

Plasma antioxidant status after high-dose chemotherapy: a randomized trial of parenteral nutrition in bone marrow transplantation patients¹⁻³

Carolyn R Jonas, Alan B Puckett, Dean P Jones, Daniel P Griffith, Elaina E Szeszycki, Glen F Bergman, Celeste E Furr, Cleve Tyre, Joanne L Carlson, John R Galloway, Jeffrey B Blumberg, and Thomas R Ziegler

ABSTRACT

Background: Chemotherapy and radiation therapy result in increased free radical formation and depletion of tissue antioxidants. It is not known whether parenteral nutrition (PN) administered during bone marrow transplantation (BMT) supports systemic antioxidant status.

Objective: The aims of the study were to determine 1) whether high-dose chemotherapy decreases concentrations of major circulating antioxidants in patients undergoing BMT and 2) whether administration of standard PN maintains systemic antioxidant concentrations compared with PN containing micronutrients and minimal lipids alone.

Design: Twenty-four BMT patients were randomly assigned to receive either standard PN containing conventional amounts of dextrose, amino acids, micronutrients, and lipid (120 kJ/d) or a solution containing only micronutrients (identical to those in standard PN) and a small amount of lipid (12 kJ/d). Plasma antioxidant status was measured before conditioning therapy and serially at days 1, 3, 7, 10, and 14 after BMT.

Results: Plasma glutathione (GSH) and α - and γ -tocopherol concentrations decreased and the GSH redox state became more oxidized after conditioning chemotherapy. Plasma cysteine concentrations were unchanged, whereas cystine concentrations increased. Plasma vitamin C and zinc concentrations and GSH peroxidase activity increased over time. Plasma α -tocopherol concentrations were lower in patients given standard PN. There were no differences in other plasma antioxidants between groups.

Conclusions: A significant decline in GSH–glutathione disulfide, cysteine–cystine, and vitamin E status occurs after chemotherapy and BMT. Standard PN does not improve antioxidant status compared with administration of micronutrients alone. Further evaluation of PN formulations to support patients undergoing high-dose chemotherapy and BMT are needed. *Am J Clin Nutr* 2000;72:181–9.

KEY WORDS Parenteral nutrition, antioxidants, glutathione, bone marrow transplantation, cysteine

INTRODUCTION

High-dose chemotherapy administered in bone marrow transplantation (BMT) regimens commonly induces nausea, emesis,

oropharyngeal inflammation, abdominal pain, and diarrhea. These adverse gastrointestinal effects limit oral food intake and provide a basis for the routine use of parenteral nutrition (PN) to meet metabolic needs. However, the clinical efficacy of PN therapy in patients receiving high-dose chemotherapy during BMT protocols remains unclear (1–6).

Chemotherapy and radiation therapy are associated with increased formation of reactive oxygen species and depletion of critical plasma and tissue antioxidants (7–11). Human peripheral polymorphonuclear leukocytes from patients receiving chemotherapy for hematologic and solid malignancies produce more hydrogen peroxide and superoxide anion in vitro than do those of healthy control subjects (8). Increased plasma lipid hydroperoxides and thiobarbituric acid–reactive substances also suggest oxidative stress in patients receiving chemotherapy (11–14).

In patients undergoing BMT, high-dose chemotherapy has been shown to decrease plasma nutrient antioxidant concentrations, including concentrations of vitamin C, α -tocopherol, and β -carotene (13, 15). In addition to micronutrient antioxidants, the tripeptide glutathione (γ -glutamyl-cysteine-glycine; GSH) is a critical endogenous antioxidant that acts directly as a free radical scavenger and in conjunction with the detoxification enzymes glutathione peroxidase (GSHPx) and glutathione-S-transferase (16). Studies in animal models and in humans showed that plasma and hepatic GSH concentrations are markedly reduced by chemotherapeutic agents, such as busulfan, cermustine (BCNU), and cisplatin (17–19). However, the effect of high-dose chemotherapy on the GSH antioxidant system in

¹From the Departments of Medicine, Biochemistry, and Surgery and the Nutrition and Health Sciences Program, Emory University School of Medicine, Atlanta, and the US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston.

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³Address reprint requests to TR Ziegler, Department of Medicine, Division of Endocrinology and Metabolism, The Emory Clinic, Room A-4327, 1365 Clifton Road NE, Atlanta, GA 30322. E-mail: tzieg01@emory.edu.

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TABLE 1
Patient characteristics¹

Characteristic	Treatment group	
	Std-PN (n = 11)	Mod-PN (n = 13)
Age (y)	41 ± 3 ²	38 ± 3
Women	7	4
Men	4	9
Body weight (kg)	77.0 ± 6.3	76.7 ± 5.2
Diagnosis		
Hodgkin disease	2	2
Non-Hodgkin lymphoma	4	7
Chronic myelogenous leukemia	4	4
Acute myelocytic leukemia	1	0
Transplantation type		
Autologous	6	9
Allogeneic	5	4
Clinical infections	6	4
Energy intake from PN (kJ/d) ³	389 ± 41	11 ± 1
Energy intake from food during PN (kJ/d)	70 ± 21	98 ± 13

¹Std-PN, standard parenteral nutrition; Mod-PN, modified parenteral nutrition. There were no significant differences between groups by Fisher's exact test or unpaired *t* test.

² $\bar{x} \pm SE$.

³1 kJ = 4.18 kcal.

BMT patients has not been studied. Furthermore, GSH concentrations, which are known to be influenced by vitamin C and vitamin E status (20, 21), may be decreased as a result of vitamin C and E depletion in these patients.

In the limited studies available in BMT patients, the administration of standard PN did not prevent decreases in plasma micronutrient antioxidants, such as vitamin E and β -carotene (13, 15). In addition to an oxidative stress state during chemotherapy and BMT, there is evidence to suggest that antioxidant requirements may be increased in PN-dependent patients. Several studies showed that the lipid emulsions and L-amino acids used in standard PN solutions are susceptible to oxidation under usual storage conditions and produce reactive lipid peroxides and hydrogen peroxide (22–26). These oxidative products may stimulate oxidative processes in vivo (22–26). It is not known whether macronutrient solutions provided in PN support contribute to the oxidative burden and antioxidant depletion in BMT patients receiving high-dose chemotherapy.

The purpose of this double-blind, controlled, randomized clinical trial was to test the hypothesis that 1) plasma pools of the major antioxidant thiols [GSH and glutathione disulfide (GSSG) and cysteine and cystine] and plasma concentrations of other antioxidant nutrients (α -tocopherol, γ -tocopherol, vitamin C, and zinc) decline over time in patients undergoing BMT; and 2) substrates present in standard PN formulations support plasma concentrations of these antioxidants in this catabolic stress state.

