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Comparison of L-arginine and L-citrulline oral supplementation in head trauma ICU patients receiving enteral nutrition: A randomized clinical trial

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A B S T R A C T

Objective: This study aimed to compare the effect of L-arginine and L-citrulline supplementation on overall prognosis of critically ill patients.

Methods: A total of 105 head trauma ICU patients were randomized to three groups (Arginine, Citrulline, and Control). Patients in the treatment groups took 10 gr per day of oral L-arginine or L-citrulline for 10 days. Demographic characteristics and measurements were recorded. Nitric oxide (NO), prealbumin, pro-oxidant–antioxidant balance (PAB), fasting blood sugar, lipid profile, hepatic enzymes, serum electrolytes, blood urea nitrogen, creatinine, and serum amino acids were measured. Gastrointestinal complications, overall time on a ventilator, length of hospital stay, and 28-day mortality rate were recorded.

Results: We observed no significant changes in NO and PAB ($p = 0.8$, $p = 0.1$ respectively). There was a significant increase in serum LDL ($p = 0.02$), which was greater in the control group after 10 days of supplementation. There was a nonsignificant increase in serum L-arginine in all three groups ($p = 0.36$). However, the change in serum L-arginine was only significant in the citrulline group ($p = 0.048$). Serum L-citrulline was higher in the citrulline group compared with the arginine group ($p = 0.04$).

Conclusion: L-arginine and L-citrulline supplementation did not increase NO levels beyond that observed in the control group. Also, PAB balance was not different among the intervention groups and the control group. L-arginine and L-citrulline had no significant effects on length of hospital stay, mortality rate, overall time on a ventilator, and other factors evaluated in this study.

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Introduction

The mortality rate in patients admitted to the intensive care unit (ICU) still remains high in spite of better availability of appropriate diagnostic tests and treatments. Adequate and timely nutritional support improves clinical and metabolic outcomes of ICU patients [1].

In recent years, the use of immune-modulating formulas in the critical care setting has become a part of nutritional care [2]. These immunonutrition formulas contain specific nutrients such as arginine, n-3 fatty acids, glutamine, antioxidants, and nucleic acids. Arginine, the key component of these formulas, has gained specific attention [2].

L-arginine is a nonessential amino acid in healthy people but is considered essential during illness and hypermetabolic status [3]. Arginine plays a crucial role in the intermediary metabolism of critically ill patients [4, 5]. It is a substrate of protein synthesis with broad physiological functions owing to its role in proline synthesis, hormonal secretion, insulin sensitivity [6, 7], and endothelial function. L-arginine supplementation has been shown to have beneficial effects on immune regulation and wound healing [8].

A meta-analysis showed that immunonutrition (IMN) formulas containing arginine decreased the length of hospital and ICU stay [9, 10], number of days of mechanical ventilation support [9], and the risk of infection [9, 10], but had no effect on mortality rate [10]. Another study indicated that L-arginine improved posttraumatic cerebral blood flow [11].

L-citrulline (C6H13N3O3) is a nonprotein amino acid that was first identfied from Citrullus vulgaris (watermelon) in the 1930s, hence the name [12]. Until recently, it was considered only an intermediate in the urea cycle. However, recent studies have indicated the importance of this amino acid in cellular metabolism and organ function [12]. L-citrulline metabolism is greatly associated with that of L-arginine, thus it could be an alternative to L-arginine supplementation [12]. Most studies evaluating the effectiveness of L-arginine supplementation in ICU patients have used arginine-rich immunonutrition formulas containing other substances [13-15], and the results of these studies are controversial. Given the close association of L-arginine metabolism with that of L-citrulline, the aim of the present study was to evaluate the effects of oral L-arginine and L-citrulline supplementation on overall prognosis, oxidative stress, and clinical and metabolic outcomes of critically ill head trauma patients. Moreover, we assessed the effect of these two supplements on serum amino acid levels.

