Controlled study of enteral arginine supplementation in burned children: impact on immunologic and metabolic status

Veronica B. Marin, M.D.[a,b,*], Lorena Rodriguez-Osiac, M.D.[a], Liana Schlessinger, M.D.[a], Jorge Villegas, M.D.[c], Marcelo Lopez, M.D.[a], and Carlos Castillo-Duran, M.D.[a]

[a] *INTA, University of Chile, Santiago, Chile*
[b] *Department of Pediatrics, University of Chile, Santiago, Chile*
[c] *Department of Pediatric Surgery, Luis Calvo Mackenna Childrens Hospital, Santiago, Chile*

Abstract

Objective: We compared the effects of an arginine-supplemented diet with those of an isocaloric isonitrogenous diet on immune and metabolic response of children with burns.

Methods: This was a double-blind, randomized, placebo-controlled trial in a burn treatment center of a pediatric hospital in Santiago, Chile. All children (1–5 y of age) admitted within 48 h of a moderate to deep burn injury covering 10% to 40% of total body surface area were evaluated. Twenty-eight children met the criteria and were randomly assigned to receive an arginine-supplemented diet (AG; $n = 14$) or an isocaloric isonitrogenous diet (CG; control, $n = 14$) for 14 d. Samples were collected at admission (baseline) and on days 7 and 14 for lymphoproliferative response to mitogens, plasma interleukins (interleukin-1, interleukin-6, tumor necrosis factor-α), plasma arginine and ornithine levels, serum C-reactive protein, prealbumin, albumin, glucose, and total urinary nitrogen.

Results: The AG enhanced lymphoproliferative responses (analysis of variance, $P < 0.05$), which were 72% of normal at baseline in both groups; by day 7 responses increased to 144% in the AG group and decreased to 56% in the CG group; both groups returned to normal by day 14. Baseline interleukin-6 was significantly increased in all children. There were no differences in plasma concentrations of interleukin-1, interleukin-6, tumor necrosis factor-α, C-reactive protein, prealbumin, albumin, or glucose between the AG and CG groups. On day 7 plasma ornithine levels increased significantly in the AG versus CG group ($P < 0.05$); arginine levels showed no change.

Conclusions: An exclusively AG improves mitogen-stimulated lymphocyte proliferation in burned children. The benefits of arginine for the immune system do not appear to be related to a metabolic response. The biological significance of this finding remains to be determined.

Keywords: Enteral nutrition; Arginine; Immune response; Metabolic response; Burn; Children

Introduction

Burn injury produces a hypermetabolic response characterized by increased metabolic rate, increased minute ventilation, increased cardiac output, increased gluconeogenesis that is resistant to glucose infusion, and increased visceral and skeletal muscle catabolism [1]. Patients become hypermetabolic and hypercatabolic with a negative nitrogen balance; this may delay wound repair until wound closure is complete and for a variable period thereafter. Adequate support of this physiologic repair process is essential [2–4].

In contrast, burn injury patients exhibit depressed immune function and are at high risk of developing nosocomial infections. Injury-induced anergy has been considered a major determinant for the high susceptibility of burned trauma victims to serious infections and multiple organ dysfunction syndrome [5]. It is commonly agreed that ther-

This study was funded in part by grant 1990081 from FONDECYT.
* Corresponding author. Tel.: +56-2-758-8574; fax: +56-2-630-0474.
E-mail address: vmarin@uec.inta.uchile.cl (V.B. Marin).

mal injury is associated with suppression of cellular mitogen-stimulated lymphocyte proliferation [6,7] and a diminished ability to propagate and maintain a normal immunoglobulin G (IgG) antibody response, despite the presence of normal or increased numbers of antigen-specific B cells [8,9]. Inflammatory cytokines are overproduced, inducing an excessive inflammatory response, sepsis, multiple organ failure, and even death [10–12]. Early enteral nutritional support has proved effective in providing metabolic-nutritional substrates in such catabolic patients, but there is still a need to define qualitatively the role of specific pharmaconutrients in moderating the inflammatory response. Hopefully, this should lead to enhanced immune function and improved survival [13–17]. The so-called immune-enhanced diets containing or supplemented with glutamine or arginine are able to decrease septic complications and shorten hospital stay [18–20].

Arginine is a non-essential amino acid in healthy adults, but may be considered conditionally essential during critical illness [21,22]. Previous studies in burned patients have demonstrated a limited net rate of arginine de novo synthesis despite a significantly increased arginine turnover, thus fulfilling the definition of a conditionally indispensable amino acid after major burns [23]. Experimental studies have suggested that dietary arginine supplementation decreases mRNA expression of inflammatory cytokines in organs and improves survival rate after thermal injury [24]. Some studies have used complex formulas that combine arginine, nucleic acids, and ω-3 fatty acids; therefore, the specific role of arginine is unclear [25,26]. Considering the above and the lack of data for pediatric burn patients, we studied the effects of supplemental dietary arginine versus an isocaloric and isonitrogenous placebo diet to clarify specific roles of arginine in immune and metabolic responses in burned children.

