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ORIGINAL RESEARCH

The Effect of L-Citrulline Supplementation on Outcomes of Critically Ill Patients under Mechanical Ventilation; a Double-Blind Randomized Controlled Trial

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Abstract: **Introduction:** Effective parenteral and enteral amino acid replacement is crucial for critically ill patients with altered amino acid metabolism. This study aimed to assess the effects of l-citrulline supplementation on the clinical and laboratory outcomes in critically patients. **Methods:** This was a double-blind placebo-controlled randomized clinical trial. 82 critically ill patients who were expected to receive mechanical ventilation for more than 72 hours were selected. The patients were assigned to either a placebo or an intervention group. The patients in the placebo group received 10 gr of microcrystalline cellulose and the ones in the intervention group were given l-citrulline daily for 7 days. Serum levels of fasting blood sugar (FBS), lipid profile, hepatic enzymes, serum electrolytes, urea nitrogen, creatinine, and C-reactive protein (CRP) were evaluated before and after the intervention. Duration of invasive ventilation, intensive care unit (ICU) length of stay, ventilator-free days, and 28-day mortality rate were recorded and compared between groups. **Results:** Eighty-two patients completed the trial. No statistically significant differences were observed between the two groups in terms of age ($p = 0.46$), sex ($p = 0.49$), body mass index (BMI) ($p = 0.41$), Sequential Organ Failure Assessment (SOFA) Score ($p = 0.08$), Clinical Pulmonary Infection Score (CPIS) score ($p = 0.76$), Acute Physiology and Chronic Health Evaluation (APACHE II) score ($p = 0.58$), risk factors ($p = 0.13$), ICU stay before randomization ($p = 0.32$), and reason of admission ($p = 0.50$) before the intervention. Citrulline group had a notable reduction in FBS ($p = 0.04$), total cholesterol (TC) ($p = 0.02$), low density lipoprotein (LDL-C) ($p < 0.001$) and high-sensitivity CRP (hs-CRP) ($p < 0.001$). Also, a significant increase in lactate dehydrogenase (LDH) concentration ($p < 0.001$) was observed in the intervention group at the end of the trial. Total duration of invasive ventilation and the mean SOFA score on 7th day were significantly lower in the citrulline group compared to the control group. Moreover, a significant increase in days alive and ventilator-free days within 28 days after admission was found in the citrulline group at the end of the trial. Also, there were no significant differences between the groups in terms of mortality rate during intervention, serious adverse events, endotracheal intubation, the use of tracheotomy or non-invasive ventilation after extubation, length of ICU stay, ICU-free days at 28 days, and CPIS and APACHE II scores. For mortality, in the citrulline group, there was two deaths compared to eight deaths in the control group. This resulted in an absolute risk reduction (ARR) of 14.05% (95% CI: 0.39–27.71%) and a number needed to treat (NNT) of 7.1 (95% CI: 3.6–29.5), regarding mortality. **Conclusion:** The results of the present study demonstrated the probable positive effects of citrulline supplementation on lipid profile, hs-CRP levels, duration of invasive ventilation, and SOFA score. Also, l-citrulline consumption may increase the probability of survival without mechanical ventilation.

Keywords: L-citrulline; critical illness; ventilation; intensive care units; treatment outcome; clinical trial

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1. Introduction

Providing the appropriate nutrition therapy in an intensive care unit (ICU), especially for critically ill patients, is essential to improve the metabolic and clinical outcomes (1). Critical illnesses, such as major trauma and sepsis are characterized by a high level of stress/inflammation. Regulation of amino acid metabolism is altered by neuroendocrine changes and cytokine effects due to stress and inflammation, respectively (2). The 2019 European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines recommend nutrition therapy to all critically ill patients staying in the ICU for more than 48 hours (1). Catabolic conditions in critically ill and injured patients cause an increase in energy expenditure and muscle wasting (3, 4). Muscle protein degradation and loss of contractile protein can affect the diaphragm, which is the main inspiratory muscle. People with respiratory problems stay on ventilators for prolonged periods (5).

For critically ill and injured patients, amino acids are crucial for nutritional and metabolic support (6). Citrulline, which has gained attention recently, is included in these immune-nutrition (IMN) formulas, along with arginine, n-3 fatty acids, glutamine, antioxidants, and nucleic acids (7). As our knowledge of altered amino acid metabolism in such patients increases, it is crucial to develop more effective parenteral and enteral amino acid replacement products. Following trauma and surgery, arginine and citrulline levels appear to decrease in critically ill patients (7-10). Moreover, these amino acids are reversely correlated with levels of cytokines and inflammatory markers (11). A growing body of evidence shows that cytokines can contribute to the emergence of critical illness (12). Cytokines are strongly related to higher disease severity in these states, while the persistence in the spread of the cytokinesis is associated with improvement in multiple organ failure (MOF) (13). Orally ingested L-citrulline can result in the biosynthesis of L-arginine and, L-citrulline (14) ,and also enhance arginine bioavailability in the circulation, which acts as a precursor for nitric oxide (NO) formation (15). NO level could regulate vasodilation, blood flow, and muscle oxygenation (16, 17).

L-citrulline has been found to be more effective than L-arginine supplementation for formation of NO, which can be due to the fact that arginine might be metabolized by arginase and transformed to urea and ornithine, making it less available to nitric oxide synthase for producing NO and citrulline(18)(19).

