Proline Precursors to Sustain Mammalian Collagen Synthesis$^{1,2}$

Adrian Barbul$^*$

Department of Surgery, Sinai Hospital of Baltimore and Johns Hopkins Medical Institutions, Sinai Hospital, Baltimore, MD 21215

Abstract

Biochemically, one-third of the collagen molecule is composed of glycine. The next largest amino acid component is formed by proline (PRO) and hydroxyproline, which together comprise ~23% of the collagen molecule. The best method to support wound collagen biosynthesis is to provide adequate host nutrition, assuring adequate provision of calories and protein. However, despite adequate nutrition, clinically, there is a need to enhance collagen synthesis and research has focused on methods to enhance collagen precursor availability. PRO biosynthesis is related to both the citric acid cycle and the urea cycle. During the early phases of wound healing, wound fluid PRO levels are at least 50% higher than plasma levels, suggesting active import of PRO into the wound. Providing additional PRO in the diet to enhance PRO bioavailability for collagen biosynthesis does not result in increased collagen accumulation. Provision of other citric cycle precursors such as glutamine also does not enhance wound collagen synthesis. In looking at other PRO biosynthetic pathways, the arginine (ARG) \(\rightarrow\) ornithine (ORN) \(\rightarrow\) glutamic semialdehyde \(\rightarrow\) PRO pathway looks the most promising. ARG administration in quantities above those required for growth and reproduction results in a marked enhancement in wound collagen deposition. This effect is also shared by ORN, which cannot replace ARG for growth requirement but shares many of its biological and pharmacological activities. Several mechanisms have been postulated to explain the positive effect of ARG on wound healing, although none have been firmly proven. In conclusion, ARG and ORN supplementation are most effective in increasing collagen deposition, but whether this is accomplished by conversion to PRO is uncertain. J. Nutr. 138: 2021S–2024S, 2008.

Introduction

Except for superficial supra-dermal injuries, wounds in most tissues heal by scar formation. The main component of scar tissue is collagen. Collagen synthesis and remodeling is the final, longest, and most critical step in the process of wound healing \((1)\). Metabolically, the process requires energy and amino acid precursors, as well as various trace minerals, vitamins, and oxygen as cofactors. Therefore, nutritional provision of adequate energy and protein is an essential first step in ensuring successful wound fibroplasia. The relationship between successful wound healing and adequate nutrient intake has been appreciated for several decades, most succinctly stated as “wound nutrition is in fact whole-body nutrition” \((2)\). Patient nutritional status is 1 of the major determinants for wound outcome, whether measured by successfully completed healing or by the lack of infectious and other complications \((3)\).

However, there has always been an interest in the question of whether the administration of certain nutrients can specifically enhance collagen synthesis. In this regard, the administration of various amino acids has been studied most extensively.

One approach is to examine the structure of collagen and pursue provision of substrates based on its molecular composition. Biochemically, the collagen molecule contains 1-third glycine \((\text{Fig. 1})\), so that every 3rd amino acid is a glycine molecule, according to the formula GLY-X-Y. The next largest amino acid component is constituted by proline (PRO) and its derivative, hydroxyproline (HYP), which frequently occupy either the X or Y position and together comprise ~23% of the amino acid content of the collagen molecule \((4)\). Approximately 99.8% of the body’s stores of HYP are found in collagen, which renders assays of this amino acid useful as a marker for the total amount of collagen present. The hydroxylation of PRO occurs post-translationally and is carried out by the enzyme prolyl hydroxylase, which requires oxygen, ascorbate, and iron as cofactors for successful activity. Both PRO and HYP are vital for

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* To whom correspondence should be addressed. E-mail: abarbul@jhmi.edu.

Abstract used: HYP, hydroxyproline; iNOS, inducible nitric oxide-synthase NO, nitric oxide; OKG, ornithine-\(\alpha\)-ketoglutarate.

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collagen biosynthesis, structure, and strength. Their cyclic structure constrains the rotation of the polypeptide collagen chain and creates and strengthens the helical characteristic of the molecule (4).

Although plasma levels of PRO can be quite variable, its importance in human nutrition is not clear. True PRO deficiency probably only occurs in proline deficiency, an autosomal inborn metabolic error, where lack of the enzyme does not allow for the degradation of the imino-dipeptides (PRO-containing) generated during collagen lysis, with large excretion of these dipeptides in the urine. Patients with proline deficiency have low PRO levels and a variety of wound-healing deficits (5).

