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Efficacy and Safety of Glutamine-Supplemented Parenteral Nutrition in Surgical ICU Patients: An American Multicenter Randomized Controlled Trial

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Abstract

Objective—To determine whether glutamine (GLN)-supplemented parenteral nutrition (PN) improves clinical outcomes in surgical ICU (SICU) patients.

Summary Background Data—GLN requirements may increase with critical illness. GLN-supplemented PN may improve clinical outcomes in SICU patients, but data in patient subgroups are limited.

Methods—A parallel-group, multicenter, double blind, randomized, controlled clinical trial in adults after gastrointestinal, vascular, or cardiac surgery who required PN and SICU care. Subjects were without significant renal or hepatic failure or shock at entry. All received isonitrogenous, isocaloric PN [1.5 g/kg/d amino acids (AA) and energy at $1.3 \times$ estimated basal energy expenditure]. Controls (n = 75) received standard GLN-free PN (STD-PN); the GLN group (n = 75) received PN containing alanyl-GLN dipeptide (0.5 g/kg/d), proportionally replacing AA in control PN (GLN-PN). Enteral nutrition (EN) was advanced and PN weaned as indicated. Hospital mortality and infections were primary endpoints.

Results—Baseline characteristics, days on study PN and daily energy and amino acid/protein intakes via PN and EN were similar between groups. There were 11 hospital deaths (14.7%) in the GLN-PN group and 13 deaths in the STD-PN group and (17.3%; difference, -2.6% ; 95% confidence interval -14.6 to 9.3% ; $P = 0.66$). The 6-month cumulative all-cause mortality was 31.4% in the GLN-PN group and 29.7% in the STD-PN group ($P = 0.88$). Incident bloodstream infection rate was 9.6 and 8.4 per 1000 hospital days in the GLN-PN and STD-PN groups, respectively ($P = 0.73$). Other clinical outcomes and adverse events were similar.

Conclusions—PN supplemented with GLN dipeptide was safe but did not alter clinical outcomes among SICU patients.

Mini-Abstract

Glutamine requirements may increase in catabolic states. This multicenter, double blind, randomized, controlled clinical trial evaluated the safety and efficacy of glutamine (GLN) dipeptide-supplemented parenteral nutrition (PN) in 150 adults requiring postoperative surgical intensive care unit (SICU) admission. PN supplemented with GLN was safe but did not alter clinical outcomes.

Introduction

Over the past several decades, numerous studies in animal models of catabolic and critical illness indicate that parenteral nutrition (PN) supplemented with the non-essential amino acid glutamine (GLN) may enhance protein anabolism, gut-associated barrier functions, systemic immunity and gut mucosal repair, potentially via GLN use as an important fuel substrate and via upregulation of cytoprotective pathways (1–5). Concomitantly, small randomized controlled trials (RCTs) in postoperative and/or critically ill medical and surgical patients demonstrated that PN supplemented with L-GLN (or water soluble and heat-stable GLN dipeptides) improved nitrogen balance, gut barrier function, indexes of immunity and/or clinical outcomes (including reduced hospital-acquired infections, length of stay, and mortality) (6–12).

Earlier experimental data showed that organ GLN uptake was increased during catabolic states by the splanchnic bed, immune cells, and other tissues (1). GLN levels in human skeletal muscle decreased markedly after major surgery and in critically ill patients admitted to intensive care units (ICU) (2, 6–7, 13). In addition, low GLN levels early in the ICU course were associated with adverse clinical outcomes (14).

Relatively small RCTs of GLN-supplemented PN in patients after major operation and/or critical illness suggest beneficial effects on hospital infections (15–17), length of hospital stay (18) and 6-month mortality (11, 19). However, other prospective trials in post-operative patients showed no significant clinical outcome benefits with intravenous GLN (20–22). Further, intent to treat analysis of several recently published, double-blind, multicenter RCTs in mixed ICU patients showed no benefit on clinical outcomes with GLN-supplemented PN (23–25). Most RCTs have not focused on specific types of critically ill medical or surgical patients; thus, characteristics of those who may benefit (and the effective GLN dose) remain an area of uncertainty (26–30). This is reflected in the results of several meta-analyses on efficacy of GLN-supplemented PN in critical illness, which often have shown improved specific clinical outcome parameters, but with trial heterogeneity and data uncertainty (31–35).

No RCT of GLN-supplemented PN to date has suggested any adverse effects due to GLN supplementation. However, the recently published large REDOXs RCT enrolled primarily medical ICU patients with multiple organ failure and shock, who were given a high dose of combined enteral and parenteral GLN, with or without antioxidants, beginning on the day of ICU admission and independent of enteral or parenteral nutrition support (36). The study showed no effects of GLN on organ failure or overall infection rates; however, the GLN-treated arms demonstrated a modest, but statistically significant, increase in hospital and 6-month mortality (36). This was particularly true in patients with early renal failure (37). Therefore, it is important to define the safety of parenteral GLN in ICU settings and in specific subgroups of patients who potentially may benefit from GLN supplementation.

This RCT (the “GLND” trial) was a prospective, randomized, controlled, double blind, parallel-group, intent-to-treat, multicenter investigator-initiated Phase III study designed to define the safety and clinical efficacy of GLN dipeptide-supplemented PN in SICU patients

after cardiac, vascular or intestinal surgery. The study design and power was determined by an earlier single-center trial in which GLN dipeptide-supplemented PN significantly decreased nosocomial infections in the subgroup of patients following cardiac, vascular, and colonic surgery (17). We hypothesized that the current trial would decrease hospital mortality and new hospital-acquired infections (primary endpoints).