Several studies have examined the effects of L-arginine supplementation on ICU patients using arginine-rich IMN formulas, including other compounds (20-22). Based on a recent study on critical care, citrulline plasma levels are extremely low in patients with sepsis and even lower in those with acute respiratory distress syndrome (ARDS) (12). According to one viewpoint, plasma citrulline levels could be linked with better vision in critically ill patients. Most importantly, plasma citrulline levels are low in the majority of these patients (23-25). Low plasma citrulline levels are related to poor prognosis (24, 25). Plasma citrulline and C-responsive protein (CRP) fixations are conversely connected (24-26). Although several clinical studies have demonstrated the favourable effects of citrulline on critically ill patients (27, 28), it is yet not confirmed to be effective as a solitary treatment and further controlled clinical trials are needed to examine the effects of citrulline on clinical outcomes, such as the respiratory capacity and the duration of ventilation in these patients. Therefore, the aim of the current study was to evaluate the effect of oral L-citrulline supplementation on FBS, lipid profile, hepatic enzymes, serum electrolytes, urea nitrogen, creatinine, CRP, duration of invasive ventilation, ICU length of stay, ventilator-free days, and 28-day mortality rate in ventilated intensive care unit patients.

2. Methods

This was a randomized clinical trial performed on intensive care unit (ICU) patients admitted to Imam Reza Hospital affiliated with the AJA University of Medical Sciences, Tehran, Iran, from December 21, 2021 to May 27, 2022. The Research Ethics Committee of AJA University of Medical Sciences approved the study protocol (IR.AJAUMS.REC.1400.269). We registered the present trial on the Iranian Registry of Clinical Trials website (http://www.irct.ir, identifier: IRCT20210920052530N1). All patients' families provided written informed consent after a full explanation of the study.

2.1. Participants

This study was performed on adult critically ill patients. Only subjects that were under invasive ventilation either through intubation or tracheotomy tube and required ventilation for at least 72 hours after study entry and expected to survive and remain in the ICU for at least 96 hours after were included. The study exclusion criteria were as follows: less than 18 years old, pregnancy, previous allergy to citrulline or arginine, a history of gastrointestinal disease, digestive tract surgery, intestinal obstruction, paralytic ileus, intestinal ischemia, septic patients, and hyperthyroidism.

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2.2. Randomization and allocation

Random allocation software (RAS) was used for randomization. During the first 24 hours of invasive ventilation, the subjects were randomly assigned to each group in a centralized, blinded manner by means of an assignment sequence that was generated by computer. In addition, the Acute Physiology, and Chronic Health Evaluation (APACHE) II (score dichotomized as ≤ 15 or > 15) was used to assess severity of disease at the time of inclusion. Random block sizes were utilized to balance the list. Furthermore, investigators, patients, and research staff did not know the group of the participants.

2.3. Study protocol

Participants in placebo (n=42) and Citrulline (n=40) groups received 10 gram/day Microcrystalline cellulose and L-Citrulline powder (Karen Pharma & Food Supplement Co., Iran) for 7 days, respectively. Blood samples were drawn before and after the 7-day intervention. Acute-phase proteins (APPs) as immunology factors, complete blood count (CBC), blood urea nitrogen (BUN), creatine, albumin, glycemic status, lipid profile, and liver function were determined before and after the intervention.

Demographic characteristics, physiological variables, and other clinical and laboratory data were collected. Data on the days with mechanical ventilation were collected within 28 days.

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

2.4. Measurements

Clinical outcomes

The number of days free from mechanical ventilation for at least 48 consecutive hours and alive is defined as ventilator-free days within the first 28 days. If patients were discharged prior to the end of study period, they are considered as alive without mechanical ventilation. Also, for subjects who died, the ventilator-free days are omitted.

Data on all-cause mortality, ICU-free days, and mechanical ventilation duration at 28 days plus both Sequential Organ Failure Assessment (SOFA) score and Clinical Pulmonary Infection Score (CPIS) on the first and seventh days were measured in addition to other outcomes. The severity of illness was evaluated using APACHE II on the day of admission

Blood sample collection

Fasting blood samples (10 mL) were taken at baseline (day 0) and at the end of the trial (day 7) 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

CRP was measured via the method of agglutination of latex particles on the slide (ENISON Co kits). Albumin was assessed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abcam). BUN was 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).

Sample size and statistical methods

The minimum sample size was determined as 30 subjects in each group. The calculation is based on the mean ±SD of hs-CRP determined by Barkhidarian et al. (19) considering α = 0.05 and power of 80%. Considering a possible dropout of 35%, 40 subjects were included in each group.

Data were presented as mean ± SD. We used the Kolmogrov-Smirnov test to examine the normal distribution of variables. Log transformation was conducted for nonnormally distributed variables. Independent sample t-test and paired Student's t-test were employed to identify the effect of the intervention on outcome variables. The chi-square test was applied to compare the relative or absolute frequency. Analysis of covariance (ANCOVA) was used for comparisons between the two groups post-intervention after adjusting for baseline values. Paired t-test was used to compare differences from baseline to post-intervention period within groups.

Absolute risk reduction (ARR) and the number needed to treat (NNT) were also calculated (29). The Statistical Package for the Social Sciences (SPSS, version 19; Chicago, IL) was utilized to carry out the statistical analysis, and a p-value of < 0.05 was regarded statistically significant.

