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Supplementation of Essential Amino Acids May Reduce the Occurrence of Infections in Rehabilitation Patients With Brain Injury

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Abstract

Background: To investigate whether supplementation with oral essential amino acids (EAAs) may reduce the occurrence of nosocomial infection among patients with brain injury (BI: stroke, trauma, anoxic coma). *Methods:* Patients (n = 125; 77 men, 48 women; mean age 63 ± 15 years) with stroke (68.8%), subarachnoid hemorrhage (17.6%), traumatic BI (7.2%), and anoxic BI (6.4%) 88 ± 15days after the index event. Patients were randomly assigned to 2 months of oral EAAs (n = 63; 8 g/d) or placebo (n = 62). *Results:* Over the first month of rehabilitation, there were 60 infections in the whole population of 125 patients (48%); however, the rate was 23.2% lower in the EAA group (23 episodes/63 patients; 36.5%) than in the placebo group (37 episodes/62 patients; 59.7%) ($P < .01$). The types of infection were similarly distributed between the 2 groups. Serum levels of prealbumin <20 mg/dL and C-reactive protein (CRP) >0.3 mg/dL were the best predictors of future infection (prealbumin: odds ratio [OR] = 4.17, confidence interval [CI] 1.84–9.45, $P < .001$; CRP: OR = 3.8, CI 1.71-8.44, $P < .001$). *Conclusion:* Supplementary EAAs may reduce the occurrence of nosocomial infections in rehabilitation patients with BI. Prealbumin and CRP are the best predictors of future infections. (*Nutr Clin Pract*. 2012;27:99-114)

Keywords

amino acids, essential; infection; brain injuries

Infection often complicates the rehabilitation period of patients with brain injury (BI), whether the cause is ischemic/hemorrhagic stroke (ST), subarachnoid hemorrhage (SH), or anoxic traumatic insult (A/T). Infection affects functional outcomes, increases length of stay,[1] and negatively influences both patients' life and functional prognosis.[2]

The incidence rate of infection during rehabilitation has been reported to range from 16.5%[3]–34.5%[4] in patients with ST and to be 45% in post-A/T[5]; infection in patients with SH undergoing rehabilitation has not been documented, whereas the infection rate is 46% in patients with acute SH.[2]

In our tertiary care rehabilitation institute for patients with BI, the rate of development or exacerbation of infection within the first month after admission is about 60% (unpublished data). This high infection rate reflects the severity of the patients' clinical and functional compromise[6] following their primary disease but also suggests a relative inadequacy of the measures adopted to prevent infections.[7-9]

Rehabilitation patients with BI are particularly prone to the development or exacerbation of infection because of their immunodeficiency state.[10-12] Central nervous system injury induces a disturbance of homeostatic mechanisms between the immune system and the brain, responsible for immunodeficiency and infectious complications.[10] The processes contributing to this immunity-brain disturbance include post–acute injury systemic inflammatory response syndrome (SIRS)[13-15] and counterinflammatory response syndrome (CIRS),[16] which both play major roles. Additive

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factors may contribute to further impair patients' immunological defences. These include abnormal mental status, coma, aspiration,[2] the provenience of patients (neurological and neurosurgical intensive care units),[2] blood transfusion,[17] length of stay in the hospital,[18] metabolic stress response,[19] a hypermetabolic state in the acute stage of BI,[20] poor nutrition,[21] possible multiple trauma (in traumatic BI),[22] and use of concomitant medical devices.[2]

The implementation of preventive measures aimed at reducing the infection rate of rehabilitation patients with BI is, therefore, of clinical importance and can reduce the financial burden for hospitals. The central hypothesis of the present study is that, in addition to preventive measures, enhancing/improving patients' defense capacities could play a critical role in limiting the occurrence of infection in rehabilitation patients with BI and that this reinforcement of patients' immune responses could be achieved by supplementation of essential amino acids (EAAs).

This hypothesis is based on four main considerations. First, EAAs are the building block of every process of protein synthesis[23]: the shortage of even only 1 EAA can reduce the rate of protein synthesis. Second, protein synthesis is necessary and increased during the post–acute period following BI characterized by systemic and intracranial inflammatory responses.[24,25] Third, protein synthesis is necessary for the high rate of proliferation, duplication,[26] and phagocytosis[27,28] of immune cells. Last, the efficacy of EAAs has been documented in octogenarian patients undergoing intensive rehabilitation for diseases other than BI,[7] among whom the occurrence of infection was reduced by 30%.

In the present study, therefore, we investigated the effect of EAA supplementation on the rate of infections among rehabilitation patients with BI. In addition, we identified possible risk factors for developing infection.

Methods

Clinical Setting

The policy of our clinical rehab division is to admit all BI patients independently of the time the acute event arises, as soon as their functional status worsens and/or neurocognitive level deteriorates despite a rehab program carried out in other hospitals.

Clinical interventions also aim to highlight clinical and neurological factors that cause patient worsening and to indicate secondary prevention measures for infection at the time of patient discharge from the rehab setting.

Population

One hundred seventy-five patients with BI (88.3 ± 110 days after acute event; range, 5-447; median 43) were consecutively admitted to our rehab division from October 1, 2009, to September 30, 2010, and were enrolled in this randomized, double-blind, placebo-controlled study. Criteria for exclusion were the presence or occurrence of intracerebral hypertension, hydrocephalus, complications from neurosurgery, admission antibiotic therapy, pressure ulcer with infection clinically evaluated by the presence of purulent fluid, presence of fever with a temperature >36.8°C, chronic heart failure, acute coronary artery disease, acute and chronic renal failure (creatinine clearance <30 mg/100 mL), cancer, or surgery for cancer.

We adopted these selection criteria to avoid existing infection and diseases that, per se, represent a very high risk for infection and/or are life threatening. The patients had been admitted from general intensive care units (ICUs; 16%), neurosurgery ICUs (14.8%), stroke units (31.4%), a neurological setting (18.4%), and the home (because of worsening of neurological symptoms) (19.4%).

The study was approved by the ethical, technical-scientific committee of our institute. Written informed consent was obtained from participants after the nature of the study had been explained. For those patients with cognitive dysfunction, informed consent was obtained from the patients' caregivers, who were carefully informed that EAAs should not be considered drugs but rather as nutrition substrates with the potential of acting on immunological defenses.

