

## Effects of Arginine and Citrulline supplementation on inflammatory markers in critically ill patients

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### ABSTRACT

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**Background:** Citrulline is a nonessential amino acid that is a main precursor of arginine and therefore could be a best alternative for arginine supplementation. The current study aimed to assess the effects of L-arginine and L-citrulline administration on inflammatory factors (C-reactive protein (CRP), interleukine-6 (IL-6)).

**Methods:** A total of 102 patients in 5 trauma intensive care units (ICU) were divided into three groups: Arginine, Citrulline and Control. Patients received 10 g/d L-arginine powder (Arginine Group) or 10 g/d L-citrulline powder (Citrulline Group) for 10 days. Serum level of CRP and IL-6 were determined in all patients before (day 0) and after intervention (day 11).

**Results:** There were significant reduction in IL6 levels only in Citrulline group ( $P=0.009$ ). Moreover, serum levels of CRP decreased in both experimental group but it was elevated during study period in Control group (mean changes in control group were  $0.28 \pm 19.66$ , in Citrulline group 11.16 and in arginine group it was 1.12) which was only significant in Citrulline group ( $P=0.025$ ).

**Conclusion:** L-citrulline supplementation could have modulatory effects on inflammatory biomarkers.

### Introduction

Amino acids are key components of the nutritional and metabolic care of critically ill and injured patients. As our current knowledge of the altered regulation of amino acid metabolism in such patient increases, the formulation and administration of more

effective parenteral and enteral therapeutic feeding regimens increases. The use of specific amino acids in pharmacologic doses and in special combinations is likely to be beneficial in improving outcome in these patients.

Several studies show that plasma levels of arginine and citrulline decreases in critically ill patients after trauma and surgery (1-4) and also there is reverse association between levels of these amino acids with cytokines and inflammatory markers (5).

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**Table 1.** Patients characteristic

	Control	Citrulline	Arginine	P value
Age	32.71	34.59	33.06	P= 0.85
Gender (Men/Women)	29/6	23/9	30/5	P= 0.32
Height	171.6±10.17	172.75± 7.61	175.6± 6.08	P= 0.11
Energy requirement	2227 ± 375.01	2311.80 ± 319.14	2380.69± 25.48	P= 0.14
APACHEII	14.25± 4.42	13.37± 4.26	13.76± 5.03	P=0.89

APACHE II; Acute Physiology and Chronic Health Evaluation.

There is accumulating evidence that cytokines are implicated in the pathogenesis of critical illness (6). This is based on a great amount of studies on cytokine patterns in individual acute conditions, primarily in sepsis (7, 8), but also in trauma (9, 10), surgery (9, 11), pancreatitis (12), burns (13), or cardiogenic shock (14). In these states cytokines correlate positively with severity of underlying disease, and their persistence in the circulation is associated with the development of multiple organ failure (MOF). Surgery and malnutrition (which is frequent in these patients) have also been found to depress the immune system (15). Although immune dysfunction is multifactorial, the immune system may be modulated by specific nutritional substrates, such as arginine (16). Arginine is an amino acid (AA) with unique properties and with a key-role in the metabolic, immune and reparative response to trauma and sepsis.

To our knowledge there is no clinical trial that evaluates effect of L-arginine as a single agent in critically ill patient or compares it with L-citrulline as a main precursor of arginine. So the aim of our study was to compare the effects of oral L-arginine and L-citrulline supplementation on inflammatory factors (CRP, IL6) in critically ill trauma patients.

### Materials and methods

This was a randomized clinical trial that conducted in five intensive care unit (ICU) in Shahid Kamyab Hospital, Mashhad, Iran, between March 2010 and March 2011. The study was approved by the ethics committee of the Mashhad University of Medical Sciences (code: 89479). All patients'

families gave written informed consent after a full explanation of the study.

### Subjects

The study was performed on 102 critically ill patients. Subjects were randomly assigned for each groups based on simple randomization method. Patients were considered eligible for the study if they were 18-60 years old, GCS= 4-10 without any underlying diseases.

### Study protocol

Enteral feeding started for all patients in their 24-48 hour of admission in hospital. All subjects received same Hospital Prepared Enteral Formulation (HPF), that contained % 42.8 carbohydrates, %16.6 protein and % 34.2 fat.

Patients in Arginine (n=35) and Citrulline (n=32) groups received 10 gram/day L-arginine and L-Citrulline powder (in two separated 5 gram doses) respectively for 10 days. Patients in control group (n=35) received no amino acid supplements. Blood samples were drawn before (days0) and after 10 days' intervention (days 11). Serum IL6 levels as an inflammatory cytokine, acute phase response proteins CRP were determined before and after intervention.

### Measurements

The IL-6 was determined using commercially available ELISA kits manufactured by eBioscience Co. CRP was measured by the method of agglutination of latex particles on slide (ENISON Co kits).

