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Enteral nutrition supplemented with L-glutamine in patients with systemic inflammatory response syndrome due to pulmonary infection

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ABSTRACT

Objective: To evaluate the effect of enteral nutrition (EN) supplemented with L-glutamine on glycolytic parameters, inflammation, immune function, and oxidative stress in moderately ill intensive care patients with sepsis.
Methods: Thirty patients received EN. Fifteen patients received EN supplemented with glutamine (30 g; GLN group) for 2 d followed by EN supplemented with calcium caseinate (30 g, CAS group), also over 2 d. The other 15 patients received EN with calcium caseinate (30 g; CAS group) for 2 d followed by EN with glutamine (30 g; GLN group), also over 2 days. One washout day with only EN was provided between every 2-d period of EN plus supplementation to all patients. Blood samples were taken before and after supplementation.
Results: There were no changes in glycolytic parameters in either group. Leukocytes decreased in the two groups (from 13 650 to 11 500 in the CAS group, $P = 0.019$; from 12.850 to 11.000 in the GLN group, $P = 0.046$). Lymphocytes increased in the GLN group (from 954 to 1916, $P < 0.0001$) and were more numerous after glutamine supplementation (from 1916 to 1085, $P < 0.0001$, GLN versus CAS). No significant changes were observed in interleukin levels, but urea levels were higher in the GLN compared with the CAS group (50.0–47.0, $P = 0.030$). Glutathione plasma concentrations did not differ significantly between the groups. No significant changes were observed in the plasma glutamine and glutamate concentrations.
Conclusions: The EN supplemented with glutamine increased the lymphocyte count and helped to decrease lipid peroxidation but presented no effect on the antioxidant glutathione capacity and on cytokine concentrations or glycolytic parameters.

Introduction

Clinical critical illness leads to a deficiency in glutamine and its intravenous supplementation has been demonstrated to decrease complications and infections that are correlated with mortality in the intensive care unit (ICU) [1,2]. Clinical trials have shown that glutamine-enriched parenteral nutrition in critically ill patients increases serum heat-shock protein [3] and improves antioxidant defenses [4]. L-Alanyl-glutamine–enriched parenteral nutrition has been shown to augment glucose use in critically ill patients [5] and to decrease insulin resistance in patients with severe trauma in the ICU [6], thus stimulating glycolysis and possibly representing a nutritional tool to help maintaining glucose control [5,6]. However, a recent study on intravenous nutrition supplemented with L-alanyl-glutamine ($0.4\ g \cdot kg^{-1} \cdot d^{-1}$) in severely critically ill patients with Second Acute Physiology and Chronic Health Evaluation (APACHE II) scores from 22 to 26 has shown no immunomodulatory benefit, without significant difference in lymphocytes count, B-lymphocytes, and T-lymphocytes, and their subgroups (helper T-lymphocytes, cytotoxic T-lymphocytes) [7].

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Enteral supplementation with glutamine is clinically well tolerated and free of side effects in intensive care patients [8–10]. A review study has suggested that glutamine-enriched enteral nutrition (EN) with nutraceutical doses of glutamine (20–30 g/d) should be initiated early and maintained for at least 5 d in critically ill and septic patients and multiple-trauma and post-surgical patients [9]. In contrast, a study involving patients in the ICU has reported that glutamine ($0.6\ g \cdot kg^{-1} \cdot d^{-1}$) added to a standard or an immunomodulatory enteral diet should not be routinely administered to surgical ill patients (APACHE score 18) to decrease in-hospital mortality [11].

There appears to be a lack of information on the effects of glutamine-enriched EN in a less severely ill subpopulation of patients in the ICU. The objective of the present study was to assess the effect of L-glutamine (30 g/d) enriched EN on glycolytic parameters, oxidative stress, immune response, and inflammation markers in moderately/severely ill patients with systemic inflammatory response syndrome (SIRS) caused by pulmonary infection.