Procedure

Within the first week after admission and at discharge, the following assessments were made:

a. Anthropometric characteristics: body weight (kg), determined using a mechanical weight lifter; height (m), calculated from knee height[29]; and body mass index (BMI), calculated as kg/m^2
b. Biohumoral variables: after overnight fasting, at 8:00 AM, blood samples from an arm peripheral vein were drawn to determine the following:
    1. Routine variables
    2. Biomarkers of body inflammatory status including serum levels of: interleukin-6 (IL-6), determined in duplicate using a high-sensitivity commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit from Mabtech (Agilent Technologies GmbH, Boblingen, Germany); C-reactive protein (CRP), determined by an immunoturbidimetric method; acute phase reactant proteins (fibrinogen, haptoglobin); and nonreactant proteins (albumin, prealbumin, and transferrin) were also measured.
    3. Plasma EAAs: leucine, valine, isoleucine, methionine, phenylalanine, tryptophan, lysine, and threonine. The concentration of free amino acids in plasma was measured by an AminoQuant II

(Milan, Italy) amino acid analyzer, based on the HP 1090 HPLC system, with fully automated precolumn derivatization, by using both ortho-phthalaldehyde and 9-fluorenyl-methyl-chloroformate reaction chemistries according to the manufacturer's protocol. Determinations were performed essentially by injecting 1 μL of the derivatized mixture and measuring absorbance simultaneously at 338 and 262 nm. Plasma concentrations were expressed as μmol/L. In our laboratory, a normal value of the sum of the EAAs is 862 ± 122 μmol/L (range, 710–1066 μmol/L) and 32.3% ± 4.5% of total amino acids (range, 27.6%–39.7%). Although we have determined all the amino acids (nonessential and essential amino acids) both at admission and discharge times, we discussed only the EAA at admission of patients to our institute.

c. Functional status: the functional status of patients was evaluated by means of a functional independence measure (FIM)[30] or disability rating scale (DRS) when appropriate.[31]
d. Nutrition intakes: for self-feeding patients (85%), a 3-day alimentary diary was kept by rehabilitation nurses who had been previously trained ad hoc. The nurses recorded the type and weight of cooked or uncooked food selected by patients from the hospital menu on a diet sheet for 3 days before and after the patients' meals. The amount of food really ingested was converted (by R.A.) to the raw equivalent, when necessary, using appropriate tables.[32] Nutrition analysis, carried out using a computer program designed by our group,[33] was used to calculate actual ingested calories, macro-/micronutrients, and amino acids. In our laboratory, dietary ingestion of EAAs is 25%–35% of total ingested amino acids.

Randomization of Patients

After completing all these procedures, the patients were assigned to treatment according to a randomized allocation procedure. A randomization list was generated using SAS statistical software (SAS Institute, Cary, NC). A and B were the identifiers of the blinded treatment. The list was made available both to physicians (M.B., MP.A., N.A.) and to the hospital pharmacists. The physicians sequentially allocated patients to treatment A or B according to a randomization list. The second author (R.A.) who interpreted all results was blinded to the patients' allocation. The experimental group (EAA group) received an oral (or via feeding tube in tube-fed patients) nutrition mixture supplement that provided 8 g of EAAs/d (Aminotrophic, Professional Dietetics, Milan, Italy; Table 1; 4 g in the morning + 4 g in the afternoon diluted in half a glass of water) for 60 days. The placebo group (control group) was given a similar isocaloric product containing maltodextrin instead of EAAs. Rehabilitation nurses assisted every patient during the intake of placebo or EAAs to be certain about the patients' compliance.

Table 1. Nutrition Composition of an Individual Packet of Supplementation, Containing 4 g of an Amino Acid Mixture, Used in This Study[a]

kcal	20.6
KJ	86.1
Total amino acids, including the following:	4 g
L-leucine	1250 mg
L-lysine	650 mg
L-isoleucine	625 mg
L-valine	625 mg
L-threonine	350 mg
L-cysteine	150 mg
L-histidine	150 mg
L-phenylalanine	100 mg
L-methionine	50 mg
L-tyrosine	30 mg
L-tryptophan	20 mg

[a]Treated patients were given 2 packets daily (8 g essential amino acids).

The nurses were blinded to the type of supplementation (maltodextrin or EAAs) given the patients because the packets containing the products were identical but numbered as 1 or 2. The contents were known only to the physicians (M.B., MP.A., N.A.) and the pharmacists (1 = placebo; 2 = EAAs). The product content in the packets 1 and 2 had similar color and taste.

For patients receiving enteral nutrition (EN), the aqueous solution of EAAs was supplied through the feeding tube (nasogastric or percutaneous endoscopy gastrostomy).

The duration of the study was 60 days from the randomization procedure. After randomization, patients were surveyed daily by rehabilitation nurses for evidence of infection. A diagnosis of infection was made by a physician on the basis of described criteria for initiating antibiotics.

Criteria for Diagnosing Infection[2]

Pneumonia: diagnosed according to American College of Chest Physicians criteria in patients who had a new infiltrate on chest x-ray plus 1 of the 3 following conditions: elevated white blood cell count (more then 10,000 cells/mL), fever, or purulent sputum[34]

Urinary tract infection: was defined as pyuria with more than 5 white blood cells/hpf with a positive urine culture with more than 100,000 colonies[35]

Bloodstream infection:

(a) Microbiologically documented: recognized pathogen in the blood not related to an infection at another

site or fever (>37.5°C), chills, or hypotension and any of the following: common skin contaminant isolated from (1) at least 2 positive blood cultures drawn on separate occasions or (2) a positive blood culture in a patient with an intravascular device. The organism is not related to infection at another site.

(b) Clinically diagnosed: when the patient had fever, hypotension, or oliguria plus (1) no microorganism cultured or isolated from the blood, (2) no infection at another site, or (3) physician instituted appropriate antimicrobial therapy for sepsis[36]

Gastroenteritis: one of the following criteria had to be met:

(a) 2 or more loose or watery stools over a day
(b) 2 or more episodes of vomiting in a 24-hour period
(c) both of the following: (1) stool culture positive for a pathogen, including *Clostridium difficile* (toxin) and (2) at least 1 symptom compatible with a gastrointestinal tract infection (nausea, vomiting, abdominal pain, or diarrhea)

Statistical Analysis

Sample size. Assuming type I and type II errors of 0.05 and 0.20, respectively ($\alpha = 0.05$, $\beta = 0.80$) and a 1-sided test, the number of patients required to prove a 20% reduction in the occurrence of infections between the group receiving placebo and that receiving supplementation with EAAs was 62 patients per group. Taking into account a dropout rate of 10%, at least 136 patients had to be enrolled overall.