3. Results

3.1. Baseline characteristics of studies cases

From December 21, 2021 to May 27, 2022, a total of 90 patients were randomly assigned to the intervention (n=45) and placebo (n=45) groups. After randomization, there was drop out throughout the investigation and finally 40 subjects in the citrulline group and 42 subjects in the placebo group completed the trial. 5 and 3 participants in the citrulline and placebo groups, respectively, were excluded after randomization due to the poor adherence to the intervention (Figure 1). Table 1 shows the baseline characteristics of the patients in the L-citrulline group and the placebo group. The mean age of the participants in the L-citrulline and the placebo groups were 52.2 ± 18.4 and 49.9 ± 19.0 years, respectively. All baseline characteristics of study participants were well balanced between groups and no statistically significant differences were observed between the two groups in terms of age (p = 0.46), sex (p = 0.49), BMI (p = 0.41), SOFA score (p = 0.08),

CPIS score (p = 0.76), APACH II score (p = 0.58), risk factors (p = 0.13), ICU stay before randomization (p = 0.32), and reason of admission (p = 0.50) before the intervention.

3.2. Comparing the outcomes of intervention

Laboratory parameters

Among the laboratory variables, as presented in Table 2, citrulline group had a notable reduction in FBS (p = 0.04), total cholesterol (TC) (p = 0.02), low density lipoprotein (LDL-C) (p <0.001) and high-sensitivity CRP (hs-CRP) (p <0.001). Also, a significant increase in LDH concentration (p <0.001) was observed in the intervention group compared to the control group after adjusting for baseline values at the end of the trial. Non-significant differences were found between groups in term of albumin (p = 0.58), triglyceride (TG) (p = 0.68), high density lipoprotein (HDL-C) (p = 0.59), AST (p = 0.22), ALT (p = 0.75), and BUN (p = 0.40). Serum concentrations of creatine (p = 0.01) significantly decreased during 7 days in patients receiving citrulline, while such effect was not observed in the placebo group. Considering that no adverse side effects were reported, citrulline was well tolerated.

Disease severity and disposition

As demonstrated in Table 3, the total duration of invasive ventilation and the mean SOFA score on the 7th day were significantly lower in the citrulline group compared to the control group. Moreover, a significant increase in days alive and ventilator-free days within 28 days after admission was found after citrulline supplementation. Also, there were no significant differences between the groups in terms of mortality rate during intervention, serious adverse events, endotracheal intubation, the use of tracheotomy or non-invasive ventilation after extubation, length of ICU stay, ICU-free days at 28 days, and CPIS and APACHE II scores. Regarding mortality, in the citrulline group, there was two deaths compared to eight deaths in the control group. This resulted in an absolute risk reduction (ARR) of 14.05% (95% CI: 0.39–27.71%) and a number needed to treat (NNT) of 7.1 (95% CI: 3.6–29.5), regarding mortality.

4. Discussion

Based on the obtained findings, L-citrulline supplementation significantly decreased the serum levels of FBS, LDL-C, TC, and hs-CRP, duration of invasive ventilation, and SOFA score. Also, serum LDH levels and days alive and ventilator-free days within 28 days after admission were significantly increased by L citrulline supplementation. However, there was no difference in terms of other assessed variables after L citrulline supplementation.

As mentioned above, serum levels of LDL-C and TC in the intervention group were lower than the placebo group. Based on a prior study that was conducted on type 2 diabetes mellitus patients, an eight-week supplementation with L-Citrulline powder showed a remarkable improvement on glucose homeostasis, some lipid profiles, and inflammatory biomarkers, which is consistent with our results (30). Also, the efficacy of L-citrulline supplementation on lipid profile was assessed in numerous animal models. For example, Kudo et al.

investigated the effect of nine weeks of 1 gr/kg L-citrulline administration on the serum level of TG in rats. In this study, authors concluded that L-citrulline powder does not affect serum levels of TG (2). Moreover, notable reduction in cholesterol levels without affecting TG concentration were detected as a result of L-citrulline (0.5 g/kg) supplementation for 11 weeks in rats with high-fat diet (31). The plausible hypolipidemic effects of L-citrulline may justify liver lipid metabolism improvement. L-citrulline can particularly suppress the expression of sterol regulatory element-binding protein 1 (SREBP-1). SREBP-1 expression is decreased with activation of adenosine monophosphate-activated protein kinase alpha (AMPKalpha) (32). AMPK is a cell strength sensor that merges different physiological indicators for power balance restoration. Specifically, it induces silencing of SREBP-1 cleavage, its target gene expression, and the nuclear translocation. This results in the reduction of lipid accumulation and synthesis of fatty acids in hepatocytes (33). Furthermore, it has been demonstrated that intervention with citrulline stimulates visceral fat lipolysis. Hormone-sensitive lipase phosphorylation and down-regulation of glyceroneogenesis might be induced by L-citrulline, which leads to accelerated fatty acid launch from the adipose tissue (34). Moreover, it is known that the phosphorylation and activation of AMPKα in various tissues are raised through NO produced by L-citrulline (35, 36). Not finding notable variation in other lipid profiles can be justified by various factors such as small sample size, insufficient dosage, and short intervention interval.

In contrast with the study that found a significant increase in serum LDH levels, an animal study performed by Villareal et al. demonstrated a decrease in the expression of LDH after L-citrulline supplementation (37). Regarding hepatic enzymes including ALT and AST, in opposition to our results that recommended no remarkable effect of L-citrulline, a clinical trial done by Darabi et al. indicated a statistically significant decrease in ALT levels following L-citrulline supplementation (38). Also, regarding kidney function, contrary to our results that presented no favourable effect by L-citrulline, a recent animal study by Hashemi et al. concluded that L-citrulline could significantly reduce the levels of BUN and creatinine in rats (39). The discrepancies between aforementioned findings may result from the different nature of the studies and dissimilar dosage and duration of L-citrulline supplementation.

