Glutamine-enriched Intravenous Feedings Attenuate Extracellular Fluid Expansion After a Standard Stress

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A double-blind, randomized controlled trial was performed to determine the effect of glutamine (GLN)-enriched intravenous feedings on the volume and distribution of body fluids in catabolic patients. Subjects with hematologic malignancies in remission underwent a standard treatment of high-dose chemotherapy and total body irradiation before bone marrow transplantation. After completion of this regimen, they were randomized to receive either standard parenteral nutrition (STD, n = 10) or an isocaloric, isonitrogenous nutrient solution enriched with crystalline L-glutamine (0.57 g/kg/day, GLN, n = 10). Extracellular water (ECW) and total body water (TBW), determined by bromide and heavy water dilution techniques, were measured before the conditioning treatment and after termination of the intravenous feedings that were administered for 27 ± 1 days. In addition electrical resistance (R, in ohms, Ω) and reactance (X_c, Ω) of the body to a weak alternating current were measured at these time points. Both study groups were comparable for age, weight, height, sex, and diagnosis. Initial TBW was highly related to electrical resistance ($r = -0.93$, $p < 0.001$). After conditioning therapy, bone marrow infusion, and intravenous feedings, a 20% expansion in ECW was observed in the STD group (ECW: 18.0 ± 1.1 L *vs.* 14.9 ± 1.0, $p = 0.012$), and this fluid retention was associated with a marked decrease in electrical resistance (R: 514 ± 28 Ω *vs.* 558 ± 26, $p < 0.05$). In contrast the extracellular fluid compartment in patients receiving GLN-supplementation did not change (ECW: 15.8 ± 0.9 L *vs.* 15.4 ± 0.8, $p = 0.49$), and the body's resistance was maintained (R: 552 ± 27 Ω *vs.* 565 ± 23, $p = 0.42$). Expansion of ECW could not be related to differences in fluid or sodium intake, or to the use of diuretics or steroids. Patients receiving the STD solution, however, exhibited a greater number of positive microbial cultures ($p < 0.01$) and a higher rate of clinical infection compared with the GLN patients (5/10 *vs.* 0/10, $p < 0.05$); the fluid expansion in infected STD patients was greater compared with uninfected individuals (Δ ECW: + 5.0 ± 1.4 *vs.* 0.7 ± 0.5, $p = 0.007$). In this model of catabolic stress, fluid retention and expansion of the extracellular fluid compartment commonly observed after standard total parenteral nutrition can be attenuated by administering glutamine-supplemented intravenous feedings, possibly by protecting the host from microbial invasion and associated infection.

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RETENTION OF SODIUM and water and the concomitant expansion of body fluid compartments are characteristic sequelae of injury.[1] These changes are thought to result from the enhanced release of catabolic mediators[2] and hormones such as vasopressin[3] and aldosterone.[4] This hormonal response is proportional to the severity of injury and is accentuated by blood loss[5] and severe infection.[6] Although the cause of the redistribution in body fluids is incompletely understood,[7] it has generally been found that "stress" is associated with expansion of the extracellular fluid compartment.[8]

A regimen of enteral feedings often is not possible in catabolic patients. For example patients undergoing allogeneic bone marrow transplantation (BMT) and associated chemoradiotherapy often exhibit intense nausea, vomiting, oral mucositis, and diarrhea, which preclude adequate enteral food intake.[9] Thus intravenous nutrition is commonly administered in this patient population. Administration of total parenteral nutrition (TPN), however, is associated with expansion of the extracellular fluid compartment that persists at hospital discharge.[10]

Usual intravenous feedings do not contain glutamine (GLN), although this amino acid may be a conditionally

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essential nutrient during catabolic states.[11,12] After abdominal irradiation in rodents, GLN-supplemented feedings decreased toxicity and death.[13] In addition GLN-supplemented parenteral feedings enhance cellularity of the gastrointestinal mucosa and improve immunologic function.[14,15] In preliminary studies patients receiving bone marrow transplants and GLN-supplemented parenteral nutrition demonstrated improved nitrogen retention when compared with matched individuals receiving standard GLN-free intravenous feedings.[16] No deleterious effects of GLN administration were observed. The purpose of this study was to evaluate the effects of GLN-enriched intravenous solutions on body fluid compartments in patients undergoing bone marrow transplantation.

Materials and Methods

Subjects with hematologic malignancies in remission (n = 20) who enrolled in allogeneic BMT protocols in the Brigham and Women's Hospital were studied. After hospital admission patients received central venous catheters, followed by a study of body composition, which was performed 1 week before the BMT (referred to as "initial" measurement, day −7, Fig. 1). The individuals then underwent a program that greatly reduced the endogenous flora of skin and gut, including placement in laminar airflow rooms and oral ingestion of gentamycin, vancomycin, and nystatin. A conditioning regimen was initiated using cytosine arabinoside (3 g/m^2, twice a day for 3 days), cyclophosphamide (1800 mg/m^2, once a day for 2 days), and whole-body irradiation (175 cGy twice a day for 4 days, a total dose of 1400 cGy, n = 18) over a 7-day period. Two additional subjects received only cyclophosphamide (3.6 g/m^2) and busulfan (16 mg/kg). Graft-*versus*-host (GVHD)-prophylaxis consisted of *ex vivo* lymphocyte depletion with ST-1 immunotoxin (Sanofi Research, Inc., Montpelier, France)[17] or a combination of methotrexate and cyclosporin.

