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White Paper: L-Citrulline

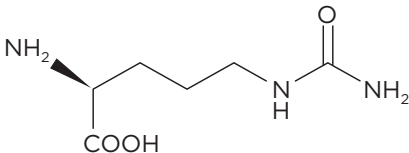
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L-Citrulline is a non-essential amino acid that previously was thought of as just an intermediate metabolite. However, over the past decade research on L-Citrulline has dramatically increased due to the understanding and importance of L-Citrulline's metabolism. L-Citrulline is converted to L-Arginine in the body, leading to increases in both L-Arginine and nitric oxide. Increased production of nitric oxide (NO) promotes vascular dilation, which helps support normal oxygen and blood circulation throughout the body.³ Since L-Citrulline is not a component of proteins unlike most other amino acids, dietary proteins cannot be a direct source of L-Citrulline to the body. As a result, interest in this nutrient is emerging in various applications for improving health and wellness. In this paper, we will specifically discuss L-Citrulline's role in nitric oxide production, vascular health, muscle protein synthesis, ammonia elimination, and immune function.

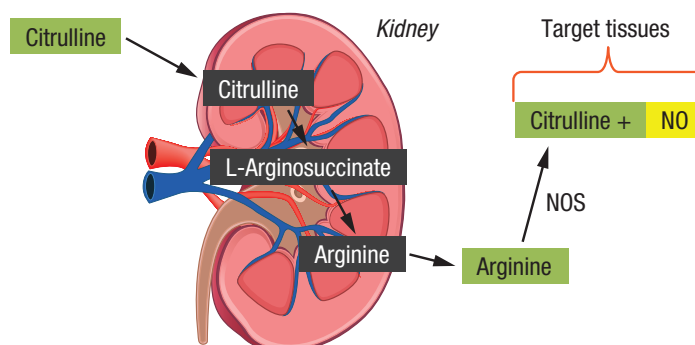
Properties of L-Citrulline

Table 1. Physical properties of L-Citrulline¹¹

Synonym	L-2-amino-5-ureidovaleric acid
CAS No.	372-75-8
Structural formula	
Molecular formula	C ₆ H ₁₃ N ₃ O ₃
MW	175.19
Solubility	Freely soluble in water (200 g/L)
Hygroscopicity	None (0.02% at 75% relative humidity for 7 days)
Taste	Slightly bitter taste
Odor	Unique slight odor
Appearance	White powder

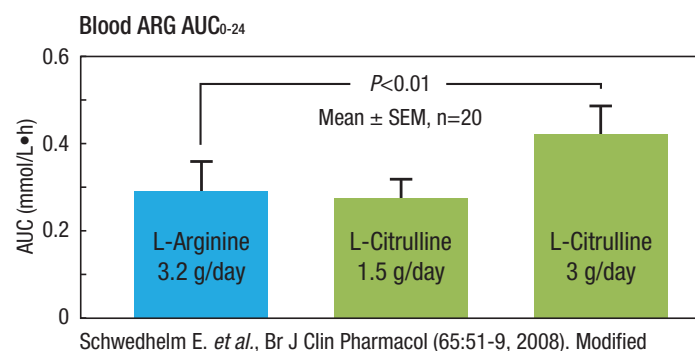
Biosynthesis, metabolism and pharmacokinetics of L-Citrulline

L-Citrulline is made from L-Ornithine and carbamoyl phosphate, is a component of the urea cycle in the liver. L-Citrulline is also synthesized from L-Arginine and L-Glutamine in enterocytes. The majority of L-Citrulline is converted to L-Arginine in the kidney.³ **Once in circulation, L-Arginine is readily converted into L-Citrulline and nitric oxide, which in turn serves as an L-Arginine precursor (see figure below).**¹⁰



