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Effectiveness and Safety of a Novel Gel Dressing in the Management of Neuropathic Leg Ulcers in Diabetic Patients: A Prospective Double-Blind Randomized Trial

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Abstract

Neuropathic leg ulcers (NLUs) affect more than 10% of diabetic patients with peripheral neuropathy and represent the most common cause of ulceration of the leg in these patients. Though their pathogenesis is well known, related to the chronic neuropathic edema, the management of NLUs, mainly based on elastocompression, is still controversial, with lower healing rates than nondiabetic venous leg ulcers. The authors tested if a novel gel formulation, containing amino acids and hyaluronic acid (Vulnamin® gel; Errekappa, Milan, Italy), will improve the outcomes of NLUs when used together with elastocompression. Thirty patients affected by NLU were randomized into 2 groups, both treated with 4-layer elastocompressive bandaging: patients in group A were topically treated with the application of Vulnamin® gel, whereas patients in group B received only the inert gel vehicle. The healing rate at 3 months was evaluated as the primary endpoint, whereas the secondary endpoints were healing time, reduction in ulcer area and ulceration score in 4 weeks, number of infective complications, and overall satisfaction of patients. Healing rate was significantly ($P < .05$) higher in patients in group A when compared with those in group B; healing time, patients' satisfaction, and reduction in ulcer area and ulceration score in 4 weeks were also higher in patients in group A. However, no significant differences were found in the prevalence of infections and other adverse events. The use of Vulnamin® gel with elastocompression is safe and effective in the management of NLUs of diabetic patients.

Keywords

Diabetes; Ulceration; Wound healing; Aminoacids; Gel

Diabetic foot ulceration (DFU) is the most frequent form of ulcer in the foot in industrialized countries. DFU is the major determinant of lower limb amputations in diabetic patients, because up to 85% of all major amputations in diabetes are preceded by an ulcer.[1,2] The presence of frank DFU has been reported in 4% to 7% of the general population; it is estimated that up to 15% of people with diabetes will experience DFU at least once in their lifetime.[3,4] The pathogenesis of DFU is well understood; a number of different recognized factors act in synergy leading to ulceration. Peripheral neuropathy is the most important of these factors, with both motor and sensory components as well as autonomic components being involved in the pathogenesis of DFU.[5,6]

In a neuropathic leg, chronic edema resulting from persistent vasodilation secondary to the autonomic denervation of precapillary arterioles has been associated with chronic foot ulcers.[7,8] This peculiar pattern of chronic ulceration is common among diabetic neuropathic patients, with a reported prevalence of 20% in selected populations.[9-11] Such lesions are also different from those caused by excess unrelieved plantar pressures.

The clinical course of neuropathic leg ulcers (NLUs) is similar to that of venous leg ulcers (VLUs). Their treatment based on elastic compression of the leg to reduce chronic edema is also similar to that of VLUs, although their prognosis has repeatedly reported to be less favorable with longer healing times than those associated with VLUs.[12-14]

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Local conditions have been implicated in NLUs; these conditions are likely to be related to the chronic, low-grade inflammatory state present in diabetic chronic ulceration.[15] Thus, the management of the local aspect of the lesions, together with edema reduction, may play a role in determining the healing tendencies of NLUs. This report presents the potential of a topical gel Vulnamin® (Errekappa, Milan, Italy) in the management of NLUs.

Vulnamin® Gel

Recently, a novel dressing, in the form of a metal cellulose gel containing the amino acids glycine, L-lysine, L-proline, L-leucine, and jaluronic acid, was proposed for the management of chronic ulcers of different pathologies.[16]

The favorable outcomes of Vulnamin® gel have been related both to the proenergetic role of the amino acids at the mitochondrial level and the downregulation of the proinflammatory cytokines present in the lesions. In the reparative phase, hyaluaronic acid acts like a provisional matrix scaffold and exerts an angiogeneic effect.[17-22] This report presents a prospective randomized controlled study designed to test the effectiveness and safety of Vulnamin® gel and elastic compressive bandages in the management of NLUs.