Table 1
Characteristics of enteral formula*

Nutrient	Per 100 g
Energy (kcal)	488
Proteins (g)	13.2
Total lipids (g)	22.4
Available carbohydrates (g)	58.4
Taurine (mg)	31.7
L-carnitine (mg)	3.65
Sodium (mg)	195.2
Potassium (mg)	595.4
Vitamin A (μg RE)	419.7
Vitamin C (mg)	97.6
Vitamin D (μg)	9.0
Vitamin E (mg TE)	12.0
Thiamin (mg)	1.0
Riboflavin (mg)	1.2
Niacin (mg NE)	10.0
Vitamin B6 (mg)	1.1
Folic acid (μg)	104.4
Biotin (mg)	0.05
Pantothenic acid (mg)	3.7
Vitamin K1 (μg)	52.5
Choline (mg)	34.2
Inositol (mg)	24.4
Calcium (mg)	439.2
Iron (mg)	6.3
Phosphorus (mg)	341.6
Iodine (μg)	49.1
Magnesium (mg)	73.2
Zinc (mg)	6.3
Copper (mg)	0.5
Selenium (μg)	14.7
Chromium (μg)	15.0
Molybdenum (μg)	14.6
Manganese (mg)	0.8
Chlorine (mg)	439.2

RE, retinol equivalents; TE, tocopherol equivalents.
* ADNR (Braun Laboratories, Santiago, Chile).

Materials and methods

Patients

In our service we use a classification that involves total body surface area burned and full thickness burn, relating factors, forecast, and diagnosis. Thus, the injury can be superficial (A), which will be resolved by complete epithelium treatment, moderate (AB), or deep (B).

Thirty children with moderate (AB) or deep (B) burns admitted after injury to the burn treatment center of a Chilean pediatric public hospital were entered in a double-blind, randomized study to test immune and metabolic responses to an arginine-supplemented diet versus a control isocaloric isonitrogenous base diet. Inclusion criteria were age 1 to 5 y, with a classification of AB and/or 10% to 40% B body surface area burns, and prescribed enteral nutrition.

Patients admitted after 3 d after a burn or with a non-thermal burn, severe associated trauma, hepatic or renal disease, inhalation injury, or early septic shock (<48 h) were excluded from the study.

All patients were treated and assessed by the same group of staff surgeons and intensive care physicians and received standardized care according to the protocol, including early excision and grafting. Patients were monitored daily for burn complications and complications of enteral feeding. Abdominal pain, hyperkalemia, and hypophosphatemia were considered complications of arginine supplementation [27]. The protocol was evaluated and approved by the institution's ethics committee. Written informed consent was obtained from the children's legal guardians before they entered the study.

Diets

All patients were fed with a commercially available, polymeric diet, with an energy composition of 41% fat, 48% carbohydrate, and 11% protein (Table 1; ADNR, Braun Laboratories, Santiago, Chile) with additional casein, mal-

todextrin, and medium-chain triacylglycerol oil. If there was oral tolerance, they also received solid feeding. Patients were prospectively randomized, in double-blind fashion, to an arginine-supplemented diet (2% of energy as pure arginine salt, prepared specially for this purpose and without other additives; AG; $n = 14$) or a control isocaloric isonitrogenous base diet (CG; $n = 14$).

Arginine concentration was based on previous experimental studies showing that 2% arginine was the best concentration to inhibit bacterial translocation and stimulate immune function [28,29]. The amino acid profile of the two diets differed only in arginine levels. Energy intake was prescribed according to the Galveston equation [30]: total energy = 1800 cal/m^2 total body surface area/day + 2200 cal/m^2 total burn area/day.

Enteral feeding was administered as soon as possible; parenteral nutrition was not required. Patients received the diet in a bolus or as continuous enteral nutrition depending on tolerance. The same amounts of minerals and vitamins were added to both diets, which provided 100% of nutritional requirements. Randomization was performed by using a list in blocks of six patients. Clinical investigators and patients were blinded to product identity.

Laboratory parameters

Blood and urine samples were collected at admission (day 1) and at days 7 and 14 of the study, without fasting but before surgical procedures.