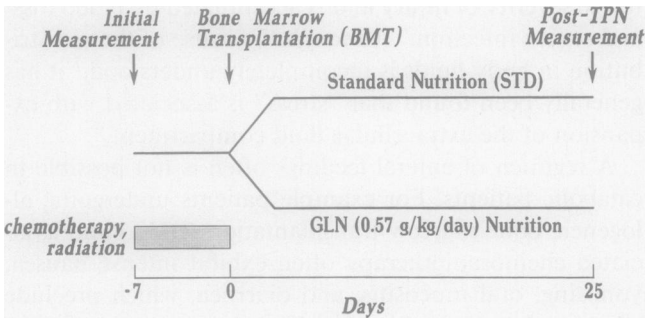

FIG. 1. Study protocol. Initial measurement of body fluid volumes using dilutional and impedance techniques was performed on day −7 followed by a standard dose of chemotherapy and total body radiation administered over the next 7 days. After bone marrow transplantation, patients were randomized to receive either standard parenteral nutrition or GLN-supplemented intravenous feedings. A second measurement of body fluid volumes (Post-TPN) was performed at the termination of TPN (day 25 ± 1).

TABLE 1. *Characteristics of Patient Groups and Healthy Controls (Mean ± SEM)*

Characteristics	STD*	GLN	CTR
N	10	10	10
Age (yr)	33 ± 3	36 ± 3	35 ± 3
Body weight (kg)	69.9 ± 4.5	70.6 ± 3.3	70.0 ± 4.5
Height (cm)	170 ± 2	171 ± 3	172 ± 2
Sex (F/M)	6/4	5/5	3/7
Diagnosis (CML/AML/misc)	5/4/1	5/4/1	—
GVHD-prophylaxis (ST1/MTX + cyclosporine)	7/3	6/4	—
Splenectomy (≤2 mo)	3	3	—

* No differences were seen between individuals receiving a standard parenteral solution (STD) compared with subjects receiving glutamine-enriched parenteral feedings (GLN) or healthy controls (CTR).

After infusion of allogeneic bone marrow on day 0, patients were randomized by the research pharmacist into those who received standard parenteral nutrition (STD) or those who received GLN-supplemented intravenous feedings (GLN). The control and the experimental groups were matched by balanced design for sex, diagnosis, and GVHD-prophylaxis (Table 1). Patients, investigators, physicians, and nurses were blinded to the randomization.

Total parenteral nutrition was initiated on the day after bone marrow infusion. The caloric requirements of the patients were based on basal energy requirements obtained from standard tables multiplied by 1.50, a calculation previously determined to meet the needs of BMT patients.[18] The protein intake was maintained at 1.5 g/kg/day; nonprotein calories were administered as a 70% glucose and 30% lipid emulsion (Intralipid, Kabi Vitrum, Stockholm, Sweden). Thus the two study solutions were isonitrogenous and isocaloric parenteral solutions. The STD solution contained a commercially available amino acid mixture (Novamine, Kabi Vitrum, Stockholm, Sweden). Crystalline L-glutamine (0.57 g/kg/day, Ajinomoto USA, Raleigh, NC) was added to another commercially available solution rich in essential amino acids (Renamine, Baxter Health Care Corp., McGaw Park, IL)[16] and sterilized by membrane filtration (Millex-GS, 0.22-μm filter, Millipore, Bedford, MA). The patients were allowed an *ad libitum* low-bacterial diet throughout their hospitalization.

The parenteral regimens were terminated when the individuals consumed more than 50% of their energy requirements by the oral route. A second body compositional analysis (referred to as "post-TPN" measurement) was performed immediately at termination of the TPN,

and patients were discharged from the hospital shortly thereafter.

Body Compositional Analysis

Volumes of body fluid compartments were assessed by two different methods. The first technique was based on the dilution of bromide and deuterium oxide (heavy water, D_2O).[19] These substances were used as nonradioactive indicators to determine the volume of extracellular (ECW) and total body water (TBW), respectively.

On the morning of study, venous blood samples were obtained to measure background concentrations of these substances. Heavy water was then administered over 1 minute through a centrally placed intravenous line at a dose of approximately 0.15 g/kg body weight (bw); bromide was given over 5 minutes at a dose of 0.75 mL/kg bw. Three hours after injection, venous blood samples were obtained and processed to determine the equilibrium concentrations of these indicators. The heavy water was sterile, pyrogen free, and 99.8% pure (Cambridge Isotope Laboratories, Woburn, MA). The quantity required was measured to the nearest 0.1 mg by determining the difference in weight of a syringe containing this dilutional marker before and after injection. A 3% solution of sodium bromide (wt/vol) was prepared 1 day before the day of study (99.6% pure NaBr, Spectrum, Gardena, CA). The required amount of this 3% solution usually approximated 50 mL and was drawn into 30-cc syringes using standard cold sterilizing techniques (Millex-GS, 0.22-μm Filter, Millipore, Bedford, MA). The weight of the injected NaBr was determined to the nearest milligram.