SUBJECTS AND METHODS

Twenty-four patients (10 women, 14 men; mean age: 40.1 ± 3.2 y; range: 26–59 y) with non-Hodgkin lymphoma (*n* = 10), chronic myeloid leukemia (*n* = 8), Hodgkin disease (*n* = 4), acute myeloid leukemia (*n* = 1), and T cell lymphoma

(*n* = 1) were recruited from patients admitted for autologous or allogeneic BMT at Emory University Hospital (EUH), Atlanta (Table 1). These study patients were a subgroup of patients from a larger, ongoing trial designed to evaluate the clinical efficacy of PN in BMT.

Most patients received a chemotherapy regimen consisting of busulfan, etoposide (VP-16), and cyclophosphamide (*n* = 16); other conditioning regimens included cytosine arabinoside (Ara-C; *n* = 5), diaziquone (*n* = 3), and thiotepea (*n* = 1). Four patients received chemotherapy combined with total body irradiation for 3 consecutive days before BMT. Eligible study patients (> 18 y of age) were selected on the basis of the following exclusion criteria: 1) active infection requiring antibiotics on admission to the hospital; 2) primary cancer diagnosis other than leukemia or lymphoma; and 3) PN not indicated during entire hospital course according to usual clinical protocols. Informed consent was obtained before the study from all patients in accordance with the EUH Human Investigations Committee.

Parenteral nutrition protocol

On entry to the study, patients were randomly assigned by blocks to receive a standard PN solution (Std-PN) or a modified PN solution containing micronutrients, essential fatty acids, and electrolytes (Mod-PN) (Table 1 and Table 2). As shown in Table 2, Std-PN patients received a solution containing conventional amounts of fluid, dextrose, L-amino acids (Novamine; Clintec, Deerfield, IL), electrolytes, vitamins and minerals (1.0–1.2 L/d), and 250 mL 20% lipid emulsion (120 kJ/d; Intralipid, Clintec). Nonprotein energy was provided as 70% dextrose and 30% lipid emulsion. The Mod-PN group received a solution containing only electrolytes, vitamins, and minerals (1.0–1.2 L/d). An isovolemic 2% lipid solution (12 kJ/d) was infused in this group of patients both to prevent essential fatty acid deficiency and to maintain the double-blind nature of the study. Thus,

TABLE 2
Composition of parenteral nutrition formulas¹

Component	Treatment group	
	Std-PN	Mod-PN
Amino acids (g·kg ⁻¹ ·d ⁻¹)	1.5	0
Dextrose (% of nonprotein energy)	70	0
Lipid (kJ/d)	120 ²	12
Total daily intravenous energy (kJ)	BEE + 20% ²	12
Vitamins (mL) ³	10	10
Vitamin C (mg) ⁴	500	500
Minerals (mL) ⁵	2	2
Electrolytes	Standard	Standard

¹Std-PN, standard parenteral nutrition; Mod-PN, modified parenteral nutrition providing only micronutrients, electrolytes, and essential fatty acids. 1 kJ = 4.18 kcal.

²BEE, basal energy expenditure estimated by the Harris-Benedict equation.

³Each 10 mL of vitamin preparation (MVI-12; Clintec, Deerfield, IL) provides 2 mg vitamin A, 4 mg vitamin D (ergocalciferol), 20 mg vitamin E (*all-rac*- α -tocopheryl acetate), 200 mg vitamin C, 6 mg thiamine, 7.2 mg riboflavin, 80 mg niacin, 8 mg pyridoxine, 10 μ g vitamin B-12, and 120 μ g biotin.

⁴In addition to MVI-12, all patients were given an additional 500 mg vitamin C.

⁵Each 1 mL of mineral preparation (MTE-6; Clintec) provides 5 mg Zn, 1 mg Cu, 0.5 mg Mn, 10 μ g Cr, 60 μ g Se, and 75 μ g I.

patients in the Mod-PN group were not given intravenous dextrose or amino acids and were given only 10% of the amount of intravenous fat emulsion administered to the Std-PN group. Both groups received identical daily intravenous multivitamin and mineral preparations, including an additional 500 mg vitamin C daily (Table 2). Energy requirements were determined by basal energy expenditure as calculated with the Harris-Benedict equation, then multiplied by a factor of 1.2 to account for energy needs associated with activity (27). The Std-PN prescription was calculated to provide protein at $1.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. PN intake was reduced in proportion to oral food intake by standard criteria.

The investigators, patients, and staff were blinded to the randomization, except for the research pharmacist, who prepared the PN solutions. PN (standard and modified) was initiated when patients consumed orally <50% of estimated maintenance energy needs for 2 consecutive days. One patient in the Std-PN group began PN 1 d before BMT during high-dose chemotherapy; the remainder of the patients received PN support after BMT. Ad libitum oral intake of energy and protein were calculated daily as part of routine care by the EUH Nutrition Support Service. The blinded PN orders were adjusted per standard protocols on the basis of serum chemistry test results. To prevent the development of wasting in the micronutrient-only group, pre hoc criteria required patients to start receiving unblinded standard PN if 1) body weight decreased by >10% from admission body weight or 2) oral intake decreased to $<1.2 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for >14 consecutive days. PN was discontinued when ad libitum oral intake was >50% of the calculated maintenance energy needs for 3 consecutive days.

To evaluate the effect of high-dose chemotherapy and PN on plasma antioxidant status over time, baseline values were determined in blood drawn 7–8 d before BMT (3–4 d before induction chemotherapy). Patients received the bone marrow transplant 1 d after completion of chemotherapy, and blood was subsequently obtained 1, 3, 7, 10, and 14 d post-BMT. Plasma obtained by centrifugation ($3000 \times g$ for 15 min at 4°C) was stored at -80°C until analyzed. Because infection may influence plasma antioxidant indexes, clinical foci of infection were defined pre hoc by positive blood cultures or by signs and symptoms compatible with localized infection, with or without positive microbial cultures of the affected site and prompting initiation or alteration of nonprophylactic antibiotic administration.

Plasma antioxidant and micronutrient assays

Plasma thiol concentrations and reduction potential values

Plasma GSH, GSSG, cysteine, and cystine concentrations were measured by HPLC with fluorescence detection. Briefly, blood was collected by using a 19-gauge syringe with heparin (to prevent hemolysis), then immediately placed in preservation buffer containing bathophenanthroline disulfonate. After centrifugation ($12000 \times g$ for 30 s at 22°C), equal volumes of plasma and ice-cold 10% perchloric acid in $10 \mu\text{mol } \gamma\text{-glutamyl-glutamate/L}$ were combined, and samples were stored at -80°C until assayed. Prior stability studies showed that plasma thiols are stable under these storage conditions for ≤ 8 wk. The acid-soluble fraction was separated and derivatized with iodoacetic acid and dansyl chloride (28).