Our study exhibited that L-citrulline powder notably decreased the duration of invasive ventilation. This finding is consistent with the results of a study conducted by Lauterbach et al. in 2018, which measured the effectiveness of L-Citrulline supplementation in treating pulmonary hypertension. This study finally proposed that treatment with L-citrulline may be an alternative and potentially useful treatment in the prevention or treatment of chronic pulmonary hypertension in infants (40). Also, an animal study investigated the efficacy of L-citrulline on alveolar development and lung condition in newborn rats. The results of this research indicated that L-Citrulline therapy prevents lung damage caused by hyperoxia and high blood pressure in newborn rats (41). Furthermore, L-Citrulline may be a new therapeutic alternative for inhaled NO and, prevent bronchopulmonary dysplasia. In addition, oral L-citrulline, as a precursor of NO, improved symptoms of sickle cell disease in children and reduced pulmonary blood pressure after surgery for congenital heart disease (42). Orally ingested L-citrulline can also enhance arginine bioavailability in the circulation and act as a precursor for nitric oxide (NO•) formation (15). NO• levels could regulate vasodilation, blood flow, and muscle oxygenation (16, 17). Although no side effects were previously reported for L-citrulline, oral administration of L-arginine in high doses could result in nausea, vomiting, diarrhea, headache, flushing, and numbness, owing to intestinal and hepatic conversion of L-arginine to ornithine and urea. Orally ingested L-citrulline can mainly have a more beneficial effect compared to L-arginine (43).

The SOFA score evaluates blood pressure and the function of neurological system, blood, liver, and kidney. A higher SOFA score indicates a higher chance of mortality (44). According to the findings of the present study, the SOFA score in the group that received L-citrulline was notably lower in comparison to the control group. Based on previous investigations, L-Citrulline therapy induced an anti-inflammatory profile and obviously protected against kidney dysfunction through improving the function of glomerulus and its associated tubule (45). In addition, L-citrulline amino acid consumption exerts beneficial effects on cardiometabolic health, glucose homeostasis, and protein and lipid metabolism through direct and indirect pathways (46-48). Moreover, L-citrulline intake may truncate the required time for complete myocardial depolarization and repolarization. Thus, L-citrulline ingestion transiently modifies myocardial blood supply and improves the energy supply required for faster recovery in restoring ATP-dependent ionic exchanges. Taking this supplement can even control blood pressure in people with hypertension. The findings of a systematic review and meta-analysis showed that L-citrulline supplementation reduced the systolic blood pressure by 4 mmHg (49).

Hs-CRP, a measurable protein in the blood, increases in the body during systematic inflammation. Measurement of this index in the blood is used to determine the risk of cardiovascular diseases and other inflammatory-related complications. Based on our results, L-Citrulline supplementation significantly decreased hs-CRP levels. In line with our findings, Abbaszadeh et al. found a notable drop in hs-CRP levels in patients who received 10 g L-citrulline daily for 10 days (50). Also, another study demonstrated that an increase in L-citrulline concentrations caused a considerable reduction in hs-CRP production in obese rats with diabetes (51). Several in vitro and in vivo models proposed that L-citrulline supplementation caused a remarkable reduction in inflammation by inhibiting the expression of NF-kB as an important transcription factor involved in the inflammatory response (52).

5. Limitations

Among the limitations of this study was the short duration of the follow-up period as well as the budget restrictions, which prevented us from examining the complete profile of inflammatory biomarkers. Moreover, our participants were ill patients over 18 years old, and consequently, our findings may not apply to people of other ages and health conditions. Finally, although several potential confounders were considered in the statistical analysis, some other unknown confounders could affect the obtained results and lead to bias.

6. Conclusions

In conclusion, our finding proposed that L-citrulline supplementation decreased the serum levels of FBS, LDL-C, TC, and hs-CRP, duration of invasive ventilation, and SOFA score. On the other hand, serum LDH levels and days alive and ventilator-free days within 28 days after admission significantly increased with L citrulline supplementation. Although obtained findings suggested that L citrulline supplementation could improve some clinical outcomes in critically ill patients, further well-designed clinical trials with larger sample size and longer follow-up period are required to verify its favourable effects in ventilated intensive care unit patients.

7. Declarations

7.1. Acknowledgments

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7.2. Conflict of interest

The authors declare that they have no conflicts of interest.

7.3. Fundings and supports

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7.4. Authors' contribution

MRA, SH, SMM, EH and VA studied concept and designed the study. MRA, EH and FBY collected data. MRA, SH, SMM, MMA and VH analyzed and interpreted data. MRA drafted whole of the manuscript. All authors read and approved the final version of manuscript.

7.5. Ethics approval

The protocol of the study was approved by the Research Ethics Committees of AJA University of Medical Sciences (IR.AJAUMS.REC.1400.269).

7.6. Consent for publication

All participants provided written informed consent.

