and GSH before Gln supplementation for the three subjects were 521 ± 44.8 μM, 72.32 ± 16 μM, and 869.56 ± 54.5 μM, respectively. After Gln supplementation, mean levels were 629 ± 49.6 μM for Gln, 156.7 ± 44 μM for Glu, and 542.8 ± 56 μM for GSH. All assessments represented 4-h measurements over 24 h (Figs. 1 to 3).

When the data from all subjects were pooled and Gln and Glu concentrations before and after Gln supplementation were compared, we found a significant trend toward increasing Gln (20%; $P = 0.046$) and a significant rise in Glu concentration (116%; $P = 0.006$). However, when GSH concentrations were compared before and after Gln supplementation, there was a significant decrease by 37% ($P = 0.00091$).

DISCUSSION

Cellular Response to Oxidative Injury

The production of reactive oxygen intermediates (e.g., peroxinitrite, O_2^-, and $OH^{\bullet}$) during acute and chronic diseases results in

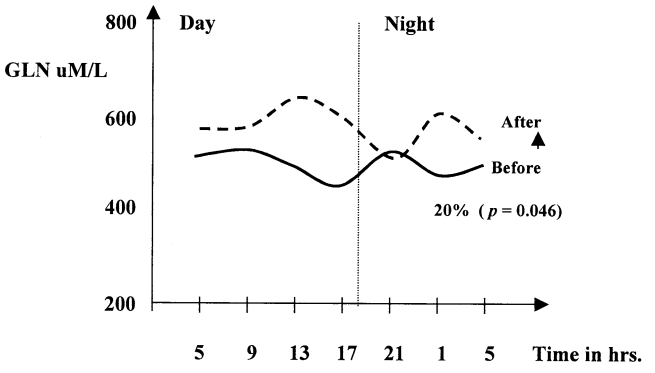

FIG. 2. Plasma Gln circadian variations changes before and after Gln supplementation. GLN, glutamine.

cell injury.[24–31] The reactive oxygen intermediates generated react with polyunsaturated fatty acids of the cell membrane, leading to cell membrane lipid peroxidation[24,25,32,33] and cell death.[34–37]

Enterocytes, endothelial cells, inmmunologic cells, and red blood cells have multiple mechanisms for limiting oxidative damage. The first phase is the reduction of O_2^- to H_2O_2 and catalysis of O_2 by superoxide dismutase. The H_2O_2 formed is then reduced to H_2O and O_2 by intracellular catalase.[34] Lastly, GSH peroxidase uses GSH to reduce H_2O_2, lipid hydroperoxides, peroxinitrate (ONOO), and a variety of other free radicals.[34,38–40] Here, GSH peroxidase reduces the various peroxides by catalyzing their reaction with reduced GSH to form oxidized GSH disulfide and H_2O. These three antioxidant defenses prevent the various reactive oxygen intermediates from participating in further oxidative reactions, which could generate even more volatile oxidants. However, if the oxidant stress overwhelms the GSH defense network, severe cell injury can occur because there are no intrinsic enzymes to reduce the extremely reactive radicals ($OH^\bullet$ and ONOO) formed during oxidation.[41]

Gln and Glu Augmentation

Nutritional supplementation can assist in protecting enterocytes, hepatocytes, red blood cells, and immune system cells in a variety of pathologic states. Gln is a major energy source for the gastrointestinal tract and other cells providing nitrogen and carbon for nucleotide synthesis.[42] Newsholme et al.[43] also found that in lymphocytes the rate of Gln use is significantly in excess of biosynthetic requirements, and Boza et al.[44] reported that plasma

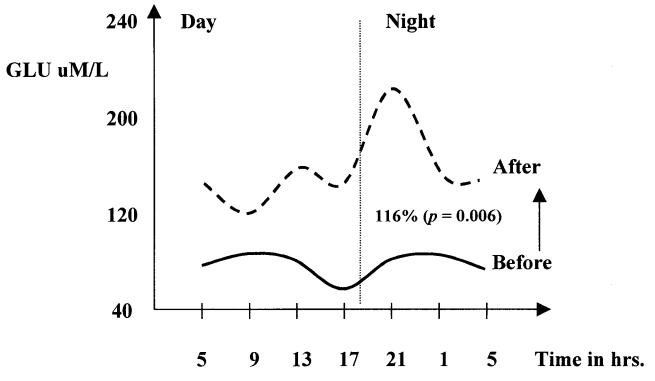

FIG. 3. Plasma Glu circadian variations changes before and after glutamine supplementation. GLU, glutamate.