The dansyl-derivatized compounds, including GSH, GSSG, cysteine, and cystine, were separated as described previously (28) on a 3-aminopropyl column (5 m; $4.6 \text{ mm} \times 25 \text{ cm}$; Custom LC, Houston) by using a Waters 2690 HPLC apparatus and

autosampler system (Waters, Milford, MA). Fluorescence detection of thiols and disulfides was performed by using bandpass filters (305–395 nm excitation and 510–650 nm emission; Gilson Medical Electronics, Middleton, WI). Plasma thiol and disulfide concentrations were calculated on the basis of integration relative to the internal standard, $\gamma\text{-glutamyl-glutamate}$.

As an index of the plasma thiol redox state, the reduction potential (E_h) of the plasma GSH-GSSG pool was calculated by using the Nernst equation:

$$E_h = E_o + RT/2F \ln[(\text{GSSG})/(\text{GSH})^2] \quad (1)$$

where R is the gas constant, T is the absolute temperature, F is the Faraday constant, and E_o is the standard potential for the GSH-GSSG redox couple. A value of -0.264 V was used for E_o assuming a blood pH of 7.4 (29, 30). Although blood pH values were not determined in these subjects, serum carbon dioxide concentrations were similar between groups on the days of GSH determination, ranging from 3.1 to 3.4 kPa in the Std-PN group and from 3.2 to 3.4 kPa in the Mod-PN group (NS). Because none of the patients had evidence of chronic respiratory acidosis, the similarity of carbon dioxide concentrations indicated that pH values were comparable in the 2 groups. The final equation used for these data was as follows:

$$E_h = -0.264 + 0.03 \log[(\text{GSSG})/(\text{GSH})^2] \quad (2)$$

The same equation was used for calculations of redox potential for the cysteine-cystine redox couple (E_o of -0.250 V), with the oxidized form (cystine) and the reduced form (cysteine) used in the numerator and denominator, respectively.

Plasma tocopherols

Plasma α - and γ -tocopherol concentrations were determined by reversed-phase HPLC as described previously (31). Briefly, plasma samples were extracted with hexane and centrifuged at $3000 \times g$ for 15 min at 4°C , and the hexane layer was evaporated under nitrogen. The lipid fraction was dissolved with a mixture of methanol and ethyl ether and separated by using a reversed-phase HPLC apparatus, consisting of a 510 pump, a 710B WISP autosampler, and a 490 multiwavelength detector set to 292 nm (Waters). Retinyl acetate was used as an internal standard, and tocopherol concentrations were calculated on the basis of α - and γ -tocopherol external standards.

Plasma vitamin C

Plasma ascorbic acid concentrations were determined by using methods described by Behrens and Madere (32). After complete reduction of dehydroascorbic acid with the addition of DL-homocysteine, 0.50 mol perchloric acid/L was added to the deproteinized plasma sample, and the mixture was separated on a Biosil octadecylsilane (5S 150 \times 4 mm column; Bio-Rad, Richmond, CA) by using a Waters HPLC apparatus with a model 710B autosampler and a Bioanalytical Systems LC4B electrochemical detector with amperometric detection (Bioanalytical Systems, West Lafayette, IN). The ascorbic acid was eluted with a mobile phase of 40 mmol sodium acetate/L, 0.25 mol *n*-octylamine/L, and 0.2 g EDTA/L at pH 4.

Plasma glutathione peroxidase and zinc

GSHPx activity was measured by enzymatic assay as described previously (33) using a Cobas Fara II spectrophotometric centrifugal analyzer (Roche Diagnostics, Nutley, NJ). This assay

TABLE 3Effect of bone marrow transplantation conditioning regimens on plasma antioxidants¹

	Prechemotherapy	Postchemotherapy
Glutathione ($\mu\text{mol/L}$)	1.88 ± 0.12	1.54 ± 0.07^2
Glutathione reduction potential (mV)	-116.0 ± 1.9	-113.9 ± 1.7^3
α -Tocopherol ($\mu\text{mol/L}$)	19.8 ± 1.9	23.9 ± 1.9
γ -Tocopherol ($\mu\text{mol/L}$)	5.0 ± 0.9	3.9 ± 0.6
Vitamin C ($\mu\text{mol/L}$)	48.3 ± 9.0	49.4 ± 5.1
Zinc ($\mu\text{mol/L}$)	11.5 ± 0.5	15.4 ± 0.7^3
Glutathione peroxidase (U/L)	234 ± 12	253 ± 14

¹ $\bar{x} \pm \text{SE}$. Prechemotherapy values were measured at the time of admission, and postchemotherapy values were determined 1 d after the completion of conditioning therapy.

^{2,3}Significantly different from prechemotherapy (paired *t* test): ² $P < 0.05$, ³ $P < 0.01$.

measures GSHPx activity on the basis of the catalysis of reduced GSH and hydrogen peroxide conversion to oxidized GSSG, which is coupled to the oxidation of NADPH by glutathione reductase. Serum zinc was analyzed on a Perkin-Elmer 5000 atomic absorption spectrometer (Roche Molecular Systems, Inc, Branchburg, NJ) with standard settings for zinc and flame atomization as described by Weinstock and Uhlemann (34).

Statistical analysis

Results are expressed as means \pm SEs. Two-tailed *P* values for Fisher's exact test or unpaired *t* tests were used to compare the 2 treatment groups for age, sex, weight, diagnosis, type of BMT, and incidence of infection. Differences in plasma antioxidants and nutrients before and after chemotherapy were analyzed by paired *t* test. Changes in plasma indexes over time, both for the combined Std-PN and Mod-PN groups and as a function of the

PN group, were analyzed by using one- and two-way repeated-measures analysis of variance (ANOVA) with interaction, respectively. Data from 2 patients who received PN for <3 d were excluded from analyses of changes in plasma antioxidants over time. *P* values <0.05 were considered statistically significant.

