

# **Citrulline: An Innovative Pharmaconutrient that Accelerates the Healing of Wounds**

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Nutrition support for wound treatment has traditionally been supplemental protein, arginine, vitamin C, and zinc with positive results. This article sheds light on the potential use of citrulline as a therapeutic agent in accelerating wound healing by enhancing arginine availability, modulating nitric oxide (NO) production. The added cost of nutritional support represents only a small component of the management care plan but could significantly reduce the costs associated with prolonged hospitalization as well as decrease the pain, suffering and discomfort accompanying wounds.

A decade of research has shown that NO is essential to wound healing. NO is an integral part of the inflammatory phase, possesses cytotoxic properties, functions as a regulatory mechanism to mediate epithelialization, angiogenesis, and collagen deposition crucial to the proliferative phase. NO functions as a signal molecule in vasodilatation and angiogenesis (1) and acts as a host-protective agent by killing pathogens and increasing blood flow to wounds (2). NO activates wound macrophages and neutrophils, causes vasodilation, and increases vascular permeability. It indirectly provides the wound area with sufficient O<sub>2</sub> and nutrition. This stimulates fibroblasts to synthesis collagen and keratinocytes to proliferate and thus NO takes part in building up repair tissue in the wound. A plethora of published articles are available with regard to the role of NO in wound healing.

Due to its chemical nature, NO has a short half-life (3). It is highly reactive and quickly converted by oxygen and water to its stable end products. The metabolism of NO is critically dependent on the metabolism of arginine since this amino acid is the sole substrate for NO synthesis. The availability of arginine is one of the rate-limiting factors of cellular NO production (4). Levels of arginine become critically low after wounding (5, 6). Wound-repair cells, such as macrophages, fibroblasts, endothelial cells and keratinocytes, also depend on extracellular arginine concentration for their NO synthesis (7, 8). Therefore, it is important that the wound is supplied with sufficient arginine.

Reasons for arginine depletion can be (1) an insufficient supply of arginine or (2) an increased use of arginine. In humans, it is common knowledge that protein deficiency or malnutrition impairs wound healing and arginine supplementation increases wound collagen deposition. Chronic NO synthesis is increased during wound healing limiting the supply of arginine (9). Furthermore, arginine can be competitively metabolized by arginase. An enhanced expression of arginase is seen in wound-

derived fibroblasts, diabetes-impaired healing and psoriatic skin lesions (10-12). Therefore, increased arginine is needed to heal wounds.

In some people, high oral doses of arginine (>9g/d) are associated with nausea, gastrointestinal discomfort, and diarrhea (13,14). Excessive arginine supplementation has been shown to cause additional renal problems in patients with compromised kidneys (15). Additionally, oral administration of arginine, is currently not recommended for patients with myocardial infarction because of a possible increase in mortality (16, 17) likely due to an abrupt increase in NO production.

A solution to the challenges associated with dietary arginine supplementation may be the alternative use of citrulline, a “non-standard” amino acid and an effective precursor for arginine synthesis (18). Citrulline increases plasma concentrations of arginine (19) and may represent an innovative strategy to accelerate wound healing. Citrulline supplementation is an efficient alternative to oral administration of Arginine in raising its plasma levels and modulating NO production.

Provision of citrulline from supplements offers a unique advantage over the enteral supply of arginine for the following reasons. Firstly, 40% of dietary arginine is catabolized by enterocytes in the first pass (20), whereas citrulline undergoes limited degradation (21). On the same equal molar basis, the entry of dietary citrulline into circulation is much greater than that of dietary arginine. Secondly, arginine is largely metabolized by the liver whereas citrulline is not taken up by the liver and therefore nearly all the citrulline absorbed from the small intestine bypasses the liver and enters the systemic circulation (22). Thirdly, the synthesis of arginine from citrulline is the only pathway for its utilization by extrahepatic tissues (23).

In summary, citrulline circulation in the bloodstream is first converted to arginine and then in cells to citrulline and NO. The conversion of citrulline to arginine occurs continuously, as long as citrulline is circulating in the bloodstream. As a result, circulation citrulline makes it possible to maintain elevated concentrations of arginine over time, which in turn makes it possible to maintain a steady release of NO in cells to accelerate wound healing.