Materials and Methods

All the patients presenting for the first time at our diabetes foot clinic during the calendar year 2007 were screened. The inclusion criteria were the following:

- More than 18 years of age
- Type 1 or type 2 diabetes mellitus for 5 years
- Have a frank NLU for more than 3 weeks
- Have all the 4 pulses palpable at the ankle

Exclusion criteria were ankle brachial pressure index <0.9 and infection. Infection was defined as the presence of 3 of the following signs:

- Local necrosis or secretion
- Local pain or tenderness
- Increase of local skin temperature
- Local edema or redness
- Systemic signs such as fever or leucocytosis

Others exclusions were left ventricular dysfunction (class III or IV according to the New York Heart Association classification), any other actual or previous ulcers in the foot or the leg in the past 6 months, current or previous history of deep venous thrombosis, serum creatinine >2 mg/dL, recent episodes of ketoacidosis or glycated hemoglobin >10% (normal values 4.1% to 6.1%), cancer, HIV positive, hepatitis C virus positive, and any systemic disease potentially interfering with the clinical course of tissue repair.

Forty-seven patients fulfilled the inclusion criteria, but only 30 were enrolled and participated in the study. Among the nonparticipants, 12 had concomitant limb ischemia, 10 had local infection or necrosis, 8 refused to give informed consent, 6 had previous ulcerations in the same leg, and 3 had serum creatinine >2 mg/dL.

After giving informed consent patients underwent a local clinical evaluation. Lesions were surgically debrided under local anesthesia to remove any nonviable tissue, opening all the sinuses and fistulae present after which hemostasis was performed. Ulcer contours were measured using a Visitrak® (Smith & Nephew, Hull, UK) planimetry system, photographed, and swabs taken for microbiology using the technique previously described to exclude the presence of infection.[23,24] Edema was assessed by measuring the circumference of the legs both at the ankle (at the intermalleolar line) and sural (5 cm below the fibular condilus) levels. All the measurements were performed by the same person using a metric tape with the patients lying supine.

The patients were then randomized into 2 different groups using a computer-generated randomization list. Patients in group A were treated with the local application of Vulnamin® gel and polyurethane film (Biocclusive® Gel (Systagenix Wound Care, Gargrave, UK), whereas patients in group B were treated with the inert vehicle of the gel and covered using the film. All patients received 4-layer compressive bandaging (Profore®, Smith & Nephew) using the technique described by Dale et al.[25] None of the patients had received 4-layer bandaging prior to entering the study. Both the active gel and the inert vehicle were prepared by the manufacturers and were identical, making it difficult to distinguish one from the other.

All clinical and local evaluations and measurements were carried out by a blinded trained podiatrist, with physicians being unaware of the treatment.

Patients were instructed to reduce the daily sodium intake and to reduce daily activities that required them to stand for long periods of time. Patients were followed-up at weekly intervals. At each control visit adverse events were recorded, measurements of ulcer contour and leg circumferences were repeated, and local clinical conditions for the presence of infection or any other complication examined. An evaluation of the local condition of the lesion was performed at baseline and at each control visit to determine a "lesion score" that included the following:

- Perilesional redness or edema (0 = *intense*; 1 = *moderate*; 2 = *absent*)
- Granulation tissue (0 = *absent*; 1 = *scarcely present*; 2 = *well represented*)
- Epithelization (0 = *absent*; 1 = *initial*; 2 = *evolute*)
- Margins (0 = *infiltrated*; 1 = *not infiltrated*)
- Presence of debris or necrosis (0 = *present*; 1 = *absent*)

The compressive bandage was removed at the beginning of each control visit and a new one applied at the end of the

Table 1. Demographic and Clinical Features of Patients in Both Groups

	Group A	Group B	ANOVA
N (DM1/DM2)	15 (2/13)	15 (3/12)	NS
Age (years)	61.8 ± 8.9	62.4 ± 7.4	NS
Duration of diabetes (years)	21.9 ± 6.7	19.8 ± 4.2	NS
HbA1c (%)	8.8 ± 1.0	8.6 ± 1.2	NS
Ankle/brachial systolic ratio (ABPI)	1.1 ± 0.2	1.0 ± 0.1	NS
Circumference at malleolus (cm)	25.8 ± 8.3	24.4 ± 9.1	NS
Circumference at calf (cm)	28.4 ± 5.5	29.7± 4.2	NS

NOTES: DM1 = diabetes mellitus type 1; DM2 = diabetes mellitus type 2; NS = not significant; HbA1c = glycated hemoglobin; ABPI = ankle brachial pressure index.

