available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/burns

# Intravenous arginine and human skin graft donor site healing: A randomized controlled trial

*Iris B.J.G. Debats* [a,*], *Margot M. Koeneman* [a], *Darren I. Booi* [a], *Otto Bekers* [b], *Rene R.W.J. van der Hulst* [a]

[a] *Department of Plastic, Reconstructive and Hand Surgery, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands*
[b] *Dept. of Clinical Chemistry, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands*

ARTICLE INFO

*Article history:*
Accepted 24 June 2010

*Keywords:*
Arginine
Wound healing
Donor sites
Nutritional therapy
Pharmaconutrients

ABSTRACT

*Background and aims:* Studies evaluating the effect of arginine supplementation in human wound healing are inhomogeneous with conflicting results. This study aims to clarify the role of arginine supplementation in the healing of human skin graft donor sites.
*Methods:* 35 subjects undergoing skin autografting were randomly assigned to receive intravenous arginine ($n = 16$) or placebo ($n = 19$) for 5 days in a dose of 30 g of arginine or an isovolumetric amount of placebo (25.2 g of alanine). Wound healing was evaluated at the donor sites by objectifying angiogenesis, reepithelialization and neutrophil influx. Plasma amino acid concentrations were measured to evaluate our intervention.
*Results:* The two groups were comparable in age, morbidity and nutritional, metabolic and inflammatory state. Plasma arginine and alanine levels increased significantly upon supplementation in the two groups, respectively. No differences were found between the arginine supplementation group and the placebo group in the studied parameters. Placebo vs. arginine; mean ± SD: neutrophil influx on day 2: 6.67 ± 3.0 vs. 6.57 ± 3.3, $p = 0.66$; angiogenesis on day 10: 8.0 ± 2.8 vs. 8.9 ± 3.1; reepithelialization in % on day 10: 81 ± 8.5 vs. 85 ± 7.1.
*Conclusion:* Intravenous arginine supplementation does not improve angiogenesis, reepithelialization or neutrophil influx in healing of human skin graft donor sites.


## 1. Introduction

The effect of arginine supplementation on wound healing has been extensively studied in animals and has shown beneficial effects. A dietary lack of arginine leads to impaired wound healing in rodents [1,2], whereas arginine supplementation leads to increased wound collagen synthesis, wound breaking strength and reepithelialization [3–7]. While human studies are relatively scarce and show methodological flaws, data led to the production commercially available oral nutritional supplements, enriched with arginine, to increase wound healing. Until today however, the effect of arginine supplementation on the clinical outcome of wound healing in man remains to be clarified. In general, the effect of nutrition on wound healing, is well established [8]. In contrast, the role of immunonutrition and its specific ingredients to enhance wound healing is less established [9]. Human studies concerning the use of arginine as immunonutrition show conflicting results. While some studies show no beneficial effect of arginine supplementation on clinical or biochemical parameters in injured and post-surgical patients [10–12], others indicate improved healing of diabetic ulcers [13],

* *Corresponding author*. Tel.: +31 43 3876543; fax: +31 43 3875485.
E-mail address: ibjgdebats@gmail.com (Iris B.J.G. Debats).
0305-4179/$36.00 
doi:10.1016/j.burns.2010.06.003

enhanced immune function, reduced postoperative infections, reduced postoperative wound complications and length of hospital stay in post-surgical patients [14–22]. Experimental studies evaluating specific parameters of wound healing and arginine supplementation, such as collagen deposition and epithelialization, closest to our study objective, indicate enhanced wound healing [23,24]. Furthermore, most other studies have focused on patients in depleted state, suffering from malignancy, diabetes, pressure ulcers and severe trauma. These studies are often limited in size or express wound healing in terms of infection. Moreover, studies addressing the effect of arginine supplementation in wound healing often use combinations of arginine and other immunonutrients, which makes the interpretation of the single effect of arginine supplementation impossible [25–32]. Skin grafts are frequently used in reconstructive and burn wound surgery. Knowledge of possible means to enhance wound healing is particularly relevant in the treatment of burn wounds, as regrafting of a donorsite is often required [33]. In addition, a donor site is a very homogenous wound healing model. Therefore, the effect of oral arginine supplementation on healing of skin graft donor sites in relatively healthy humans was studied in a pilot study previously conducted at our department. No beneficial effects of arginine supplementation were found, on angiogenesis, reepithelialization and leukocyte influx as parameters of wound healing in patients undergoing skin autografting [34]. The beneficial effects, as suggested by previous literature prompted us to conduct a study that overcomes the possible shortcomings of our previous pilot study. In this double blinded, randomized controlled trial we included a larger study population and administered arginine intravenously, in order to rule out any influence on study results due to intake problems. Wound healing was objectively evaluated as primary end point, using angiogenesis, reepithelialization and neutrophil influx as key parameters.