Data analysis. Descriptive statistics were performed for all recorded variables. The mean and standard deviation were reported for continuous variables; qualitative variables were summarized as frequencies and percentages. The occurrence of infections between the EAA and placebo groups was compared using Pearson's χ^2 test. Baseline characteristics between EAA and placebo groups were compared using an unpaired Student *t* test or χ^2 test, as appropriate. Trends over time from baseline to discharge were compared between the 2 groups by means of an analysis of variance model with 1 factor. Single and multiple correlation analyses were performed to test the association between FIM/DRS levels at admission and discharge and serum albumin, prealbumin, transferrin, and CRP concentrations. A stepwise procedure was applied to select independent variables highly correlated with FIM/DRS.

Conditional logistic regression analysis was used to test the association between the occurrence of infection and hypothetical risk factors recorded at baseline. Risk factors were initially included as continuous variables in univariate models: variables that were statistically associated with the outcome were dichotomized to identify a risk and reference category and included in a multivariate model. A stepwise procedure was applied to identify the risk factors with the highest predictivity for developing infections. Results were expressed in terms of unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs).

Results

From a total of 175 screened patients with BI, 136 met the inclusion criteria and were included in the study (Figure 1). Fifteen patients were not included because of complications from neurosurgery or hydrocephalus or intracerebral hypertension, 6 because of advanced chronic renal failure (creatinine clearance <30 mg/dL), 1 because of acute renal failure, 4 because of chronic heart failure, and 13 because they were on antibiotic therapy at the time of admission to the rehabilitation unit.

Of 136 patients, 125 completed the study protocol: 11 patients (5 in the treated group and 6 in the placebo group) discontinued the study. The reasons for discontinuation were self-discharge (n = 5), need for surgical intervention (n = 2), myocardial infarct (n = 2), hemorrhagic gastric ulcer (n = 1), and brain hemorrhage recurrence (n = 1). Thus, 63 patients on EAAs and 62 patients on placebo completed the 60-day protocol and were analyzed.

Time from injury event was 85 ± 15 days for placebo patients and 98 ± 25 for EAA patients. From medical referral, 46% of patients allocated in treatment group and 40.3% of the placebo group had suffered from infections after index events over the period prior to their admission to our rehabilitation institute.

Of 125 patients analyzed, 31 (25%) with type II diabetes mellitus and taking insulin or oral hypoglycemic drugs were similarly distributed between the groups (14 in the EAA group, 17 on placebo). Ten (8%; 4 in the placebo group and 6 in treated group) had moderate renal impairment (creatinine clearance 30–59 mL/min).

Baseline Characteristics

After randomization, the 2 groups of patients were similar in demographics, anthropometric characteristics, types of primary brain insult, medical advice, and nutrition intakes (by spontaneous diet or tube-feeding nutrition; Table 2).

Among nutrition intakes, the ingestion of EEAs in the diet was within the range of normal values of our laboratory (25%–35%) and similar between the 2 groups, being 27.1 ± 3.5 g/d (34.45% ± 6% total ingested amino acids) in placebo patients and 27.78 ± 4.1 g/d (39% ± 5.8% of total ingested amino acids) in the EAA group (*NS*). However, when supplemental EAA in the experimental group was considered (8 g/d), the total intake of EAA in the experimental group was 35.78 g/d or 43.8% ± 6% of total ingested amino acids ($P < .02$ vs placebo).

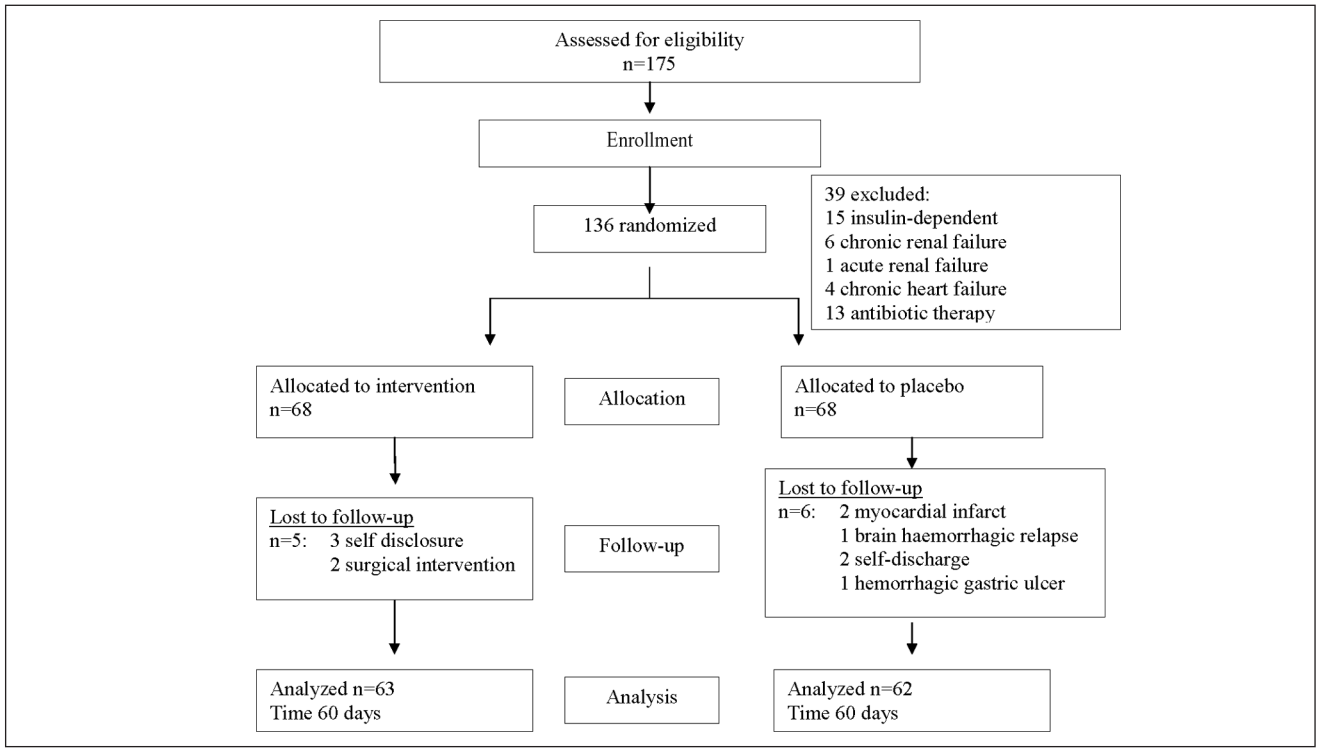

Figure 1. Flow diagram indicating the number of patients recruited and registered.