Methods

Informed Consent

The Institutional Review Boards of Emory University (Atlanta, GA, USA), Vanderbilt University (Nashville, TN), Miriam Hospital (Providence, RI), University of Wisconsin-Madison (Madison, WI) and University of Colorado (Aurora, CO) approved this study [[Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00248638) identifier: NCT00248638]. All subjects or their legally authorized representatives signed site-specific approved consent forms prior to randomization.

Study coordination and oversight

The GLND Steering Committee included the overall Principal Investigator (PI; TRZ), the National Institute of Diabetes and Digestive and Kidney Diseases U01 grant Project Scientist (MEE), the Data Coordinating Center (DCC) Director (KAE) and the site PIs (AKM, HCS, KAK, PEW) and was the main oversight body of the study responsible for the implementation, coordination and management of the trial. The Steering Committee reviewed and analyzed progress of the trial, monitored performance at individual clinical centers, and responded to recommendations from the NIDDK-convened Data and Safety Monitoring Board (DSMB).

The GLND Data Coordinating Center (DCC), located at the Emory University Rollins School of Public Health, Department of Biostatistics and Bioinformatics, was responsible for data management and quality control, case report form (CRF) generation, and statistical analysis. The DCC also prepared interim and final analyses blinded and open semi-annual DSMB reports.

An independent DSMB, established by the NIDDK, consisted of four experts in SICU nutrition support and a biostatistician. The DSMB met twice yearly with the Emory-based study leadership and the NIDDK Program Official.

Patient eligibility for enrollment and recruitment

Adult patients were screened for enrollment if the following criteria were met: 1) patient required admission to the surgical intensive care unit (SICU) following cardiac, non-neurologic vascular, or complete or partial esophageal, gastric, or intestinal surgery or after exploratory laparotomy to identify a source of peritonitis when evidence of a bowel perforation was present; and 2) patient deemed by the investigator team (each led by an expert in SICU nutrition support) and attending physician to likely require parenteral nutrition (PN) for ≥ 7 subsequent days. Informed consent was obtained from all study participants or their legally authorized representative.

Inclusion criteria were: 1) age 18–90 years; 2) body mass index (BMI) < 40 kg/m² prior to surgery; 3) requires current SICU care and is > 14 days postoperative from the following open (non- laparoscopic) surgical procedures: CABG, cardiac valve, vascular (non-neurological), complete or partial esophageal, gastric, small bowel, colon and/or rectal resection or exploratory laparotomy to identify a source of peritonitis when evidence of a bowel perforation was present; 4) deemed to require central venous PN for > 7 subsequent days after entry; 5) central venous access for administration of the study PN in place by entry; 6) patient's primary physician(s) will allow the investigative team to manage the study PN and enteral feedings during the current hospitalization.

Exclusion criteria were: 1) pregnancy; 2) current clinical sepsis, defined as an unstable blood pressure despite vasopressor agent support AND mean arterial pressure (MAP) < 60 mm Hg on at least 3 consecutive readings within a 3-hour period during the 24 hours prior to study entry; 3) current malignancy requiring surgery as the study qualifying operation or receiving an active regimen of chemotherapy and/or radiotherapy to treat a previously diagnosed malignancy; 4) history of seizures or pre-existing seizure disorder; 5) current encephalopathy; 6) known history of cirrhosis or a serum total bilirubin level > 10.0 mg/dL; 7) history of chronic renal failure requiring dialysis, or significant renal dysfunction (defined as serum creatinine > 2.5 mg/dL and not receiving continuous renal replacement therapy (CRRT) or the patient requires acute hemodialysis postoperatively; 8) concomitant burn or trauma injury; 9) previously organ transplant; 10) history of HIV/AIDS; 11) administration of any investigational drug within 60 days prior to study entry; 12) administration of enteral or parenteral enteral feedings enriched in arginine and/or glutamine within 30 days prior to study entry; and 13) subject unable or unwilling to participate in study procedures such as longitudinal blood draws and outpatient follow-up visits. The most recent available blood renal and hepatic function test results in the medical record were used to determine eligibility.

Randomization of study subjects

Following informed consent, the study team at each site calculated the Acute Physiology and Chronic Health Evaluation II (APACHE II) score (38). Treatment assignments were stratified according to clinical center and on the illness severity (APACHE II score dichotomized as ≤ 15 or > 15). The APACHE II score CRF was received at the DCC by the DataFax data management system using optical character recognition software to create data records from the CRFs (www.datafax.com). Treatment assignments were generated using a pseudo-random-number generator with randomly permuted blocks. The un-blinded PN pharmacist manager at each clinical center maintained two color-coded sets of sealed, sequenced, opaque envelopes containing the treatment assignment (APACHE II stratum ≤ 15 or > 15). Each envelope uniquely identified the clinical center and illness severity stratum and the sequence number. All other individuals involved in the study were blinded to the randomization, with the exception of DCC biostatisticians (TL, GAC, ESR) who prepared biannual closed reports to the DSMB. TL served as the unblinded DCC biostatistician during the closed session of each DSMB meeting.