Table 2. Ulcer Characteristics in Both Groups

	Group A	Group B	ANOVA
Ulcer area (cm^2)	25.9 ± 8.8	27.3 ± 10.4	NS
Ulcer duration (weeks)	30.8 ± 16.7	22.9 ± 18.6	$P < .05$
Granulation tissue (%)	15.4 ± 5.5	18.3 ± 10.1	NS

NOTE: NS = not significant.

visit; patients were followed-up for 3 months or until the lesion had healed.

At the end of treatment patients were requested to express their satisfaction regarding the treatment using a visual analogue scale ranging from 0 (*not satisfied at all*) to 10 (*maximally satisfied*).

The primary endpoint considered was the healing rate of the lesion at 3 months, whereas the secondary endpoints considered were healing time, reduction in ulcer area and ulceration score in 4 weeks, number of infective complications, and overall satisfaction of patients.

Data, expressed as mean ± 1 standard deviation, were analyzed by means of analysis of variance and Mann–Whitney test for nonparametric data for continuous variables; χ^2 test was used for dichotomic variables using a commercial statistical software (Statview, SAS Institute, Cary, IL) running on a personal computer.

Results

The baseline clinical characteristics of patients randomized to the 2 groups are shown in Table 1. In Table 2, the features of the lesions after the initial debridement are shown at baseline for both groups. There were no significant differences in terms of demographics and clinical features of patients in both groups. Patients randomized in group A had a longer duration of ulcers ($P < .05$) than those in group B; lesional areas overlapped between the groups.

Duplex scanning evaluation confirmed the absence of deep venous thrombosis in all patients and yielded evidence

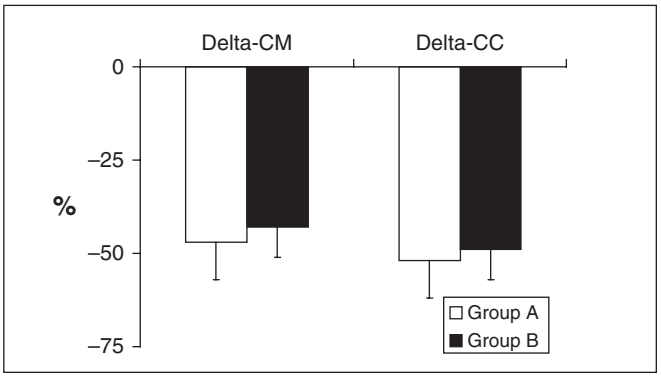

Figure 1. Percentage variation (delta) in circumference at malleolus (CM) and at calf (CC) in both groups at 4 weeks compared with baseline

of the presence of venous insufficiency, which was taken to be the cause of ulceration. Compressive bandaging was effective in reducing the edema of the legs in both groups. In Figure 1, the changes of circumference at malleolus and at calf are shown from baseline to 4 weeks later for both groups. Fourteen of 15 (93%) lesions healed in patients in group A in 3 month with 1 degree of freedom when compared with 9 of 15 (60%) in patients in group B ($\chi^2 = 6.9$; $P = .0084$).

Healing times were faster in patients in group A when compared with those in group B (60.4 ± 24.8 vs 79.9 ± 18.6 days, $P < .05$), whereas no differences were observed in the number of infective complications in the 2 groups (4 in group A and 5 in group B) as well as in the number of the adverse events (see Table 3).

All the infections were treated with systemic antibiotic (amoxicillin clavulanate, 1 bid) and healed in less than 1 week in all cases; in no case did this require any interruption of the study protocol.

Ulcer areas after 4 weeks of treatment showed a significant ($P < .05$) reduction in patients in group A (from 25.9 ± 8.8 cm^2 to 10.7 ± 9.4 cm^2, –58.7%), but not in patients in group B (from 27.3 ± 10.4 cm^2 to 20.9 ± 12.6 cm^2, –23.4%), as shown in Figure 2.

Table 3. Adverse events in both groups

	Group A	Group B	ANOVA
Infective episodes (n)	4	5	NS
Pain/discomfort (report)	3	2	NS
Maceration (n/changes)	0.3 ± 0.1	0.4 ± 0.1	NS
Skin sensitization (n)	1	2	NS
Odor (n)	0	1	NS

NOTE: NS = not significant.