7.7. Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Ginguay A, De Bandt JP, Cynober L. Indications and contraindications for infusing specific amino acids (leucine, glutamine, arginine, citrulline, and taurine) in critical illness. Curr Opin Clin Nutr Metab Care. 2016;19(2):161-9.
2. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. Int J Mol Sci. 2019 Nov 28;20(23):6008.
3. Herndon DN, Hart DW, Wolf SE, Chinkes DL, Wolfe RR. Reversal of catabolism by beta-blockade after severe burns. N Engl J Med. 2001;345(17):1223-9.
4. Hofford JM, Milakofsky L, Vogel WH, Sacher RS, Savage GJ, Pell S. The nutritional status in advanced emphysema associated with chronic bronchitis. A study of amino acid and catecholamine levels. Am Rev Respir Dis. 1990;141(4 Pt 1):902-8.
5. Ottenheijm CA, Heunks LM, Sieck GC, Zhan WZ, Jansen SM, Degens H, et al. Diaphragm dysfunction in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2005;172(2):200-5.
6. Barkhidarian B, Hashemi SI, Nematy M, Rahbar A, Ranjbar R, Safarian M. Effects of arginine and citrulline supplementation on inflammatory markers in critically ill patients. JNSD. 2017;2(2).
7. Zhou M, Martindale RG. Immune-modulating enteral formulations: optimum components, appropriate patients, and controversial use of arginine in sepsis. Curr Gastroenterol Rep. 2007;9(4):329-37.
8. Luiking YC, Poeze M, Ramsay G, Deutz NE. Reduced citrulline production in sepsis is related to diminished de novo arginine and nitric oxide production. Am J Clin Nutr. 2009;89(1):142-52.
9. Zhou M, Martindale RG. Arginine in the critical care setting. J Nutr. 2007;137(6 Suppl 2):1687s-92s.
10. Morris SM, Jr. Recent advances in arginine metabolism. Curr Opin Clin Nutr Metab Care. 2004;7(1):45-51.
11. van Waardenburg DA, de Betue CT, Luiking YC, Engel M, Deutz NE. Plasma arginine and citrulline concentrations in critically ill children: strong relation with inflammation. Am J Clin Nutr. 2007;86(5):1438-44.
12. Oberholzer A, Oberholzer C, Moldawer LL. Cytokine signaling–regulation of the immune response in normal and critically ill states. Crit Care Med. 2000;28(4 Suppl):N3-12.
13. Von Blomberg-van der Flier B, Riezebos R, Scholten P, Quak J, Snow G, Van Leeuwen P. Differences in immune status between well-nourished andmalnourished head and neck cancer patients. Clin Nutr. 1998;17(3):107-11.
14. Windmueller HG, Spaeth AE. Source and fate of circulating citrulline. Am J Physiol. 1981;241(6):E473-80.
15. Schwedhelm E, Maas R, Freese R, Jung D, Lukacs Z, Jambrecina A, et al. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. Br J Clin Pharmacol. 2008;65(1):51-9.
16. Jones AM, Thompson C, Wylie LJ, Vanhatalo A. Dietary Nitrate and Physical Performance. Annu Rev Nutr. 2018;38:303-28.
17. Margaritelis NV, Paschalis V, Theodorou AA, Kyparos A, Nikolaidis MG. Redox basis of exercise physiology. Redox Biol. 2020;35:101499.
18. Curis E, Nicolis I, Moinard C, Osowska S, Zerrouk N, Bénazeth S, et al. Almost all about citrulline in mammals. Amino Acids. 2005;29(3):177-205.
19. Bahareh B, Seyed Issac H, Sakineh S, Mohsen N, Ashraf R, Roya R, et al. Comparison of L-arginine and L-citrulline oral supplementation in head trauma ICU patients receiving enteral nutrition: A randomized double blind clinical trial. JNSD. 2018;4(2).
20. Galbán C, Montejo JC, Mesejo A, Marco P, Celaya S, Sánchez-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(3):643-8.
21. Atkinson S, Sieffert E, Bihari D. A prospective, randomized, double-blind, controlled clinical trial of enteral immunonutrition in the critically ill. Guy's Hospital Intensive Care Group. Crit Care Med. 1998;26(7):1164-72.
22. 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(4):531-40; discussion 40-3.
23. Poole A, Deane A, Summers M, Fletcher J, Chapman M. The relationship between fasting plasma citrulline concentration and small intestinal function in the critically ill. J Crit Care. 2016;19(1):1-8.
24. Piton G, Manzon C, Monnet E, Cypriani B, Barbot O, Navellou J-C, et al. Plasma citrulline kinetics and prognostic value in critically ill patients. Intensive Care Med. 2010;36(4):702-6.
25. Piton G, Belon F, Cypriani B, Regnard J, Puyraveau M, Manzon C, et al. Enterocyte damage in critically ill patients is associated with shock condition and 28-day mortality. Crit Care Med. 2013;41(9):2169-76.
26. Cynober L. Citrulline: just a biomarker or a conditionally essential amino acid and a pharmaconutrient in critically ill patients? Critic care. 2013;17(2):1-3.
27. Barkhidarian B, seyedhamzeh S, Hashemi SI, Nematy M, Rahbar A, Ranjbar R, et al. Effects of Arginine and Citrulline supplementation on inflammatory markers in critically ill patients. JNSD. 2017;2(2).
28. Vermeulen MA, van de Poll MC, Ligthart-Melis GC, Dejong CH, van den Tol MP, Boelens PG, et al. Specific amino acids in the critically ill patient–exogenous glutamine/arginine: a common denominator? Crit Care Med. 2007;35(9 Suppl):S568-76.
29. Weeks DL, Noteboom JT. Using the number needed to treat in clinical practice. Arch Phys M. 2004;85(10):1729-31.
30. Azizi S, Mahdavi R, Mobasseri M, Aliasgharzadeh S, Abbaszadeh F, Ebrahimi-Mameghani MJPR. The impact of L-citrulline supplementation on glucose homeostasis, lipid profile, and some inflammatory factors in overweight and obese patients with type 2 diabetes: A double-blind randomized placebo-controlled trial. Phytother Res. 2021;35(6):3157-66.
31. Kudo M, Yoshitomi H, Momoo M, Suguro S, Yamagishi Y, Gao MJB, et al. Evaluation of the effects and mechanism of L-Citrulline on anti-obesity by appetite suppression in obese/diabetic KK-Ay mice and high-fat diet fed SD rats. Biol Pharm Bull. 2017;40(4):524-30.
32. Capel F, Chabrier G, Pitois E, Rigaudière JP, Plenier SL, Durand C, et al. Combining citrulline with atorvastatin preserves glucose homeostasis in a murine model of diet-induced obesity. Br J Pharmacol. 2015;172(20):4996-5008.
33. Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, et al. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell Metab. 2011;13(4):376-88.
34. Joffin N, Jaubert A-M, Durant S, Barouki R, Forest C, Noirez P. Citrulline counteracts overweight-and aging-related effects on adiponectin and leptin gene expression in rat white adipose tissue. Biochimie open. 2015;1:1-5.
35. Abudukadier A, Fujita Y, Obara A, Ohashi A, Fukushima T, Sato Y, et al. Tetrahydrobiopterin has a glucose-lowering effect by suppressing hepatic gluconeogenesis in an endothelial nitric oxide synthase–dependent manner in diabetic mice. Diabetes. 2013;62(9):3033-43.
36. Cardaci S, Filomeni G, Ciriolo MR. Redox implications of AMPK-mediated signal transduction beyond energetic clues. J Cell Sci. 2012;125(9):2115-25.
37. Villareal MO, Matsukawa T, Isoda H. L-Citrulline Supplementation-Increased Skeletal Muscle PGC-1α Expression is Associated With Exercise Performance and Increased Skeletal Muscle Weight. Mol Nutr Food Res. 2018;62(14):e1701043.
38. Darabi Z, Darand M, Yari Z, Agah S, Hrkmat doust A. Effects of Citrulline on Non-alcoholic Fatty Liver Disease: A Randomized-controlled Clinical Trial. Iranian-J-Nutr-Sci-Food-Technol. 2019;14(3):1-9.
39. Hashemi SR, Arab HA, Seifi B, Muhammadnejad S. A comparison effects of l-citrulline and l-arginine against cyclosporine-induced blood pressure and biochemical changes in the rats. Hipertens Riesgo Vasc. 2021;38(4):170-7.
40. Lauterbach R, Pawlik D, Lauterbach JPJSOMCR. L-citrulline supplementation in the treatment of pulmonary hypertension associated with bronchopulmonary dysplasia in preterm infant: A case report. SAGE Open Med Case Rep. 2018;6:2050313X18778730.
41. Sopi RB, Zaidi SI, Mladenov M, Sahiti H, Istrefi Z, Gjorgoski I, et al. L-citrulline supplementation reverses the impaired airway relaxation in neonatal rats exposed to hyperoxia. Respir Res. 2012;13(1):1-9.
42. Vadivel A, Aschner JL, Rey-Parra GJ, Magarik J, Zeng H, Summar M, et al. L-citrulline attenuates arrested alveolar growth and pulmonary hypertension in oxygen-induced lung injury in newborn rats. Pediatr Res. 2010;68(6):519-25.
43. Mahboobi S, Tsang C, Rezaei S, Jafarnejad S. RETRACTED ARTICLE: Effect of l-citrulline supplementation on blood pressure: a systematic review and meta-analysis of randomized controlled trials. J Hum Hypertens. 2019;33(1):10-21.
44. Cuartas PA, Santos HT, Levy BM, Gong MN, Powell T, Chuang E. Modeling Outcomes Using Sequential Organ Failure Assessment (SOFA) Score-Based Ventilator Triage Guidelines During the COVID-19 Pandemic. Disaster Med Public Health Prep. 2022:1-4.
45. Romero MJ, Yao L, Sridhar S, Bhatta A, Dou H, Ramesh G, et al. L-citrulline protects from kidney damage in type 1 diabetic mice. Front immunol. 2013;4:480.
46. Adam J, Brandmaier S, Leonhardt J, Scheerer MF,