Bioelectrical impedance analysis was used as a second method of fluid determination. This technique is based on the principle that electrical conduction is proportional to the quantity of water present in a substance. A weak electrical current is passed through the body and is opposed by the body's tissues[20]; this opposition is termed impedance (Ohms, Ω). The impedance signal consists of two components: resistance (R, Ω) and reactance (X_c, Ω). Whole body R is reciprocally related to the volume of TBW,[21,22] whereas the body's X_c may reflect integrity of cellular membranes and associated volume of ECW.[23] A high ratio of X_c to R (X_c/R) is found during health, whereas low values are observed during critical illness.[24] Impedance measurements were performed across the body by attaching electrodes to a hand and a foot as described by others[21] and applying a 50-KHz alternating current generated by a plethysmograph (Model 101A, RJL Systems, Mt. Clemens, MI)

A group of healthy subjects (n = 10) served as normal controls for the patients (CTR, Table 1). These individuals were fasted after midnight, and on the study morning venous blood samples were obtained. Heavy water and bromide were taken as a drink from a glass using a straw. The glass was rinsed multiple times with distilled water at a fixed dose of 2 ml/kg bw, and this additional volume was ingested to ensure total administration of the indicators. Equilibrium blood samples were obtained 4 hours later. Subjects were not allowed to eat or drink during the tracer equilibrium period. Body impedance measurements were performed during this period.

Patient Assessment

Vital signs, body weight, and standard blood tests were monitored daily. In addition, the intake of parenteral and enteral nitrogen and calories was calculated daily. Significant clinical events, including infections, were recorded. In all cases clinical infection was associated with fever >38.5°C. Pneumonia was diagnosed by chest-roentgenogram, and bacteremia was associated with positive blood cultures. Soft tissue infection was reflected by clinical signs and symptoms of redness, pain, and local necrosis. Throat and stool were cultured routinely two to three times per week for aerobic bacteria, and additional cultures of blood, urine, sputum, and other sites were obtained when clinically indicated. The number of positive results of all these cultures was calculated between day 0 and the day of intestinal recontamination (mean interval: 25 ± 1 days). The total quantity of blood products (platelets, red blood cells), steroids, and diuretics received was tabulated. Also calculated were the number of nonprophylactic antibiotic agents administered daily and the total number of days of antibiotic use. In addition, the number of hospital days after transplantation was determined for each patient.

Blood Processing

Heparinized blood was centrifuged for 15 minutes at 3000 rpm and the plasma stored in sealed plastic tubes at −20°C. Concentrations of heavy water were determined using mass spectrometry (Model 3/60, Nuclide, Bellefonte, PA).[25] Additional venous blood was allowed to clot for 60 minutes, centrifuged (3000 rpm for 15 minutes), and stored in plastic tubes at −20 C. Before analysis samples again were centrifuged for 4 hours at 3000 rpm using a filter for deproteination (Amicon 30, Amicon Corp., Lexington, MA). Concentrations of bromide were assessed by high-pressure liquid chromatography (HPLC Model LC-6A, Shimadzu Corp., Columbia, MD) using an ultraviolet detector; the technique is a modification of a method used by others.[26] A Partisil SAX-10 column (Whatman Inc., Clifton, NJ) was used at room temperature, and the mobile phase was 25 mmol/L (or less) KH_2PO_4 to assure a retention time for bromide between 17.5 and 19 minutes. The detection wavelength was 195 nm, absorbance was 0.08, and the attenuation was 5. The injection volume

TABLE 2. *Measurements of Body Fluid Compartments (Mean ± SEM)*

Measurement	STD*	GLN	CTR
ECW (L)	14.9 ± 1.0	15.4 ± 0.8	15.2 ± 1.2
TBW (L)	40.3 ± 1.8	41.1 ± 2.4	43.6 ± 2.3
Resistance (R, Ω)	558 ± 26	565 ± 23	523 ± 25
Reactance (Xc, Ω)	67 ± 3	62 ± 3	71 ± 3
Xc/R	0.121 ± 0.004	0.110 ± 0.006	0.137 ± 0.005†

* Volumes of extracellular water (ECW), total body water (TBW), electrical resistance (R), and reactance (Xc) of patients before a standard stress were not different compared with those of healthy controls. However, when the ratio of Xc to R was determined, a significantly smaller value was obtained in both patient groups when compared with controls.
† $p < 0.05$ *vs.* STD, GLN by Fisher's PLSD post-hoc.

was 20 μL, and the samples were analyzed in triplicate. Correlation coefficients for the standard curve were ≥0.998.

Calculations and Definitions

Heavy water dilution space was calculated from the quantity of administered tracer and the venous heavy water concentration after complete distribution.[27] Total body water was calculated from these values by using a correction factor of 1.04, as previously described.[28,29] Extracellular water also was calculated according to the dilution principle, but was corrected for intracellular bromide distribution and the Donnan equilibrium.[30]

Statistical Analysis

The data were analyzed using standard statistical software (StatView #512, Abacus Concepts, Inc., Berkeley, CA) for the Macintosh SE personal computer. Analysis of variance (with Fisher's PLSD *post hoc* test) was used to determine differences between groups at initiation of the study. Linear regression analysis was used to describe the relationship between dependent and independent variables. Paired or unpaired t tests compared parametric data; the Mann-Whitney U tested nonparametric data when appropriate for single comparisons. A chi square and the Fisher's exact test were used to determine differences between expected and observed frequency. A p-value < 0.05 was considered significant. Results are expressed as mean ± standard error of the mean (SEM).