With respect to biohumoral variables, there were no significant intergroup differences except for blood glucose and serum prealbumin levels, which were, respectively, higher ($P < .01$) and lower ($P < .05$) in treated patients and patients on placebo.

The 2 groups were similar for plasma content in EAAs: 901 ± 181 µmol/L in the placebo group and 983 ± 193 µmol/L in the EAA group.

The degree of disability evaluated by FIM or DRS was similar between treated and nontreated patients.

EAAs and Occurrence of Infections

The study showed that when considering all patients as an entire group, 60 of 125 (48%) had single infection events over the first month. It should be emphasized that each patient developed a single infection event over the first month. Over the total period of rehabilitation (2 months), the infection rate was 77 of 125 (61.6%).

When the 2 groups were analyzed separately, the incidence of infections in the first month of rehabilitation was 23.2% lower in the EAA group (23/63 patients; 36.5%) than in the placebo group (37/62 patients; 59.7%) ($P < .01$). The types of infection were similarly distributed between treated and placebo subgroups (Table 3).

Stratifying the entire study population in relation to 2 groups of etiology—that is, the group of ischemic stroke patients (n = 86) and the group of hemorrhagic stroke + anoxic coma + traumatic brain injury patients (n = 39)—we observed that within each group, the occurrence of infection in placebo patients was about 2-fold that of patients on EAAs. Indeed, in the ischemic stroke group, the infection rate was 54.7% in the placebo patients vs 27.5% in treated patients; in the other group, the infection rate was 70% in placebo patients and 37% in patients treated with EAAs.

With respect to the occurrence of infections over the second month of the hospital admission, 10 patients on placebo developed 1 infection, and 1 developed 3 infections. Among the treated group, 2 had another infection and 1 patient had 2 other episodes of infection. Intergroup differences in the recurrence of infection were not important ($P = .26$). Thus, over 2 months of hospital stay, the total number of infections was 50 in the placebo group and 27 in the treated patients.

The timing of infection was 15 ± 3 days after randomization in 45% of the patients and more than 20 days after randomization in the remaining 55% of the patients.

Antibiotics used for treating infections were cotrimoxazole (12.2%), penicillin and derivates (25.6%), quinolones (26.6%), amikacin (18.4%), imipenem (6.1%), teicoplanin (2%), cephalosporin (2%), and metronidazole (4.1%). Nonsignificant differences were found between the 2 subgroups with respect to the type of antibiotic being used.

Table 2. Demographic, Clinical, Biohumoral, Anthropometric, and Nutrition Variables and Medical Devices in 2 Groups of Patients After Randomization to Placebo or Supplementation With Essential Amino Acids (EAAs)

Variables	Normal Values	Placebo Group (n = 62)	%RDA	EAA-Supplemented Group (n = 63)	%RDA	*P* Value
Demographic						
Male/female		37/25		40/23		*NS*
Age, y		63.9 ± 17		62.7 ± 12.4		*NS*
Clinical						
Etiology						
Stroke						
Ischemic (n = 86; 68.8%)		45		41		*NS*
Hemorrhagic (n = 22; 17.6%)		9		13		*NS*
Traumatic brain injury (n = 9; 7.2%)		5		4		*NS*
Cardiac arrest/anoxic coma (n = 8; 6.4%)		3		5		*NS*
Anthropometric						
Body weight, kg		72.29 ± 15.26		71.63 ± 15.61		*NS*
Body mass index, kg/m^2		25.86 ± 4.6		25.38 ± 4.55		*NS*
Blood						
Hemoglobin, g/dL	F >12 M >13	12.94 ± 1.9		13.09 ± 1.2		*NS*
Urea, mg/dL	20–40	39.8 ± 21.8		40.9 ± 20.1		*NS*
Creatinine, mg/dL	0.7–1.2	0.96 ± 0.3		0.99 ± 0.26		*NS*
Glucose, mg/dL	80–110	101.77 ± 26.1		118.26 ± 43.2		<.01
Total white cells, n/mm^3	4000–9000	6479 ± 1923		6856 ± 1984		*NS*
Lymphocytes, %	20–40	27.3 ± 8.83		27.8 ± ±10.3		*NS*
Albumin, g/dL	3.5–4.8	3.41 ± 0.54		3.26 ± 0.56		*NS*
Prealbumin, mg/dL	18–30	22.04 ± 5.9		19.94 ± 5.39		<.05
Fibrinogen, mg/dL	230–500	401.3 ± 101.4		377 ± 91.93		*NS*
C-reactive protein, mg/dL	<0.3	1.48 ± 3.03		1.27 ± 1.67		*NS*
Daily nutrition intake[a]						*NS*
Energy						
kcal	≥25	1960 ± 340		1835 ± 278		*NS*
kcal/kg		26.2 ± 4.5	105	24.3 ± 3.66	97.2	*NS*
Proteins						
g		78.4 ± 8.5		73.7 ± 9.1		*NS*
g/kg	≥1.1	1.08 ± 0.23	98	1.03 ± 0.22	94	*NS*
Lipids						
g		66.6 ± 7.1		64.3 ± 5.9		*NS*
g/kg	≤1	0.92 ± 0.19	92	0.89 ± 0.19	89	*NS*
Carbohydrates						
g		266 ± 61		256 ± 54		*NS*
g/kg	2.5–4	3.7 ± 0.77		3.57 ± 0.77		*NS*

(continued)

Table 2. (continued)