## 2. Materials and methods

### 2.1. Design and subjects

Between July 2006 and July 2009 a randomized double blind, placebo-controlled study was performed at our department. The Medical Ethical Committee of Maastricht University Hospital approved the study protocol, and informed consent was obtained for each subject. The sample size for this study was calculated based on mean total healing time of donor sites of 10 days, with 80% confidence and accepting an $\alpha$ of 5%. In order to detect a increased healing of 15%, 25 subjects per group would have to be included. All subjects received skin autografting as part of reconstructive surgery. Body mass index and weight loss within 6 months prior to surgery was measured to determine clinical nutritional status according to the Espen guidelines. Exclusion criteria were age younger than 16 or older than 75 years, kidney or liver failure, pregnancy, use of steroids, immune deficiency diseases, and diabetes mellitus. Before operation subjects were randomly assigned to arginine ($n = 16$) or placebo treatment ($n = 19$), by an independent clinical pharmacist, using numbered envelopes. A block randomization was chosen to equally divide subjects in both groups. Based on literature at the initiation of the study, the highest tolerable amount of arginine was administered. Subjects received intravenous supplementation of arginine or placebo (Bufa, Uitgeest, The Netherlands) during 5 days, starting during surgery, in order to evaluate the different processes during the initial phases of wound healing (inflammatory and proliferative phase). Intravenous supplementation consisted of either a daily dose of 30 g of arginine, dissolved in 1000 ml 0.9% NaCl and adjusted to pH 7.2 using 10% HCL (net nitrogen intake 45.7 mmol/l) or a placebo treatment consisting of a daily dose of 25.2 g of alanine, dissolved in 1000 ml 0.9% NaCl (net nitrogen intake 44 mmol/l). To be able to perform a double blinded study the infusions were made isovolumetric and isonitrogenous. Although it was not possible to make them isocaloric, the arginine infusion accounted for 120 kcal and the placebo infusion for 100.8 kcal. However, in a metabolic stress situation, these small differences are often neglected. Patients received a daily infusion of 1000 ml in two doses of 500 ml equally divided over the day. Oral food intake was allowed as desired, in order to maintain the clinical applicability of arginine as a potential immunonutrient treatment.

#### 2.1.1. Wound model

All clinical wound procedures were performed at the Department of Plastic Surgery, University Hospital Maastricht, The Netherlands. Under general anesthesia and aseptic conditions, split skin grafts were obtained using an electric dermatome (Aesculaap®) with a thickness of 0.3 mm. The donor sites were used to evaluate wound healing. Wound fluid was collected from these donor sites by covering it with a layer of Gordasoft® (homemade sterilized polyester fabric), followed by a polyvinyl alcohol sponge (Coldex®, Taureon, Rijswijk, The Netherlands) and a transparent dressing (Tegaderm®, 3M Nederland B.V., Zoeterwoude, The Netherlands) on top as previously described.

The surfaces of all donor sites were measured. Twenty-four hours before wound fluid collection the wound dressing was changed by removing the transparent dressing and the sponge, followed by reapplying a new sponge and transparent dressing. After 24 h the sponge was removed, and stored on ice until further processing. Using this protocol a 24 h wound fluid sample was used each time for analysis. From each patient wound fluid samples were taken using this method on days 2, 5 and 10. From the central part of the donor site 3-mm punch biopsies were taken. Before the excision of the biopsies, lidocaine was locally injected. Subsequently a venous blood sample was drawn from a major vein in the cubital fossa.

### 2.2. Sample processing and analysis

Heparinized blood was centrifuged at 4 °C for 10 min at 4000 rpm within 1 h after sampling. After centrifugation, 500 μl of plasma was deproteinized using 20 mg dry sulphosalicylic acid (SSA), vortexed and frozen in liquid nitrogen. Samples were stored at −80 °C until analysis. Wound fluid was obtained by centrifuging the sponges for 10 min at 4 °C (11,000 rpm). After centrifugation, 500 μl of wound fluid was treated similar to the plasma. The recovery of fluid from the sponges was validated and found to be constant.