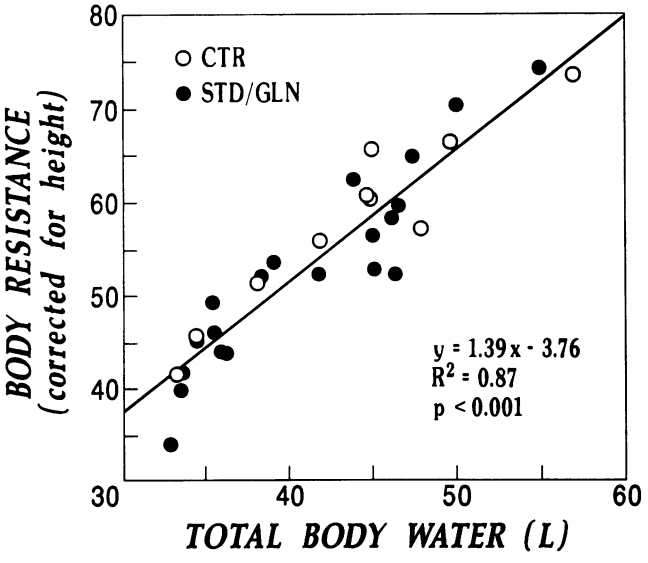

FIG. 2. At baseline, a close relationship was seen between TBW measured by heavy water dilution and body resistance (corrected for height, in cm^2/Ω) in all subjects. Patients and controls were equally distributed along the regression line.

Results

Initial Characteristics of Subjects

The patients groups were similar with respect to age, weight, height, sex, diagnosis, conditioning regimen, or GVHD-prophylaxis (Table 1). Initial ECW, TBW, R, and X_c obtained from all patients were similar and comparable to measurements obtained in healthy controls (NS [not significant] by ANOVA [analysis of variance], Table 2). The ratio of X_c/R obtained in healthy subjects, however, was significantly greater when compared with both patient groups. At baseline an inverse relationship was present between measured TBW and R in all subjects studied ($R^2 = 0.72$, $p < 0.001$). Correcting R for subject's height^2 (in cm^2/Ω) improved this relationship (Fig. 2).[20]

Intravenous Feedings

Both patient groups received comparable amounts of parenteral and enteral calories, parenteral amino acid, and oral nitrogen (Table 3). Likewise both groups of subjects received TPN for a similar duration after BMT (STD: 28 ± 2 *vs.* GLN: 25 ± 2 days, $p = 0.25$). The period between the initial and the post-TPN body compositional assessment was identical (32 ± 2 *vs.* 32 ± 2 days).

TABLE 3. *Nutrient Intake During Period of Parenteral Nutrition (Mean ± SEM)**

Intake	STD	GLN
Intravenous calories/day	2330 ± 70	2347 ± 93
Oral calories/day	265 ± 25	379 ± 70
Intravenous nitrogen (g/day)	13.9 ± 0.4	14.5 ± 1.0
Oral nitrogen (g/day)	1.1 ± 0.4	1.5 ± 0.3
Days of administration	28 ± 2	25 ± 2

* Intake of parenteral nutrients was comparable between groups with respect to calories by either route; intravenous and oral nitrogen intake was also similar.

TABLE 4. *Alterations in Body Weight During TPN Administration (Mean ± SEM)*

	Initial	Maximal	Post-TPN	Δ Total	Δ Last 8 days
STD	69.9 ± 4.4	74.4 ± 4.2	71.6 ± 4.4*	+1.8 ± 0.9	−2.8 ± 0.9
GLN	70.6 ± 3.3	74.9 ± 3.0	69.9 ± 3.0*	−0.7 ± 1.0†	−5.0 ± 1.1

Δ Total, difference between the post-TPN and initial body weight measurements; Δ Last 8 days, change in body weight over 8 days preceding the post-TPN determination.

* $p < 0.001$ over time by ANOVA.
† $p < 0.05$ vs. STD by Mann-Whitney U-test.

Alterations in Body Weight And Fluid Compartment Volumes

Body weight measured in the STD group was maximal on day 18 ± 1 after BMT and then decreased over the consequent 8 days by 2.8 ± 0.9 kg to 71.6 ± 4.4 ($p < 0.001$ by ANOVA, Table 4). Body weight determined in GLN patients peaked on day 16 ± 2, followed by a more pronounced weight loss of 5.0 ± 1.1 kg. At the post-TPN measurement, STD patients had increased their BW by 1.8 ± 0.9 kg compared with the initial measurement, which was significantly different compared with GLN patients ($p < 0.05$ by Mann-Whitney). With termination of TPN, subjects who had received STD solution retained more than 3 L of ECW (baseline ECW: 18.0 ± 1.1 L *vs.* 14.9 ± 1.0, $p = 0.012$, Fig. 3). This expanded ECW volume was associated with a tendency to increase TBW (42.2 ± 1.8 L *vs.* 40.3 ± 1.8, $p = 0.075$). In contrast patients who received GLN-enriched parenteral nutrition maintained ECW (baseline ECW: 15.8 ± 0.9 L *vs.* 15.4 ± 0.8, $p = 0.49$) and TBW (40.2 ± 2.0 L *vs.* 41.1 ± 2.4, $p = 0.45$).

