Acute L-Citrulline Supplementation

Subjects: Agriculture, Dairy & Animal Science

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The present study aimed to investigate whether acute L-citrulline supplementation would affect inspiratory muscle oxygenation and respiratory performance. Twelve healthy males received 6 g of L-citrulline or placebo in a double-blind crossover design. Pulmonary function (i.e., forced expired volume in 1 s, forced vital capacity and their ratio), maximal inspiratory pressure (MIP), fractional exhaled nitric oxide (NO•), and sternocleidomastoid muscle oxygenation were measured at baseline, one hour post supplementation, and after an incremental resistive breathing protocol to task failure of the respiratory muscles. The resistive breathing task consisted of 30 inspirations at 70% and 80% of MIP followed by continuous inspirations at 90% of MIP until task failure. Sternocleidomastoid muscle oxygenation was assessed using near-infrared spectroscopy. One-hour post-L-citrulline supplementation, exhaled NO• was significantly increased (19.2%; $p < 0.05$), and this increase was preserved until the end of the resistive breathing (16.4%; $p < 0.05$). In contrast, no difference was observed in the placebo condition. Pulmonary function and MIP were not affected by the L-citrulline supplementation. During resistive breathing, sternocleidomastoid muscle oxygenation was significantly reduced, with no difference noted between the two supplementation conditions. In conclusion, a single ingestion of 6 g L-citrulline increased NO• bioavailability but not the respiratory performance and inspiratory muscle oxygenation.

Keywords: nitric oxide ; L-citrulline ; NIRS ; respiratory muscles ; sternocleidomastoid ; ergogenic supplements ; fatigue

1. Introduction

L-citrulline is a nonessential, non-coded alpha-amino acid that has a key role during the urea cycle in the liver [1]. In the urea cycle, L-citrulline is synthesized from ornithine and metabolized by argininosuccinate synthetase [1]. During this cycle, there is no release of citrulline in the circulation, and also, hepatocytes are unable to uptake citrulline from the circulation [2][3]. Thus, citrulline synthesis in the liver is compartmentalized to the urea cycle and independent of the other metabolic pathways of citrulline. Until recently, it was considered that the primary precursor for citrulline synthesis was the consumption of L-glutamine. However, it appears that the contribution of L-glutamine to citrulline synthesis is minor [4]. Orally ingested L-citrulline mainly leads to the biosynthesis of L-arginine. L-citrulline, released from the small intestine into the circulation, bypasses hepatic metabolism and is absorbed by the proximal tubular cells of the kidneys [3]. Then, citrulline is converted into arginosuccinate, which is then converted into arginine [1]. The increased arginine bioavailability in the circulation serves as a precursor for the formation of nitric oxide ($NO^{\bullet}$) [5]. L-citrulline can also be synthesized via the $NO^{\bullet}$ cycle. This formation of $NO^{\bullet}$ from arginine is catalyzed by nitric oxide synthase with citrulline release [1].

$NO^{\bullet}$ is a signaling molecule that has a vital role in regulating vasodilation, blood flow, and muscle oxygenation [6][7]. Briefly, it regulates vascular tone and blood flow by activating soluble guanylate cyclase in the vascular smooth muscle [8] and controls mitochondrial cellular respiration by inhibiting cytochrome c oxidase [9]. Indeed, the increase of blood flow and oxygen delivery to skeletal muscle during exercise is achieved by vasodilators such as $NO^{\bullet}$ formed locally in muscle tissue [10][11]. Thus, supplementation with $NO^{\bullet}$ precursors such as L-citrulline and L-arginine has been considered as an ergogenic aid [12][13][14][15]. Compared to L-arginine, evidence supports that oral L-citrulline supplementation is a more efficient intervention for increasing $NO^{\bullet}$ synthesis [5][16]. This is because L-citrulline bypasses hepatic metabolism [3] by inhibiting arginase enzymes [17].

During exercise, blood flow in skeletal muscles is increased to match exercise's oxygen demands and remove metabolic by-products [18][19]. Physiologically, the increase in inspiratory and expiratory muscle work leads to an increased demand for blood flow and oxygen delivery to the respiratory muscles that must be sustained during exercise [20]. A mismatch between oxygen supply and demand increases the rate of fatigue development and decreases exercise performance [21][22]. More specifically, inspiratory muscle fatigue decreases exercise tolerance and impair working locomotor muscle performance [23][24][25][26]. This impairment in performance is probably due to reduced blood flow and oxygen supply to the inspiratory and locomotor muscles [27][28][29][30]. Even though several mechanisms have been implicated in regulating blood flow during exercise (e.g., increased cardiac output; vascular smooth muscle relaxation), $NO^{\bullet}$ appears to be the key

molecule in regulating blood flow during exercise [31]. Hence, several NO• precursors (e.g., L-citrulline, L-arginine) have been prescribed with the aim to increase resistance to fatigue and improve exercise performance by modulating blood flow and oxygen metabolism.