During the first 10 d of healing, wound PRO levels are 30–50% higher than plasma levels, suggesting that import of PRO into the wound occurs actively or that biosynthesis of PRO takes place in the wound environment (6). Conversely, some of the PRO could also be generated from the lysis of collagen, which occurs in parallel to synthesis. The high demand for PRO during wound repair could cause a local PRO deficiency; theoretically, therefore, enhancing PRO bioavailability would be an attractive strategy to optimize collagen biosynthesis.

The most obvious approach is to provide additional PRO in the diet. However, the provision of supplemental PRO or HYP to a complete diet does not result in increased wound breaking strength (7). We now know that ingested HYP is degraded and that synthesis of HYP only occurs post-translationally once PRO has been incorporated into the collagen molecule. In experiments from our laboratory, male Sprague Dawley rats (250–300 g body weight) were given a 1% dietary supplement of PRO in addition to a complete nonpurified diet, adequate for growth and reproduction. All animals underwent dorsal skin incision and subcutaneous implantation of polyvinyl alcohol sponges. Ten d postwounding, we found no difference in wound breaking strength or in wound collagen deposition as assessed by the amount of OPH present in the subcutaneously implanted sponges (unpublished data). The failure of supplemental dietary PRO to enhance wound collagen may reflect metabolic disposal that does not lead to increased availability in the mitochondria where collagen molecule translation and transcription occurs.

Another approach would be to examine PRO biosynthetic pathways and the means by which they can be exploited to enhance PRO bioavailability for collagen synthesis. PRO is linked metabolically with the tricarboxylic acid cycle and the urea cycle. The common element of the 2 pathways is glutamate-γ-semialdehyde; this spontaneously converts to Δ1-pyrroline-5-carboxylate, which is then reduced to PRO (Fig. 2).

Glutamate/glutamine

The production of glutamate via the citric acid cycle links it to the biosynthesis of PRO. Large amounts of dietary glutamate are poorly tolerated. On the other hand, glutamine has been extensively studied. Glutamine is the most abundant amino acid in the body, accounting for ~20% of the total circulating free amino acid pool and 60% of the free intracellular amino acid pool. Glutamine is a critical fuel for many cells, including fibroblasts, macrophages, neutrophils, and lymphocytes, all of which participate in wound healing (8). Most cells require glutamine for in vitro growth and function.

Given the abundant roles of glutamine in cells involved in wound healing, it is not surprising that there is a rapid decrease in plasma and muscle glutamine levels after injury (9). This decrease is greater than that of any other amino acid. Although efficacy of supplemental glutamine administration has been shown in some clinically important conditions, it has not proved to have any noticeable effect on wound healing (3). No report has ever shown any effect of supplemental glutamine on wound collagen synthesis and/or accumulation. Most of the benefits of supplemental glutamine appear to involve improvements in gut permeability, normalization of serum levels, improved protein synthesis, and decreased hospital length of stay [see e.g (10)].

Arginine/ornithine

The urea cycle can also provide precursors for PRO biosynthesis. Arginine (ARG) is converted to ornithine (ORN) through the action of arginase, a key enzyme of the urea cycle. ORN, through the action of ORN γ-aminotransferase, is converted to glutamic γ-semialdehyde, the link to PRO synthesis. In naturally occurring proteins, ARG is by far more prevalent and available than ORN.

In humans, ARG is synthesized in adequate quantities to sustain muscle and connective tissue mass but probably in insufficient quantities for optimal collagen biosynthesis and healing following injury. In situations of stress or injury, body stores of ARG decrease rapidly. It is during these times, in which endogenous synthesis is insufficient to meet the demands of increased protein turnover, that ARG becomes an indispensable amino acid for wound healing and the maintenance of a positive nitrogen balance (11).

The role of ARG in wound healing was first shown in animals fed an ARG-deficient diet for 4–6 wk that were subjected to the...
minor trauma of a dorsal skin incision and closure. The animals manifested increased postoperative weight loss, increased mortality (in a trauma model that normally has 0% mortality), and a notable decrease in wound breaking strength and wound collagen accumulation compared with animals fed a diet containing ARG (12). Subsequent experiments using stock diet-fed rats that were not ARG deficient and were then fed a diet containing an additional 1% ARG demonstrated enhanced wound healing as assessed by wound breaking strength and collagen synthesis compared with stock diet-fed controls (11). Similar findings were observed in parenterally fed rats given an amino acid mixture containing high doses (7.5 g/L) of ARG (13).

Two studies in healthy human volunteers examined the effects of ARG supplementation and demonstrated that doses of 17–24.8 g/d ARG enhanced collagen deposition in healthy adult and elderly human volunteers (14,15). ARG supplementation did not affect the rate of epithelialization of a superficial skin defect, indicating that the predominant effect of ARG is on wound collagen deposition (15).