Alterations in Electrical Properties of the Body

The extracellular fluid retention observed in STD patients was associated with a significant fall in R when compared with baseline values (baseline: 558 ± 26 Ω *vs.* post-TPN: 514 ± 28, $p < 0.05$, Fig. 4). Furthermore X_c was decreased by approximately 25% in these patients ($p < 0.001$). In contrast GLN patients maintained R (565 ± 23 Ω *vs.* 552 ± 27, $p = 0.42$). The fall in X_c was significant but smaller ($p < 0.05$) in GLN patients (62 ± 3 Ω *vs.* 53 ± 3, $p < 0.001$) when compared with the STD patients (67 ± 4 Ω *vs.* 50 ± 4, $p < 0.001$).

High ratios of reactance to resistance (X_c/R) are found in healthy individuals, whereas low values of X_c/R are associated with disease.[24] A significant decrease in X_c/R after BMT occurred in both patient groups combined (initial: 0.116 ± 0.004 *vs.* post-TPN: 0.098 ± 0.004, $p < 0.001$). The $\Delta X_c/R$ was −0.024 ± 0.003 in the STD group, which tended to be greater than the $\Delta X_c/R$ of −0.013 ± 0.005 observed in GLN group ($p = 0.09$). A positive relationship was present between $\Delta X_c/R$ and cumulative dose of steroids administered ($p < 0.02$), total antibiotics used ($p < 0.04$), number of units of packed cells transfused ($p < 0.02$), and days of TPN ($p < 0.04$, Fig. 5). The relationship between length of hospital stay and fall in this ratio approached significance ($p = 0.06$).

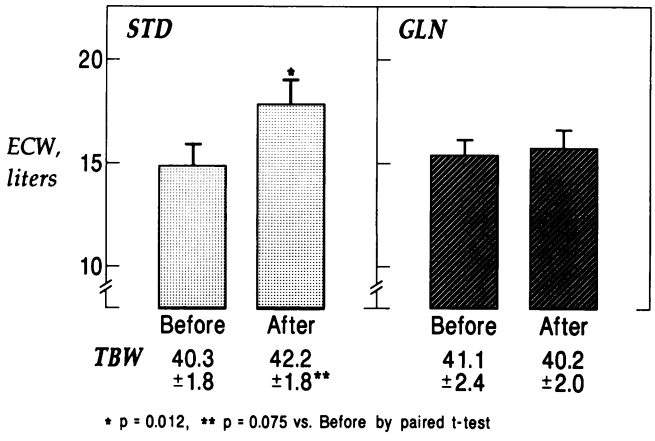

FIG. 3. Alterations in ECW and TBW. With termination of TPN (*after*), subjects who received STD solution retained more than 3 L of ECW (18.0 ± 1.1 L *vs.* 14.9 ± 1.0), which was associated with a tendency to increase TBW. In contrast, patients who received GLN-enriched parenteral nutrition showed stable ECW (15.8 ± 0.9 L *vs.* 15.4 ± 0.8 L) and TBW values. Numbers are mean ± SEM. All values in liters.

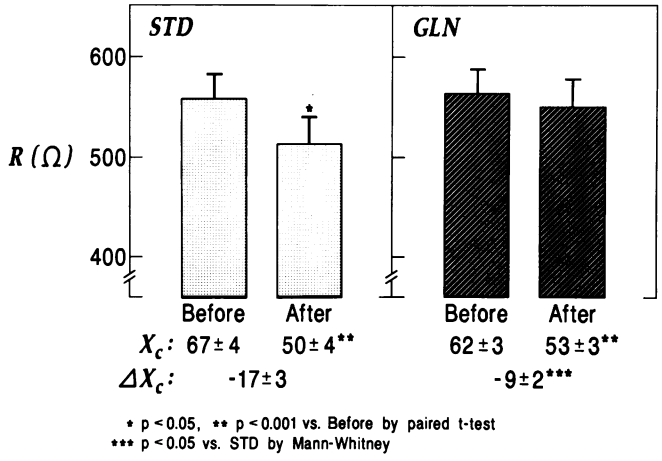

FIG. 4. Alterations in electrical properties of the body. The fluid retention observed in the STD patients was also reflected by a decrease in electrical resistance measured across the body (R: 558 ± 26 Ω *vs.* 514 ± 28, $p < 0.05$). In contrast, resistance in patients who received GLN-enriched TPN was comparable when compared with pretreatment values (R: 565 ± 23 Ω *vs.* 552 ± 27, $p = 0.42$). The decrease in electrical reactance (X_c, in Ω) was significantly attenuated in the GLN patients ($p < 0.05$). Numbers are mean ± SEM.