Methods

Participants

A total of 105 head trauma patients at Shahid Kamyab University Hospital, Mashhad, Iran, were recruited for the study. Patients were eligible if they were 18-60 years old with grade 3 head trauma (score 3-8 on the Glasgow Coma Scale [GCS]) who were admitted to ICU and had normal GI motility. Patients with diabetes, immune disorders, internal hemorrhage, pancreatitis, metabolic disorders, liver and kidney disorders, sepsis, a nothing-by-mouth (NPO) diet for ≥ 3 days, and any disorder that would make it impossible to use enteral nutrition support within 24 to 48 hours were excluded from the study.

The participants were recruited between March 2010 and March 2011. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (code: 89479) and registered on www.irct.ir as IRCT201108027199N1. Written informed consent was obtained from families of the patients at the start of the study. The flow diagram for patient recruitment is shown in Figure 1.

The patients' personal characteristics such as age, sex, height, and clinical symptoms were recorded before the beginning of the intervention. Intake of calorie and nutritional supplements, days of NPO, and supplemental parenteral feeding were recorded on a daily basis.

Study design and interventions

A randomized clinical trial was performed. The sample size for each group was estimated at 30, as reported in our previous article [16], to ensure a 10% difference and 80% power with $\alpha = 0.05$. We set the number of patients in each group at 35 to account for possible dropouts. The simple randomization method using computer-generated random numbers was used to allocate the subjects to the study groups.

For patients in the arginine and citrulline groups, 5 grams of oral L-arginine (Karen Pharma & Food Supplement Co., Iran) or L-citrulline (Now Food Co., US) supplements were administered twice a day for 10 days. L-arginine and L-citrulline supplements were similar in size, shape, and color. Patients in the control group received no supplements. Care providers were blinded to the study groups. All patients received

a standard hospital-prepared formula (standard HPF).

Measurements

Severity of illness

The severity of illness was evaluated using the Acute Physiology and Chronic Health Evaluation (APACHE) II score at the day of admission.

Calorie estimation

The patients' daily calorie requirements were calculated based on the standard value of 25 kcal/usual body weight.

Blood sample collection

Fasting blood samples (10 mL) were taken at baseline (day 0) and at the end of the trial (day 11) early in the morning and after an overnight fast. Serum was immediately separated by centrifugation at 3400 rpm for 3 minutes. Serum samples were stored at -80°C until assayed.

Laboratory investigation

Nitric oxide (NO) was measured as plasma nitrite plus nitrate concentration using the Griess colorimetric method. Pro-oxidant–antioxidant balance (PAB) was assessed using Tetramethylbenzidine (TMB) in two different reactions: one was an enzymatic reaction in which TMB was cationized with H2O2, and the other was a cationic reaction in which uric acid was reduced by antioxidants. PAB was assessed using a procedure previously used in a study by Alamdari and colleagues [17].

Prealbumin was assessed with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abcam). The concentrations of serum L-arginine and L-citrulline were determined using high-performance liquid chromatography (HPLC).

Glycemic status, lipid profile, liver enzymes, serum electrolytes, and blood urea nitrogen

FBS, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, triglycerides, and blood urea nitrogen (BUN) were determined using enzymatic methods. Alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) were measured based on the method recommended by the IFCC (International Federation of Clinical Chemistry). Serum sodium and potassium were measured using flame photometry. Phosphorus was measured with phosphomolybdate assay. Colorimetry was used to assess serum calcium, magnesium, albumin, and creatinine. Serum total protein was determined using the biuret test. All measurements were done using Selectra automated analyzer and ELITech kits.

Features of the nutritional formula

For all patients, bolus feeding of a standard enteral formula was started in the first 24 to 48 hours of admission. The formula was prepared based on the exchange list to provide calorie and nutrient requirements similar to standard commercial formulas. Physical properties (osmolality and viscosity) and macronutrient content of the formula were assessed. The formula provided 0.96 calories per ml and contained 45.36% carbohydrates, 16.64% protein, and 34.2% fat. The osmolality of the formula was 345 mOsmol/kg and the viscosity was 43 cps.