Materials and methods

From February 2008 through April 2009, a randomized, controlled, double-blind, cross-over study was conducted at the clinical ICU of Casa de Saúde e Maternidade São Raimundo (Fortaleza, Brazil) in patients with SIRS and sepsis receiving EN. The study was previously approved by the research ethics committee of the Federal University of Ceará (protocol 055.06.02, December 4, 2006) and conducted in accordance with Brazilian legislation (National Health Council/Brazilian Ministry of Health Resolution 196/96) and the tenets of the Declaration of Helsinki and subsequent revisions. Informed written consent was obtained from patients' legal representatives and physicians before study entry.

One hundred eighty-six patients admitted to the ICU during the study period were initially assessed to participate in the trial. The inclusion criteria included a diagnosis of SIRS, hemodynamic stability, no recent use of vasopressors, and an indication for EN. Patients with septic shock, diabetes, acute or chronic renal disease, liver diseases, heart disease, or using vasopressors were excluded. Sample randomization was performed with software from the Statistics and Epidemiology Laboratory, Instituto Dante Pazzanese, São Paulo, Brazil [12].

Patients were diagnosed with SIRS when two or more of the diagnostic clinical symptoms described in the literature were observed [13], when they presented an evident need for EN based on clinical judgment, and the preselected APACHE II score range [14].

After recruitment, 36 patients (30 to 92 y old, 22 men and 14 women, APACHE score >10 to <20) who met eligibility criteria were randomly assigned to receive supplemented EN. Thirty patients completed the study protocol. Fifteen patients received EN supplemented with glutamine (GLN group) followed by EN supplemented with calcium caseinate (CAS group), whereas the other 15 patients were provided with EN supplemented with calcium caseinate (CAS group) followed by EN supplemented with glutamine (GLN group) for 2 d. A washout period of 1 d was established between every 2-d feeding period with non-supplemented EN. The nutritional intervention started up to 48 h after admission and was maintained for 5 d. Four blood samples per patient were drawn according to standard procedures before (T_0) and after (T_1) each 2-d supplemented EN.

The supplements used in the study were L-glutamine (Ajinomoto Interamericana Indústria e Comércio Ltda., São Paulo, Brazil) and calcium caseinate (Kerry do Brasil Ltda., Três Corações, MG, Brazil) in the form of white crystalline powder synthesized from dried skim milk treated with dietary glycerol monooleate. Both supplements were administered at a nutraceutical dose of 30 g/d added to 1 L of Nutri Enteral diet (Nutrimed Industrial Ltda., Fortaleza, Brazil).

Nutri Enteral is a complete diet for enteral or oral use (1 L) composed of 50 g of protein, 175 g of carbohydrates, and 33 g of lipids, providing 1.2 kcal/1 mL. With the addition of a 30-g supplement (glutamine or caseinate), the diet provided 80 g of protein, 175 g of carbohydrates, and 33 g of lipids, providing 1.32 kcal/1 mL. This polymeric complete diet does not present L-glutamine in its protein source composition (Table 1).

To ensure the blinded design of the study, neither patients nor those responsible for their care and evaluation knew which supplemented enteral feed the patients were receiving. The powder supplements were added to 1 L of liquid at an ultrahigh temperature, which was sterilized at an enteral clinical compounding unit and supervised by a dietitian who kept the randomization records and organized the order of every 2-d supplemented diet to each selected patient and the 1-d washout period in which each patient received 1 L of the non-supplemented polymeric enteral diet.