### Statistical analysis

**Table 2: Variable changes before and after intervention between and within groups**

		Control	Citrulline	Arginine	P value
IL6	Day 0	14.20 ±21.80	22.83 ± 16.30	18.35 ±19.93	P=0.217
	Day 11	12.30 ±13.54	11.96 ± 12.52	19.60 ±27.80	
		P=0.82, t=0.22	P=0.009, t=2.90	P= 0.30, t=0.75	
CRP	Day 0	21.71 ±14.63	39.13 ± 24.86	25.68 ±20.45	P=0.08
	Day 11	22.00 ±21.15	27.97 ± 30.51	24.56 ±23.51	
		P=0.65, t= -0.44	P=0.025, t=-2.23	P= 0.41, t=-0.80	

IL6; Interleukin 6, CRP; C - reactive protein

Normal distribution was assessed using the Kulmogorov Smirnov test. The Chi square test was used to compare the qualitative variables and in order to compare means in the normal quantitative variables we used the one-way variance analysis. To compare non-normal variables Krauskal Wallis test or Mann Whitney test was applied. To compare differences within each group paired t-test or Wilcoxon used. Correlation coefficients were obtained using Pearson test.

### Results

As shown in Table 1, there were no significant differences between three groups in patients' characteristic.

Serum concentration of IL-6 was reduced in Citrulline and Control groups but it shows slight increases in Arginine group (the mean changes was  $-1.9 \pm 25.71$ ,  $-10.87 \pm 16.40$ ,  $1.24 \pm 26.32$  in Control, Citrulline and Arginine groups, respectively). There was significant reduction in IL6 levels just in Citrulline group compared with control group ( $p=0.009$ ) (Table 2).

In addition, serum levels of CRP decreased in both experimental group compared to control group (mean changes in control group were  $0.28 \pm 19.66$ , in Citrulline group  $-11.16$  and in Arginine group it was  $-1.12$ ) which was only significant in Citrulline group ( $p=0.025$ ) (Table 2).

### Discussion

This clinical trial in critically ill patients with trauma shows significant decrease in IL6 and CRP in citrulline group, although there were no significant differences between changes in groups.

Our data indicates that average level of IL6 at first day of study (24 to 48 hours after admission) was higher than normal levels; this finding is similar to studies suggesting that the level of IL6 increases immediately after trauma injury (17, 18). Results of this study also showed that IL6 level has decreased in Citrulline group but it is elevated in Arginine group. Overall, these results indicate that L-citrulline supplementation may be effective in reducing IL6 levels and regulating inflammatory response. Several studies suggest the association of elevated IL6 with infection and inflammation (18). Its reduction in the Citrulline group may be indicative of the effect of L-citrulline on the regulation of inflammatory reactions.

In contrast to our findings, the study of Khorana et al. (18) showed that in patients with head injuries after surgery that received Immuno-nutrition formula (IMN) rich in arginine or standard formula, the level of IL6 had significant decrease in IMN group, whereas such change was not observed in the control group. Another study in patients with gastric, small intestine and colon cancer showed that IL-6 concentrations were higher in the control group than in the IMN group after 8 days (19).

In the study of the role of L-citrulline in IL6, Asgeirsson et al. (20) showed that in sepsis mice, half of whom received enriched formulas with 8% Citrulline for 3 weeks, IL6 levels that were measured 3 days after surgery in this group was less than the control group. These results are in line with our study and confirm the possible effect of L-citrulline on inflammatory status by reducing IL6 level.

We also evaluated the effect of L-arginine and L-citrulline on CRP level. CRP is acute phase protein, synthesized by hepatocytes, and increases by several cytokines, such as IL6, some hours after infection and inflammation (21, 22). The results of our study showed that during the intervention, CRP levels decreased in the Citrulline groups. These results demonstrate that L-citrulline supplementation may have a beneficial effect on the reduction of CRP levels in comparison with the other two groups. Studies have shown that high levels of CRP in patients admitted to the ICU which leads increasing length of hospital stay and higher mortality rate. Then, evaluating CRP level in the first 48 hours of admission In ICU may help to decide on the type of treatment needed and then to assess the success of the treatment (23). Based on our findings, patients may benefit with L-citrulline supplementation to achieve lower the likelihood of organ failure and improving the prognosis of these patients. However, it should be noted that studies that have evaluated the effect of arginine-rich IMN on the CRP level, have contradictory results. The study of de Luis et al. (24) on patients with oral and laryngeal cancer who received arginine- rich formulas or isonitrogen isocalorie standard formulations showed that CRP levels decreased in both groups. Schilling et al. (25) also obtained similar results and showed that there was no significant difference in CRP level between arginine-rich IMN and control group. In contrast to these results, a study by Taylor et al. (26) demonstrated that CRP level in a group that received arginine-rich IMN formulas were lower than those receiving standard formulas.

### Conclusion

Our data suggests that L-citrulline could have modulatory effects on inflammatory response and was more effective than L-arginine. In order to confirm the results obtained in our study, further studies with the aim of investigating the role of these two

amino acids on other inflammatory factors in head injury patients are suggested.

### Conflict of Interests

The authors declared no conflicts of interests.

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