RESULTS

Patients

Patient age, sex, body weight, primary diagnosis, and transplantation type were not significantly different between the 2 PN protocols (Table 1). The Std-PN group included 1 patient undergoing matched unrelated donor allogeneic BMT, whereas the Mod-PN group included 3 patients who received matched unrelated donor allogeneic BMT (NS). An equal number of patients ($n = 2$) from each PN group received total body irradiation. The number of clinical infections (Table 1) and days of receiving antibiotics (data not shown) were not significantly different between the 2 groups. Patients continued to receive their full prescribed PN regimen during the study period, with the exception of 3 patients from the Std-PN and 3 from the Mod-PN groups who met pre hoc criteria for oral food intake and discontinued PN (at days 11, 12, and 12 and 10, 13, and 13 postchemotherapy, respectively). The number of days that subjects received the study PN solutions was not significantly different between the 2 groups: 13.0 ± 0.5 d (Std-PN) and 13.3 ± 0.5 d (Mod-PN). As designed, patients in the Std-PN group received significantly more intravenous energy than did the Mod-PN patients ($P < 0.001$). Intravenous protein intake averaged 1.3 ± 0.1 g L-amino acids $\cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in Std-PN patients, whereas patients in the Mod-PN group received no intravenous amino acids. Oral energy intake, primarily as carbohydrate-containing liquids, was similar between the 2 groups during PN (Table 1).

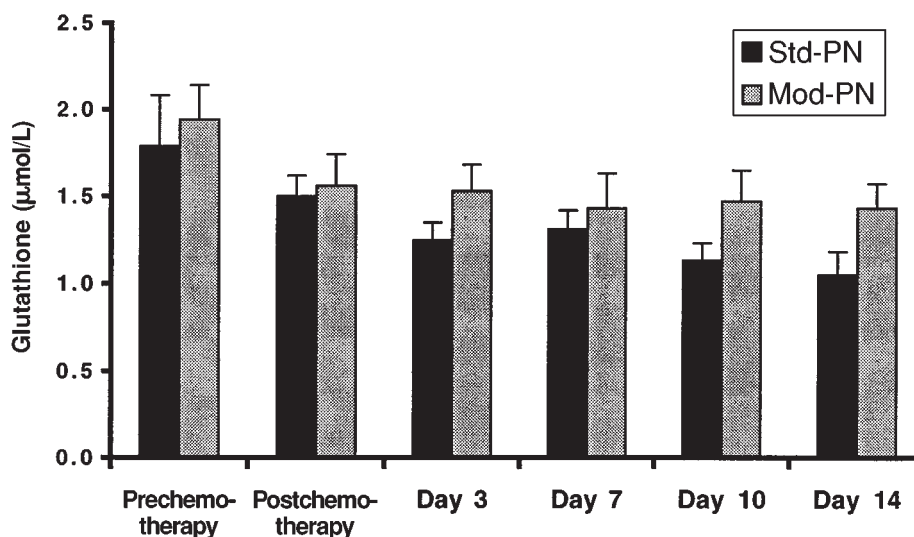


FIGURE 1. Mean (\pm SE) plasma glutathione concentrations over time for the standard parenteral nutrition (Std-PN) group ($n = 11$) and the modified PN (Mod-PN) group ($n = 13$). Prechemotherapy values were obtained at the time of admission and postchemotherapy values were obtained on days 1, 3, 7, 10, and 14 after conditioning therapy (day 1 postchemotherapy = day of bone marrow transplantation). Glutathione concentrations were measured as described in Methods. Glutathione concentrations decreased significantly over time for the entire patient group ($n = 24$; $P < 0.001$); values in the Std-PN group tended to be lower than those in the Mod-PN group (NS). There was a significant main effect of time by two-factor ANOVA, but no effect of PN treatment and no significant treatment-by-time interaction.

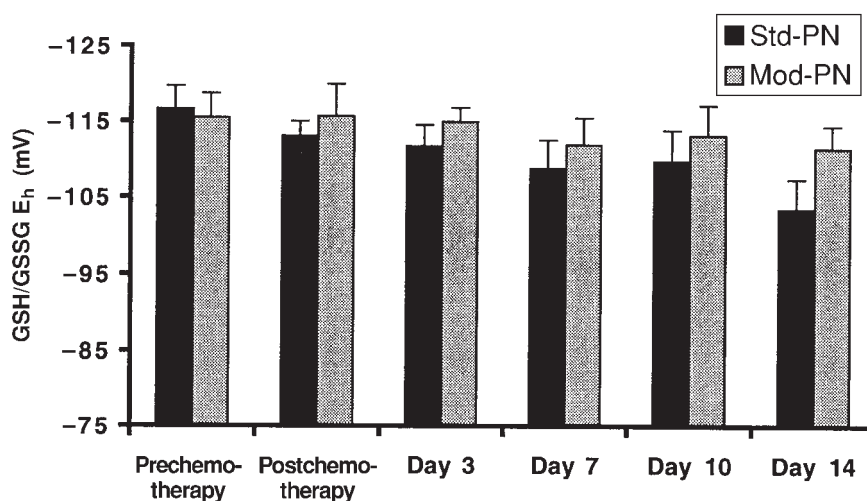


FIGURE 2. Mean (\pm SE) plasma glutathione–glutathione disulfide (GSH/GSSG) reduction potential (E_h) over time for the standard parenteral nutrition (Std-PN) group ($n = 11$) and the modified PN (Mod-PN) group ($n = 13$), which were calculated by using plasma glutathione and glutathione disulfide concentrations as described in Methods. GSH/GSSG E_h values increased significantly (ie, became less negative) over time for the entire patient group ($n = 24$), indicating a progressively more oxidized glutathione pool, by day 14 ($P < 0.001$). The E_h was not different between PN groups and there was no significant treatment-by-time interaction by two-factor ANOVA.

Plasma antioxidant status

Acute changes in plasma antioxidants were assessed by comparing prechemotherapy blood values with values obtained 1 d after chemotherapy (Table 3). High-dose chemotherapy reduced plasma GSH concentrations by $\approx 20\%$ ($P < 0.05$) and resulted in a significantly more oxidized GSH plasma pool, as indicated by more positive E_h values ($P < 0.01$). Plasma zinc concentrations rose by $\approx 30\%$ after chemotherapy for the entire patient group ($P < 0.01$). There were no significant differences in plasma α - or γ -tocopherol, vitamin C, or GSHPx values immediately after the cytoreductive therapy regimens.

Plasma GSH concentrations decreased significantly (from 1.85 ± 0.14 to 1.19 ± 0.08 $\mu\text{mol/L}$) during the 14-d post-chemotherapy period for the entire patient group ($P < 0.001$). The GSH values reached their lowest values by day 14 post-BMT, a concentration well below our laboratory's normal reference range for healthy adult subjects (35). For each serial measure, the Std-PN group tended to have lower plasma GSH concentrations than did the Mod-PN group (Figure 1), but values between PN groups were not significantly different by two-factor ANOVA. Of interest, the 4 patients receiving total body irradiation had a dramatic 60% decrease in plasma GSH concentrations by 21 d post-BMT (data not shown).