Immunologic evaluation

Lymphoproliferative response to concanavalin A was performed by using lymphocytes separated by dextran sedimentation and Ficoll Hypaque gradient centrifugation 22. Mononuclear cells were incubated in RPMI-1640, glutamine, antibiotics, and autologous human serum at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 72 h. Eighteen hours before harvesting, cultured cells were incubated with 18.5 GBq of tritiated thymidine (specific activity 247.9 GBq/mol) and harvested in an automatic cell harvester [31]. Results were expressed as net counts per million (cpm), i.e, counts per minute of stimulated lymphocytes with concanavalin A minus counts per minute of unstimulated lymphocytes. C_3, C_4, and IgG were measured by a radial immunodiffusion technique using the Kallestad commercial method (Sanotsky Diagnostic Pasteur, Minneapolis, MN, USA). Determination of serum proinflammatory cytokines interleukin-1, interleukin-6 (IL-6), and tumor necrosis factor-α were measured with commercial kits from R&D Systems (Minneapolis, MN, USA).

Because published data on normal parameters of lymphoproliferative response or interleukins for children are not available, a group of 12 eutrophic children 1 to 5 y of age, were studied. They did not differ from children in the AG, CG, and healthy groups (analysis of variance [ANOVA], P not significant), had no history of significant morbidity, and underwent minor optional surgery. Plasma cytokine concentration and lymphoproliferative responses were evaluated in these children by using the same presurgical blood sampling and with previously obtained written informed consent from each child's guardian.

Metabolic measurements

Blood samples drawn for serum glucose, C-reactive protein, albumin, and prealbumin were collected in tubes without anticoagulant. Samples were centrifuged within 20 min and serum was stored at 20°C until assayed. Serum C-reactive protein concentration was measured by a liquid phase immunoprecipitation assay with nephelometric detection (Turbox, Orion Diagnostic, Espoo, Finland). Serum albumin and prealbumin concentrations were also measured by a nephelometric technique (Orion Diagnostic). Total urinary nitrogen was determined by the micro-Kjeldahl method. Blood amino acid measurements of arginine and ornithine were performed by cation exchange liquid chromatography (Biotronik LC-2000; Biotronik GmbH & Co., Berlin, Germany, amino acid analyzer with Cromatopac C R5A integrator; Shimadzu Corporation, Kyoto, Japan).

Statistical analysis

Data were analyzed with Excel (Microsoft, Redmond, WA, USA) and Statistica (Statsoft, Inc., Tulsa, OK, USA). Statistical analyses included two-way ANOVA for repeated measurements to analyze changes in immunologic and metabolic parameters through days, between days, and between groups. In addition, Fisher's exact test and Student's t test were used to determine significant differences between groups in clinical, biochemical, and immunologic parameters at baseline and on days 7 and 14. Results are presented as mean ± SD; $P < 0.05$ was considered statistically significant. To calculate sample size, we used published data [6,10] concerning metabolic and immune responses, with an 80% (1-B error) chance of showing a difference ≥1 SD between means of both groups, based on Student's t test for independent samples and an α error of 0.05. Based on these considerations, a sample of 12 subjects for each group was estimated as adequate. Randomization was performed in blocks of six subjects by closed-envelope method, by an individual not involved in the rest of the study, indicating subject assignment to the study or control group until the sample was completed.

Results

Thirty patients were included, 15 in the AG group and 15 in the CG group; two patients, one from each group, developed early septic shock (<48 h) and were excluded from

Table 2
Characteristics of patients upon entry

	AG (n = 14)	CG (n = 14)
Age (y)*‡	2.3 (1–5)	2.4 (1–5)
Male/female‡	6/8	10/4
W/H (%)†‡	104 ± 11	101 ± 12
H/A (z score)†‡	0.8 ± 1.4	0.7 ± 0.8
TBSA AB and/or B (%)*‡	18 (13–30)	18 (16–40)

AG, arginine-supplemented group; CG, control group; H/A, height for age; TBSA, total burn surface area; W/H, weight for height
* Values expressed as median (range), chi-square and Fisher's tests.
† Values expressed as mean ± SD, Student's t test.
‡ Not significant.

analysis. Clinical characteristics and burn surface areas of 28 patients are presented in Table 2. There was no difference between groups with respect to age, sex ratio, anthropometric measurements, and burn surface area. Total burn surface area, including superficial (A) burns was 20.6 ± 3.9% in the AG group versus 21.6 ± 8% in the CG group (P not significant).

Nutritional status on admission, expressed as percentage of weight for height by World Health Organization standards [32], showed that 2 patients had mild malnutrition, 19 were normal, 1 was overweight, and 1 was obese. No differences were found between groups.

Nutritional intake

All patients were fed exclusively with enteral diets; 61% received enteral nutrition through a nasogastric tube, in a bolus, or as a continuous infusion depending on tolerance, and 39% were orally fed only (31% for AG group versus 42% for CG group, P not significant). Nutritional support was prescribed immediately after admission and reached 100% of total requirements within 48 h (range 12–48) without differences between groups. Real intake average was close to 95%, without significant differences between groups (91% for AG group versus 99%). No dropouts occurred due to intolerance. Mean caloric and protein intakes were 93 ± 14 versus CG: 93 ± 20 $kcal \cdot kg^{-1} \cdot d^{-1}$ and 4.19 ± 0.67 versus 4.2 ± 0.94 $g \cdot kg^{-1} \cdot d^{-1}$ for the AG and CG groups, respectively, with no differences between groups.