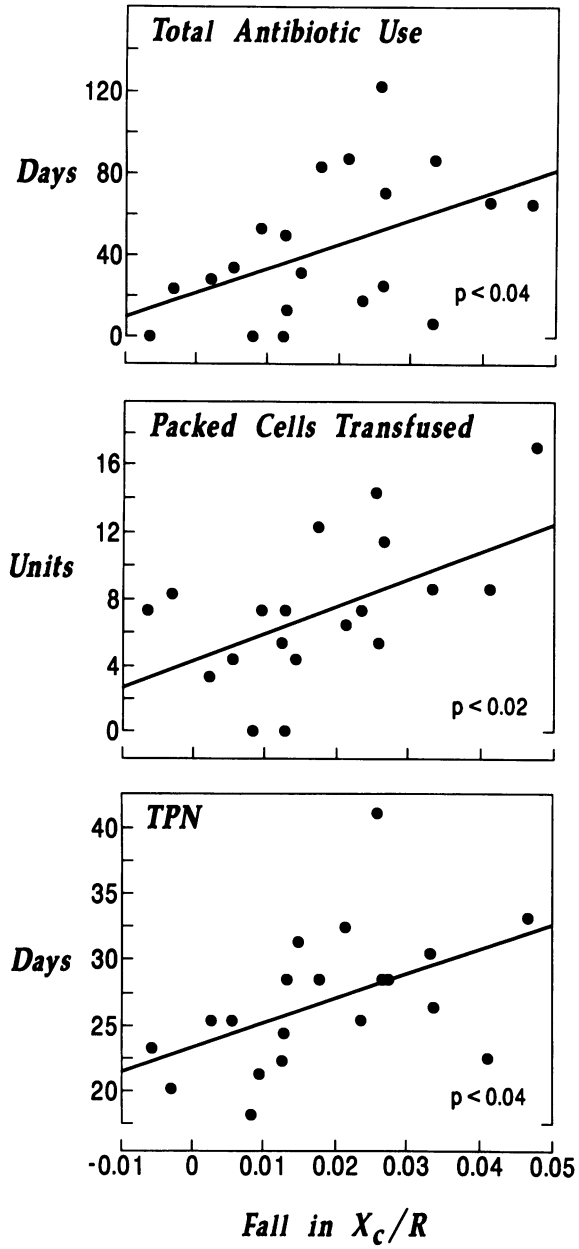

FIG. 5. Associations between decrease in X_c/R ($=\Delta X_c/R$), quantity of antibiotics used, units of red blood cells transfused, and days of TPN received. These associations suggest that this ratio is correlated with the severity of illness.

Treatment Comparability

Daily intravenous and oral fluid intake and electrolytes administered were similar for both groups during the study period. In addition, mean daily fluid losses and fluid balance were comparable between groups (Table 5). No differences were observed between the number of units of blood products (platelets, packed cells) received ($p = 0.96$), or quantity of diuretics ($p = 0.76$) or steroids ($p = 0.65$) administered.

TABLE 5. *Fluid and Medication Administered During TPN (Mean ± SEM)**

Treatment	STD	GLN
Fluid intake (L/day)†	5.553 ± 0.359	5.429 ± 0.278
Fluid output (L/day)†	4.643 ± 0.431	4.557 ± 0.318
Fluid balance (L/day)†	0.911 ± 0.101	0.872 ± 0.152
Packed cells/platelets (units)	15 ± 2	16 ± 3
Lasix (mg prescribed)	106 ± 45	127 ± 51
Steroids (mg cortisol)	168 ± 43	247 ± 152

* No differences were observed between groups with respect to fluid intake, output, and balance; in addition, blood products and medication prescribed were similar.

† Determined over first 18 days of TPN administration, since dropouts after day 18 rendered further comparisons invalid.

Results of Cultures and Clinical Infection

Cultures obtained from the throat, stool, urine, and blood indicated that 45 of 155 in the STD group were positive for the growth of aerobic bacteria (29%, Table 6). In contrast only 29 of 188 cultures (15%) in the GLN group demonstrated bacterial growth (chi square = 9.3, $p < 0.01$). An association was present between increase in ECW and number of positive cultures ($r = 0.47$, $p < 0.05$); the fall in X_c and increase in ECW were also positively correlated ($r = 0.7$, $p < 0.001$).

The occurrence of clinical infections was more frequent in the STD group: Pneumonia was diagnosed in two patients, necrotizing soft tissue infection in two additional individuals, and bacteremia in a fifth patient. In contrast clinical infection was not present in patients receiving GLN ($p = 0.033$ by Fisher's exact test). The mean expansion of ECW in clinically infected STD patients was 5.0 ± 1.4 L, which was significantly more than the mean of 0.7 ± 0.5 L observed in uninfected patients ($p = 0.007$ by Mann-Whitney, Fig. 6); alterations in uninfected STD patients were not different from values determined in GLN subjects (1.2 ± 0.8 *vs.* 0.4 ± 0.5, NS).

Discussion

The purpose of this study was to examine the effects of GLN-enriched parenteral feedings on distribution of body fluids in a homogenous group of patients undergoing

TABLE 6. *Cultures and Clinical Infection*

	STD	GLN
Positive	45	29
Total	155	188
%	29	15*
Patients with clinical infection	5/10	0/10†

* $p < 0.01$ *vs.* STD by chi square.

† $p < 0.05$ *vs.* STD by Fisher's exact test.