Baseline data collection

Baseline clinical data was obtained on the day of randomization and before initiation of study PN. These data included demographic data, dates of initial hospitalization and specific index operation, APACHE II score (38) upon admission to the SICU, indication for PN, days in SICU prior to study entry, entry day Sepsis-related Organ Failure Assessment (SOFA) score (39) preoperative body weight, height, body mass index [BMI; weight (kg)/height (m²)] presence of acute respiratory distress syndrome (ARDS), current requirement for mechanical ventilation and evidence of nosocomial infection postoperatively (prior to study entry), based on Centers for Disease Control and Prevention (CDC) definitions for healthcare associated infections (40). Use of PN and/or enteral tube feedings within the 30-day period before entry and the number of days of these nutritional interventions were also recorded. Test results available in the electronic medical record (EMR) were recorded: white blood cell count (WBC), serum renal function tests [blood urea nitrogen (BUN) and creatinine concentrations] and hepatic function tests [total bilirubin, alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT)].

Blood was collected in the AM (0800–1000 h) and serum and plasma aliquots stored at –80°C, for later batch analysis of serial study samples within individual subjects for GLN and glutamate concentrations and blood chemistry levels from all clinical centers at Emory University. Serum was also obtained for later batch analysis for concentrations of glucose, BUN, creatinine, total bilirubin, alkaline phosphatase, ALT and AST, performed using standard chemical analyzer methods at Emory University Hospital, Atlanta, GA. Amino acid analysis was performed using a Beckman System 6300 High Performance Amino Acid Analyzer (Beckman Instruments, Inc. Palo Alto, CA) at the Emory Genetics Laboratory. In the latter 3 years of the study, AA were analyzed using a Biochrom 30 Amino Acid Analyzer (Biochrom US, Holliston, MA).

Baseline (entry day) blood glucose (BG) was recorded from the EMR at three predefined time points (between 4 and 6 AM using the hospital laboratory value if obtained), and subsequent representative laboratory or hospital floor point-of-care BG level obtained between 2–4 PM and again between 10 PM–12 AM. If a BG value was not recorded for the specified time intervals, the most recent to the outside time point was recorded.

Study PN formulations

After randomization, PN calorie (kcal), amino acid (AA), dextrose, and fat emulsion composition for both control (STD-PN) and experimental (GLN-PN) formulas was calculated by the blinded study team. This data was faxed to the PN pharmacist manager at each clinical center for preparation of the appropriate study PN solution.

Study PN was given for a maximal time of 28 days after entry. Conventional methods for PN administration and daily composition adjustment for critically ill patients were used (41–42). Subjects who received GLN-free standard PN before and/or after surgery were eligible.

The overall total daily kcal intake goal after entry was 1.3 times basal energy expenditure (BEE) from study PN, plus any kcal provided by dextrose-containing IV fluids (when > 500mL/day), propofol and clevidipine, and enteral nutrition (EN). BEE was calculated via

the Harris-Benedict equation (43). The initial total amino acid intake goal from study PN was 1.5 gm/kg/day (41–42). A conventional GLN-free complete PN amino acid formula (15% Clinisol® Baxter Inc., Deerfield, IL) was used in STD-PN (1.5 g/kg/day). In GLN-PN, a 20% alanyl-GLN dipeptide solution (Dipeptiven®, Fresenius-Kabi, Bad Homburg, Germany) was admixed to provide alanyl-GLN dipeptide at 0.5 g/kg/day and thus 15% Clinisol® at 1.0 g/kg/day was used such that the two formulas provided the same total PN amino acid dose/kg body weight.

If the subject was > 125% of ideal body weight (IBW), adjusted body weight [ABW= IBW + (current weight-IBW x 0.25)] was used to calculate energy and amino acid requirements (42). In both STD-PN and GLN-PN, dextrose initially comprised 70% of study PN non-amino acid kcal and a standard soybean oil-based fat emulsion (20% Intralipid®, Fresenius Kabi, Uppsala Sweden) initially comprised 30% of study PN non-amino acid kcal daily. Conventional formulations of vitamins (10 mL of MVI Adult™, Mayne Pharma, Paramus, NJ) and trace elements (1 mL of Multitrace™-5 Concentrate, American Regent, Shirley, NY) were added to both STD-PN and GLN-PN daily (42).

The study PN was continued after the subject was discharged from the SICU to the regular floor if PN was deemed to be indicated by the investigators and attending physician. If the study PN was initially discontinued before the 28 day time point from entry, but later reinitiated on clinical grounds, the subject was placed on the same study PN type as previously administered (i.e., STD-PN or GLN-PN), until 28 days of study PN administration, after which non-study PN was administered as indicated, at the discretion of the attending physicians.

Enteral tube feeding, using conventional formulas that were not GLN- or arginine-enriched, were initiated and advanced at each site via feeding tube as clinically indicated. The amount of study PN administered was proportionally decreased as a function of EN (tube feeding and oral diet) intake to maintain the daily kcal and amino acid/protein goals. Study PN was discontinued when the subject received more than 50% of their caloric intake goal enterally for a consecutive 48-hour period.

Study procedures overview

Subjects were followed for a total of 6 months after entry. Blood sampling for GLN, glutamate, glucose, renal function and hepatic function was performed at entry (day 1, prior to initiation of study PN) and serially, when possible, on study days 3, 7, 14, 21 and 28 after entry. Subjects were contacted via telephone two, four and six months after randomization to determine vital status and whether they had been re-hospitalized or readmitted to the SICU. The overall study schema is shown in Supplemental Figure 1.