Immunologic studies

To evaluate the effect of arginine on sequential changes in lymphoproliferative response, we considered only the first two samples (days 1 and 7) because eight patients (three in the AG group and five in the CG group) had incomplete data for day 14. Results of statistical analyses did not change when these were included. The AG diet significantly increased the lymphoproliferative response to concanavalin A by repeated measures ANOVA ($F_{1,15}$ = 4.41, P = 0.05). Values for both groups were 72% of normal at entry compared with healthy children measured in our laboratory (mean 20 683 ± 12 646 for burned children versus 28 591 ± 20 237 for healthier children, not significant). AG increased the response to 151% by day 7 versus a decrease to 66% with CG (mean 35 995 ± 29 046 cpm for AG versus 17 576 ± 14 996 cpm for CG; Student's t test, P = 0.01), as shown in Figure 1. Both groups returned to normal by day 14.

At admission serum IL-6 levels were significantly elevated in both groups compared with levels in healthy children (median 30.7 pg/mL, range 3.4–182, versus median 2.75 pg/mL, range 0.6–5.9, respectively; Kruskal-Wallis, P = 0.000). Circulating IL-6 increased significantly after injury and was closely correlated with burned surface area (r = 0.62, $P < 0.05$). There were no significant differences in IL-6 levels on days 7 and 14 between the AG and CG groups (repeated measures ANOVA, P not significant; Fig. 2). Serum levels of interleukin-1 and tumor necrosis factor were undetectable in most cases; thus, statistical analysis was not possible. Serum IgG levels were below the normal limit for age and sex in 78% of cases (21 of 28) and returned to normal progressively, without differences between groups. Complement studies showed early consumption of C_3 in 86% of children in both groups, which was normalized by day 7. C_4 was low only in 40% of patients upon entry and all normalized by day 7.

Metabolic measurements

The trend toward hyperglycemia (glycemia >120 mg/dL) expected in stressed patients [33,34] was found in six patients on day 1, with a mean of 111 ± 37.5 for the entire group, and decreased significantly by days 7 and 14 (90.6 ± 17.1 and 91.3 ± 24.6 mg/dL, respectively; ANOVA, $P <$ 0.01), without differences in treatment groups. In contrast, although there were no undernourished children upon entry, most patients had serum albumin levels <3.5 g/dL (25 of 28) and prealbumin levels <0.1 g/dL (21 of 28). In both groups, albumin and prealbumin values returned to normal by day 7 in >90% of patients. C-reactive protein was slightly elevated (median 11 mg/dL, range 5–86) and only two patients showed values >75 mg/dL in the first sample, as seen in severely stressed patients [35]. In both groups values increased by day 7 (median 24 mg/dL, range 5–170) and most returned to normal by day 14 (median 5 mg/dL, range 5–86), without differences between diet groups.

Total urinary nitrogen was elevated from day 1 in both groups (mean 297 ± 137 $mg \cdot kg^{-1} \cdot d^{-1}$) without significant changes by day 7 (mean 288 ± 143 $mg \cdot kg^{-1} \cdot d^{-1}$) and day 14 (mean ± 134 $mg \cdot kg^{-1} \cdot d^{-1}$) for the AG and CG groups. Arginine supplementation had no effect on the evolution of sequential changes in glycemia, albumin, prealbumin, C-reactive protein, and total urinary nitrogen (repeated measures ANOVA; Table 3).

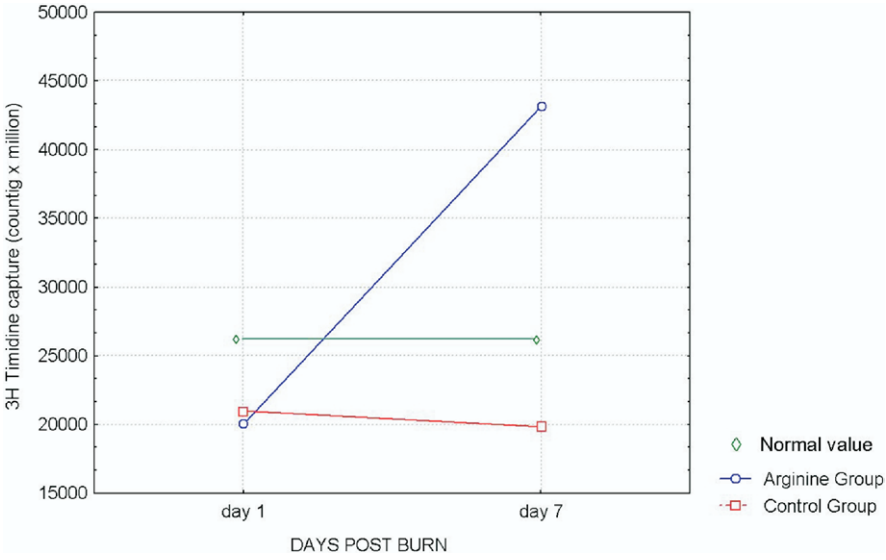

Fig. 1. Sequential changes in lymphoproliferative response. Arginine-supplemented group versus control group by repeated measures analysis of variance.