Gln increases up to 40% after a single dose of free L-Gln added to caseinate. Clinical interest in Gln metabolism was prompted by the fact that Gln deficiency occurs in various states of critical illness and that this deficiency might be a major determinant of clinical outcome in these circumstances.[45,46] Several studies have demonstrated the upregulation of GSH in various host tissues by oral or intravenous Gln[47–48] and have hypothesized that excess Gln use may provide intracellular Glu to synthesize GSH.[49] Even though enteral Gln has benefits in the critically ill, we wondered whether Gln also functions as a precursor to increasing antioxidant defense and investigated the role of Gln in GSH synthesis and the ability of oral Gln to enhance plasma Gln and GSH in red blood cells (whole blood). Our experiments were performed on healthy subjects who were not depleted and showed no evidence of a deficiency in the potential GSH precursor amino acids, i.e., plasma Gln and Glu levels were within normal ranges before Gln ingestion. However, plasma levels of Gln and Glu were significantly elevated within the first hour and during the 10 d of oral Gln ingestion. This finding is consistent with that of a previous volunteer study.[50] Human red blood cells transport Glu poorly, so a rise in plasma Glu would result only in a minimal rise of intracellular Glu in erythrocytes. The increase in plasma Glu concentration thus may be the result of splanchnic bed (enterocytes) uptake of Gln to produce Glu during the 10-d period. Splanchnic conversion of Gln to Glu can be up to 53%.[51,52]

GSH as an Antioxidant

GSH is a tripeptide consisting of Glu, cysteine, and glycine. Glu is transported poorly across cell membranes, but Gln, which is easily transported across cell membranes, is assumed to serve as the intracellular source of Glu.[53] However, the conversion of Gln to Glu depends on sufficient glutaminase, a mitochondrial enzyme. Because mature erythrocytes do not contain mitochondria, intracellular Glu and thus GSH might not rise, despite Gln supplementation. Nevertheless, Gln depletion led directly to a decrease in cell GSH content, suggesting that an adequate supply of Gln is important for the synthesis of GSH.[54–56] Our findings did not appear to support this concept for healthy subjects. Gln should be a precursor of GSH, according to basic biochemistry, but our data indicated that GSH red blood cell levels actually decrease after L-Gln supplementation. Under normal resting conditions, the bonding of Glu with cysteine is the rate-limiting step in the synthesis of GSH. This decrease in whole-blood GSH therefore may be as a result of two factors. First, high Glu concentrations might inhibit cysteine synthesis (another GSH precursor) in red blood cells.[57] The enzyme γ-glutamylcysteine synthase, which catalyzes the first step of GSH biosynthesis, is regulated by a negative feedback from GSH. As a consequence, neither Gln nor Glu may be able to significantly increase intracellular GSH. Second, red blood cells have limited Gln (20%) and Glu (4%) exchange.[58] Therefore, we suggest that Gln is not the only precursor required to raise GSH concentrations within red blood cells, at least in healthy individuals, and that different cells may have different precursor requirements for intracellular GSH.

In conclusion, we accept that there are limitations in a study with such a small number of subjects. Nevertheless, even with this small sample size, there were significant differences between assessments before and after treatment that are interesting and could be useful to compare with data from more extensive future research, which certainly is warranted. The results of this pilot study suggest that whole-blood GSH levels are not maintained in healthy subjects receiving oral Gln. However, our findings indicated that oral Gln is a safe, easy, and inexpensive method to increase Gln (and Glu) levels in normal plasma and possibly tissue stores. Although we could not demonstrate that whole-blood GSH increases over time with this oral Gln dose, we cannot rule out the possibility that Gln increases GSH in other tissues in healthy

subjects or patients with Gln and/or GSH depletion. Therefore, the question of whether this approach to cellular protection will prevent cell oxidation in different organs and promote increased survival remains unanswered, and we still do not know whether these variations are likely to have any clinical repercussions.