Progressive oxidation of the GSH-GSSG pool, indicated by more positive (less reducing) GSH E_h values, occurred in all BMT patients; the greatest decline in GSH-GSSG reducing capacity was observed 14 d post-BMT ($P < 0.001$). As with plasma GSH concentrations, plasma GSH E_h values tended to be more positive (oxidized) in patients receiving Std-PN than in patients receiving Mod-PN throughout the study period (Figure 2; NS). There was no significant treatment-by-time interaction by two-factor ANOVA.

Plasma cysteine concentrations did not change acutely with high-dose chemotherapy and were 15.8 ± 2.5 $\mu\text{mol/L}$ before chemotherapy and 16.5 ± 2.2 $\mu\text{mol/L}$ after chemotherapy (NS). Plasma cysteine also did not change over time (18.5 ± 3.2 ,

18.1 ± 2.6 , 17.1 ± 3.0 , and 14.9 ± 2.5 $\mu\text{mol/L}$ on days 3, 7, 10, and 14, respectively; NS). However, plasma cystine, the oxidized disulfide form of cysteine, increased significantly over time from 57.1 ± 5.4 $\mu\text{mol/L}$ (before chemotherapy) to 75.3 ± 8.2 $\mu\text{mol/L}$ on day 14 after chemotherapy ($P < 0.05$). The calculated E_h values for cysteine-cystine tended to become more oxidized (-113 ± 4 mV before chemotherapy compared with -103 ± 3 mV by 14 d after chemotherapy; NS). No differences in cysteine or cystine concentrations or the cysteine-cystine E_h value were observed between the Std-PN and Mod-PN groups.

Plasma α - and γ -tocopherol, vitamin C, and zinc concentrations and GSHPx activity were evaluated in a subset of the study patients ($n = 6$ Std-PN, $n = 6$ Mod-PN). The 2 groups were not significantly different with regard to age, sex, body weight, primary diagnosis, transplantation type, enteral energy intake, or infection (data not shown). In the entire patient subgroup ($n = 12$), plasma α -tocopherol concentrations decreased significantly from 19.8 ± 1.9 $\mu\text{mol/L}$ at baseline to 15.3 ± 1.4 $\mu\text{mol/L}$ by day 14 ($P < 0.05$), but values remained in the low-normal range (12–42 $\mu\text{mol/L}$). Patients receiving Std-PN had significantly lower plasma α -tocopherol concentrations over time than did patients receiving Mod-PN (Figure 3; $P < 0.05$). Plasma γ -tocopherol concentrations decreased significantly over time for the entire patient group from 5.0 ± 0.9 to 3.0 ± 0.2 $\mu\text{mol/L}$ ($P < 0.001$). Values for γ -tocopherol were not significantly different between the 2 groups over time.

Plasma values for vitamin C and zinc were at the lower end of the normal ranges (vitamin C: 23–125 $\mu\text{mol/L}$; zinc: 11–20 $\mu\text{mol/L}$) before conditioning chemotherapy and approached the midpoint of the normal range during PN administration in each study group. Plasma vitamin C, plasma zinc, and GSHPx activity increased significantly over time in the entire patient group (Table 4). Plasma vitamin C concentrations tended to be lower in the Mod-PN group, but values were not significantly different from those in patients receiving Std-PN. Plasma zinc and GSHPx activity were not significantly different between treatment groups.

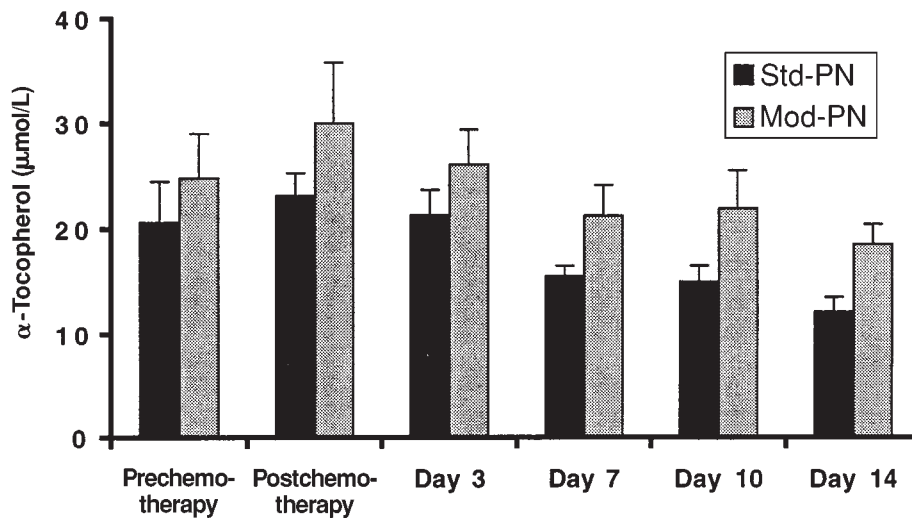


FIGURE 3. Mean (\pm SE) plasma α -tocopherol concentrations over time for the standard parenteral nutrition (Std-PN) group ($n = 6$) and the modified PN (Mod-PN) group ($n = 6$). Plasma α -tocopherol concentrations were determined as described in Methods. Plasma α -tocopherol concentrations decreased significantly over time for all patients ($n = 12$; $P < 0.025$) and were significantly lower in the Std-PN group than in the Mod-PN group ($P = 0.032$). There was a significant effect of time and treatment but no time-by-treatment interaction by two-factor ANOVA.

DISCUSSION

Several investigations in animals showed that chemotherapeutic agents routinely used in BMT patients are associated with significant depletion of GSH in the liver, the primary site of synthesis (17, 36, 37). Data available from small groups of patients suggest that chemotherapy and radiation treatment decreases circulating GSH concentrations acutely (10, 38). The results of the current study are also limited by small sample size but show that high-dose chemotherapy administered in BMT regimens causes an acute and significant decrease in plasma GSH concentration that persists for ≥ 14 d. Depletion of plasma and tissue GSH appears to contribute to chemotherapy-induced organ toxicity (37, 39, 40). In a dog model of venoocclusive disease, which is a major complication of BMT therapy, decreased concentrations of plasma and hepatic GSH were associated with increased hepatic toxicity of cytotoxic agents (37). In mice, depletion of tissue GSH exacerbated cisplatin-induced nephrotoxicity, whereas augmentation of tissue GSH concentrations reduced renal injury after cisplatin treatment (39, 40).