The percentage of lesional area covered by granulation tissue after 4 weeks of treatment was significantly higher in patients in group A when compared with those in group B (62.8 ± 14.7% vs 28.3 ± 10.2%, $P < .01$). Lesion score was significantly higher ($P < .01$ for trend) in patients in group A when compared with those in group B, as shown in Figure 3. The overall satisfaction expressed by the patients was significantly higher in patients in group A than those in group B (8.2 ± 1.1 vs 6.7 ± 2.2, $P < .05$). Figure 4A-C shows a case of NLU treated with Vulnamin® gel.

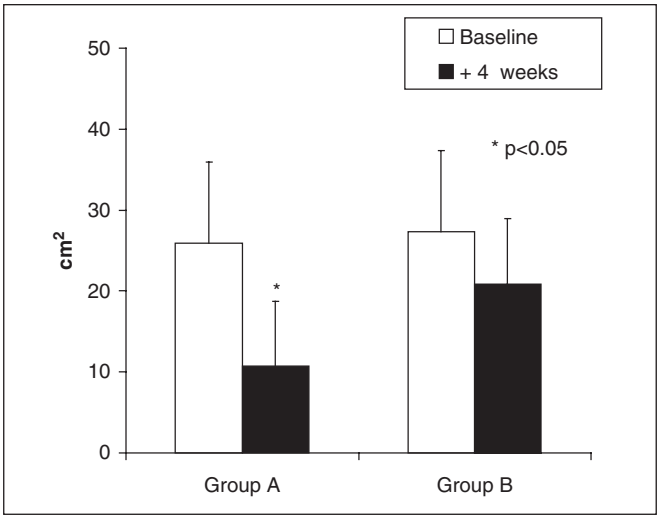

Figure 2. Area of ulceration at baseline and after 4 weeks of treatment. Although patients in group A showed a significant reduction in lesional areas (−58.7%, $P < .05$), patients in group B showed no significant changes in this parameter

Discussion

This study demonstrates that Vulnamin® gel used with a 4-layer elastocompressive bandaging system is safe. At the 4-week time point, there was significant reduction in edema and ulcer area reduction in patients in group A. Also, at the same time point, more of the lesions were covered, which is in accord with reduction in ulcer areas. Over 12 weeks, there was significantly better ulcer healing in patients in group A when compared with those in Group B, who received the inert vehicle and 4-layer bandaging ($P = .0084$).

As other studies have demonstrated, diabetic chronic ulcers are probably the most difficult to heal among chronic ulcerations, with a rate of healing that is lower than similar conditions in nondiabetic patients and with a higher frequency of recurrences and complications.[12,14] It is accepted that good compression bandaging (the 4-layer system is an excellent example) is associated with a healing rate of the order of 40% of VLUs. In this study, we observed healing rates of 93% and 60% in patients in groups A and B, respectively, which suggests that both groups benefited from compression bandaging treatment.

Despite a common pathogenesis, though via different causes, NLUs are less prone to heal than VLUs and have an average healing time that is significantly longer, even when all the therapeutic strategies are correctly applied.

There are increasing evidences that the local biology of the chronic ulcer play a determining role in conditioning the clinical course of the lesions; the evolution toward a reparative phase, with a shift from a chronic attitude to a more healing pattern, is delayed and even ended in patients with DFU.[12]

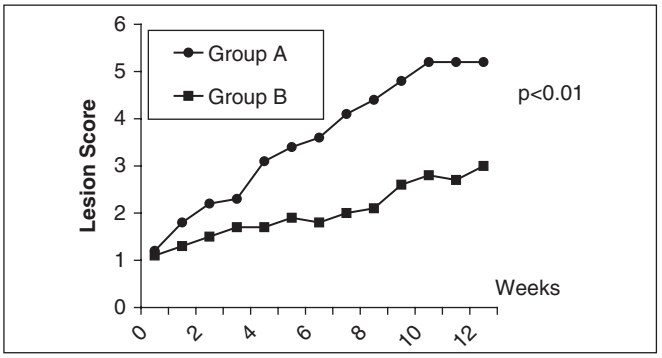

Figure 3. Lesion scores during the study in the 2 groups. Patients in group A showed a faster improvement than those in group B for all the items that composed the score (see text for details)