REFERENCES

1. Furst P, Albers S, Stehle P. Dipeptides in clinical nutrition. Proc Nutr Soc 1990;49:343
2. Jackson NC, Carroll PV, Russell-Jones DL, et al. The metabolic consequences of critical illness: acute effects on glutamine and protein metabolism. Am J Physiol 1999;76:E163
3. Jackson NC, Carroll PV, Russell-Jones DL, et al. Effect of glutamine supplementation, GH, and IGF-I on glutamine metabolism in critically ill patients. Am J Physiol 2000;278:E226
4. Welbourne TC, Joshi S. Interorgan glutamine metabolism during acidosis. JPEN 1990;14:77S
5. Souba WW, Austgen TR. Interorgan glutamine flows following surgery and infection. JPEN 1990;14:90S
6. Hall JC, Heel K, McCauley R. Glutamine. Br J Surg 1996;83:305
7. Calder PC. Glutamine and the immune system. Clin Nutr 1994;3:2
8. Higashigushi T, Hasselgren P-O, Wagner K, Fisher JE. Effect of glutamine on protein synthesis in isolated intestinal epithelial cells. JPEN 1993;7:307
9. Meister A, Anderson ME. Glutathione. Annu Rev Biochem 1983;2:711
10. Sagui M, Tapel AL. Lipid peroxidation by some halomethanes as measured by in vivo pentane production in the rat. Toxicol Appl Pharmacol 1979;49:283
11. TF Slater. Free-radical mechanisms in tissue injury. Biochem J 1984;222:1
12. Smith MT, Thor H, Orrenius S. The role of lipid peroxidation in the toxicity of foreign compound to liver cells. Biochem Pharmacol 1983;32:763
13. Younes M, Siegers CP. Interrelation between lipid peroxidation and other hepatotoxic events. Biochem Pharmacol 1984;3:2001
14. Comporti M. Biology of disease: lipid peroxidation and cellular damage in toxic liver injury. Lab Invest 1985;3:599
15. Girotti W. Mechanisms of lipid peroxidation. J Free Radic Biol Med 1985;1:87
16. Halliwell B, Gutteridge JMC. The importance of free radicals and catalytic metal concentrations in human diseases. Mol Aspects Med 1985;8:89
17. Sevanian A, Hochstein P. Mechanisms and consequences of lipid peroxidation in biological systems. Annu Rev Nutr 1985;5:365
18. Boughattas NA, Li XM, Filipski J, et al. Modulation of cisplatin chronotoxicity related to reduce glutathione in mice. Hum Exp Toxicol 1996;15:563
19. Ortolani O, Conti A, Gudio AR, et al. The effect of glutathione and *N*-acetylcisteine on lipoperoxidase damage in patients with early septic shock. Am J Respir Crit Care Med 2000;161:1907
20. Alonso de la Vega J, Diaz J, Serrano E, Carbonell LF. Plasma redox status relates to severity in critically ill patients. Crit Care Med 2000;28:1812
21. Winterbourn CC, Buss H, Chan TP, et al. Protein carbonyl measurements show evidence of early oxidative stress in critically ill patients. Crit Care Med 2000; 28:143
22. Lund P. L-glutamine, and L-glutamate. UV-method with glutamines and glutamate dehydrogense. In: Bergmeyer HU, Grassl M, eds. *Methods of enzymatic analysis, Vol 13.* Basel, Switzerland: Verlag Chemie, 1983:1987
23. Akerboom TPM, Sies H. Assey of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods Enzymol 1981;77:373
24. Van Kuijk FJGM, Sevanian A, Handelman GJ, Dratz EA. A new role for phospholipase A_2: protection of membranes from lipid peroxidation damage. Trend Biochem Sci 1987;2:31
25. Powell SR, Tortolani AJ. Recent advances in the role of reactive oxygen intermediates in ischemic injury. J Surg Res 1992;53:417
26. Winterbourn CC, Buss IH, Chan TP, et al. Protein carbonyl measurement shows evidence of early oxidative stress in critically ill patients. Crit Care Med 2000; 28:143
27. Alonso de Vega JM, Diaz J, Serrano E, Carbonell LF. Plasma redox status relates to severity in critically ill patients. Crit Care Med 2000;28:1812
28. Ishii Y, Partridge CA, Del Vecchio PJ, Malik AB. Tumor necrosis factor-alpha mediated decreases in glutathione increases the sensitivity of pulmonary vascular endothelial cells to H_2O_2. J Clin Invest 1992;89:794