Pharmacokinetic advantages

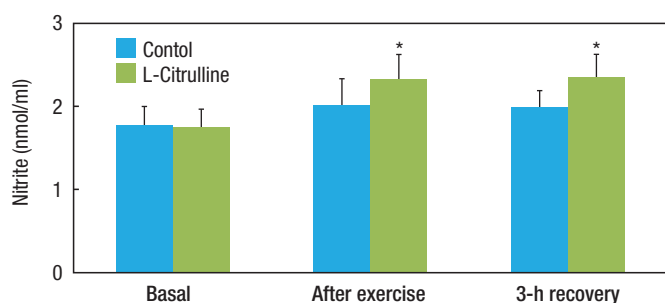
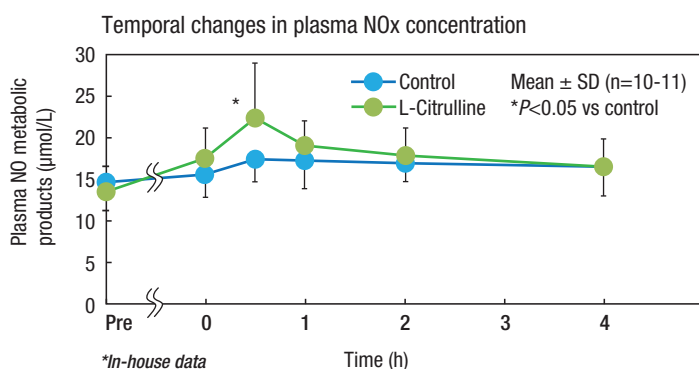
The pharmacokinetic advantage of L-Citrulline is that it is not subject to any elimination by the liver prior to entering the bloodstream. Conversely, L-Arginine is subject to extensive elimination by the gut wall and liver.²⁶ The main organ responsible for L-Citrulline conversion is the kidney. In fact, it is metabolized solely by the kidneys into L-Arginine. The kidneys will release L-Arginine in response to the uptake of L-Citrulline. Thus, the release of L-Arginine is a factor of the level of L-Citrulline in the blood. Oswoska et al (2004) showed that oral L-Citrulline administration was more potent at raising plasma L-Arginine levels in rats with a massive intestinal resection and was able to restore nitrogen balance over that of L-Arginine supplementation. **Schwedhelm et al (2008) confirmed in humans that oral supplementation of L-Citrulline was able to raise plasma levels of L-Arginine more effectively than L-Arginine itself and in a dose-dependent manner.**



Schwedhelm E. *et al.*, Br J Clin Pharmacol (65:51-9, 2008). Modified

Nitric oxide effects

As the natural precursor of L-Arginine, L-Citrulline plays an important role in the metabolism and regulation of nitric oxide. Nitric oxide is synthesized from L-Arginine as mentioned previously. An in-house study demonstrated that L-Citrulline oral administration as compared to a control was able to effectively increase nitric oxide in SD rats.¹¹



Sureda *et al.*, Eur J Appl Physiol (2010). Modified

In a human study, male cyclists were supplemented with L-Citrulline vs. placebo and NO production was measured as nitrite plasma concentration. Nitrates in the plasma were significantly increased after the cycling stage in the supplemented group and maintained high during recovery.²⁵ A more recent study by Ochiai *et al.*, confirmed that oral L-Citrulline significantly increased serum nitric oxide and NO metabolic products as compared to placebo. Altogether, this evidence helps to confirm that L-Citrulline plays a critical role in NO production.

Ammonia detoxification

L-Citrulline is involved in ammonia detoxification as an L-Ornithine cycle amino acid. L-Citrulline, along with L-Arginine and L-Ornithine, has various functions as an amino acid in the L-Ornithine cycle. The effect of supplemental L-Citrulline on young animals fed arginine-deficient diets is similar to that of supplemental L-Arginine. In rat experiments, L-Citrulline was shown not only to promote the metabolism of ammonia accumulated in muscle during exercise, but also to elevate the survival rate of rats intraperitoneally injected with a lethal dose of ammonia.¹⁸

Effects on muscle protein metabolism

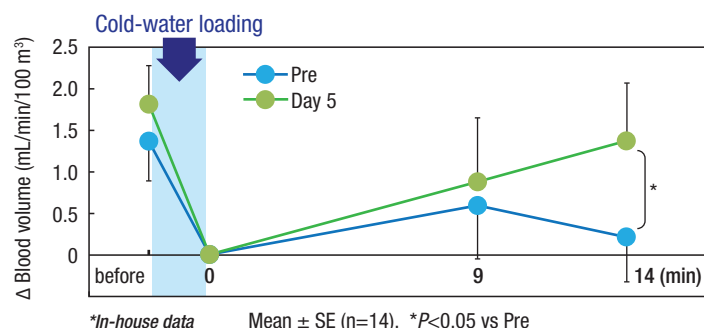
A series of studies have proven that L-Citrulline can increase muscle protein content and protein synthesis in animal models. Osowska *et al.*, found in malnourished aged rats that were fed a L-Citrulline-supplemented diet, there was an increase in protein synthesis and protein content in the muscle as compared to the standard diet fed rats. It was also found that L-Citrulline improved nitrogen balance in rats with short bowel syndrome. In addition, L-Citrulline stimulates muscle protein synthesis in fasted adult rats.¹³ Most recently, Faure *et al.*, found that L-Citrulline supplementation increases expression of the main myofibrillar proteins and seems to induce a switch in muscle energy metabolism, from aerobia toward anaerobia.

It has been confirmed in a human clinical study that L-Citrulline was effective at stimulating muscle protein synthesis without affecting whole body protein turn over.⁹ Further studies in humans are warranted.