Procedures for insulin administration and BG management

The BG (BG) goal range for the trial was 80–130 mg/dL. Conventional SICU methods were used to achieve goal BG levels over time (44).

Longitudinal data collection

Daily energy and amino acid intake from study PN and EN was determined daily during the 28 days after enrollment or until hospital discharge, whichever came later. Daily BG data obtained from baseline to day 28 was determined. Subjects were monitored for clinical outcomes while hospitalized, including the incidence of new nosocomial infections, daily SOFA score while in the SICU, body weight, concomitant medication use, SICU and hospital length of stay (LOS), presence or absence of ARDS, and ventilator free days (VFD), defined as the number of days within the first 28 days after enrollment on which the patient was alive and breathing without ventilator assistance for ≥ 48 h (45). Conventional methods for mechanical ventilation and sedation were employed at all clinical study sites (46–48).

Assessment and surveillance for hospital-acquired infections

Hospital-acquired infections were diagnosed based on standardized CDC criteria (www.cdc.gov/ncidod/dhqp/pdf/nNIS/NosInfDefinitions.pdf) (40). Incident nosocomial infections were not diagnosed until > 48 hours after Day 1 study PN initiation to minimize the chance that the infection was actually prevalent (but undiagnosed) prior to study PN entry. All prevalent and incident infections were adjudicated by review of the required pertinent data from all clinical sites in a blinded fashion by an infectious disease specialist co-investigator (HMB) at Emory University (40).

In addition to total new (incident) hospital-acquired infections (second primary endpoint), secondary hospital infection endpoints monitored included: 1) Rate of new BSIs and other site-specific infections; 2) Rate of new hospital infections attributed to Gram-positive microorganisms; 3) Rate of new hospital infections attributed to Gram-negative microorganisms; and 4) Rate of new infections attributed to fungal pathogens.

Six-month mortality determination

Six-month mortality was determined via telephone contact with the patient's home at 2, 4 and 6 months after enrollment.

Adverse event (AE) and serious adverse event (SAE) monitoring and reporting

All serious adverse events (SAEs) and unexpected and expected adverse events (AEs), as well as significant clinical or surgical events as narrative data, were recorded in the CRF. SAEs and AEs were reported up to and including 30 days after study PN discontinuation.

Sample size and power considerations

Our pilot RCT in SICU subjects following cardiac, vascular, or colonic surgery showed that the hospital mortality rate was 22% (6 deaths) for the combined 27 subjects: [STD-PN 5/12 (42%) and GLN-PN 1/15 (7%), respectively; $P=0.06$] (18). GLND goal enrollment was determined to be 150 subjects (75 subjects in each arm), to provide 90% statistical power to detect a 25% difference in hospital mortality (42% versus 17%) with a two-sided Fisher's exact test and type I error rate of 5%. Our pilot RCT showed that 83% of the STD-PN and 64% of the GLN-PN group developed new hospital-acquired infections after entry (18).

GLND group sample sizes of 75 subjects in the STD-PN group and 75 subjects in the GLN-PN for GLND achieved 90% power to detect a 25% difference between study groups in the percentage of subjects with new infections (83% versus 58%).

Statistical analysis

The primary analyses of the data were performed according to patients' original treatment assignment [i.e., intention-to-treat (ITT) analyses] and the inclusion of all data from all patients randomized in the final analysis. Hospital mortality was compared between treatment groups using a χ^2 test. Confidence intervals (95%) were calculated for hospital mortality rates within each study cohort and for the observed difference in hospital mortality rates. Hospital-acquired infection rates per 1000 hospital days were estimated and compared between treatment groups, using exact methods based on the Poisson distribution. Cumulative mortality was estimated with the Kaplan-Meier method and compared between treatment groups with the log-rank test.

Repeated-measures analyses of daily SOFA scores, daily BG concentrations and serial GLN, glutamate and blood chemistry concentrations were analyzed with a means model with SAS Proc Mixed (version 9, mixed linear models) providing separate estimates of the means by time on study (daily for SOFA scores, baseline and days 3, 7, 14, 21 and 28 days for GLN, glutamate and blood chemistry concentrations; daily with morning, afternoon and evening measurements of BG concentrations) and treatment group. A compound-symmetric variance-covariance form among the repeated measurements was assumed for each outcome and robust estimates of the standard errors of parameters were used to perform statistical tests and construct 95% confidence intervals. The model-based means are unbiased with unbalanced and missing data, so long as the missing data are non-informative (missing at random). Reported P values are two-sided.

The primary outcomes were hospital mortality and incident total hospital-acquired infections. Relative risks were calculated to measure the degree of association between APACHE II score at SICU admission (quartiles) and hospital mortality. Additionally, the relative risk for hospital mortality was estimated with a log-binomial regression model including treatment group and APACHE-II score as covariates. The potential association between clinical outcomes (hospital mortality; incident infection) and the entry plasma glutamine concentration was evaluated with a chi-square test. Other secondary clinical outcomes (ventilator-free days, ICU and hospital LOS after entry) were compared between treatment groups with the Wilcoxon rank-sum test.