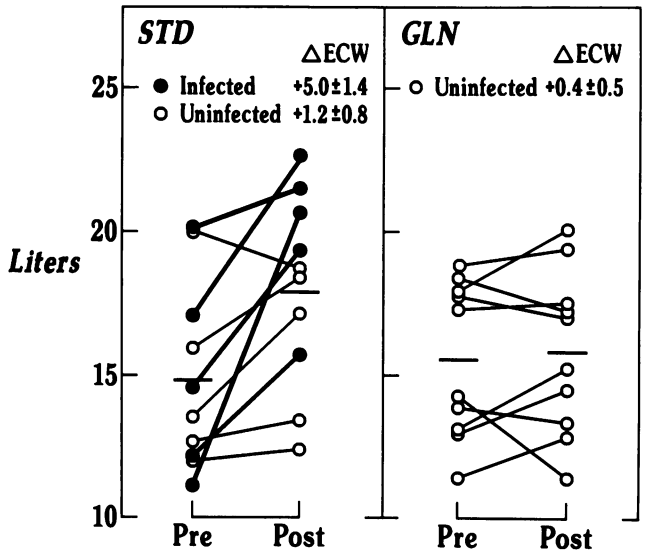

FIG. 6. The relationship between ECW expansion and infection. A clinical infection (pneumonia, soft-tissue infection, or bacteremia) was present in half of the patients who received the STD solution (●); these infected patients had expanded their ECW by 5.0 ± 1.4 L. The remainder of the STD patients did not show infection (○), and Δ ECW in these patients was significantly lower (+1.2 ± 0.8 L). In contrast, infection in patients receiving the GLN solution was absent, whereas ECW volumes were stable (+0.4 ± 0.5 L).

a standard catabolic insult. Our studies confirm previous reports that the administration of commercially available intravenous feedings is associated with expansion of ECW in a similar patient population.[10] In our study patients receiving the STD-TPN retained more than 3 L of ECW, which also was reflected by a marked fall in the electrical resistance of the body. In contrast matched subjects who received the GLN-enriched solution demonstrated normal ECW volumes and stable electrical resistance at the termination of the TPN. Because the two patient groups received similar quantities of sodium, water, calories, protein, blood products, diuretics, and steroids, exogenous or treatment factors did not appear to contribute to this effect.

Glutamine may affect fluid balance by multiple mechanisms. For example GLN is taken up by the kidney and donates NH_3, which couples with H^+ to form urinary NH_4^+. Under physiologic conditions 30% to 50% of the daily acid load is excreted by this mechanism.[31] Excretion of H^+ is coupled with mandatory reabsorption of Na^+ and associated water. Patients receiving the STD-TPN demonstrated a higher frequency of positive bacterial cultures and clinical infection, which may be associated with increased generation of acid substances. If this occurred the increased H^+-load may have enhanced Na-reabsorbtion and contributed to the subsequent ECW expansion in the STD patients. A positive correlation between increased ECW and number of positive cultures ($p < 0.05$) and the greater expansion of ECW in clinically infected patients are both suggestive of this mechanism. Venous HCO_3^- was not different between groups (STD: 24 ± 1 vs GLN: 23 ± 1), however, and urinary sodium excretion measured in selected patients also was comparable.

An alternative explanation may be that GLN ameliorated the increase in capillary permeability, which typically occurs after chemotherapy, irradiation, or severe infection. Injury to endothelial cells may be the result of increased production of free oxygen radicals, and GLN may be important in providing antioxidant protection. For example GLN appears to be an essential precursor in the synthesis of glutathione (GSH).[32] During specific conditions GSH is a major antioxidant that protects tissues from free oxygen radical damage.[33,34] Glutathione depletion may occur in the STD patients, and GSH may have been maintained in patients receiving GLN. Hinshaw et al.[35] suggested an additional mechanism of protection associated with GLN. They found that GLN added to culture media protected endothelial cells after H_2O_2 exposure by serving as a fuel for adenosine triphosphate generation.[35] Others have reported that GLN suppresses inflammation and the associated edema formation that occur after various inflammatory stimuli.[36] Glutamine therefore may diminish extravasation of fluids by preventing "leakage" through endothelial cells and subsequently may blunt the expansion of the extracellular volume. Body weight gain was similar for both groups, however, over the first 16 to 18 days after BMT. Similar increases in body weight may indicate that a comparable expansion of the extracellular fluid compartment occurred in both groups, and that after these events GLN-supplemented nutrition facilitated a more efficient diuresis associated with recovery. Such effects suggest that GLN may be associated with modulating the neuroendocrine control of sodium and water homeostasis.[37]

The effects of GLN on elaboration of arginine vasopressin, renin, or aldosterone are unknown, although GLN is thought to have effects on the central nervous system.[38] Furthermore GLN may stimulate increased elaboration of glucagon.[39] Animal studies have shown an association between portal infusion of glucagon and increased glomerular filtration rate.[40] Previous studies in normal volunteers, however, did not show elevations of glucagon in peripheral blood samples after intravenous administration of GLN[41]; concentrations of glucagon were not measured in our patients.

Restoration of extracellular fluid volumes also may be related to the beneficial effects of GLN on gut metabolism. Glutamine attenuates atrophy of gut mucosa, which is associated with prolonged administration of intravenous feedings,[14] and this amino acid serves as an important fuel for enterocytes, colonocytes, macrophages, and lymphocytes. Increased availability of GLN during the regen-

eration phase of the mucosa after gastrointestinal injury[9] may have facilitated repair and accelerated mobilization of bowel and mesenteric edema. Moreover, GLN administration may have facilitated lymphocyte proliferation and enhanced phagocytosis, as demonstrated by others.[42] The combined responses of mucosal repair and immunoenhancement may have improved host defense and attenuated microbial invasion and subsequent infection, which frequently originates from the gastrointestinal tract in this patient population.[43]

Before treatment the body fluid compartments were similar in patients and controls: these groups also demonstrated comparable body resistance (R) and reactance (X_c). The ratio of X_c to R, however, was significantly lower in patients compared with normals. Several observations have suggested a relationship between a low X_c/R and the presence of illness. For instance, progressive malnutrition in acquired immune deficiency syndrome (AIDS) patients was associated with a declining ratio.[44] In addition another quantative measure of malnutrition also suggests that X_c falls with nutritional depletion in surgical patients.[45] We also have observed that this ratio falls markedly in surgical patients[24] and returns to normal with recovery. In the present study, the fall in the ratio was generally related to increased numbers of therapeutic interventions such as the dose of steroids and antibiotics administered, units of blood products transfused, days of TPN administration, and length of hospital stay. These associations suggest that the X_c/R ratio may be an objective measure of severity of illness and possibly may predict recovery.