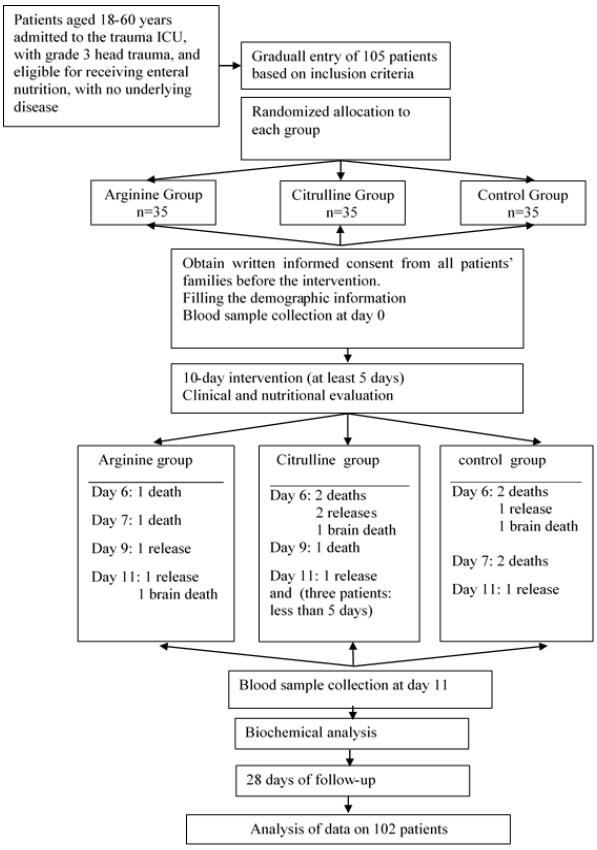

Figure 1: Flow chart of the study design

Statistical analysis

Data were expressed as mean ± SD. The normality of the distribution of data was determined using the Kolmogorov-Smirnov test. The chi-square test was used to compare the categorical variables among the three groups. A repeated-measures analysis of variance

(ANOVA) was used to evaluate time × group interactions, with time and group as factors. In case of significant time-group interaction, between-group comparisons of changes at day 11 were done using ANOVA, followed by Tukey's post hoc tests, or a Mann-Whitney test. When the time effect was significant, the within-subject comparison of values was performed using a paired-samples t test or a Wilcoxon signed rank test. The Statistical Package for the Social Sciences (SPSS, version 15; Chicago, IL) was used to perform the analyses, and a p value of < 0.05 was considered significant.

Results

As shown in Figure 1, data were analyzed for 102 patients. During the follow-up period, no gastrointestinal complications or adverse effects on FBS, liver enzymes, lipid profiles, or serum electrolytes were seen.

Baseline characteristics

Baseline characteristics of the patients are presented in Table 1. There were no statistically significant differences among groups before the intervention.

Biochemical measurements

Table 2 shows the comparison of biochemical parameters among the arginine, citrulline, and control groups with the p values for "treatment" and "time" factors and their interaction.

The mean NO concentration increased during the intervention period in all three groups, and the largest increase was observed in the control group (mean changes: 5.34 ± 25.96 vs 2.38 ± 14.16 in the arginine group and 3.09 ± 12.31 in the citrulline group). However, these changes were not significant when considering time effects ($p = 0.102$), or time-group interactions ($p = 0.268$) (Table 2).

PAB showed incremental changes over time ($p < 0.001$). Within-subject changes were also significant ($p = 0.01$), although when considering time-group interaction, there was no significant difference ($p = 0.13$) (Table 2).