Mohney RP, Xu T, et al. Metformin effect on nontargeted metabolite profiles in patients with type 2 diabetes and in multiple murine tissues. Diabetes. 2016;65(12):3776-85.
47. Irving BA, Carter RE, Soop M, Weymiller A, Syed H, Karakelides H, et al. Effect of insulin sensitizer therapy on amino acids and their metabolites. Metabolism. 2015;64(6):720-8.
48. Irving BA, Spielmann GJD. Does citrulline sit at the nexus of metformin's pleotropic effects on metabolism and mediate its salutatory effects in individuals with type 2 diabetes? Diabetes. 2016;65(12):3537-40.
49. Kameda N, Okigawa T, Kimura T, Fujibayashi M, Asada T, Kinoshita R, et al. The effect of L-citrulline ingestion on ECG QT interval and autonomic nervous system activity. J Physiol Anthropol. 2011;30(2):41-5.
50. Abbaszadeh F, Azizi S, Mobasseri M, Ebrahimi-Mameghani M. The effects of citrulline supplementation on meta-inflammation and insulin sensitivity in type 2 diabetes: a randomized, double-blind, placebo-controlled trial. Diabetol Metab Syndr. 2021;13(1):1-9.
51. Breuillard C, Bonhomme S, Couderc R, Cynober L, De Bandt J-P. In vitro anti-inflammatory effects of citrulline on peritoneal macrophages in Zucker diabetic fatty rats. Br J Nutr. 2015;113(1):120-4.
52. de Winther MP, Kanters E, Kraal G, Hofker MH. Nuclear factor kappaB signaling in atherogenesis. Arterioscler Thromb Vasc Biol. 2005;25(5):904-14.