In conclusion, catabolic patients receiving a standard glutamine-free intravenous solution after intensive chemotherapy and total body irradiation demonstrated expanded extracellular fluid compartments coupled with low values of electrical resistance; these events were associated with a greater number of positive microbial cultures and more clinical infection. In contrast matched patients who received GLN-enriched intravenous feedings demonstrated unaltered fluid compartments and a stable body resistance; number of positive cultures was diminished and clinical infection was absent in GLN patients. Intravenous feedings supplemented with GLN aid in restoring normal body fluid distribution after critical illness, possibly by attenuating microbial invasion and infection of the host.

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Discussion

DR. EDWARD M. COPELAND, III (Gainesville, Florida): There is little question that glutamine is metabolically beneficial during critical illness. For example endotoxin, tumor necrosis factor, and interleukin-1 (IL-1) all stimulate glutamine uptake by endothelial cells threefold. Perhaps the endothelial cell requirement for glutamine during critical illness is increased to support metabolism and repair, as shown by both Dr. Wilmore's laboratory and by ours.

Likewise Tom Austgen and Chip Souba from our laboratory have shown that preinfusion of glutamine to rats decreases the amount of pulmonary edema that develops after standard endotoxin challenge.

Dr. Scheltinga has investigated the effect of glutamine on maintenance of water homeostasis in a group of young, nutritionally stable patients who are challenged with systemically toxic doses of chemotherapy and with whole-body irradiation. These patients received an average of 5½ L fluid daily for 3 to 4 weeks, along with Lasix, platelets, blood, and steroids. Both groups of patients gained an equal amount of weight for the first 16 days, but the glutamine group returned to baseline by day 25. The standard TPN group appeared sicker, because they had more infections and a lower ratio of reactance to resistance, which correlated with the use of steroids, antibiotics, and platelets.

Dr. Scheltinga, I must ask you, with all of these variables in this patient population, how do you know that glutamine was an effective compound, or was the standard therapy group just more ill and the return to baseline sooner in the glutamine group the consequent expected outcome?

You must have other measures of nutritional state, such as serum albumin concentration, retinol-binding protein, etc., that demonstrate better metabolic maintenance in the glutamine group. Share these with us.

Why did the two groups receive different amino acid solutions, particularly one high in essential amino acids? Did not this add another confounding variable?

I want to believe, and in fact do believe, that glutamine ameliorates capillary fragility, lowers infection rate, provides antioxidant protection, and repairs endothelial cell injury. However it is difficult for me to extrapolate these conclusions solely from your study of change in body fluid compartments and in bioelectrical impedance in this patient population.

DR. JOHN R. BORDER (Buffalo, New York): This is an extremely important paper. It is important not because of the fluid balance changes in the abstract but because those fluid balance changes reflect septic complications. The radiation and chemotherapy delivered to these patients effectively destroy their gut mucosa. To prevent septic complications, the patient and his gut are largely sterilized before radiation and chemotherapy and the patient is thereafter maintained in isolation with as much sterility as possible. During this time there is severe diarrhea and emesis plus the isolation procedures that make many standard study techniques practically impossible. The electrical impedance technique was used here as a practical study technique.

During the period of isolation, not only is there regrowth of gut mucosa but also of the gut bacterial population. The septic problems reported here are almost undoubtedly gut-origin septic states. The essence of the paper is that glutamine-enriched amino acid solutions as compared with standard amino acid solutions greatly reduced the gut-origin septic state problems. This is to be expected granted the importance of glutamine as a gut mucosa energetic fuel.

It must be clearly noted in these patients that the critical maneuver after destruction of the gut mucosa is to achieve regrowth of the gut mucosa before the bacteria repopulate the gut. This requires both gut mucosa energetic fuels and amino acids. Glutamine is one such energetic fuel. The ketone bodies and short-chain fatty acids are others, and these are clearly of most benefit to the colonic gut mucosa. These agents can now easily be given in quantity as their mono or diglyceride ester. The *in vivo* esterases then release the ketone body as short-chain fatty acid and glycerol.

This paper confirms in a practical clinical way the existence of the gut origin septic states and suggests that support of these unfortunate young people can be greatly improved. However because the gut-origin septic states are probably very common in all critically ill people, the information obtained may have a much broader application then in the patients studied.

DR. BASIL A. PRUITT, JR (San Antonio, Texas): This report is yet another in the series of studies from Dr. Wilmore's laboratory demonstrating that hormonal manipulation and the addition of specific dietary constituents can influence the risk of infection, preserve lean body mass, and alter the duration and velocity of convalescence in surgical patients.