The hyperglycemia and hypoglycemia rates (episodes per 1000 hospital days) by treatment group were estimated and compared by performing a generalized estimating equation Poisson regression analysis of the daily counts, implemented using SAS Proc Genmod, using an exchangeable correlation structure for the repeated daily counts within patient. Each serious adverse event and each adverse event was counted once per patient when first identified and compared between treatment groups with a chi-square or Fisher's exact test.

One planned interim analysis for all-cause mortality was performed before the final analysis. A Lan-DeMets spending function was used, with stopping boundaries corresponding to the

O'Brien-Fleming stopping rule. The DSMB regularly reviewed accrual, quality control, safety and efficacy and approved the interim analysis plan proposed by the DCC.

Additional details on Methods are provided in the on-line Supplemental Material.

Results

Subjects

A total of 1,247 subjects were assessed for eligibility (Figure 1). A total of 150 subjects were randomized to receive either STD-PN (n=75) or GLN-PN (n=75). Demographic and clinical characteristics were comparable between the two study groups (Table 1). One hundred subjects completed 6-months of follow-up. Forty-five patients died. One subject withdrew consent on day 7 and four were lost to follow up at days 39, 59, 64 and 121 days.

Amino acid/protein and caloric intake from study PN and EN

Non-study PN was administered within 30 days prior to enrollment in 122/150 (81%) subjects for a median of 3 days. Non-study EN (tube feeds) was administered within 30 days prior to enrollment in 20/150 (13%) subjects for a median of 3 days. The average number of days subjects received study PN was similar between the groups (STD-PN, 10.6±5.2 days; GLN-PN, 11.0±5.0, respectively). The mean GLN dose administered during study PN days was 22.7 g/day or 0.30±0.04 g/kg/day, respectively, in the GLN-PN group.

Overall, *pre hoc* goal amino acid + protein and caloric intakes were achieved in both study groups (Supplemental Figure 2A and 2B). The median (25th and 75th percentile) intake for combined amino acid/protein intake during the 28 days after entry was 1.5 (1.4–1.5) g/kg/day in both study groups. Caloric intake was also similar; the STD-PN group received 26.7 (23.8–29.6) kcal/kg/day and the GLN-PN group 26.7 (23.9–29.5) kcal/kg/day, respectively.

Study PN amino acid and kcal administration was similar between the STD-PN and GLN-PN groups for the first 14 study days (Figure 2A and 2B) and also for the entire 28-day period of observation (not shown). The advance of EN-derived protein and kcal during the initial 14 days was also similar between groups and is shown in Supplemental Figure 3A and 3B.

Plasma glutamine and glutamate concentrations

Mean plasma GLN concentrations were in the low to low-normal range at entry in both groups (Figure 3) (49). Plasma GLN levels rose significantly (≈33%) by day 3 with GLN-PN compared to plasma GLN levels the STD-PN group. Plasma GLN concentrations remained in this range and were significantly higher than in STD-PN subjects through day 14. With STD-PN, plasma GLN levels rose slowly and modestly over time. Plasma GLN concentrations were similar between the two study groups at the day 21 and 28 time points, reflecting transition from study PN to EN (Figure 3). Plasma glutamate concentrations rose slightly from baseline on days 3 and 7 and were similar in both groups over time (data not shown).

Blood glucose concentrations

Mean BG levels remained within the target GLND range (80–130 mg/dL) and were similar in both the STD-PN and GLN-PN groups over time at the morning, afternoon and evening time-points (Supplemental Figure 4, **panels A, B and C**). As shown in Supplemental Table 1, **panel A**, the rate of hyperglycemic episodes ($\geq$ BG 250 mg/dL)/1000 hospital study days (mean and 95% confidence interval) was significantly lower in the GLN-PN group compared to the STD-PN group ($p=0.04$). Rates of BG > 180 mg/dL/1000 hospital days and hypoglycemia (< 50 mg/dL/1000 hospital days) were similar between groups. (Supplemental Table 1, **panels B and C**).

SICU illness severity

There were no differences between the two study groups for changes in SICU illness severity over time by sequential SOFA scores (Supplemental Figure 5).

Healthcare-associated infection rates

Thirty-nine patients (26.0%) had a prevalent infection at entry and 61 patients (40.7%) had an incident healthcare-associated infection after entry. Incident infections included 35 patients with Gram-positive bacterial infections, 29 patients with Gram-negative bacterial infections and 24 patients with fungal pathogen as the putative causal microorganism. There were no differences between the STD-PN and GLN-PN groups for total number of new (incident) healthcare-associated infections after entry, infection rates/1000 hospital days, site- specific infections, or causative microorganism class (Table 2).

Mortality

A total of 45 subjects died during 6-month follow up. There were no differences between the groups for any index of mortality (Figure 4). Total hospital mortality was 13/75 (17.3%) with STD-PN compared to 11/75 (14.7%) with GLN-PN ($P=0.66$). Mortality at 28 days after entry was also similar [STD-PN, 12/75 (16.0%) versus GLN-PN, 11/75 (14.7%); $P=0.82$]. The 6-month cumulative all-cause mortality was 31.4% in the GLN-PN group and 29.7% in the STD-PN group ($P = 0.88$; Figure 4) and the estimated hazard ratio for treatment was 1.05 (95% CI: 0.58–1.88, $P=0.88$). Hospital mortality rates between groups did not differ by Apache II score at SICU admission, although, as expected Apache II score did predict hospital mortality (Supplemental Table 2). Similarly, hospital mortality did not differ by group in subjects with an SICU admission Apache II score at or below the median score of 23 (not shown). In subjects with an SICU admission Apache II score of 24 or greater, 8/34 (23.5%) of subjects in the STD-PN group died in the hospital, compared to 6/37 (16.2%) of subjects randomized to GLN-PN [relative risk = 0.69 (0.27–1.78), $P = 0.44$].