Variables	Normal Values	Placebo Group (n = 62)	%RDA	EAA-Supplemented Group (n = 63)	%RDA	*P* Value
Calcium, mg	1000	1333 ± 225	133	1285 ± 194	128	*NS*
Phosphorus, mg	1000	1333 ± 204	133	1285 ± 175	128	*NS*
Potassium, mg	3100	2900 ± 650	93	2796 ± 592	90	*NS*
Sodium, mg	ND	1725 ± 152		1663 ± 103		*NS*
Iron, mg	10	12 ± 2.5	120	11 ± 1.5	110	*NS*
Zinc, mg	10	9.8 ± 1.9	98	7.6 ± 0.8	76	*NS*
Copper, mg	1.2	1.1 ± 0.3	92	0.9 ± 0.3	75	*NS*
Iodide, mcg	ND	108 ± 14.5		104 ± 20.5		*NS*
Devices						
Endotracheal tube		4/4		4/4		*NS*
Bladder catheter		19/41		22/41		*NS*
Venous catheter						*NS*
Central		7/15		8/15		*NS*
Peripheral		19/32		13/32		*NS*
Tube feeding		8/19		11/19		*NS*

Data are expressed as mean ± SD unless indicated otherwise. Statistical analysis: unpaired *t* test and χ^2 test when appropriate. Placebo group vs essential amino acid–supplemented group. RDA, recommended daily allowance; ND, not defined; *NS*, not significant.

[a]The values represent the average of combined spontaneous diet and tube-feeding nutrition.

Table 3. Number of Single Infections Developed in the Placebo and Treated Groups Over the First Month of Rehabilitation

	Total, No. (%)	Placebo Group	EAA-Treated Group	*P* Value
No. of infections	60	37	23	<.01
Distribution				
Urinary tract	44 (73.3)	26	18	*NS*
Respiratory tract	8 (13.3)	5	3	*NS*
Skin	2 (3.3)	1	1	*NS*
Gastrointestinal Tract	2 (3.3)	2	0	*NS*
Blood	4 (6.7)	3	1	*NS*

Anatomical distribution of infections is described. Statistical analysis: χ^2 test. EAA, essential amino acid; *NS*, not significant.

Changes in Variables During the Rehabilitation Period

Over time, both groups had significant and similar reductions from baseline of blood glucose ($P < .001$), blood white cells ($P = .005$), neutrophils ($P < .0001$), haptoglobin ($P < .002$), fibrinogen ($P = .003$), and CRP ($P < .016$). In contrast, the percentage of lymphocytes ($P < .0001$) and FIM/DRS ($P < .001$) increased in both groups. The average CRP values at discharge were above the normal upper limit (<0.3 mg/dL; Table 4). The changes in all these variables were more pronounced in the EAA group than in the placebo group, although the difference was not statistically significant.

During the rehabilitation period, baseline nutrition intakes, including ingested EAAs, were practically unchanged in the 2 groups, so that the patients' body weight was preserved. This is an indicator that the nutrition intakes were adequate, particularly in proteins, iron, and zinc content, which play a major role in immune function[37] (Table 2).

Risk-Identifying Variables

The univariate logistic analyses showed that albumin, prealbumin, transferrin, and CRP concentrations were statistically significantly associated with an increased risk of developing infection. ORs for patients at risk varied from 2.43 for transferrin to 4.17 for prealbumin (Table 5). The stepwise procedure identified CRP (adjusted OR = 4.44; 95% CI, 1.74–11.32) and prealbumin (adjusted OR = 3.11; 95% CI, 1.30–7.46) as the risk factors most strongly predicting infections.

Relationship Between Measured Variables and Functional Independence

At both admission and discharge, FIM/DRS was positively correlated with albumin, prealbumin, transferrin, and hemoglobin, whereas it was negatively associated with CRP. We also found that FIM/DRS at discharge was related to FIM/DRS on admission.

In multiple regression analysis, albumin and transferrin were the variables with the strongest association with FIM/DRS level at both admission and discharge (Figure 2). The albumin level on admission was the strongest predictor of FIM/DRS at discharge (Table 6).

Discussion

The study shows that supplementary EAAs may reduce the occurrence of infection in rehabilitation patients with BI by 23% over the first month of their stay in the hospital and by a total 30% over the entire period of rehabilitation (2 months). The study also indicated that serum concentrations of prealbumin <20 mg/dL and CRP >0.3 mg/dL can allow physicians to identify patients at high risk of future infection.

EAAs, Infection, and Body Inflammation

Despite the adoption of preventive measures, adequate nutrition (including daily ingestion of EAAs), and normal EAA plasma concentration, patients with BI receiving placebo had a high frequency of infections. This suggests that both normal ingestion and plasma concentration of EAAs may not be enough to limit the development of infection. It is probable that both the persistence of long-lasting inflammation[38] and a further worsening of immune function following acute BI[10] favor the occurrence of infections.

The persistence of inflammation at the time of admission to rehabilitation may reflect the persistence of an inflammatory response to brain ischemia[39,40] and/or postacute complications and hospitalization.

Indeed, in the post–acute phase of BI, patients usually develop 1 or more complications, causing a condition of chronic stress responsible for immunosuppression.[41] This might help to explain why the rate of infections observed in rehabilitation patients receiving placebo was higher than that reported for individuals in ICUs.[2]

Table 4. Trends Over Time of the Variables Determined in the 2 Groups of Patients