L-citrulline has received considerable scientific attention for its potential to increase NO• bioavailability and improve exercise performance [15]. Increasing NO• bioavailability during exercise with L-citrulline supplementation appears to induce greater tissue oxygenation, oxygen uptake [12][16], and peripheral vasodilation [32] in locomotor muscles. Thus, it is interesting to examine whether similar favorable changes in blood flow and oxygenation can diminish inspiratory muscles' fatigability and enhance performance. Near-infrared spectroscopy (NIRS) is a non-invasive technique that continuously monitors regional tissue oxygenation in vivo. It has good sensitivity to detect and provide a reliable picture of muscle oxygenation changes during exercise [33][34]. Therefore, using the NIRS technique, this study aimed to investigate whether acute L-citrulline supplementation would affect sternocleidomastoid muscle oxygenation and respiratory performance. We chose to examine the sternocleidomastoid muscle since it is a crucial muscle for pressure generation during inspiration [35]. We hypothesized that an increase in NO• synthesis would increase sternocleidomastoid muscle oxygenation and respiratory performance during resistive breathing to task failure.

2. L-Citrulline Supplementation and NO• Bioavailability

NO• has a vital role in regulating vasodilation, blood flow, and muscle oxygenation; thus, increasing NO• bioavailability positively affects performance during exercise and recovery [13][36][37]. Consequently, supplementation with NO• precursors such as L-citrulline and L-arginine to augment nitric oxide bioavailability and enhance performance is a common practice followed by athletes and physically active individuals. Of interest, L-citrulline supplementation appears to be more efficient in enhancing NO• bioavailability compared to L-arginine because it bypasses hepatic metabolism, increasing this way the levels of extracellular L-arginine [3][5][17]. Indeed, previous studies reported that chronic L-citrulline supplementation might increase nitric oxide bioavailability [5][38]. In the present study, we found that a single dose of 6 g of L-citrulline increased exhaled NO• one hour after the supplementation, which is supported by relevant studies that showed that after oral L-citrulline supplementation, L-arginine concentrations peak around one hour later [39][40] in a dose-dependent manner [5]. Thus, based on our results, a single dose of 6 g of L-citrulline supplementation 1 hour before exercise is sufficient for increasing NO• bioavailability.

Certainly, considering the different origins of nitric oxide in skeletal muscle (i.e., neuronal, and endothelial) and exhaled air (i.e., epithelial) [41], some plasma or skeletal muscle measurements of nitric oxide production and/or metabolism could have added insightful mechanistic information. In the present study, our purpose was to examine whether acute L-citrulline supplementation would affect sternocleidomastoid muscle blood flow, oxygenation, and respiratory performance. Thus, the lack of any NO• measurements in skeletal muscle is a significant limitation of the present study. Yet, we have particularly focused on the respiratory system and pulmonary function, and have chosen, therefore, nitric oxide in exhaled air. Additionally, blood pressure and heart rate after L-citrulline supplementation were not measured since changes in these parameters were observed after chronic L-citrulline supplementation [42]. Moreover, alterations in blood pressure after L-citrulline supplementation were observed mainly in participants with health issues. Such as obese postmenopausal women [43][44], elderly individuals [32], and heart failure patients [45], while in the present investigation, young, healthy individuals were recruited.

3. Inspiratory Muscle Performance and Resistance to Fatigue

Inspiratory muscle dysfunction is a primary contributor to ventilatory failure during fatiguing conditions such as exercise [46]. During intense exercise, inspiratory dysfunction can occur due to increased work of breathing and/or insufficient blood flow and oxygen delivery to the respiratory muscles, progressively leading to fatigue and impairing exercise performance [25]. The sternocleidomastoid muscle is a crucial accessory muscle [47] that is highly active during exercise, supporting the primary inspiratory muscles' work [48]. Additionally, sternocleidomastoid muscle deoxygenation has been reported to be progressively increased during incremental inspiratory loading [49]. Thus, it was hypothesized that enhancing sternocleidomastoid muscle blood flow and oxygen delivery through L-citrulline supplementation might favorably affect respiratory muscle performance and resistance to fatigue during incremental resistive breathing.