Statistically significant time-treatment interaction (with time factor being statistically significant) was observed for LDL-cholesterol level ($p = 0.02$). The increase in LDL-cholesterol concentration was observed in all groups (Table 2). Tukey's post hoc test showed that this difference was significant between the arginine group and the control group ($p = 0.01$). Regarding serum amino acid levels, L-arginine levels increased in all three groups during intervention (mean changes: 9.63 ± 114.34, 53.84 ± 120.44, and 34.28 ± 83.59 in the arginine, citrulline and control group, respectively, $p = 0.01$) (Table 2), but there was no significant difference when considering time-group interaction ($p = 0.36$). The results of the paired-samples t test showed that the increase from pre to post intervention in L-arginine values was significant only in the citrulline group ($p < 0.05$). The p values for the arginine group and control group were 0.7 and 0.05, respectively. The level of serum L-citrulline decreased during the period of intervention in the arginine group and increased in the citrulline and control groups. The results of the repeated-measure analysis showed that the interaction of time and group was marginally significant

Table 1: Baseline characteristic of patients

		Arginine (n = 35)		Citrulline (n = 35)		Control (n = 35)		P value
		Mean ± SD		Mean ± SD		Mean ± SD		
Age (year)		33.06 ± 15.01		34.59 ± 14		32.71 ± 12.94		0.85
Sex	Male	30		23		29		0.32
	Female	5		9		6		
Height (cm)		175.6 ± 6.08		172.75 ± 7.61		171.6 ± 10.17		0.11
Weight (kg)		71.25 ± 5.24		71.95 ± 8.51		70.22 ± 9.47		0.86
APACHE II		13.76 ± 5.03		13.37 ± 4.26		14.25 ± 4.42		0.89
Calorie requirement (kcal)		1781.22 ± 148.63		1798.80 ± 212.90		1755.70 ± 236.99		0.86
Diagnosis		n	percent	n	Percent	n	percent	0.99
SAH		7	20	5	15.6	7	20	
SDH		9	25	9	25	7	20	
Contusion		10	28.6	10	28.6	9	25.7	
IVH		4	11.4	5	15.6	6	17.1	
SAH + SDH		5	14.3	6	17.1	6	17.1	

Table 2: Comparison of the initial and final values of the variables under study in the RCT