Provision of amino acid substrates that may be utilized for GSH synthesis (glycine, glutamate, and methionine) in our Std-PN formulation did not prevent the decline in plasma GSH in this group of BMT patients. Cysteine availability is considered rate-limiting for GSH synthesis (41); however, plasma cysteine concentrations did not change significantly in the Std-PN patients. Although methionine (a cysteine precursor) was provided with Std-PN, plasma cysteine concentrations were not significantly different from those in patients receiving no intravenous amino acids.

Glutamine may also become rate-limiting for GSH synthesis during states of metabolic or oxidative stress via intracellular conversion to glutamate (42–44). Administration of enteral or parenteral glutamine preserved hepatic and intestinal GSH concentrations in rats subjected to acute inflammatory stimuli, including acetaminophen toxicity and ischemia-reperfusion injury (43, 44). The catabolic and oxidative stress associated with high-dose chemotherapy may increase glutamine requirements in BMT

patients. Our study showed that administration of glutamine-free parenteral amino acids does not support GSH status after BMT. To date, GSH status has not been reported in the clinical trials evaluating glutamine-enriched nutritional support. Studies on GSH antioxidant capacity after supplementation with glutamine or other GSH substrates in BMT patients would be of interest (3).

The decrease in plasma GSH could have been due to decreased synthesis or increased utilization but does not necessarily reflect a decline in GSH reductive capacity because the latter is also dependent on GSSG concentrations. To assess the reducing power of the GSH pool, we calculated the GSH-GSSG redox potential, E_h . The results show that E_h becomes oxidized as a function of time after chemotherapy and BMT, supporting previous interpretations that there is a generalized oxidative stress associated with these BMT regimens. A similar degree of oxidation was apparent in the cysteine-cystine pool, as evidenced by the significant increase in plasma cystine and the corresponding cysteine-cystine E_h values. The use of standard PN and micronutrients did not prevent the loss of GSH reducing power in plasma, suggesting that additional efforts may be needed to protect against oxidation in patients requiring intravenous nutrition.

In contrast with the decreased plasma GSH concentrations, plasma GSHPx activity increased over time after high-dose chemotherapy. This increase may reflect a compensatory homeostatic response to the increased need for peroxide detoxification during the enhanced generation of reactive oxygen species induced by chemotherapy. Elevated plasma GSHPx activity that increases plasma GSH utilization may contribute to the decline in GSH concentrations. However, kinetic studies have raised questions concerning the importance of plasma GSHPx in peroxide elimination (45).

Plasma α - and γ -tocopherol concentrations declined progressively and were lowest 14 d after chemotherapy, a pattern consistent with the oxidation of the GSH and cysteine pools. There is evidence to suggest that low α -tocopherol concentrations for this prolonged period may increase the risk of organ toxicity induced by chemotherapy (7, 46, 47). In rat models of cardiotoxicity, adriamycin administration enhanced free radical generation in



TABLE 4
Effect of parenteral nutrition formulation on plasma antioxidants over time¹

	Prechemotherapy	Postchemotherapy	Day 3	Day 7	Day 10	Day 14
Vitamin C ($\mu\text{mol/L}$) ²						
Std-PN	39 \pm 8	51 \pm 10	69 \pm 8	77 \pm 7	77 \pm 3	70 \pm 7
Mod-PN	44 \pm 11	49 \pm 6	64 \pm 9	56 \pm 9	55 \pm 10	50 \pm 7
Zinc ($\mu\text{mol/L}$) ³						
Std-PN	11.3 \pm 0.6	14.6 \pm 0.1	15.5 \pm 2.2	15.5 \pm 1.7	15.5 \pm 1.3	14.8 \pm 1.4
Mod-PN	11.1 \pm 0.7	16.3 \pm 1.0	15.3 \pm 1.6	13.3 \pm 1.8	13.3 \pm 1.6	12.8 \pm 1.9
Glutathione peroxidase (U/L) ³						
Std-PN	227 \pm 25	252 \pm 29	270 \pm 36	266 \pm 30	265 \pm 30	272 \pm 33
Mod-PN	238 \pm 14	262 \pm 22	274 \pm 27	257 \pm 28	293 \pm 28	268 \pm 26

¹ $\bar{x} \pm \text{SE}$. Std-PN, standard parenteral nutrition ($n = 6$); Mod-PN, modified parenteral nutrition ($n = 6$).

^{2,3}Significant effect of time (ANOVA): ² $P < 0.01$, ³ $P < 0.05$.

perfused heart and increased membrane lipid peroxidation in intact cardiac muscle cells (46). Additional studies showed that anthracycline-induced cardiac injury in animals is aggravated by vitamin E deficiency, whereas vitamin E supplementation decreases lipid peroxide formation and cardiac cell injury (7).

Both treatment groups in our study received 20 mg α -tocopherol/d intravenously. These data, and those of others, suggest that provision of vitamin E in amounts twice those recommended for intravenous vitamin E (10 mg/d; 47) is not sufficient to maintain plasma α -tocopherol concentrations in either critically ill or stable PN-dependent patients (22). However, changes in plasma concentrations of vitamin E and other nutrients do not themselves define deficiency. Thus, further studies are needed to clarify the adverse effects, if any, of reduced plasma antioxidant nutrient concentrations in BMT patients.


A potentially important finding of the present study is that BMT patients receiving the standard PN formulation showed a significantly greater decline in plasma α -tocopherol than did those given a modified formulation containing no amino acids and one-tenth the amount of fat emulsion. Several investigators have shown that conventional lipid emulsions containing largely unsaturated fatty acids are possible sources of peroxidative products (22–25). In PN-dependent and healthy adults, short-term lipid infusions administered alone significantly elevated lipid peroxidation, as indicated by breath-pentane output (23). Another study showed increased breath-pentane output in premature infants administered a 10% lipid emulsion (24). Furthermore, the infused lipid emulsions contained significant amounts of the oxidative products pentane and malondialdehyde, which formed during standard storage conditions (24). Amino acid solutions may also be susceptible to oxidative degradation, producing hydrogen peroxide as a result of photooxidation (26). Infusion of the photooxidized amino acid and micronutrient solutions in animals was associated with depleted plasma GSH and hepatic injury (48). Further studies are needed to identify whether specific components of PN solutions or methods of delivery induce oxidative stress in this and other clinical settings.

Vitamin C deficiency was found previously to decrease plasma GSH concentrations in healthy individuals (49). Plasma vitamin C concentrations were initially low in our study patients but steadily increased during PN administration. In contrast with previous studies in BMT patients given lower vitamin C doses (13), our intravenous vitamin C dose of 700 mg/d maintained plasma vitamin C concentrations within the normal range. Thus,

vitamin C deficiency did not appear to account for the reduced plasma GSH concentrations observed in our patients.