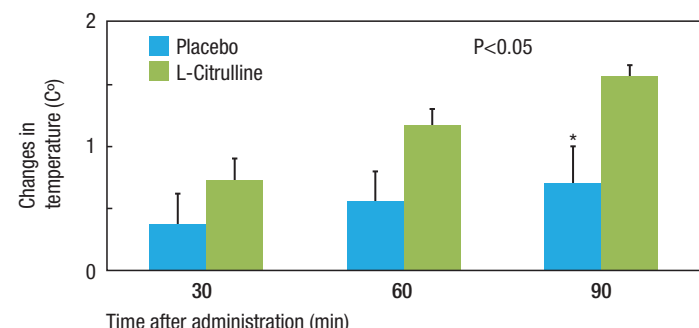
Vascular health

Because L-Citrulline is an effective precursor of L-Arginine for NO synthesis, it has been implicated to help support vascular health. More recently, many studies have been published exploring its use in various vascular disorders. It has not yet been found effective at controlling blood pressure. More studies are necessary in this area. However, Ochiai *et al.*, observed the effects of L-Citrulline on arterial stiffness. Brachial-ankle pulse wave velocity (baPWV) was measured in 15 healthy male subjects. Compared to the placebo group, baPWV was significantly reduced in the L-Citrulline group. The serum nitrogen oxide and NO metabolic products were significantly increased in the L-Citrulline group as well. Most recently, Morita *et al.* observed 21 subjects with diagnosed vasospastic angina. They were treated with 800 mg/day of L-Citrulline for 8 weeks. Flow-mediated dilation (FMD) of the brachial artery and oxidized LDL was measured. Compared with baseline values, FMD percent was significantly increased at 4 and 8 weeks as well as 4 weeks supplementation ended. At the same time, a reduction in serum oxidized LDL was reported. It was concluded that L-Citrulline supplementation improves endothelial dysfunction, probably due to potentiating NO-dependent reactions and decreasing the state of lipoprotein oxidation in humans.

In an in-house human clinical study, L-Citrulline was administered to women with poor blood flow. Peripheral blood flow was monitored using the back of the hands using laser imaging. **Compared to placebo, there was an improvement of peripheral blood flow after cold water emersion in the L-Citrulline group (see chart below).** Another in-house study observed, L-Citrulline was administered to healthy adults and body surface temperature was measured using thermography. The study showed that L-Citrulline was effective in raising body surface temperature at the neck, shoulders, and right palm.



Changes in surface temperature of neck/shoulder/palm area after L-Citrulline administration



Another area of interest is for supporting normal erectile function. A most recent pre-clinical study, Shiota et. al. found that oral L-Citrulline supplementation improves erectile function in rats with acute arteriogenic erectile dysfunction. Corneo et al. explored the role of L-Citrulline in humans for supporting normal erectile function. Compared to placebo, the L-Citrulline group reported mild improvement in erection hardness and an increase in sexual activity.

Immune function

L-Citrulline may be a safe means of immunomodulation that preserves the anti-inflammatory mediator response. Asgeirsson et al., observed the efficacy of L-Citrulline supplementation on systemic response mediators and cytokines in Wistar rats induced with sepsis. **They concluded that L-Citrulline may decrease the proinflammatory response (IL-6 and resistin) without impairing the secretion of anti-inflammatory mediators (IL-10 and adiponectin).**

Watermelon studies

Watermelon is known to have high concentrations of L-Citrulline and consuming watermelon is known to elevate both plasma L-Citrulline and L-Arginine.⁷ Watermelon extract displays similar effects as L-Citrulline on vascular health. Figueroa et. al. observed significant reductions in ankle and brachial blood pressure in middle aged adults supplemented with watermelon extract. **A more recent study demonstrated that watermelon juice containing 1.17 g of L-Citrulline was capable of reducing the recovery heart rate and muscle soreness after 24 hours in athletes as compared to placebo.**²⁷

Recommended intake of L-Citrulline

L-Citrulline dosing varies greatly among in the literature and depends on the intended use. The general range is 1-6 grams per day. A reasonable median is 2-3 grams depending on the intended use. More specific doses will be found in the studies pertaining to certain physiological effects.

Safety information about L-Citrulline

Because it occurs naturally in the living bodies of animals and humans where it plays an important role, L-Citrulline is regarded as a highly safe ingredient. Studies in humans have demonstrated that L-Citrulline is substantially innocuous. In an experiment in patients with lysinuric protein intolerance, 19 subjects were given 2.0-2.2 g (depending on body weight) of L-Citrulline each day for two years. Blood hemoglobin values, and plasma albumin and L-valine concentrations were increased, suggesting an improvement in protein turnover. No side effects were identified.²¹

An acute toxicity test in mice indicated the LD₅₀ value for oral administration of L-Citrulline is 5,000 mg/kg (B.W.) or more.¹¹

Conclusion

L-Citrulline is a substance present in the body and is used as a highly safe ingredient. Recent research has helped in understanding the importance of L-Citrulline's metabolism and role in the body. As mentioned throughout this paper, L-Citrulline is converted to L-Arginine in the body, enhancing the production of nitrogen monoxide (NO). Additionally, current research is helping our understanding of its positive role in vascular health, muscle protein synthesis, ammonia elimination, and immune function. There is still more to learn and discover about this important amino acid. Kyowa Hakko U.S.A., Inc. provides pure, high-quality L-Citrulline.

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