Other clinical outcomes

There were no differences between the study groups for median VFD [STD-PN 26.0 (12.0, 28.0), GLN-PN 24.0 (6.0–28.0)], ICU LOS [STD-PN 6.0 (3.0–13.0), GLN-PN 8.0 (3.0–18.0)], or hospital LOS [STD-PN 17.0 (10.0–28.0), GLN-PN 19.0 (14.0–28.0)].

Relationship of plasma GLN levels to mortality and infection endpoints

There was no relationship between entry (Day 1) plasma GLN concentrations and hospital mortality or development of any new infection when examined by GLN concentration quartile or dichotomized as GLN concentration < 420 or ≥ 420 μM (49) (Supplemental Table 3).

Safety of GLN-PN

Serum renal and liver function tests

There were no significant effects of treatment (study PN) or an interaction between treatment and time (blood chemistry values from entry, day 3, 7, 14, 21, and 28) on renal function tests (serum BUN and creatinine concentrations, respectively; Supplemental Figure 6, **panels A and B** and Supplemental Table 4) or on liver function tests (serum total bilirubin, alkaline phosphatase, AST, and ALT concentrations, respectively; Supplemental Figure 7, **panels A, B, C and D**).

SAEs

No SAE was reported to be possibly or definitely related to study PN treatment. SAEs were similar between the STD-PN and GLN-PN groups, except for a higher incidence of cardiopulmonary arrest in the STD-PN group (Supplemental Table 5).

AEs

Overall, AEs were similar between the STD-PN and GLN-PN groups.

Additional details on Results are provided in Supplemental Material.

Discussion

The results of this Phase III, double blind RCT of GLN dipeptide-supplemented PN demonstrate that this approach is safe, but did not improve clinical outcomes in SICU patients requiring PN after gastrointestinal, vascular or cardiac surgery in comparison to conventional GLN-free PN. The results do not confirm our previous double-blind pilot RCT study, in which patients receiving GLN dipeptide-supplemented PN demonstrated decreased new hospital infection rates compared to subjects receiving GLN-free PN after colonic, vascular or colonic surgery (18). These results are also in contrast to a number of multicenter European RCTs indicating that complete PN supplemented with GLN decreased hospital-acquired infections in adult mixed medical/surgical ICU patient populations (17, 26), as well as beneficial clinical effects with GLN-PN observed in several previous RCTs in other types of critically ill medical and surgical patients (8, 10–12, 16, 20).

Despite an initial PN GLN dose similar to current European recommended guidelines for ICU patients [0.33 g/kg/d L-GLN (50)], we found no significant differences in new healthcare-associated infections, SICU illness severity, mortality indexes, LOS indexes or use of mechanical ventilation after entry versus STD-PN controls who received no GLN in PN. In addition, low plasma GLN levels at entry did not correlate with increased

hospital mortality, as suggested in some studies in which GLN levels were determined on the day of ICU admission (14, 49). However, GLND patients were enrolled a median of 3–4 days after SICU admission, thus our results are not strictly comparable to these previous reports (14, 49).

We rigorously assessed the safety of GLN dipeptide-supplemented PN using strict inclusion/exclusion criteria (which excluded those subjects in shock or with severe, acute renal or hepatic failure at entry), site performance monitoring, serial monitoring of AEs and SAEs up to 30 days after study PN discontinuation, and regularly evaluating potential adverse events over time by both a central Medical Monitor and an active DSMB with expertise in ICU nutrition support. Our data clearly demonstrate the safety of the approaches we used for administration of GLN-supplemented PN in patients with moderately severe critical illness.

Strengths of the GLND study included performance of the trial in 5 major independent medical centers located in different regions of the U.S., rigorous concealment of random treatment allocation, appropriate blinding implementation, and the ITT design. The study was also unusual among prior studies of GLN-PN in ICU patients in that it focused on subset of SICU patients after specific major types of surgery, excluded cancer as a primary diagnosis, and was informed by the results of a similar pilot study. Other strengths include the clinically matched study groups at entry (e.g. demographic criteria, index operations, underlying illness severity, entry GLN concentrations, presence of ARDS and need for mechanical ventilation) and the similar tight BG control between groups. Study procedures ensured double blind and adequate intake of conventional PN and EN, which provided nearly identical amounts of calories, protein/AA and micronutrients between groups up to 28 days, initially as study PN, with subsequent transition to EN in a pragmatic manner. Also, all healthcare-associated infections were adjudicated by an infectious disease specialist using well-defined *pre hoc* CDC criteria.

To minimize heterogeneity in clinical outcomes, we also excluded patients with malignancy, as in the pilot study, but it is unclear whether such patients may benefit from GLN administration (21–22, 35). One may hypothesize that oncology patients may have a greater chance of benefit from GLN-treatment due to greater GLN depletion from excessive consumption of GLN and other nutrients by the tumor as has been previously hypothesized (37). GLND was designed to study the efficacy of PN GLN supplementation alone (i.e. it was not a study of PN timing, non-GLN macronutrient doses, or route of feeding). The investigators' practices were to initiate PN several days after SICU admission, but to generally continue PN immediately post-operatively in patients receiving PN preoperatively. Thus, the GLND enrollment window was a 2 to 14 day period after the index surgical operation. Although entry after the index surgery and duration of study PN were similar between the study groups, it is unclear whether the wide enrollment window after the index surgery may have introduced variation.