Several mechanisms have been postulated to explain the positive effect of ARG on wound healing. Although ARG comprises a small amount of the collagen molecule (<5%) (Fig. 1), it is possible that supplemental ARG provides substrate for the ARG → ORN → glutamine semialdehyde → PRO pathway. This hypothesis is supported by the observation that ARG levels are essentially undetectable within the wound during the later phases of healing when fibroplasia predominates, indicating a local wound ARG-deficient state (16).

Further support for this pathway comes from experiments using supplemental ORN. Mice fed a stock diet supplemented with 1% ORN demonstrated enhanced wound breaking strength and collagen deposition, similar in magnitude to the effect in mice supplemented with 1% ARG (17) (Fig. 3A,B). In wound fluid, ORN levels are higher than in the plasma, but there is also high arginase activity. The feeding of ORN supplements further significantly elevates plasma and wound fluid ORN levels. The rate of ORN conversion to PRO has been estimated in vivo at <5%; in vitro studies using whole cartilage and cell preparations estimate a 20% conversion of ORN into the total PRO incorporated into protein (18–20). These differences are significant and may be reflective of differences between in vivo tracer methodology and in vitro incorporation experiments.

The other amino acid in the urea cycle of possible importance is citrulline. Citrulline is a precursor for ARG in the urea cycle and can nutritionally replace ARG in those species that require ARG for optimal growth (21). In addition, dietary citrulline can be used to elevate plasma ARG levels in animals with short-gut (22). On the other hand, citrulline does not share any of the other biological or pharmacologic effects of ARG, nor does it have any effect on wound healing or collagen synthesis (11). Of interest, ORN cannot replace ARG for growth requirement but shares many of its pharmacologic effects in addition to the stimulation of wound healing, as shown above.

ARG is also a unique substrate for the generation of nitric oxide (NO), which plays a critical role in wound healing (23). The functional loss of the inducible NO-synthase (iNOS) gene abrogates the beneficial effect of ARG in wound healing (24) (Fig. 3A). On the other hand, ORN increases wound breaking strength and collagen accumulation in iNOS-knockout mice to the same extent as in wild-type animals (17) (Fig. 3B). This finding suggests that the iNOS pathway is at least partially responsible for the enhancement of wound healing observed with the administration of ARG, but not of ORN (24). Furthermore, these findings do not invalidate the roles played by both ARG and ORN as precursors for PRO synthesis in wounds.

Both ARG and ORN are also strong pituitary secretagogues of growth hormone, which may mediate their positive effect on wound collagen deposition. Both are also known stimulators of T-cell function. T lymphocytes are essential for normal wound healing and can be detected immunohistochemically in distinct patterns throughout the various phases of wound healing (25). How much these effects contribute to the positive effects of ARG and ORN on wound healing is not known.

In a series of experiments, we examined if simultaneous dietary administration of ARG and PRO administration results in enhanced collagen deposition. In male adult Sprague Dawley rats (250–300 g body weight) undergoing dorsal skin incision and subcutaneous placement of polyvinyl alcohol sponges, no additive effect of PRO was noted beyond the effect of ARG alone, suggesting that ARG provides sufficient precursor for PRO biosynthesis (unpublished observation).

Lastly, a compound that could provide both citric acid and urea cycle precursors for PRO biosynthesis is orn-α-ketoglutarate (OKG). This compound has been studied extensively and is noted to have salutary effects on nitrogen balance, protein metabolism, and recovery from trauma and sepsis. It has also been shown to reduce the time to healing following burn injury in both animals and humans. OKG administration also elevates plasma PRO levels in both healthy volunteers as well as in burn patients (26). Unfortunately, the possible effect of OKG on collagen synthesis has, to our knowledge, never been studied (L. Cynober, Paris Descartes University and Clinical Chemistry, Hôtel-Dieu hospital, Paris, France, personal communication). Such studies are currently being planned in our laboratory, because there is solid theoretical basis to believe that a synergistic effect may be present.

In conclusion, the best method to support wound collagen biosynthesis is by providing adequate host nutrition. ARG and

![FIGURE 3](https://example.com/figure3.png)

**FIGURE 3** Effect of 14-d dietary supplementation with 1% ARG (A) or ORN (B) on wound breaking strength and wound collagen deposition in wild-type and iNOS-knockout male mice. *P < 0.05; **P < 0.01 (Student’s t test). Composite from (16) and (22) with permission. n = 10–12/group.
ORN supplementation are most effective in increasing collagen deposition, but whether this is accomplished by conversion to PRO remains to be confirmed. The failure of citric acid precursors/ amino acids to improve collagen synthesis remains puzzling and deserves further elucidation.

Other articles in this supplement include references (27–37).

Literature Cited