Zinc concentrations were near the lower limit of normal values at baseline and increased over time. Zinc has several biochemical functions, including cytoprotective and antioxidant properties related to its role as a cofactor in Cu/Zn superoxide dismutase and metallothionein and other less clearly defined mechanisms (50). Elevated serum zinc concentrations may have been due to tissue zinc mobilization in response to chemotherapy. Whether increased serum zinc enhances antioxidant function or influences treatment outcomes in BMT patients requires further study.

There are limited data on the benefits of antioxidant supplementation in BMT protocols. In a recent case report, oral glutamine (20 g/d) and vitamin E (400 mg/d) were associated with reversal of hepatic venoocclusive disease after BMT (51). Combination treatment with β -carotene (45 mg), α -tocopherol (825 mg), and ascorbic acid (450 mg) for 3 wk before BMT conditioning improved plasma β -carotene and α -tocopherol concentrations and reduced lipid peroxide concentrations (52). A pilot study of vitamin E, vitamin C, and *N*-acetylcysteine supplementation in patients receiving chemotherapy or irradiation for various malignancies suggested that supplementation reduced the cardiotoxicity associated with therapy (53). In patients with advanced gastric cancer, cisplatin-induced neurotoxicity was reduced by the intravenous and intramuscular administration of GSH (54). Additional studies to determine the efficacy of antioxidant regimens as methods to attenuate oxidant-mediated injury in BMT patients appear to be indicated.

In summary, these data from a small group of patients provide evidence that conventional PN formulations do not maintain plasma concentrations of essential body antioxidants for several weeks after BMT. A limitation to intensifying chemotherapy regimens has been the severity of toxicity to host tissues. Antioxidant supplementation as a means of protecting healthy tissue from injurious free radicals has been approached with caution because of the potential of the antioxidants to reduce the therapeutic efficacy of the cytotoxic regimens. The current double-blind study showed the need to further evaluate PN formulations in larger groups of individuals receiving high-dose chemotherapy or irradiation and to evaluate the efficacy of nutrition interventions that support plasma antioxidant status in these patients. 

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REFERENCES

1. Klein S, Koretz R. Nutrition support in patients with cancer: what do the data really show? *Nutr Clin Pract* 1994;9:91-100.
2. Weisdorf S, Lysne J, Wind D, et al. Positive effect of prophylactic total parenteral nutrition on long-term outcome of bone marrow transplantation. *Transplantation* 1987;43:833-8.
3. Ziegler TR, Young LS, Benfell K, et al. Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition following bone marrow transplantation: a randomized, double-blind, controlled study. *Ann Intern Med* 1992;116:821-8.
4. Hays DM, Merrit RJ, White L, Ashley J, Siegel SE. Effect of total parenteral nutrition (PN) on chemotherapy and survival of acute leukemia in childhood. *Med Pediatr Oncol* 1983;11:134-40.
5. Weisdorf S, Hofland C, Sharp H, et al. Total parenteral nutrition in bone marrow transplantation: a clinical evaluation. *J Pediatr Gastroenterol Nutr* 1984;3:95-100.
6. Szeluga D, Stuart R, Brookmeyer R, Utermohlen V, Santos GW. Nutritional support of bone marrow transplantation recipients: a prospective, randomized clinical trial comparing total parenteral nutrition to an enteral feeding program. *Cancer Res* 1987;47:3309-16.
7. Weijl NI, Leton FJ, Osanto S. Free radicals and antioxidants in chemotherapy-induced toxicity. *Cancer Treat Rev* 1997;23:209-40.
8. Sangeetha P, Das UN, Koratkar R, Suryaprabha P. Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. *Free Radic Biol Med* 1990;8:15-9.
9. Durken M, Agbenu J, Finckh B, et al. Deteriorating free radical-trapping capacity and antioxidant status in plasma during bone marrow transplantation. *Bone Marrow Transplant* 1995;15:757-62.
10. Lauterburg BH, Nguyen T, Hartmann B, Junker E, Kupfer A, Cerny T. Depletion of total cysteine, glutathione, and homocysteine in plasma by ifosfamide/mesna therapy. *Cancer Chemother Pharmacol* 1994;35:132-6.
11. Clemens MR, Ladner C, Schmidt H, et al. Decreased essential antioxidants and increased lipid hydroperoxides following high-dose radiochemotherapy. *Free Radic Res Commun* 1989;7:227-32.
12. Ladner C, Ehninger G, Gey KF, Clemens MR. Effect of etoposide (VP16-213) on lipid peroxidation and antioxidant status in a high-dose radiochemotherapy regimen. *Cancer Chemother Pharmacol* 1989;25:210-2.
13. Hunnisset A, Davies S, McLaren-Howard J, Gravett P, Finn M, Gueret-Wardle D. Lipoperoxides as an index of free radical activity in bone marrow transplant recipients. *Biol Trace Miner* 1995;47:125-32.
14. Faure H, Coudray C, Mousseau M, et al. 5-Hydroxymethyluracil excretion, plasma TBARS and plasma antioxidant vitamins in adriamycin-treated patients. *Free Radic Biol Med* 1996;20:979-83.
15. Clemens MR, Ladner C, Ehninger G, et al. Plasma vitamin E and β -carotene concentrations during radiochemotherapy preceding bone marrow transplantation. *Am J Clin Nutr* 1990;51:216-9.
16. Meister A. Glutathione deficiency produced by inhibition of its synthesis and its reversal: applications in research and therapy. *Pharmacol Ther* 1991;51:155-94.
17. Mitchell JR, Smith CV, Hughes H, Lauterberg BH, Horning MG. Overview of alkylation and peroxidation mechanisms in acute lethal heptocellular injury by chemically reactive metabolites. *Semin Liver Dis* 1981;1:143-50.
18. Dwivedi C, Abu-Ghazaleh A, Guenther J. Effects of diallyl sulfide and diallyl disulfide on cisplatin-induced changes in glutathione and glutathione-S-transferase activity. *Anticancer Drugs* 1996;7:792-4.
19. Bhuvaramurthy V, Balasubramanian N, Govindasamy S. Effect of radiotherapy and chemotherapy on circulating antioxidant system of human uterine cervical carcinoma. *Mol Cell Biochem* 1996;158:17-23.
20. Meister A. On the antioxidant effects of ascorbic acid and glutathione. *Biochem Pharmacol* 44:1905-15.
21. Siow RCM, Sato H, Leake DS, Pearson JD, Bannai S, Mann GE. Vitamin C protects human arterial smooth muscle cells against atherogenic lipoproteins. *Arterioscler Thromb Vasc Biol* 1998;18:1662-70.
22. Pironi L, Ruggeri E, Zolezzi C, et al. Lipid peroxidation and antioxidant status in adults receiving lipid-based home parenteral nutrition. *Am J Clin Nutr* 1998;68:888-93.
23. Van Gossum A, Shariff R, Lemoyne M, Kurian R, Jeejeebhoy K. Increased lipid peroxidation after lipid infusion as measured by breath pentane output. *Am J Clin Nutr* 1988;48:1394-9.
24. Pitkanen O, Hallman M, Andersson S. Generation of free radicals in lipid emulsion used in parenteral nutrition. *Pediatr Res* 1991;29:56-9.
25. Neuzil J, Darlow BA, Inder TE, Sluis KB, Winterbourn CC, Stocker R. Oxidation of parenteral lipid emulsion by ambient and phototherapy lights: potential toxicity of routine parenteral feeding. *J Pediatr* 1995;126:785-90.
26. Brawley V, Bhatia J, Karp WB. Hydrogen peroxide generation in a model paediatric parenteral amino acid solution. *Clin Sci* 1993;85:709-12.
27. Szeluga DJ, Stuart RK, Brookmeyer R, Utermohlen V, Santos GW. Energy requirements of parenterally fed bone marrow transplant recipients. *JPEN J Parenter Enteral Nutr* 1985;9:139-43.
28. Jones DP, Kurtz JC, Samiec PS, et al. Glutathione measurement in human plasma. Evaluation of sample collection, storage and derivatization conditions for analysis of dansyl derivatives by HPLC. *Clin Chem Acta* 1998;275:175-84.
29. Clarke WM. The standard hydrogen half-cell and the standardization of oxidation-reduction potentials and pH numbers. In: *Oxidation-reduction potentials of organic systems*. Baltimore: Waverly Press, 1960:248-72.
30. Rost J, Rapaport S. Reduction potential of glutathione. *Nature* 1964;201:185-6.
31. Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am J Clin Nutr* 1979;32:2143-9.
32. Behrens WA, Madere R. A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic and dehydroascorbic acid in tissues, biological fluids, and foods. *Analyt Biochem* 1987;165:102-7.
33. Pleban P, Munyani A, Beachum J. Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes. *Clin Chem* 1982;28:311-5.
34. Weinstock N, Uhlemann M. Automated determination of copper in undiluted serum by atomic absorption spectroscopy. *Clin Chem* 1981;27:1438-40.
35. Samiec PS, Drews-Botsch C, Flagg EW, et al. Glutathione in human plasma: decline in association with aging, age-related macular degeneration and diabetes. *Free Radic Biol Med* 1998;24:699-704.
36. Bhanumathi P, Devi PU. Modulation of glutathione depletion and lipid peroxidation by WR-77913 and 2-mercaptopyrionylglycine in cyclophosphamide chemotherapy. *Indian J Exp Biol* 1994;32:562-4.
37. Shulman HM, Luk K, Deeg HJ, Shuman WB, Storb R. Induction of hepatic veno-occlusive disease in dogs. *Am J Pathol* 1987;126:114-25.
38. Navarro J, Obrador E, Pellicer JA, Asensio M, Vina J, Estrela JM. Blood glutathione as an index of radiation-induced oxidative stress in mice and humans. *Free Radic Biol Med* 1997;22:1203-9.
39. Appenroth D, Winnefeld K. Role of glutathione for cisplatin nephrotoxicity in young and adult rats. *Ren Fail* 1993;15:135-9.
40. Anderson ME, Naganuma A, Meister A. Protection against cisplatin toxicity by administration of glutathione ester. *FASEB J* 1990;4:3251-5.
41. Meister A. New aspects of glutathione biochemistry and transport—selective alteration of glutathione metabolism. *Nutr Rev* 1984;42:397-410.
42. Welbourne TC. Ammonia production and glutamine incorporation into GSH in the functioning rat kidney. *Can J Biochem* 1979;57:233-7.