Similar to our results, recently a number of double-blind RCTs also have not shown consistent clinical benefits of GLN-supplemented PN in ICU patients via ITT analysis (24–26). Grau et al studied 127 primarily surgical ICU patients in 12 Spanish hospitals receiving 0.5 g/kg/day alanyl-GLN dipeptide in complete PN versus GLN-free complete PN, similar

to our regimen (24). GLN-PN was administered for a median of 6 (5–8) days, but by ITT analysis, no infectious or other clinical outcome was affected, other than decreased urinary tract infections/1000 catheter days in GLN-PN cohort (24). The Scandinavian glutamine trial was a multicenter double-blind RCT in which intravenous alanyl-GLN dipeptide was given independent of EN and/or PN, as indicated, to 413 medical/surgical ICU patients for a median of 6 (3–11) days in ITT analysis and 9 (5–14) days in per-protocol analysis (> 3 days of administration) (25). No impact of GLN administration on serial SOFA scores or mortality was observed with ITT analysis, although decreased ICU mortality with GLN dipeptide was observed in per-protocol analysis (25). The SIGNET study was a 2×2 factorial multicenter double-blind RCT in 502 mixed medical/surgical patients from 10 Scottish ICUs that examined PN supplemented with 20.2 g L-GLN/day ± 500 µg selenium versus a control group with neither supplement (26). No significant effect of L-GLN-supplemented PN on hospital infection, SOFA scores, LOS, or mortality indexes was observed (26). The SIGNET study has been criticized because exact dosing of GLN in g/kg body weight was not reported and GLN-containing PN was administered for an average of only 5 days (30). In GLND, our GLN dosing regimen achieved significantly increased and sustained plasma GLN levels well above control values for 14 days and study PN was given for ≈ 11 days in each group (GLN dose = 0.30±0.04 g/kg/day, in the GLN-PN group). Thus, it cannot be argued that GLND provided an inadequate dose of GLN, given for an inadequate period of time, based on current European recommendations (50). Nonetheless, our protocol called for weaning of study PN as EN was able to be advanced clinically (pragmatic); thus, GLN administration was steadily decreased over time, particularly after the first 2 weeks after entry. It is unknown whether continued enteral GLN during (or after) hospitalization after a period of largely parenteral GLN in the ICU setting can impact clinical outcomes.

Recently, the 1,223-subject REDOXS trial, conducted in predominately medical ICU patients with shock and multiple organ failure, demonstrated a slight but statistically significant increase in hospital and 6-month mortality in patients given high-dose alanyl-GLN dipeptide (0.35 g/kg/day intravenously, plus 30 g/day enterally, independent of EN/PN delivery), started during the first 24 h after ICU admission (37). *Post hoc* analysis of REDOXS data suggested that the presence of multi-organ failure that included renal dysfunction at study entry was most strongly associated with the mortality signal with GLN administration (51). However, the current GLND trial differs from the REDOX trial in that it was conducted exclusively in post-operative SICU patients, excluded those in shock or with significant renal/hepatic dysfunction at entry, and provided a lower, more conventional GLN dose (50, 52), in conjunction with complete PN ± EN support, with GLN-PN started several days after ICU admission in resuscitated patients. Given several decades of published RCTs and numerous meta-analyses suggesting the clinical and metabolic benefits of GLN-supplemented PN (6–12, 16–20, 32–36), it remains unclear why we were unable to observe a signal of benefit in this or recent RCTs of GLN-supplemented PN (24–25). Since our pilot study (18), the index operations we included (vascular, intestinal and cardiac procedures) have increasingly been performed using minimally invasive surgical techniques, which are less catabolic than previous open procedures (53–55), while ICUs have incorporated increasing use of standardized operating procedures and clinical decision support tools (56).

However, the impact of these changes in the clinical ICU setting on GLN utilization/requirements and clinical impact of GLN administration is unknown.

Irrespective of the results reported here, it remains possible that certain subgroups of patients may still benefit from GLN-PN in ICU settings (57) or that specific metabolic or other characteristics can be identified in specific patients to predict “responders” from “non-responders” to GLN-PN, possibly by metabolic analysis, coupled with ICU-determined blood GLN levels (58–61).

In conclusion, in a rigorous, multicenter, Phase III American trial, complete PN supplemented with alanyl-GLN dipeptide at a dose of 0.5 g/kg/day was safe, but did not impact clinical outcomes in SICU patients deemed to require PN after intestinal, vascular or cardiac surgery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. CONSORT diagram

The progress through the phases (enrollment, intervention allocation, follow-up and data analysis) of a double-blind, randomized controlled trial in surgical intensive care unit (SICU) patients requiring parenteral nutrition (PN) comparing standard, glutamine (GLN)-free PN (STD-PN) with PN supplemented with alanyl-GLN dipeptide (GLN-PN) are shown.