Table 1: Comparing the baseline characteristics of studied groups

Characteristic	L-citrulline (n = 40)	Placebo (n = 42)	P value
Age (year)			
Mean ± SD	52.5 ± 18.4	49.9 ± 19.0	0.46
Gender			
Male	21 (52.5)	26 (59.09)	0.49
Female	19 (47.5)	16 (40.91)	
Body mass index (kg/m2)			
Mean ± SD	27.2 ± 8.0	26.7 ± 9.1	0.4
Disease severity			
Sequential Organ Failure Assessment	8.2 ± 0.5	8.4 ± 0.55	0.08
Clinical Pulmonary Infection Score	3.4 ± 1.6	3.1 ± 1.0	0.76
APACHE II score	16.77 ± 4.3	17.3 ± 4.6	0.58
ICU stay before randomization (day)			
Median (IQR)	1 (0-2)	1 (0-1)	0.32
Comorbidities and risk factors			
Cardiovascular disease	3 (7.5)	3 (7.1)	0.13
Diabetes Mellitus	15 (37.5)	20 (47.6)	
Hypertension	24 (60.0)	31 (73.8)	
Neurological diseases	1 (2.5)	1 (2.3)	
Respiratory disease	13 (32.5)	10 (23.8)	
Reason for admission			
Pneumonia	14 (35.0)	15 (35.7)	
Surgical	10 (25.0)	9 (21.4)	
Chronic Obstructive Pulmonary Disease	5 (12.5)	7 (16.6)	0.5
Acute Respiratory Distress Syndrome	5 (12.5)	6 (14.2)	
Stroke	2 (5.0)	2 (4.7)	
Trauma	4 (10.0)	5 (11.9)	

Data are presented as mean ± standard deviation (SD), frequency (%), or median (interquartile range; IQR). APACHE II score: Acute Physiology and Chronic Health Evaluation. II Score; ICU: intensive care unit.

Table 2: Comparing the laboratory variables between the L-citrulline-treated (n=40) and placebo (n=42) groups before and one week after intervention

Factors	Baseline	After intervention	P value
Albumin			
L-citrulline	3.67 ± 0.51	3.31 ± 0.54	0.58
Placebo	3.61 ± 0.44	3.38 ± 0.51	0.67
FBS (mg/dl)			
L-citrulline	106.93 ± 15.28	93.23 ± 9.79	0.04
Placebo	106.96 ± 16.28	100.89 ± 13.94	0.57
TG (mg/dl)			
L-citrulline	185.50 ± 44.34	136.41 ± 32.86	0.68
Placebo	164.44 ± 53.77	155.61 ± 44.12	0.52
TC (mg/dl)			
L-citrulline	181.23 ± 41.00	163.86 ± 40.21	0.02
Placebo	200.00 ± 34.06	190.77 ± 31.84	0.01
LDL-C (mg/dl)			
L-citrulline	118.78 ± 34.78	105.97 ± 37.22	<0.001
Placebo	128.73 ± 34.82	120.48 ± 35.65	0.01
HDL-C (mg/dl)			
L-citrulline	35.01 ± 11.68	37.28 ± 11.43	0.59
Placebo	33.49 ± 7.70	34.78 ± 7.78	0.35
Hs-CRP (mg/L)			
L-citrulline	3691.50 ± 644.67	2026.62 ± 246.82	<0.001
Placebo	3964.23 ± 241.09	3360.44 ± 486.36	0.01
AST (U/l)			
L-citrulline	88.43 ± 22.48	117.98 ± 34.07	0.22
Placebo	63.23 ± 24.14	117.63 ± 47.97	0.97
ALT (U/l)			
L-citrulline	66.54 ± 34.61	107.34 ± 29.21	0.75
Placebo	46.41 ± 18.79	135.22 ± 38.41	0.84
LDH (U/l)			
L-citrulline	63.93 ± 24.51	99.66 ± 31.37	<0.001
Placebo	79.04 ± 32.75	117.42 ± 33.23	0.01
BUN (mg/dl)			
L-citrulline	50.63 ± 24.42	47.25 ± 13.08	0.40
Placebo	42.07 ± 15.02	44.05 ± 13.69	0.99
Creatinine (mg/dl)			
L-citrulline	1.19 ± 0.56	0.78 ± 0.31	0.01
Placebo	1.12 ± 0.23	0.96 ± 0.52	0.13

Data are presented as mean ± standard deviation (SD). FBS: fasting blood sugar; TG: triglyceride; TC: Total Cholesterol; LDL-C: low density lipoprotein; HDL-C: high density lipoprotein; hs-CRP: high-sensitive C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; BUN: blood urea nitrogen.