Blood amino acid measurements

Both groups had slightly low levels of arginine and ornithine on entry and both progressively returned to normal (Table 4). Ornithine levels evolved significantly better in the AG group than in the CG group (repeated measures ANOVA, $F_{1.24} = 4.67$, $P \leq 0.05$.), but this was not true for arginine levels. No significant correlation was found between blood amino acid measurements and metabolic or immunologic parameters.

Clinical course

Interpretation of the clinical data is limited by the small sample. There were no complications due to arginine and no significant differences in the number of hospitalization days (median, 22.5 d; range, 11–49, for AG group versus median, 23 d; range, 11–37 for CG group) or the number of febrile days (median, 3 d; range, 0–12, for AG group versus median, 2 d; range, 0–9, for CG group).

Discussion

Much of the morbidity and mortality of severely burned patients is related to hypermetabolism and catabolism, with its accompanying impairment of wound healing and increased risk of infection. To prevent the erosion of body mass, nutritional support and other strategies to prevent catabolism have become a major focus in the care of se-

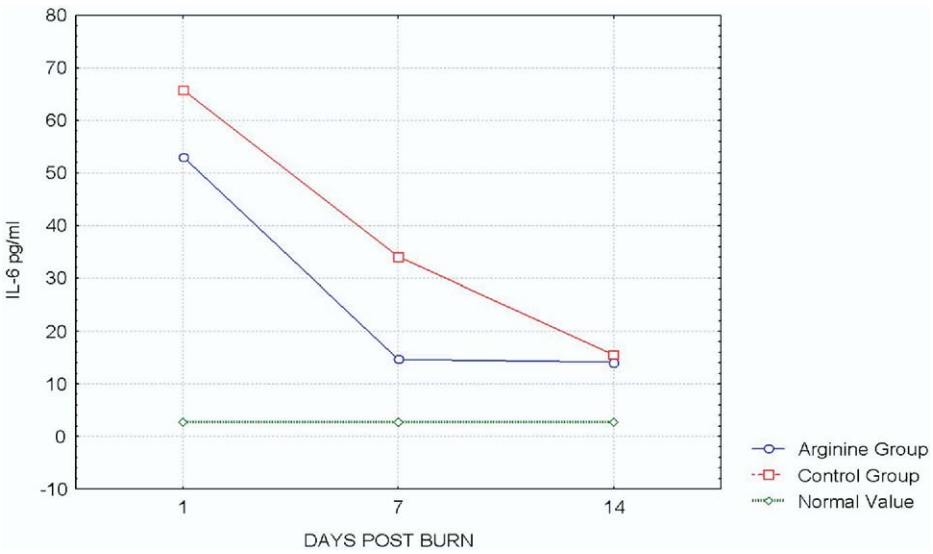

Fig. 2. Sequential changes in IL-6. Arginine-supplemented group versus control group by repeated measures analysis of variance. IL-6, interleukin-6.

Table 3
Metabolic parameters*,†

Metabolic Indicators	1 d		7 d		14 d	
	AG	CG	AG	CG	AG	CG
Glycemia (mg/dL)‡	96 (74–189)	95 (75–204)	83 (68–103)	85 (68–103)	87 (81–138)	81 (64–174)
Albumin (g/L)‡	3 (1.9–3.3)	3 (1.6–3.9)	3.5 (3.2–4.1)	3.5 (2.8–4.5)	3.8 (2.8–4.1)	3.6 (3–4.1)
Prealbumin (g/L)‡	0.08 (0.04–0.12)	0.07 (0.04–0.12)	0.10 (0.05–0.19)	0.13 (0.05–0.19)	0.15 (0.07–0.29)	0.17 (0.06–0.46)
CRP (mg/L)‡	14 (5–86)	10 (5–42)	24 (5–71)	24 (5–170)	5 (5–35)	5 (5–86)
TUN (mg N_2/kg/d)‡	250 (150–390)	260 (130–480)	420 (350–530)	360 (180–640)	400 (220–450)	400 (210–580)