	Placebo Group, Mean ± SD			EAA Group, Mean ± SD		Trend Over Time (*P* Level)	
Variables	Normal Values	Admission	Discharge	Admission	Discharge	Global	Interaction
Anthropometric							
Body weight, kg		72.3 ± 15.26	72.05 ± 14.47	71.63 ± 15.61	71.1 ± 14.72		
Body mass index, kg/m^2		25.86 ± 4.6	25.8 ± 4.36	25.38 ± 4.55	25.20 ± 4.26	.2	.37
Blood							
Erythrocyte sedimentation rate, first hour, mm	2–20	38.6 ± 30.3	32.84 ± 22.57	35.3 ± 30.3	31.27 ± 24.09	.14	.50
Hemoglobin, g/dL	F >12 M >13	12.94 ± 1.9	12.8 ± 1.5	13.09 ± 1.2	12.9 ± 1.6	.26	.92
Urea, mg/dL	20–40	39.8 ± 21.8	37 ± 16.1	40.9 ± 20.1	38.1 ± 13.6	.07	.94
Creatinine, mg/dL	0.7–1.2	0.96 ± 0.3	1.01 ± 0.33	0.99 ± 0.26	0.98 ± 0.25	.15	.08
Glucose, mg/dL	80–110	101.77 ± 26.1	90.8 ± 16.8	118.26 ± 43.2	101 ± 29	<.001	.19
Total white cells, n/mm^3	4000–9000	6479 ± 1923	6194 ± 2487	6856 ± 1987	6177 ± 1711	.005	.24
Neutrophils, %	50–70	60.19 ± 9.6	57.8 ± 9.45	59.85 ± 10.92	56 ± 10.4	<.001	.33
Lymphocytes, %	20–40	27.3 ± 8.83	29.6 ± 8.97	27.8 ± 10.3	31.12 ± 10.6	<.001	.433
Monocytes, %	2–12	8.73 ± 2.6	8.6 ± 2.64	9.23 ± 2.2	9.5 ± 2.33	.75	.31
Albumin, g/dL	3.5–4.8	3.41 ± 0.54	3.47 ± 0.63	3.26 ± 0.56	3.44 ± 0.46	<.001	.40
Prealbumin, mg/dL	18–30	22.04 ± 5.9	22.66 ± 5.8	19.94 ± 5.39	20.44 ± 5.14	.22	.93
Haptoglobin, mg/dL	420–902	195.1 ± 116.4	175 ± 90.4	193.7 ± 102.4	168 ± 73.2	.002	.80
Interleukin-6, pg/mL	1–7	11.52 ± 17.02	9.8 ± 9.03	19.92 ± 41.29	21.71 ± 79.9	.86	.50
Fibrinogen, mg/dL	230–500	401.3 ± 101.4	373 ± 66	377 ± 91.93	358 ± 72.1	.003	.96
Transferrin, mg/dL	150–300	204.23 ± 40.2	207.5 ± 41.5	205.16 ± 56	211 ± 47.5	.20	.94
C-reactive protein, mg/dL	<0.3	1.48 ± 3.03	0.75 ± 0.98	1.27 ± 1.67	0.9 ± 1.8	.003	.75
Functional							
Functional independence measure score/Disability Rating Scale	125	62.58 ± 27.9	80 ± 26.7	56.4 ± 28	77 ± 31.2	<.016	.32

Statistical analysis: repeated measures analysis of variance. Trend over time: global = difference between admission and discharge; interaction = differences in trends between groups. EAA, essential amino acid; nv, normal value.

Table 5. Results From Univariate Logistic Analysis Showing Risk-Individuating Variables Associated With an Increased Risk of Infection

Variable	Odds Ratio	95% Confidence Interval	*P* Value
Albumin (<3.8 g/dL)	3.80	1.48–9.74	.005
Prealbumin (<20 mg/dL)	4.17	1.84–9.45	.001
Transferrin (<200 mg/dL)	2.43	1.16–5.10	.019
C-reactive protein (>0.3 mg/dL)	3.80	1.71–8.44	.001

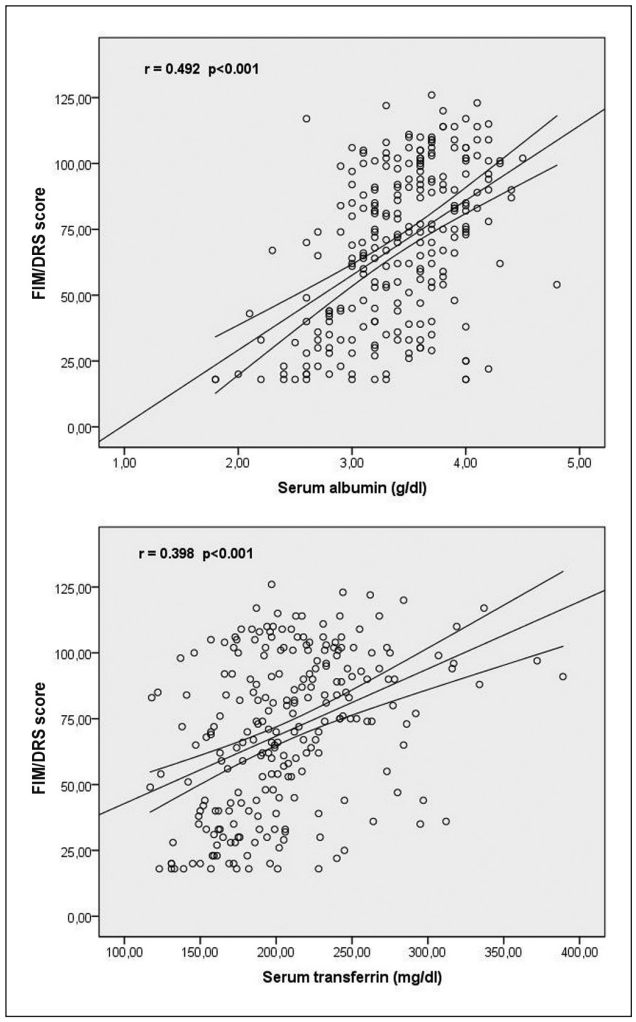

Figure 2. Correlations between circulating proteins (albumin and transferrin) and the patients' functional independence measure (FIM) or disability rating scale (DRS), when appropriate. Values at the time of admission to and discharge from the rehabilitation unit are pooled together.

The data from the current study confirm the results of our recent investigation in which we found a 30% decrease in infections in elderly individuals undergoing intensive rehabilitation for diseases other than BI but who were treated with the same EAA formula and at the same dose as that used in this study.[7] What is relevant in the present investigation is that the positive effects of EAAs occur when the amount of EAAs (as a percentage of total ingested amino acids) ingested daily is far above the normal values (around 30% in our laboratory). In treated patients, the ingestion of high amounts of EAAs could seem in conflict with normal EAA plasma concentrations. This can be reconciled when considering that the ingestion of EAAs is followed by increased EAA plasma concentration that returns to a preingestion concentration within 4 hours (R. Aquilani, unpublished data), whereas in this study, patients' blood samples for amino acid determination were drawn in the morning after overnight fasting, as indicated in the experimental section.

The decrease of infection rate in the treated group was probably due to the actions of EAAs on immune cells. Indeed, EAAs can potentiate a patient's immune response through at least 2 mechanisms: inducing protein synthesis in immune cells and directly influencing some metabolic pathways involved in immune function.