	Arginine (n = 31)			Citrulline (n = 26)			Control (n = 29)			Time	group	Time×group
Variable	**Before**	**After**	**Change**	**Before**	**After**	**Change**	**Before**	**After**	**Change**	p_1	p_2	p_3
NO (nm/l)	28.16 ± 7.85	30.62 ± 11.1	2.38 ± 14.16	32.45 ± 7.033	34.63 ± 16.68	3.09 ± 12.31	26.51 ± 11.95	32.71 ± 22.97	5.34 ± 25.96	0.102	0.268	0.846
PAB	131.28 ± 39.5	167.2 ± 27.9	32.97 ± 46.49	99.05 ± 42.16	156.33 ± 34.78	56.44 ± 41.84	116.53 ± 31.34	161.02 ± 34.53	44.32 ± 38.43	**< 0.001**	**0.011**	0.132
Prealbumin (mg/l)	9.57 ± 5.40	17.14 ± 24.67	7.57 ± 24.85	8.19 ± 6.19	6.19 ± 2.88	-2 ± 6.51	11.91 ± 6.87	17.65 ± 23.98	5.73 ± 23.04	0.097	**0.038**	0.197
Total protein	6.37 ± 1.01	6.62 ± 0.97	0.22 ± 0.91	6.72 ± 1.09	6.84 ± 0.86	0.09 ± 0.99	6.37 ± 0.76	6.64 ± 0.95	0.24 ± 1.02	0.09	0.374	0.834
Albumin	3.78 ± 0.55	3.46 ± 0.52	-0.32 ± 0.51	3.70 ± 0.52	3.34 ± 0.54	-0.38 ± 0.55	3.8 1 ± 0.45	3.48 ± 0.52	-0.33 ± 0.39	**<0.001**	0.676	0.911
FBS (mg/dl)	139.82 ± 50.50	120.58 ± 28.01	-22 ± 64.11	146.71 ± 64.21	123.96 ± 32.63	-5.4 ± 41.15	141.25 ± 36.18	124.25 ± 33.09	-22.03 ± 49.17	**0.007**	0.504	0.431
Na (mg/dl)	143.34 ± 4.27	139.68 ± 5.54	-3.65 ± 6.14	143.16 ± 5.2	140.32 ± 6.5	-2.84 ± 7.88	145.35 ± 5.8	139.82 ± 4.02	-5.53 ± 6.40	**<0.001**	0.574	0.338
P (mg/dl)	3.21 ± 1.16	3.86 ± 1.56	0.65 ± 1.59	3.15 ± 0.86	3.64 ± 1.42	0.49 ± 1.54	2.88 ± 0.88	3.59 ± 0.86	0.71 ± 0.76	**<0.001**	0.504	0.828
Ca (mg/dl)	8.39 ± 0.15	8.49 ± 0.21	0.093 ± 1.37	8.13 ± 0.84	8.28 ± 0.75	0.142 ± 0.97	8.42 ± 0.92	8.51 ± 0.02	0.85 ± 0.95	0.397	0.403	0.981
K (mg/dl)	4.14 ± 0.48	4.51 ± 0.60	0.58 ± 0.61	4.15 ± 0.48	4.73 ± 0.36	0.37 ± 0.76	4.15 ± 0.32	4.55 ± 0.35	0.39 ± 0.37	**<0.001**	0.436	0.403
Mg (mg/dl)	2.32 ± 0.48	2.46 ± 0.47	0.13 ± 0.66	2.92 ± 0.41	2.33 ± 0.44	-0.42 ± 0.70	2.37 ± 0.43	2.26 ± 0.38	-0.10 ± 0.54	0.746	0.559	0.377
BUN (mg/dl)	49.65 ± 35.00	46.17 ± 29.54	-6.55 ± 26.04	53.06 ± 32.44	45.72 ± 28.30	-6.64 ± 24.39	47.02 ± 22.50	50.07 ± 63.96	1.29 ± 63.74	0.401	0.999	0.731
Creatinine (mg/dl)	1.23 ± 1.52	0.95 ± 1.13	-0.34 ± 0.70	1.15 ± 0.94	0.73 ± 0.33	-0.36 ± 0.54	1.02 ± 0.32	0.98 ± 1.25	-0.082 ± 1.076	**0.004**	0.732	0.365
AST (U/l)	92.44 ± 93.69	116.62 ± 82.92	24.172 ± 115.73	84.83 ± 70.84	119.87 ± 124.14	35.04 ± 152.67	62.32 ± 41.44	113.76 ± 130.97	51.44 ± 134.3	**0.018**	0.638	0.757
ALT (U/l)	52.41 ± 47.16	128.34 ± 132.08	75.93 ± 138.75	55.66 ± 36.14	104.37 ± 99.22	48.70 ± 102.87	41.44 ± 29.78	131.52 ± 148.51	90.08 ± 144.29	**<0.001**	0.862	0.534
LDH (U/l)	652.5 ± 262.48	573.72 ± 278.62	-78.77 ± 257.22	557.45 ± 261.47	681.75 ± 366.02	124.3 ± 306.91	431.5 ± 284.82	514.92 ± 260.15	83.42 ± 397.51	0.340	0.193	0.136
LDL (mg/dl)	72.74 ± 30.99	80.25 ± 28.09	7.51 ± 25.91	69.33 ± 25.41	96.16 ± 37.13	26.83 ± 35.39	70.94 ± 35.27	112.47 ± 52.33	41.52 ± 57.90	**<0.001**	0.22	**0.019**
HDL (mg/dl)	37.98 ± 12.69	32.26 ± 14.06	-5.71 ± 13.59	38.30 ± 12.47	32.45 ± 14.92	-5.84 ± 17.17	37.63 ± 12.50	39.42 ± 15.49	1.78 ± 18.69	0.108	0.549	0.239
Cholesterol (mg/dl)	127.64 ± 31.09	149.48 ± 33.87	21.84 ± 39.41	130.33 ± 31.47	166.25 ± 48.04	35.91 ± 47.80	136.33 ± 35.32	163.71 ± 41.18	27.38 ± 41.47	**< 0.001**	0.378	0.519
TG (mg/dl)	117 ± 53.68	155.37 ± 81.95	38.37 ± 72.64	105.25 ± 39.78	149.20 ± 101.88	43.95 ± 100.74	121.61 ± 40.47	141.38 ± 66.82	19.76 ± 59.38	**0.001**	0.855	0.581
Arginine (µmol/l)	151.72 ± 88.59	164.98 ± 52.07	9.63 ± 114.34	137.76 ± 54.59	185.64 ± 98.65	53.84 ± 120.44	133.03 ± 50.85	173.48 ± 64.56	34.28 ± 83.59	**0.011**	0.689	0.361
Citrulline (µmol/l)	27.23 ± 12.26	21.99 ± 8.81	-5.33 ± 15.62	27.38 ± 14.23	33.32 ± 11.84	5.9 ± 20.05	20.64 ± 6.42	20.58 ± 7.89	-0.11 ± 10.38	0.895	**<0.001**	**0.054**
Ornithine (µmol/l)	120.34 ± 62.75	125.11 ± 51.74	4.7 ± 88.08	87.47 ± 31.58	123.92 ± 54.54	36.45 ± 61.27	138.24 ± 55.88	147.62 ± 54.79	9.38 ± 77.83	**0.067**	**0.004**	0.327
Glutamine (µmol/l)	569.49 ± 33.14	341.33 ± 154.46	-228.16 ± 364.67	515.96 ± 166.92	408.59 ± 205.07	-107.37 ± 271.17	586.74 ± 206.65	450.28 ± 212.24	-136.45 ± 308.49	**< 0.001**	0.353	0.517