AG, arginine-supplemented group; CG, isocaloric isonitrogenous control diet group; CRP, C-reactive protein; TUN, total urinary nitrogen
* All values expressed as median (range).
† Repeated measures analysis of variance.
‡ Not significant.

verely burned patients [36]. Several studies have documented the beneficial effects of so-called immune-enhancing diets in burn patients [16–18]. The present study is unique in the sense that a diet supplemented exclusively with arginine has been used to estimate the benefits of this amino acid on the immune system and to relate it to the metabolic response, through a double-blind, prospective, randomized, controlled, clinical trial using an isocaloric and isonitrogenous control diet. Most trauma and burn surgeons using specially designed stress formulas supplemented with arginine, ω-3 fatty acids, and glutamine have shown enhanced protein synthesis, fewer infectious complications, and decreased oxidative stress in a variety of settings, including recent studies in burns [37–39]. In 36 burned guinea pigs (30% of total body surface area), Saito et al. [27] suggested that oral dietary arginine supplementation up to 2% of energy intake versus 0%, 1%, and 4% may be beneficial after burn injury.

In humans, arginine is a component of most proteins and a substrate for several non-protein nitrogen-containing compounds with functions in immunity. The inconsistent effects of arginine supplementation on immune function may be due to numerous factors, such as the amount and timing of arginine supplementation, the animal species or strain of species, and the experimental model [40]. This study determined that an exclusively arginine-supplemented diet improves mitogen-stimulated lymphocyte proliferation in burned children and it did not seem to be related, as in other studies, to metabolic response or nitrogen balance [41,42]. The immunologic benefit observed in the AG group was attributed to increased arginine disposal and metabolism that was reflected in increased ornithine levels due to arginine pharmacokinetic characteristics, with increased turnover and oxidation, limited synthesis, and irreversible conversion to ornithine [43]. Activated macrophages have been previously shown to express arginase isoforms and nitric oxide synthase type II [21]. The common substrate used by these enzymes, L-arginine, which can be hydrolyzed by arginase to ornithine and urea, generates polyamines by the action of ornithine decarboxylase, a mechanism whereby arginine augments lymphocyte mitogenesis. In addition, arginine is oxidized by nitric oxide synthase type II to L-citrulline and nitric oxide. Nitric oxide is an ubiquitous molecule with important roles such as in the maintenance of the immune system.

Kirk et al. [44] demonstrated that dietary arginine can increase extrathymic T-cell maturation and function but cannot induce in vivo allogeneic graft recognition in athymic nude mice. These investigators also demonstrated that the increase in T-cell function is dependent not only on its thymotrophic effect but also on the polyamine system and nitric oxide production, although the exact mechanism remains unclear. Gianotti et al. [45] studied the effect of arginine administered before burn in rats and the mechanism of action; results showed that survival was 100% in the arginine-supplemented group versus 50% in the others. The benefit of arginine appears to be mediated by improved immune response and bactericidal mechanisms through the

Table 4
Plasma amino acid concentrations*

Amino acids (μmol/L)	Days after burn						
	1		7		14		$P^{\dagger}$
	AG	CG	AG	CG	AG	CG	
Arginine	71.5 (5–175)	61 (20–126)	146 (59–434)	156 (21–287)	126 (45–204)	123 (31–270)	NS
Ornithine	69.5 (39–219)	88.5 (45–214)	181 (76–333)	110.5 (64–241)	184 (115–333)	111 (66–263)	<0.05

AG, arginine-supplemented group; CG, control group; NS, not significant
* All values expressed as median (range).
† Repeated measures analysis of variance.

arginine-nitric oxide pathway [45]. In addition, enteral supplementation of L-arginine might be beneficial to resuscitation of burn shock. It might also exert a protective effect against ischemia/reperfusion injury to burn patients [46].

This study found no benefit for metabolic response, probably due to the optimal nutrition of both groups, a principal factor of hypercatabolic reaction. This differs from previous studies in burned rats that described attenuation in protein catabolism with arginine hyperalimentation [29].

The cytokine response in critically ill patients has been examined to improve our understanding of the pathophysiology and to devise new treatments [47]. In our study, we observed that circulating inflammatory cytokine, IL-6, increases greatly after severe injury and that high levels of inflammatory cytokines are closely correlated with burned surface area. Recent experimental data showed that alterations in the composition of nutrient formulas influenced the production of inflammatory mediators in stressed patients. We were not able to detect whether expression of inflammatory cytokines in plasma was influenced by the enteral AG diet. This may be due to low sensitivity of the assay.

Immunologic benefits, low cost, and digestive tolerance of enteral arginine emphasize the importance of considering supplementation of this nutrient in future clinical trials in burn patients.

Acknowledgments

The authors thank Ricardo Uauy, Pedro Herskovic, and Paulina Rojas for support received throughout the study.