Protein synthesis[42-44] is crucial to increase bacterial killing, lymphocyte proliferation, macrophage phagocytosis, and antibody and cytokine production.[26] It is interesting that the increase observed in blood lymphocyte percentage during the rehabilitation period was more pronounced, albeit not significantly so, in the treated patients (+18%) than in the placebo recipients (+4.4%). The decrease in blood white cell count was greater in treated patients (–18%) than in the placebo recipients (–4.4%). The improvement in lymphocyte counts found in this study is in line with that observed in a recent investigation carried out in individuals in an ICU treated with the same formula, at the same dose as that used in the current study.[45]

Some amino acids contained in the formula can directly and specifically affect some metabolic pathways involved in immunity. For instance, cysteine is a substrate essential for glutathione metabolism and the redox potential of immune cells[46]; methionine (and cysteine) is essential for the synthesis of nucleic bases[47] and, therefore, cell proliferation and differentiation.

The formula used in this study does not contain glutamine, the most important amino acid for immune activity.[26] Nevertheless, we observed in our laboratory (R. Aquilani, unpublished data) that healthy individuals given supplementation with 8 g of the formula had a 10% increase in their plasma

Table 6. Correlation Analysis Between Patients' Functional Independence and Biohumoral Variables

	r^a	*P* Value	Multiple Regression
FIM/DRS admission vs			
albumin at admission	0.53	<.001	
prealbumin at admission	0.35	<.001	
transferrin at admission	0.42	<.001	Albumin ($P < .001$)
CRP at admission[b]	–0.46	<.001	
hemoglobin at admission	0.35	<.001	Transferrin ($P = .015$)
FIM/DRS discharge vs			
albumin at discharge	0.423	<.001	Albumin ($P < .001$)
prealbumin at discharge	0.242	.008	
transferrin at discharge	0.385	<.001	Transferrin ($P = .001$)
CRP at discharge[b]	–0.217	.015	
hemoglobin at discharge	0.16	*NS*	
FIM/DRS discharge vs			
albumin at admission	0.400	<.001	
prealbumin at admission	0.192	.038	
transferrin at admission	0.328	<.001	
CRP at admission[b]	–0.362	<.001	
FIM at admission	0.818	.001	Albumin ($P < .001$)

CRP, C-reactive protein; DRS, disability rating scale; FIM, functional independence measure; *NS*, not significant.
[a]Pearson's correlation coefficient.
[b]Analysis performed on the log-transformed variable.

glutamine concentrations for 3 hours. The increase was not accompanied by an impairment of plasma arginine concentration, another important amino acid for immune function.[26] It is likely that leucine, the main amino acid of the formula, may stimulate glutamine formation.[48]

The study indicates that patients with BI are discharged with a subclinical inflammation even though of lower grade in comparison to that observed at admission to rehabilitation. This suggests that 5 to 6 months after the index event, inflammatory processes and an evoked immune response are still active. Indeed, high levels of IL-6 are produced by pathogen-activated immune cells.[49] In turn, IL-6 mediates the immune system response, for instance, by inducing immunoglobulin formation in B lymphocytes and differentiation of cytotoxic cells.[49]

It is relevant to this study that elevated levels of IL-6 increase the need for protein synthesis and, consequently, for EAA availability. At a first glance, the inflammation, both at admission and discharge, seems not to have evoked an acute response given the normal values of acute reactant proteins (fibrinogen, haptoglobin). However, this is misleading because the reduction at discharge of "normal" reactant proteins suggests that actually the levels of these proteins at admission were higher than they would have been in the pre-event period.

The gradual reduction of inflammation over time could explain the significant decrease in blood glucose levels during rehabilitation; during rehabilitation, the reduction of glucose means a decrease of liver gluconeogenic activity, one of the markers of the acute phase response. Very likely, the higher blood glucose levels observed in the EAA group at admission in comparison with those in the placebo group reflected a more marked acute phase response before admission for rehabilitation.

Serum Proteins as Risk Factors for Future Infection

The study indicates that serum prealbumin <20 mg/dL and CRP >0.3 mg/dL are the risk factors with the strongest potential to predict the development of a future infection.

Prealbumin is a protein synthesized by the liver and secreted into the circulation. It is a negative reactant protein during metabolic stress from trauma, injury, infection, and body systemic inflammation and is negatively influenced by inadequate nutrition. Because of its short half-life (2–3 days), prealbumin levels rapidly reflect changes in hepatic protein synthesis.[50] It is possible that the importance of prealbumin as a risk factor for infection may derive just from its ability to reflect 2 effects simultaneously: the early shift of liver protein synthesis induced by the acute phase response and inadequacy of nutrition that usually occurs with the acute phase response.

CRP is a positive reactant protein of the acute phase response following acute injury, infection, and trauma. High CRP levels reflect inflammation. This protein is synthesised in the liver under the influence of increased IL-6 production.[49] The

importance of elevated CRP as a risk factor for infection confirms the finding of our previous investigation.[7]

The value of serum albumin concentration <3.8 g/dL and transferrin <200 mg/dL as factors predicting infection should not be underestimated, particularly when measures of prealbumin and CRP are not feasible.

The patients' baseline reduction of albumin (<3.5 g/dL) can be explained by the persistence of the shift of liver protein synthesis primed by the acute phase response and, concomitantly, a possible inadequate response to nutrition in the acute and post–acute periods after the BI.[51]

It is of clinical interest that a cutoff level of albumin <3.8 g/dL to identify patients at increased risk of infection is higher than the normal lower value of our laboratory (3.5 g/dL). In this respect, the present study seems to confirm that an albumin concentration of 3.5 g/dL[52] may represent a clinically suboptimal level. Indeed, albumin <3.8 g/dL better predicts future loss of skeletal mass (sarcopenia) and poor functioning in older individuals.[52]

Last, normal transferrin levels mean a normal capacity of binding iron, which is essential for immunocompetency.[37]

Relationship Between Variables and Functional Independence

Albumin and transferrin levels had the strongest association with FIM/DRS both at admission and discharge of the patients. Importantly, 3 months after the acute event, the albumin concentration at admission had the highest predictive power for FIM/DRS at discharge. This confirms that patients' protein status, as result of nutrition/body inflammation state, plays a crucial role in brain repair.[53-57]