Table 3: Comparing the clinical outcomes (after 28 days) and disease severity between the L-citrulline-treated (n=40) and placebo (n=42) groups

Variables	L-citrulline	Placebo	P value
Mortality rate during intervention	2 (5.0)	8 (19.0)	0.61
Serious adverse events	1 (2.5)	2 (4.5)	0.82
Endotracheal intubation	40 (100.0)	41 (97.5)	0.99
NIV after extubation	13 (32.5)	16 (38.0)	0.39
All-Cause Mortality	7 (17.5)	9 (21.5)	0.85
Duration of invasive ventilation (hour)	112.5 ± 59	139 ± 61.35	0.04
Days alive and ventilator-free	6.6 ± 1.6	4 ± 1.25	< 0.001
Length of ICU stay (days)	7 ± 5.5	8 ± 5.5	0.41
ICU-free days	2.1 ± 1.75	2.0 ± 1.7	0.79
SOFA score			
1th day	8.2 ± 0.5	8.4 ± 0.55	0.08
7th day	6.1 ± 0.6	7.5 ± 1.2	< 0.001
P value	0.08	0.38	
CPIS score			
1th day	3.4 ± 1.6	3.1 ± 1.0	0.31
7th day	2.9 ± 1.1	2.7 ± 0.9	0.37
P value	0.73	0.96	
APACHE II score			
1th day	16.77 ± 4.3	17.3 ± 4.6	0.59
7th day	22.9 ± 7.6	22.0 ± 7.6	0.59
P value	0.82	0.96	

Data are presented as mean ± standard deviation (SD) or frequency (%). Abbreviations: ICU: intensive care unit; SOFA: Sequential Organ Failure Assessment; CPIS: Clinical Pulmonary Infection Score; APACHE II score: Acute Physiology and Chronic Health Evaluation II Score; NIV: non-invasive ventilation. Adverse events were considered serious when they required intensive care procedures (use of vasopressors, haemodialysis, central venous catheterization, cardiac pacing, or tube thoracostomy) or surgery, and events that prolonged hospitalization or resulted in persistent or major disability or incapacity.

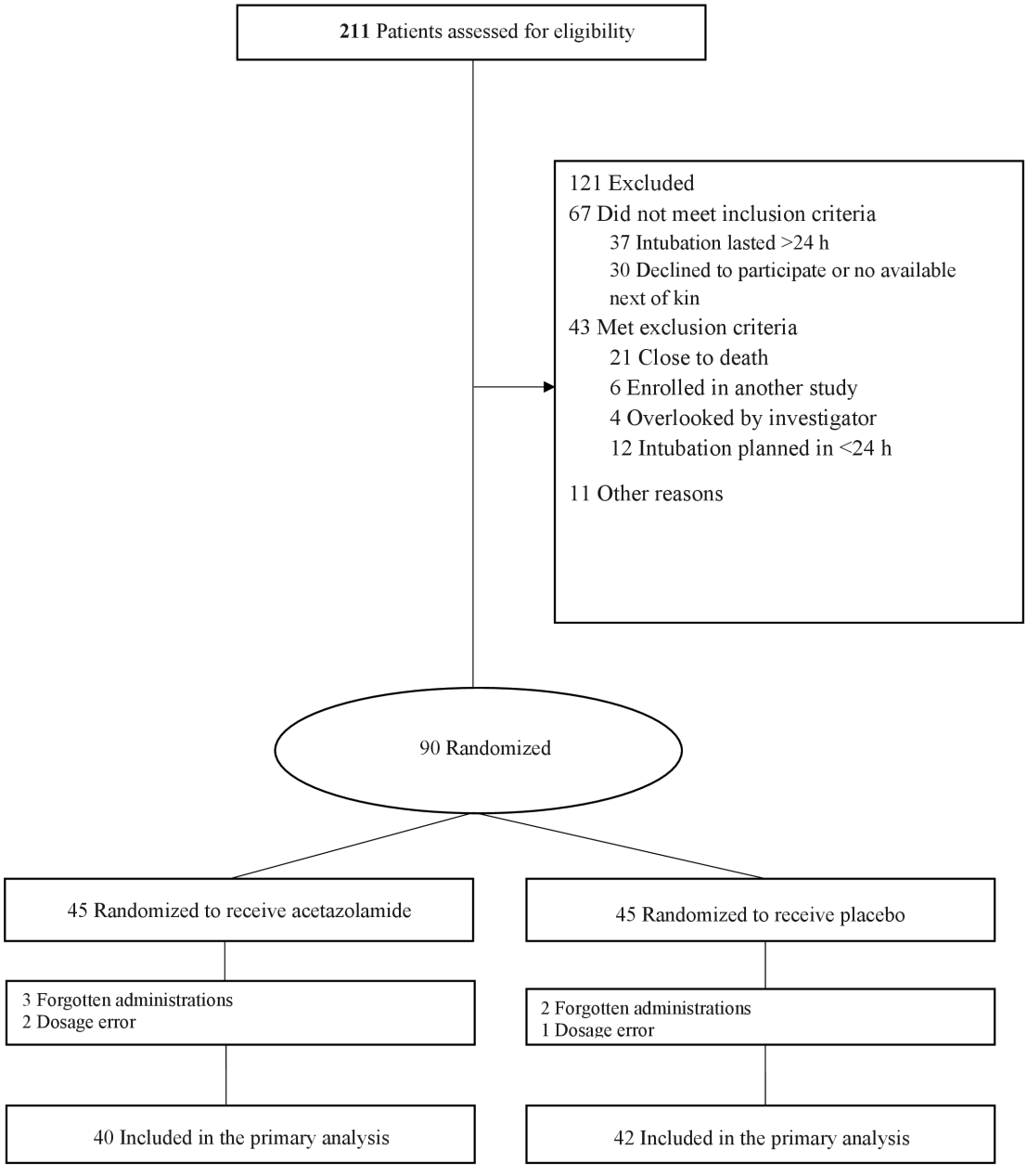

Figure 1: Flowchart of patient enrollment.