References

[1] Barak N, Wall-Alonso E, Sitrin M. Evaluation of stress factors and body weight adjustments currently used to estimate energy expenditure in hospitalized patients. JPEN 2002;26:231–8.

[2] Coss-Bu JA, Jefferson LS, Walding D, David Y, Smith EO, Klish WJ. Resting energy expenditure and nitrogen balance in critically ill pediatric patient on mechanical ventilation. Nutrition 1998;14:649–52.

[3] Milner EA, Cioffi WG, Mason AD Jr, McManus WF, Pruitt BA Jr. Accuracy of urinary nitrogen for predicting total urinary nitrogen in thermally injured patients. JPEN 1993;17:414–6.

[4] Tredget EE, Yu YM. The metabolic effects of thermal injury. World J Surg 1992;16:68–79.

[5] Askarian M, Hosseini RS, Kheirandish P, Assadian O. Incidence and outcome of nosocomial infections in female burn patients in Shiraz, Iran. Am J Infect Control 2004;32:23–6.

[6] Zedler S, Bone RC, Baue AE, von Donnersmarck GH, Faist E. T-cell reactivity and its predictive role in immunosuppression after burns. Crit Care Med 1999;27:66–72.

[7] Barlow Y. T lymphocytes and immunosuppression in the burned patient: a review. Burns 1994;20:487–90.

[8] Molloy RG, Nestor M, Collins KH, Holzeimer RG, Mannick JA, Rodrick ML. The humoral immune response after thermal injury: an experimental model. Surgery 1994;115:341–8.

[9] O'Sullivan ST, O'Connor TP. Immunosuppression following thermal injury: the pathogenesis of immunodysfunction. Br J Plast Surg 1997;50:615–23.

[10] Oberholzer A, Oberholzer C, Moldawer LL. Citokine signaling-regulation of the immune response in normal and critically ill states. Crit Care Med 2000;28:3–12.

[11] De Bandt JP, Chollet-Martin S, Hernvann A, Lioret N, du Roure LD, Lim SK, et al. Cytokine response to burn injuries. J Trauma 1994;36:624–8.

[12] Struzyna J, Pojda Z, Braun B, Chomicka M, Sobiczewska E, Wrembel J. Serum cytokine levels (IL-4, IL-6, IL-8, G-CSF, GM-CSF) in burned patients. Burns 1995;21:437–40.

[13] Bower RH. Nutrition during critical illness and sepsis. New Horiz 1993;1:348–52.

[14] Enzi G, Casadei A, Sergi G, Chiarelli A, Zurlo F, Mazzoleni F. Metabolic and hormonal effects of early nutritional supplementation after surgery in burn patients. Crit Care Med 1990;18:719–21.

[15] Dudrick PS, Souba WW. Amino acids in surgical nutrition: principles and practice. Surg Clin North Am 1991;71:459–76.

[16] Mochizuki H, Trocki O, Dominioni L, Brackett KA, Joffe SN, Alexander JW. Mechanism of prevention of postburn hypermetabolism and catabolism by early enteral feeding. Ann Surg 1984;200:297–308.

[17] Saito H, Trocki O, Alexander JW, Kopcha R, Heyd T, Joffe SN. The effect of nutrient administration on the nutritional state, catabolic hormone secretion and gut mucosal integrity after burn injury. JPEN 1987;11:1–7.

[18] Beale RJ. Bryg DJ, Bihari DJ. Immunonutrition in the critically ill: a systematic review of clinical outcome. Crit Care Med 1999;27:2799–805.

[19] Galban C, Montejo JC, Mesejo A, Marco P, Celaya S, Sanchez-Segura JM, et al. An immune-enhancing enteral diet reduces mortality rate and episodes of bacteremia in septic intensive care unit patients. Crit Care Med 2000;28: 643–8.

[20] Kudsk KA, Minard G, Croce MA, Brown RO, Lowrey TS, Pritchard FE, et al. A randomized trial of isonitrogenous enteral diets after severe trauma an immune-enhancing diet reduces septic complications. Ann Surg 1996;224: 531–43.

[21] Evoy D, Lieberman MD, Fahey TJ III, Daly JM. Immunonutrition: the role of arginine. Nutrition 1998;14:611–7.

[22] Yu YM, Sheridan RL, Burke JF, Chapman TE, Tompkins RG, Young VR. Kinetics of plasma arginine and leucine in pediatric burn patients. Am J Clin Nutr 1996;64:60–6.

[23] Cui XL. Arginine-supplemented diet decreases expression of inflammatory cytokines and improves survival in burned rats. JPEN 2000;24:89–96.

[24] Saffle JR, Wiebke G, Jennings K, Morris SE, Barton RG. Randomized trial of immune-enhancing enteral nutrition in burn patients. J Trauma 1997;42:793–802.