43. Hong RW, Rounds JD, Helton WS, Robinson MK, Wilmore DW. Glutamine preserves liver glutathione after lethal hepatic injury. *Ann Surg* 1992;215:114-9.
44. Harward TR, Coe D, Souba WW, Klingman N, Seeger JM. Glutamine serves gut glutathione levels during intestinal ischemia/reperfusion. *J Surg Res* 1994;54:351-5.
45. Flohe L. Structure and catalytic mechanism of glutathione peroxidase. In: Taniguchi N, Higashi T, Sakamoto, Meister A, eds. *Glutathione centennial: molecular perspectives and clinical applications*. San Diego: Academic Press, Inc, 1989:103-14.
46. Rajagopalan S, Politi PM, Sinha BK, Myers CE. Adriamycin-induced free radical formation in the perfused rat heart. Implications for cardiotoxicity. *Cancer Res* 1988;48:4766-9.
47. Multivitamin preparations for parenteral use. A statement by the Nutrition Advisory Group. American Medical Association Department of Foods and Nutrition, 1975. *JPEN J Parenter Enteral Nutr* 1979;3:258-62.
48. Bhatia J, Moslen MT, Kaphalia L, Rassin DK. Glutathione and tissue amino acid responses to light-exposed parenteral nutrients. *Toxicol Lett* 1992;63:79-89.
49. Henning SM, Zhang JZ, McKee RW, Swendseid ME, Jacob RA. Glutathione blood levels and other oxidant defense indices in men fed diets low in vitamin C. *J Nutr* 1991;121:1969-75.
50. Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. *Free Radic Biol Med* 1990;8:281-91.
51. Nattakom TV, Charlton A, Wilmore DW. Use of vitamin E and glutamine in the successful treatment of severe veno-occlusive disease following bone marrow transplantation. *Nutr Clin Pract* 1995;10:16-8.
52. Clemens MR, Waladkhani AR, Bublitz K, Ehninger G, Gey KF. Supplementation with antioxidants prior to bone marrow transplantation. *Wien Klin Wochenschr* 1997;109:771-6.
53. Wagdi P, Fluri M, Aeschbacher B, Fikrle A, Meier B. Cardioprotection in patients undergoing chemo- and/or radiotherapy for neoplastic disease. A pilot study. *Jpn Heart J* 1996;37:353-9.
54. Cascinu S, Cordella L, Del Ferro E, Fronzoni M, Catalano G. Neuroprotective effect of reduced glutathione on cisplatin-based chemotherapy in advanced gastric cancer: a randomized double-blind placebo-controlled trial. *J Clin Oncol* 1995;13:26-32.