Figure 2. A. Administration of amino acids (AA; g/kg/day) from study PN, shown as median and 25th and 75th percentiles, during the initial 14 days after entry
Intake was similar between the STD-PN and GLN-PN groups, and also for the entire 28-day period of observation (not shown). **B. Study PN kcal intake, shown as median and 25th and 75th percentiles.** Intake was similar between the STD-PN and GLN-PN groups during the initial 14 days and also for the entire 28-day period of observation (not shown). The steady decrease in study PN AA and kcal over time reflected the GLND standard of care protocol for weaning from PN to EN as tolerated.

Figure 3. Plasma glutamine (GLN) concentrations over time

Mean plasma GLN concentrations were in the low to low-normal range at entry in both groups (44). With GLN-PN, plasma GLN levels rose significantly ($\approx 30\%$) by day 3 but were unchanged in the STD-PN group. Plasma GLN concentrations remained significantly higher in the GLN-PN group compared to the STD-PN subjects through day 14. With STD-PN, plasma GLN levels rose slowly over time.

Figure 4. Kaplan-Meier cumulative mortality curve through 6 months after entry
There were no differences in mortality between the STD-PN and the GLN-PN groups over time.

Table 1

Baseline demographic and clinical characteristics by treatment

Characteristic *	GLN-PN (N=75)	STD-PN (N=75)
Age	60.2±13.6	60.4±13.0
Male sex	35 (46.7)	45 (60.0)
Race		
Black or African American	15 (20.0)	4 (5.3)
White	60 (80.0)	71 (94.7)
BMI (kg/m ²)	26.9±5.9	26.4±6.3
APACHE II score at study entry	16.2±5.7	15.7±7.3
Apache 15	33 (44.0)	38 (50.7)
APACHE II score on first day in SICU †	22.9±7.6	22.0±7.6
SOFA score at entry †	7.1±4.7	6.2±4.8
Index surgery		
Intestinal resection	50 (66.7)	52 (69.3)
Vascular	17 (22.7)	14 (18.7)
Coronary artery bypass	3 (4.0)	2 (2.7)
Cardiac valve	3 (4.0)	2 (2.7)
Esophageal/gastric resection	1 (1.3)	4 (5.3)
Peritonitis	1 (1.3)	1 (1.3)
Days from index surgery to randomization	4.5±3.0	4.3±2.9
On mechanical ventilation at entry	54 (72.0)	44 (58.7)
ARDS at Entry	11 (14.7)	6 (8.0)
Received any enteral tube feedings within 30 days prior to entry †	13 (17.3)	7 (9.3)
Received any PN within 30 days prior to entry †	59 (78.7)	63 (84.0)
Morning blood glucose at entry (mg/dL) ‡	136±41	143±41
WBC at entry (10 ⁹ /L) ‡	14.3±7.3	14.2±8.8
Plasma glutamate and glutamine concentrations at entry (μM) §		
Glutamate	48±35	53±32
Glutamate	438±161	427±266

* Continuous variables are reported as *mean ± SD* and categorical variables are reported as *no. (%)*.

† Sample size is 74 in the GLN-PN group and 73 in the STD-PN group

‡ Sample size is 75 in the GLN-PN group and 72 in the STD-PN group

§ Sample size is 68 in the GLN-PN group and 66 in the STD-PN group

APACHE II=Acute Physiology and Chronic Health Evaluation II; ARDS= acute respiratory distress syndrome; BMI=body mass index; PN= parenteral nutrition; SOFA= Sepsis-related Organ Failure Assessment; WBC= white blood cell

Table 2

Incident hospital-acquired infection rates by treatment

	GLN-PN* (n, %)	GLN-PN† (n, %)	STD-PN* (n, %)	STD-PN† (n, %)	P- value ^{††}
Any infection	52 (32, 43%)	28 [21–37]	39 (24, 32%)	25 [18–35]	0.70
Specific site infections					
Bloodstream	18 (17, 23%)	9,6 [6.1–15.1]	13 (11, 15%)	8,4 [4.9–14.7]	0.73
Lower respiratory**	10 (10, 13%)	5,3 [3.0–9.3]	12 (12, 16%)	7,8 [4.7–12.9]	0.32
Surgical site	9 (8, 11%)	4,8 [2.6–8.8]	9 (8, 11%)	5,8 [3.1–11.0]	0.66
Urinary tract	7 (7, 9%)	3,7 [1.8–7.9]	3 (2, 3%)	1,9 [0.5–7.9]	0.37
Gastrointestinal system	5 (5, 7%)	2,7 [1.2–5.8]	1 (1, 1%)	0,6 [0.1–4.4]	0.11
Any fungal species	21 (16, 21%)	11,1 [6.7–18.4]	13 (8, 11%)	8,4 [4.4–16.1]	0.49
Any Gram-negative bacteria	33 (16, 21%)	17,5 [12–26.8]	18 (13, 17%)	11,7 [6.9–19.8]	0.25
Any Gram-positive bacteria	35 (18, 24%)	18,6 [12.0–28.7]	32 (17, 23%)	20,8 [13.1–33.0]	0.73

* Variables reported as: number of infections (number of subjects, % of subjects), for 75 GLN-PN subjects and 75 STD-PN subjects.

† Variables reported as: Infection rate per 1000 hospital days [95% confidence interval] for GLN-PN subjects (1884 subject-hospital days) and STD-PN subjects (1539 subject-hospital days).

†† P values apply to infection rates/1000 hospital days

** Lower respiratory infections include pneumonia