NO, Nitric oxide (nitrite and nitrate); PAB, pro-oxidant–antioxidant balance; FBS, fasting blood sugar; Na, sodium, P, phosphorus; Ca, calcium; K, potassium; Mg, magnesium; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; TG, triglyceride.

P1: Probability level by repeated-measures ANOVA for difference in time course.

P2: Probability level by repeated-measures ANOVA for difference between Arginine or Citrulline supplements.

P3: Probability level by repeated-measures ANOVA for interaction between time course and Arginine or Citrulline supplements

Table 3: Effects of L-arginine and L-citrulline on clinical outcomes

	Arginine group (35)	Citrulline group (35)	Control group (35)	P value
		Percent		
Gastrointestinal complications	34.3	25	40	0.445*
Mortality rate during intervention	8.6	12.5	14.3	0.80*
28-day mortality rate	9.1	20	20	0.38*
		Mean ± SD		
Overall time on a ventilator	4.71 ± 3.82	5.28 ± 3.68	6 ± 4.42	0.421†
Length of hospital stay	45.09 ± 27.59	27.86 ± 23.22	33.77 ± 19.31	0.023†
Mean calorie intake (kcal)	1405.42 ± 445.04	1456.25 ± 580	1395.66 ± 589.72	0.89†

* Chi-square test, † One-way ANOVA

($p = 0.05$). Tukey's post hoc test revealed a significant difference in the mean pre-post changes in citrulline levels between the arginine and citrulline groups ($p = 0.04$). Also, serum L-ornithine levels increased in all three groups. Neither the time effect ($p = 0.07$) nor the time-group interaction was significant ($p = 0.327$), but changes within each group were significant ($p = 0.004$). Serum L-glutamine levels diminished in the three groups during the intervention period ($p < 0.001$), but the time-group interaction was not significant ($p = 0.5$). The mean change in glutamine concentration from baseline to post intervention was -228.16 ± 364.67 μmol/L ($p = 0.004$) for the arginine group, -107.37 ± 271.17 μmol/L ($p = 0.08$) for the citrulline group, and -136.45 ± 308.49 μmol/L ($p = 0.04$) for the control group (Table 2). The differences were not significant for other parameters.