Circulating proteins and anatomical and functional damage to the brain mutually influence each other. Indeed, the levels of serum protein are largely affected by acute phase response primed by vascular or traumatic insult to the brain,[53] and, in turn, circulating proteins influence both repair processes and functional reactivation of damaged areas of the brain.[53] Experimental investigations have demonstrated a beneficial effect of albumin on brain activity. Moderate- to high-dose human albumin therapy provides neuroprotection in animal models of cerebral ischemia and traumatic BI.[58] The benefit of albumin to the damaged brain may be due to 1 or more properties of the protein, including free radical scavenging, colloid osmotic pressure favoring blood perfusion, platelet function inhibition and antithrombotic effects, capillary membrane permeability, and the binding and transport of molecules, including zinc.[59,60] Even in healthy people, the levels of albumin are important for functional status. Indeed, observational studies found that the circulating protein influences physical performance even among nondisabled persons[61] and is positively related to functional status.[62,63] Lower levels of albumin are predictive of a greater decline in functional status.[61]

Normal levels of circulating transferrin are essential for binding brain iron to allow efflux of the mineral from the brain. An excess of free iron in brain structures occurs following neuronal damage after cerebral ischemia and trauma and contributes greatly to the progression of stroke and worsening outcome.[64]

The continued positive association of transferrin levels with functional status at the time of the patients' discharge suggests that 5 months after an acute event, the neurocognitive recovery of patients is influenced by brain iron metabolism.

Although prealbumin, hemoglobin, and CRP levels did not enter the multiple correlations in the model, we believe they merit some comments.

The positive correlation between prealbumin levels and patients' independence enhances the importance of adequate nutrition for neuromotor and cognitive recovery.[53-57] In contrast to our previous studies on stroke patients, this investigation failed to demonstrate a significant improvement in patients' disability following supplementation with EAAs in comparison to patients given a placebo. The discrepancy is probably the result of a different study design. In fact, in previous investigations, patients were enrolled within 30 days after the acute event, which is the period in which potentially 80% of future total neurological recovery occurs. In this study, patients with stroke, who represented the vast majority of the population, were enrolled later (88 days) after the index event. Difficulty of EAAs to enter brain structures can be excluded given that these substrates enter as rapidly as glucose, particularly for blood-brain barrier damage.[56,57]

By providing an oxygen supply to cerebral and extra-cerebral structures, the hemoglobin concentration favors patients' functional independence. The brain's requirement for oxygen is further increased under ischemic conditions.[64]

The negative relation of CRP with FIM/DRS suggests that subclinical body inflammation has a negative impact on the recovery of functional independence.

The study also indicates the importance of the level of neuromotor-cognitive function at admission for the patients' functional status at the end of rehabilitation.

Clinical Implications

The study suggests that certain factors may be useful for the clinical practice of patients with BI admitted at about 3 months after the index event.

First, it is possible to identify individuals with BI at increased risk of future infection by measuring the serum levels of prealbumin and CRP. If this is not feasible, albumin and transferrin concentrations can be useful.

Second, at 3 months after an acute event, it is useful to monitor patients' protein intake and circulating proteins, including CRP, given that these factors affect the recovery of functional status.

Third, at discharge from rehabilitation, CRP should always be determined, not only to predict the risk of developing infection at home but also because elevated levels of the protein per se affect both life and functional prognosis. Persistently high levels of CRP may be particularly ominous for stroke-surviving individuals. Because the protein contributes directly to the progression of atherosclerosis and the proliferation of cardiovascular events,[65] high CRP after a first cerebrovascular event can predict further adverse cardiovascular events and recurrent strokes.[66] It has been reported that carotid artery plaques are less stable in those with elevated CRP.[67] High CRP can slow the functional recovery of patients with BI because the protein diminishes angiogenesis, a key compensatory mechanism in chronic ischemia,[68] and augments the endothelial cell production of IL-6.[68]

These considerations should prompt physicians and general practitioners to reduce the risk of infection as much as possible, treat an infection immediately when it occurs, and achieve total resolution of inflammation primed by infection.

This study highlights the importance of improving patients' functional status as much as possible within the first days/weeks after the acute event. We believe that one possibility for favoring patients' recovery in this context may be better monitoring and treatment of metabolic alterations.

Limitations

This study has some unresolved issues that should be faced with appropriate investigations.

We did not consider the effects of EAAs on the prevention of a specific infection (urinary, respiratory, or gastrointestinal).

The study addressed the prevention of infection in a large population with BI independently of the type of primary disease (cerebrovascular, traumatic, or cardiac origin). At first glance, this may seem to be a limitation of the study. However, we believe that this was actually a strength of the study. In fact, our intent was to control infection independently of the underlying disease. Our starting point was that the causes of BI have a common denominator consisting of injury-induced immune dysfunction/suppression that can be further aggravated by postacute complications. Substances such as EAAs, which are potentially able to act on immune cells, could, therefore, favor reduction of infection independently of the primary disorder. This is clearly indicated in the study by the occurrence of infection that nearly halved in patients supplemented with EAAs in comparison to those on placebo, independently of the etiology group. Thus, the study suggests that each brain-injured population may benefit from EAA supplementation. In this study, we did not directly measure the activity of EAAs on immune cell function. For instance, determination of T lymphocyte subpopulations would have strengthened the discussion.

Interestingly, instead of a mixture of non-EAAs, we chose maltodextrin as the placebo because this polysaccharide has a potential immunomodulatory effect. The study demonstrated that EAAs are superior to maltodextrin in reducing infection. If we had chosen non-EAAs as placebo, we would have undoubtedly shown the superiority of EAAs to reduce the rate of infection, given that increased immunocompetence relies on a huge activity of protein synthesis. This occurs with EAAs only. Thus, we believed that a more potent competitor against EAAs would have been a substrate with a potential immunomodulatory effect.[69-71]

Bacterial Ecology and Financial Considerations

This study indicates that EAAs, by reducing the occurrence of infection, can contribute considerably to lowering the selection of more aggressive pathogens as well as antibiotic use and resistance in the rehabilitation context. Over 1 year, we spared 230 days of antibiotic therapy among 125 patients.

Appropriate studies are necessary to quantify both direct and indirect cost-savings from the use of EAA supplementation.

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