## References

1. Lee PC, Salyapongse AN, Bragdon GA, Shears LL II, Watkins SC, Edington HD & Billiar TR (1999) Impaired wound healing and angiogenesis in eNOS-deficient mice. *American Journal of Physiology* 277, H1600-H1608.
2. Stechmiller JK, Childress B, Cowan L. Arginine supplementation and wound healing. *Nutr Clin Pract.* 2005;20:52-61.
3. Taylor BS, Geller DA. 2001. Regulation of the inducible nitric oxide synthase (iNOS) gene. In: Salvemini D, Billiar TR, Vodovotz Y, editors. *Nitric Oxide and inflammation*. 3<sup>rd</sup> ed. Basel, Switzerland: Birkhauser. P 1-26.
4. Hallemeesch MM, Lamers WH & Deutz NE (2002b) Reduced arginine availability and nitric oxide production. *Clinical Nutrition* 21, 273-279.
5. Nijveldt RJ, Prins HA, Siroen MP, et al. Low arginine plasma levels in patients after thoracoabdominal aortic surgery. *Eur J Clin Nutr* 2000; 54:615-7.
6. Caldwell MD, Mastrofrancesco B, Shearer J, et al. The temporal change in amino acid concentration within wound fluid-a putative rationale. *Prog Clin Biol Res* 1991;365:205-22.
7. Jalkanen M, Larjava H, Heino J, Vihersaari T, Peltonen J & Penttinen R (1982) Arginine depletion in macrophage medium inhibits collagen synthesis by fibroblasts. *Immunology Letters* 4, 259-261.
8. Norris KA, Schrimpf JE, Flynn JL & Morris SM Jr (1995) Enhancement of macrophage microbicidal activity: supplementational arginine and citrulline augment nitric oxide production in murine peritoneal macrophages and promote intracellular killing of *Trypanosoma cruzi*. *Infection and Immunity*. 63, 2793-2796.
9. Albina JE, Mills CD, Henry WL Jr & Caldwell MD (1990) Temporal expression of different pathways of Arginine metabolism in healing wounds. *Journal of Immunology* 144, 3877-3880.
10. Witte MB, Barbul A, Schick MA, Vogt N & Becker H D (2002a) Upregulation of arginase expression in wound-derived fibroblasts. *Journal of Surgical Research* 105, 35-42.
11. Kampfer H, Pfeilschifter J & Frank S (2003) Expression and activity of arginase isoenzymes during normal and diabetes-impaired skin repair. *Journal of Investigative Dermatology* 121, 1544-1551.
12. Weller R (2003) Nitric oxide: a key mediator in cutaneous physiology. *Clinical and Experimental Dermatology* 28, 511-514.
13. Tangphao O, Chalon S, Moreno H, Hoffman BB, Blaschke TF. Pharmacokinetics of L-arginine during chronic administration to patients with hypercholesterolaemia. *Clin Sci.* 1999;96:199-207.

14. Evans RW, Fernstrom JD, Thompson J, Morris SM, Kuller LH. Biochemical responses of healthy subjects during dietary supplementation with L-arginine. *J Nutr Biochem.* 2004;15:534–9.
15. Efron DT, & Barbul A. (1999). Arginine and nutrition in renal disease. *Journal of Renal Nutrition : the Official Journal of the Council on Renal Nutrition of the National Kidney Foundation.* 9(3), 142-4.
16. Wilmore D. Enteral and parenteral arginine supplementation to improve medical outcomes in hospitalized patients. *J Nutr.* 2004; 134(6 suppl):2863S-2867S.
17. Schulman SP, Becker LC, Kass DA, Champion HC, Terrin MI, Forman S, Ernst KV, Kelemen MD, Townsend SN, et al. L-arginine therapy in myocardial infarction. The Vascular Interaction with Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. *JAMA.* 2006;295:58–64.
18. Curis, E., Nicolis, I., Moinard, C., Osowska, S., Zerrouk, N., Bbnazeth, S., & Cynober, L. (2005). Almost all about citrulline in mammals. *Amino Acids.* 29(3), 177-205.
19. Collins JK, Wu G, Perkins-Veazie P, Spears K, Claypool PL, Baker RA, Clevidence BA. Watermelon consumption increases plasma arginine concentrations in adults. *Nutrition.* 2007;23:261–6.
20. Wu G. Intestinal mucosal amino acid catabolism. *J Nutr.* 1998;128:1249–52.
21. Wu G. Urea synthesis in enterocytes of developing pigs. *Biochem J.* 1995;312:717–23.
22. Morris SM Jr. Regulation of enzymes of the urea cycle and arginine metabolism. *Annu Rev Nutr.* 2002;22:87–105.
23. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. *Biochem J.* 1998;336:1–17.