Clinical evaluation

Table 3 shows the rates of gastrointestinal complications, overall time on a ventilator, mortality during the intervention, 28-day mortality, and length of hospital stay across the three groups. According to the chi-square test, the incidence of gastrointestinal complications was not significantly different across the three groups ($p = 0.44$). The mean number of days on a ventilator ($p = 0.4$), the mortality rate during the intervention ($p = 0.8$), and the 28-day mortality rate ($p = 0.38$) were not significantly different among the three groups. The 28-day mortality rate was 9.1% in the arginine group and 20% in the citrulline and control groups. The length of hospital stay was significantly different among the three groups ($p = 0.023$), and Tukey's post hoc test revealed a significant difference between the arginine group and the citrulline group ($p = 0.02$) (45 days vs 27 days).

There was no significant difference in the number of NPO days ($p = 0.7$). Furthermore, the mean calorie intake ($p = 0.89$) and nutritional supplements intake ($p = 0.3$) were not different among the groups. Also, intralipid ($p = 0.86$) and amino acid ($p = 0.63$) intake from supplemental parenteral nutrition were similar between groups.

Discussion

Main findings

This clinical trial in critically ill patients with head trauma showed that supplementation with L-arginine and L-citrulline had no metabolic and clinical adverse effects compared with the control group. Supplementation with either amino acid did not result in increased NO levels beyond that of the control group. Nor did it cause any disturbances in PAB. Additionally, we failed to find any significant effects on albumin, prealbumin, and total protein levels.

Oxidative stress

Oxidative stress is prevalent in critically ill patients. NO radicals and proxyl nitrite are the main reactive nitrogen species [18] that are involved in oxidative damage mechanisms [19, 20]. In the present study, NO increased in all three groups, with no significant difference between them. Interestingly, the largest increase was observed in the control group, followed by the citrulline group and the arginine group. Therefore, neither L-arginine or its precursor, L-citrulline, resulted in an excessive increase of NO in these patients. Stechmiller and colleagues reported the same results in elderly people with pressure ulcers. They found that L-arginine supplementation did not increase serum NO in excess of that achieved with an isonitrogenous supplement [21]. Other studies indicated that L-citrulline raises NO production to a greater extent than L-arginine does [18, 22]. Although our study showed higher NO levels in the citrulline group compared with the arginine group, it was still lower than that of the control group, and overall changes and differences between the groups were

not significant.

Nutritional factors

Supplementation with L-arginine or L-citrulline over a period of 10 days did not affect the levels of albumin, prealbumin, and total serum protein. Prealbumin, with a half-life of 2.5 days, is considered an indicator of recent nutritional status; however, various factors such as acute phase proteins and changes in capillary permeability can affect its levels [23]. The mean prealbumin levels at both the beginning and the end of the intervention were lower than the normal range (19-38 mg/dl) for all groups. The mean prealbumin concentrations increased in the arginine and control groups and decreased in the citrulline group, although the changes were not significant. Previous studies revealed that using arginine-rich formula did not cause any beneficial effect on the values of prealbumin [15, 24]. Increased concentrations of prealbumin in ICU patients in the acute phase may indicate that at least 65% of the energy and protein requirement has been met [25]. Although there were no differences in calorie intake ($p = 0.89$) and NPO days ($p = 0.7$) between the groups in our study, we observed a decrease in prealbumin concentration in the citrulline group, suggesting that prealbumin levels may be affected by factors other than nutritional status.

Metabolic factors

We showed that the supplementation with L-arginine or L-citrulline had no effect on blood glucose levels compared with the control group. This is consistent with the findings of a study that assessed glycemic status in burn patients receiving an arginine-rich IMN formula versus a standard formula [26]. Similar results have been reported regarding the effect of L-arginine supplementation on FBS [27-29]. Regarding the effects of L-citrulline on blood glucose level, a study by Sellmann and colleagues showed that FBS was significantly lower in L-citrulline–fed mice [30].