However, contrary to our hypothesis, acute L-citrulline supplementation and the concomitant NO• increase did not improve sternocleidomastoid muscle performance and resistance to fatigue either one hour post supplementation or post-respiratory muscles resistive breathing to task failure. Comparable results have been reported in studies of the same nature using clinical and healthy populations [50][51]. However, in these investigations, the supplementation was L-arginine. L-arginine can be metabolized in the liver, contrary to L-citrulline, which mainly contributes to NO• production [3][17].

Specifically, MIP and the number of breaths at exhaustion were not different between the L-citrulline and the placebo conditions. Additionally, FEV_1, FVC, and their ratio were not affected by the L-citrulline supplementation. Even though our resistive breathing to task failure protocol induced fatigue, perhaps it was insufficient to cause extensive disturbances on respiratory performance, as observed in other studies using resistive breathing [52][53][54]. Given the fact that sub-maximal constant endurance exercise induces significant fatigue in respiratory muscles [55], it could be suggested that future studies examining inspiratory muscle fatigue should employ constant whole-body high-intensity endurance exercise protocols to compromise blood flow to inspiratory muscles and induce fatigue. Indeed, during whole-body exercise, the manipulation of inspiratory muscle work with resistors will increase the competitiveness between inspiratory and locomotor muscles for blood flow and oxygenation [23][56]. Thus, we believe that it is more likely to find any favorable effect of a supplement on fatigue when the exercise protocol involves whole-body exercise and greater fatigue levels occur in the muscle under examination.

4. Sternocleidomastoid Muscle Oxygenation

In the present study, we used NIRS that continuously monitors regional tissue oxygenation in vivo. We found that after both resistive breathing conditions (i.e., L-citrulline or placebo supplementation) and at every intensity stage (i.e., 70%, 80%, and 90% of MIP), there were a significant decrease in $\Delta[O_2Hb]$ and TSI% and a significant increase in Δ[HHb] compared to baseline in the sternocleidomastoid muscle, which is in line with previous studies [49]. The muscle's oxygenation and deoxygenation responses during loading were as expected in order to facilitate oxygen supply to the working muscles. However, despite the changes we observed after incremental inspiratory muscles resistive breathing to task failure in these parameters, L-citrulline supplementation did not affect sternocleidomastoid muscle oxygenation. Considering the role that $NO^{\bullet}$ has in blood flow and the increase we observed after L-citrulline supplementation in exhaled $NO^{\bullet}$, we expected that L-citrulline supplementation would have improved sternocleidomastoid muscle oxygenation.

Similar results were reported in a study where acute L-citrulline supplementation enhances $NO^{\bullet}$ bioavailability but had no effect on blood flow in young and older adults [57]. Additionally, no effect on blood flow was observed after ingestion of a combination of 10 g of L-citrulline with whey protein in older adults [58]. On the contrary, after longer-term supplementation with concentrate watermelon juice (providing 3.4 g/day of L-citrulline for 16 days), the tissue oxygenation index of the vastus lateralis was enhanced during moderate-intensity exercise [38]. Similarly, supplementation with L-citrulline (6 g/day for 7 days) increased VO_2 uptake kinetics of the vastus lateralis during moderate and high-intensity exercise in recreationally active adults [16]. Furthermore, muscle blood flow during exercise was improved after 14 days of L-citrulline supplementation (6 g/day) in older men [31]. Therefore, a chronic supplementation intervention could be required to successfully improve inspiratory muscle oxygenation and resistance to fatigue.

5. Conclusions

In the present study, we found that a single ingestion of 6 g of L-citrulline 1 hour before resistive breathing significantly increased $NO^{\bullet}$ bioavailability. Considering the strong ergogenic effects that $NO^{\bullet}$ has on exercise performance, this observation is of utmost importance for acute L-citrulline ingestion 1 hour before exercise events. However, neither respiratory muscle performance and resistance to fatigue nor sternocleidomastoid muscle oxygenation and deoxygenation responses were enhanced. These results imply that other reasons than $NO^{\bullet}$ bioavailability might affect sternocleidomastoid muscle performance and blood flow during resistive breathing to task failure of the inspiratory muscles. In our opinion, L-citrulline supplementation is worthy of further consideration from the scientific community, especially in patients with cardiovascular or pulmonary diseases (e.g., lower-extremity artery disease, chronic obstructive pulmonary disease) that are usually characterized by insufficiently blood flow and oxygen delivery to the muscles. It would also be interesting for future studies to examine the effect of short-term (e.g., 7-day) supplementation with L-citrulline on inspiratory muscles performance and oxygenation.

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