Table 1
Calculated amino acid profile of the protein source (milligrams of amino acid per gram of protein)

Amino acids	Enteral diet (mg)	Calcium caseinate (mg)	Glutamine (mg)	CAS group (mg)	GLN group (mg)
L-Glutamine	0	–	999	–	375.00
Isoleucine	50.50	52.20		53.00	33.47
Leucine	92.10	90.90	–	90.9	56.87
Lysine	74.60	74.70	–	75.30	47.36
Methionine	20.60	27.00	–	24.75	14.62
Phenylalanine	37.20	48.60	–	42.10	23.90
Threonine	47.40	41.40	–	49.59	34.00
Tryptophan	13.20	12.60	–	15.80	11.10
Valine	53.90	66.60	–	60.00	35.00
Histidine	21.30	28.80	–	24.10	13.30
Alanine	35.10	27.90	–	32.40	24.80
Arginine	34.40	34.20	–	31.80	19.00
Aspartic acid	85.60	65.70	–	90.34	65.70
Cysteine	12.80	3.60	–	12.90	11.59
Glutamic acid	167.70	200.70	–	187.80	112.55
Glycine	18.90	17.10	–	18.39	12.00
Proline	71.00	94.50	–	76.72	41.29
Serine	53.40	56.70	–	52.31	31.00
Tyrosine	35.30	52.20	–	42.10	22.55
Total (mg amino acids/g protein)	925	995.4	999	980.3	985.1
Total protein (g/d)	50	30	30	80	80

CAS, calcium caseinate supplementation; GLN, L-glutamine supplementation

Supplementation with free L-glutamine demands special care because of the limited solubility of the amino acid (36 g/L) [15]. These difficulties may be circumvented by using powdered L-glutamine added to commercially available enteral diets manufactured, stored, and distributed under appropriate conditions. Calcium caseinate was elected as an isoproteic control supplement because of its intact protein nature and because it does not contain L-glutamine (Table 1).

The parameters analyzed were hematocrit, leukocytes, lymphocytes, and monocytes (ABX Pentra 60, Montpellier, France) [16], fasting glycemia (hexokinase method; RA-XT Technicon, Bayer, Jersey, NJ, USA) [17], urea (Natelson-Scott-Befa Method) [18], creatinine (RA-XT Technicon), insulin (chemiluminescent assay) [16], peptide-C (chemiluminescent assay, Modular Analytics E170, Elecsys, and Cobas, Indianapolis, IN, USA) [16], prealbumin (nephelometry) [18], and lactate (enzyme colorimetric assay) [16]. The assessment of inflammation and oxidative stress was based on the serum levels of interleukin (IL)-1, IL-6, and IL-10 and tumor necrosis factor-α (TNF-α; BD OptEIA, BD Biosciences, San Jose, CA, USA), sandwich enzyme-linked immunosorbent assay (ELISA), human IL-1β ELISA set II, human IL-6 ELISA set, human IL-10 ELISA set, and human interferon-α ELISA set, thiobarbituric acid-reactive substances (TBARS; Caymann's Assay Kit, Caymann, Ann Arbor, MI, USA), and total glutathione (GSH; quantitative colorimetric analysis, Bioxytech GSH-420 Assay Kit, Bioxytech, Portland, OR, USA). Glutamine and glutamate were analyzed by a glutamine/glutamate determination kit (Sigma, St. Louis, MO, USA).

The data were compiled and organized with Microsoft Office Excel (Microsoft, Redmond, WA, 2003). Means and SDs were calculated for normally distributed variables (Kolmogorov–Smirnov test). One-way analysis of variance and the Bonferroni post-test were used for multiple comparisons. Median values (interquartile intervals) were calculated for non-parametric variables, and inter- and intragroup comparisons were performed with the Wilcoxon test. P values and the probability of a type I error (α) were calculated for all analyses. A two-tailed P less than 0.05 was considered statistically significant. All statistical tests and figures were performed with GraphPad 5.0 for Windows (GraphPad Software, San Diego, CA, USA, 2007). Results were expressed as mean/median ± SD/interquartile intervals and compiled in tables and figures.

Results

All patients were moderately ill with SIRS because of a pulmonary infection. Only 30 of the 36 selected patients completed the study protocol. Six patients died. On the first day, three of them died from leukemia, penile cancer, or Alzheimer's disease as their primary condition. Two patients presented a baseline clinical condition of cancer. The third patient presented with leukemia in remission before beginning EN. Two