[25] Bower RH, Cerra FB, Bershadsky B, Licari JJ, Hoyt DB, Jensen GL, et al. Early enteral administration of formula (Impact) supplemented with arginine, nucleotides, and fish oil in intensive care unit patients: results of multicenter, prospective, randomized, clinical trial. Crit Care Med 1995;3:436–49.

[26] Elsair J, Poey J, Issad H. Effect of arginine chlorhydrate on nitrogen balance during the three days following routine surgery in man. Biomed Express 1978;29:312–7.

[27] Saito H, Trocki O, Wang SL, Gonce SJ, Joffe SN, Alexander JW. Metabolic and immune effects of dietary arginine supplementation after burn. Arch Surg 1987;22:784–9.

[28] Adjei AA, Yamauchi K, Nakasone Y, Konishi M, Yamamoto S. Arginine-supplemented diets inhibit endotoxin-induced bacterial translocation in mice. Nutrition 1995;11:371–4.

[29] Holland KA, Gillespie RW, Lewis NM, Kachman SD. Estimating energy needs of pediatric patients with burns. J Burn Care Rehabil 1995;16:458–60.

[30] Oppenheim JJ, Rosentreich DL. In: Bloom BR, Davis JR, editors. Lymphocyte transformation: utilization of automatic cell harvesters. In vitro methods in cell-mediated tumor immunity. New York: Academic Press; 1976, p. 73–85.

[31] Mediciŏn del cambio del estado nutricional. Ginebra: World Health Organization; Ginebra 1983.
[32] Chwals WJ, Fernandez ME, Charles BJ, Schroeder LA, Turner CS. Serum visceral protein levels reflect protein calorie repletion in neonates recovering from major surgery. J Pediatr Surg 1992;27:317–21.
[33] Anand KJ, Hansen DD, Hickey PR. Hormonal-metabolic stress responses in neonates undergoing cardiac surgery. Anesthesiology 1990;73:661–70.
[34] Chwals WJ, Letton RW, Jamie A, Charles B. Stratification of injury severity using energy expenditure response in surgical infants. J Pediatr Surg 1995;30:1161–4.
[35] Andel H, Kamolz LP, Horauf K, Zimpfer M. Nutrition and anabolic agents in burned patients. Burns 1993;29:592–5.
[36] Daly JM, Reynolds J, Thom A, Kinsley L, Dietrick-Gallager M. Immune and metabolic effects of arginine in surgical patient. Ann Surg 1988;208:512–23.
[37] Jeschke MG, Herndon DN, Ebener C, Barrow RE, Jauch KW. Nutritional intervention high in vitamins, protein, amino acids, and omega-3 fatty acids improves protein metabolism during the hypermetabolic state after thermal injury. Arch Surg 2001;136:1301–6.
[38] Alexander JW. Nutritional pharmacology in surgical patients. Am J Surg 2002;183:349–52.
[39] Tsai HJ, Shang HF, Yeh CL, Yeh SL. Effects of arginine supplementation on antioxidant enzyme activity and macrophage response in burned mice. Burns 2002;28:258–63.
[40] Nieves C Jr, Langkamp-Henken B. Arginine and immunity: a unique perspective. Biomed Pharmacother 2002;56:471–82.
[41] Barbul A, Sisto DA, Wasserkrug HL, Yoshimura NN, Efron G. Metabolic and immune effects of arginine in post-injury hyperalimentation. J Trauma 1981;21:970–4.
[42] Cen Y, Luo XS, Liu XX. Effect of L-arginine supplementation on partial-thickness burned patients. Chin J Reparat Reconstr Surg 1999; 13:227–31.
[43] Yu YM, Ryan CM, Castillo L, Lu XM, Beaumier L, Tompkins RG, Young VR. Arginine and ornithine kinetics in severely burned patients: increased rate of arginine disposal. Am J Physiol Endocrinol Metab 2001;280:E509–17.
[44] Kirk SJ, Regan MC, Wasserkrug HL, Sodeyama M, Barbul A. Arginine enhances T-cell responses in athymic nude mice. JPEN 1992; 16:429–32.
[45] Gianotti L, Alexander JW, Pyles T, Fukushima R. Arginine-supplemented diets improve survival in gut-derived sepsis and peritonitis by modulating bacterial clearance. The role of nitric oxide. Ann Surg 1993;217:644–54.
[46] Yan H, Peng X, Wang P, Huang YS, Wang SL. Influence of the enteral feeding of levorotatory arginine on severely burned patients during shock stage. Zhonghua Shao Shang Za Zhi. 2005;21:251–4.
[47] Wu JZ, Ogle CK, Mao JX, Szczur K, Fischer JE, Ogle JD. The increased potential for the production of inflammatory cytokines by Kupffer cells and splenic macrophages eight days after thermal injury. Inflammation 1995;19:529–41.