Metabolic and hepatic disorders are common complications in ICU patients and are believed to be associated with nutritional support. Although these disorders are more common following parenteral nutrition, studies have shown that it could also occur after enteral nutrition [31]. In the present study, AST, ALT, and LDH were evaluated to monitor possible hepatic complications during the intervention. We found no difference in hepatic enzymes among the three groups, although a previous study in patients with sickle cell anemia showed that L-arginine at a dose of 1 g/day for six weeks caused a great reduction in serum ALT and AST [32]. In the case of lipid profile, we found significant differences in LDL changes among the study groups, with the arginine group showing the smallest increase. However, L-arginine or L-citrulline supplementation had no effect on HDL, triglycerides, or total cholesterol. Several studies evaluating the effects of L-arginine on lipid profile have shown that L-arginine has no effects on total cholesterol, LDL, triglycerides, or HDL level [28, 29, 33]. In contrast, West and colleagues indicated that L-arginine supplementation in hypercholesterolemic individuals decreased serum triglycerides level by 27% but had no effect on HDL-C level [27].

Serum amino acid level

Overall, our results indicated that L-citrulline supplementation may lead to a greater increase in serum L-arginine concentration compared with L-arginine supplementation. Osowska and colleagues showed that, in comparison with L-arginine supplementation, administration of L-citrulline resulted in greater increases in plasma L-arginine level in mice with short bowel syndrome ($p = 0.05$) [34]. In the study by Schwedhelm and colleagues, 6 g/day oral L-citrulline supplementation resulted in a dose-dependent increase in plasma L-arginine concentrations [35].

Our result also showed that L-citrulline supplementation led to a greater elevation in serum L-citrulline compared with L-arginine supplementation. This finding can be due to the fact that arginine might have been metabolized by arginase and converted to urea and ornithine, making it less available to nitric oxide synthase for producing NO and citrulline.

Clinical evaluation

We found no significant differences in the incidence of gastrointestinal complications (diarrhea and residue), mortality during the intervention (11-day mortality), and 28-day mortality among the three groups. Atkinson and colleagues [14] showed that patients who received arginine-rich IMN formulas had a mortality rate of 48% compared with 44% in the control group (not significant). Galbán and colleagues [13] reported that the mortality rate was lower in the IMN formula group compared with the control group (19% vs 32%). A

systematic review of 22 randomized clinical trials with a total of 2419 patients using standard or IMN formula in ICU showed that IMN formula was not effective in reducing mortality rate [10].

In previous studies, the length of hospital stay in the arginine-rich IMN group was significantly shorter than that in the control group [14, 15]. In contrast, our results showed no beneficial effects of L-arginine on length of hospital stay, with the shortest hospital stay being seen in the L-citrulline group. Consistent with the findings of Kudsk and colleagues [15], our data did not show any effects of L-arginine or L-citrulline supplementation on the overall time on a ventilator. However, in the study of Atkinson and colleagues, overall time on a ventilator was shorter in the arginine-rich IMN group than in the standard group (6 vs 10.5 days) [14].

Limitations

Several limitations must be considered when interpreting the results of this study. The population was limited to head trauma patients, hence we cannot generalize our results to other ICU patients. The intervention was conducted just for 10 days, using a small sample; therefore, our results need to be supported by further studies with longer duration and larger samples.

Conclusion

This study was the first randomized clinical trial to compare the effects of L-arginine and L-citrulline supplementation in head trauma ICU patients. Supplementation with either of these two amino acids (10 g/day) did not show any adverse effects. L-arginine and L-citrulline supplementation did not increase NO levels or disturb PAB balance. L-arginine and L-citrulline had no significant effects on length of hospital stay, mortality rate, overall time on a ventilator, and other factors evaluated in this study.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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