29. Buffinton GD, Doe WF. Depleted mucosal antioxidant defenses in inflammatory bowel disease. Free Radic Biol Med 1995;19:911
30. Southorn PA. Free radicals in medicine, II: involvement in human disease. Mayo Clin Proc 1988;63:390
31. Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants and human disease. Where are we now? J Lab Clin Med 1992;119:598
32. Goode HF, Webster NR. Free radicals and antioxidants in sepsis. Crit Care Med 1993;21:1170
33. Yoshikawa T, Minamiyama Y, Ichikawa H, et al. Role of lipid peroxidation and antioxidants in gastric mucosal injury induced by the hypoxanthine-xantine oxidase system in rats. Free Radic Biol Med 1997;23:243
34. Weiss SJ. Oxygen, ischemia, and inflammation. Acta Physiol Scand 1986; 48(suppl):9
35. Slater TF. Free radical mechanism in tissue injury. Biochem J 1984;22:1
36. Jacobson MD. ROS and programmed cell death. Trends Biochem Sci 1996;1:83
37. Stefanec T. Endothelial apoptosis. Chest 2000;117:841
38. Schoemberg MH, Berger HG. Oxygen radicals in intestinal ischemia and reperfusion. Chem Biol Interact 1990;76:141
39. Cha MK, Kim IH. Thioredoxin-linked peroxidase from human red blood cells. Biochem Biophys Res Commun 1995;217:900
40. Iwata S, Hori T, Sato N. Thiol-mediated redox regulation of lymphocyte proliferation. J Immunol 1994;152:5633
41. Wenk DA, Vodovotz Y, Grisham MB, et al. Antioxidant effect of nitric oxide. Methods Enzymol 1999;301:413
42. Klimberg VS. Glutamine, cancer, and its therapy. Am J Surg 1996;72:418
43. Newsholme EA, Crabtree B, Ardawi MSM. Glutamine metabolism in lymphocytes: its biochemical, physiological and clinical importance. J Exp Physiol 1985;70:473
44. Boza JJ, Maire JC, Boretto L, Ballevre O. Plasma Glutamine response to enteral administration of glutamine in human volunteers (free glutamine versus protein bound glutamine). Nutrition 2000;16:1037
45. Griffiths RD, Jones C, Palmer A. Six-month outcome of critically ill patients given glutamine-supplemented parenteral nutrition. Nutrition 1997;13:295
46. Jackson MC, Carroll PV, Russell-Jones DL, et al. The metabolic consequences of critical illness: acute effects on glutamine and protein metabolism. Am J Physiol 1999;76:E163
47. Welbourne TC. Ammonia production and glutamine incorporation into glutathione in the functioning rat kidney. Can J Biochem 1979;7:233
48. Basoglu M, Yildirgan L, Akay F, et al. Glutathione and nitric oxide concentrations in glutamine-infused rabbits with intestinal ischaemia/reperfusion. Eur J Clin Biochem 1997;35:415
49. Rouse K, Nwokedi E, Woodliff JE, et al. Glutamine enhances selectivity of chemotherapy through changes in glutathione metabolism. Ann Surg 1995;221: 420
50. Castell LM, Newsholme EA. The effects of oral glutamine supplementation on athletes after prolonged exhaustive exercise. Nutrition 1997;13:738
51. Matthews DE, Marano MA, Campbell RG. Splanchnic bed utilization of glutamine and glutamic acid in humans. Am J Physiol 1993;64:E848
52. Burrin DG, Reeds PJ. The gut and amino acid homeostasis. Nutrition 2000;16: 666
53. Hong RW, Round JD, Helton WS, Robinson MK, Wilmore DW. Glutamine preserves liver glutathione after lethal hepatic injury. Ann Surg 1992;215:114
54. Jian-Chun Y, Zhu-Ming J, De-Min LI. Glutamine: a precursor of glutathione and its effect on liver. World J Gastroenterol 1999;5:143
55. Cao Y, Feng Z, Hoos A, Klimberg VS. Glutamine enhances gut glutathione production. JPEN 1998;22:224
56. Denno R, Rounds JD, Faris R, Holejko LB, Wilmore DW. Glutamine-enriched total parenteral nutrition enhances plasma glutathione in the resting state. J Surg Res 1996;61:35
57. Eck H-P, Martens T, Rosokat H, et al. $T4^+$ cell number are correlated with plasma glutamate and cysteine levels: association of hyperglutamatasaemia with immunodeficiency in diseases with different aetiologies. Int Immunol 1992;4:7
58. Darmun D, Matthews DE, Bier DM. Glutamine and glutamate kinetics in humans. Am J Physiol 1986;251:E117