Loots et al[15] demonstrated how, compared with other models of chronic ulceration, diabetic models had a longer duration of the marker of chronic inflammatory state, characterized by the persistence of provisional matrix and expression of proinflammatory cytokines, like they were "frozen" in a low-grade inflammatory condition. The same authors, in a more recent article, showed how autologous fibroblasts taken from the lesion and compared with fibroblasts coming from other sites from the same diabetic patient were less active, had a lower proliferative rate, and produced less collagen, as if influenced by the local milieu.[26]

We now know how chronic inflammation is strictly related to diabetes and other degenerative chronic diseases, in which

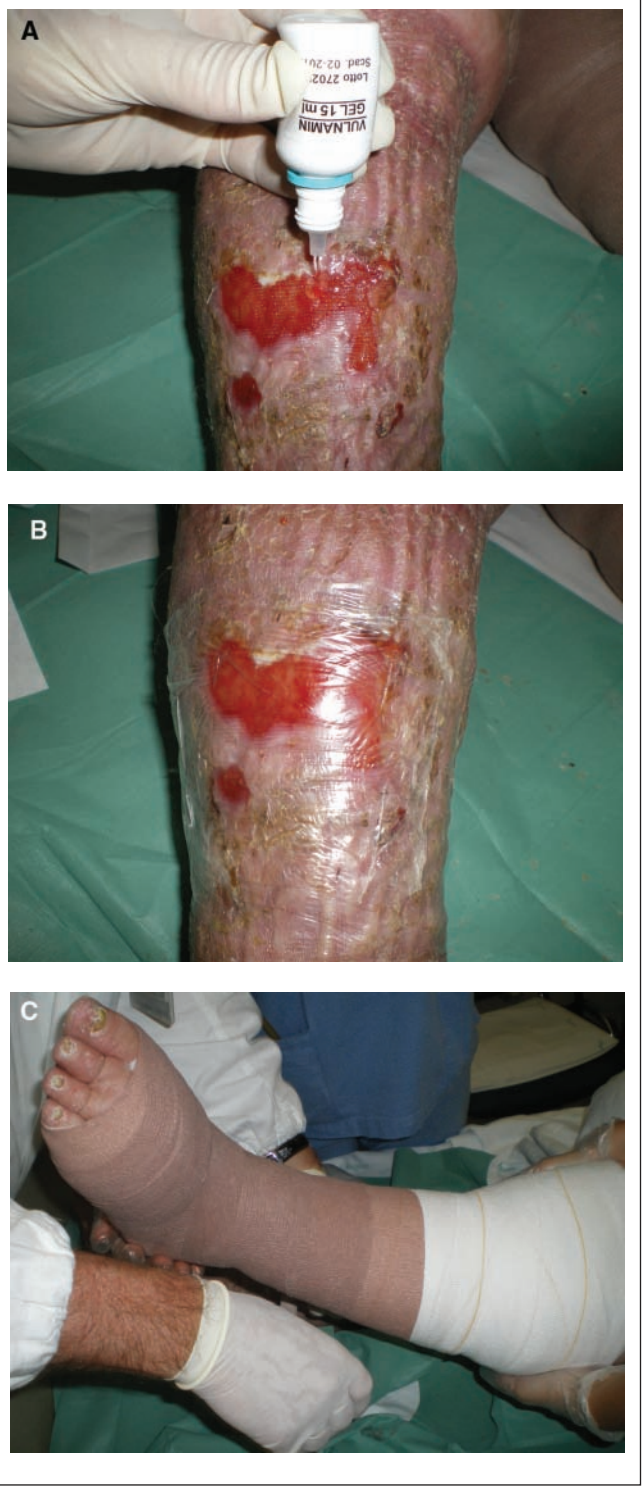

Figure 4. A clinical case treated with Vulnamin® gel. A, A lesion on the anterior surface of the leg is covered with Vulnamin® gel. B, Lesion covered with polyurethane film. C, The leg is bandaged with Profore®, a 4-layer elastocompressive bandaging system

it represents an important cofactor of morbidity and mortality. NLU is a typical complication of diabetes that relates to this model, where diabetes negatively conditions the

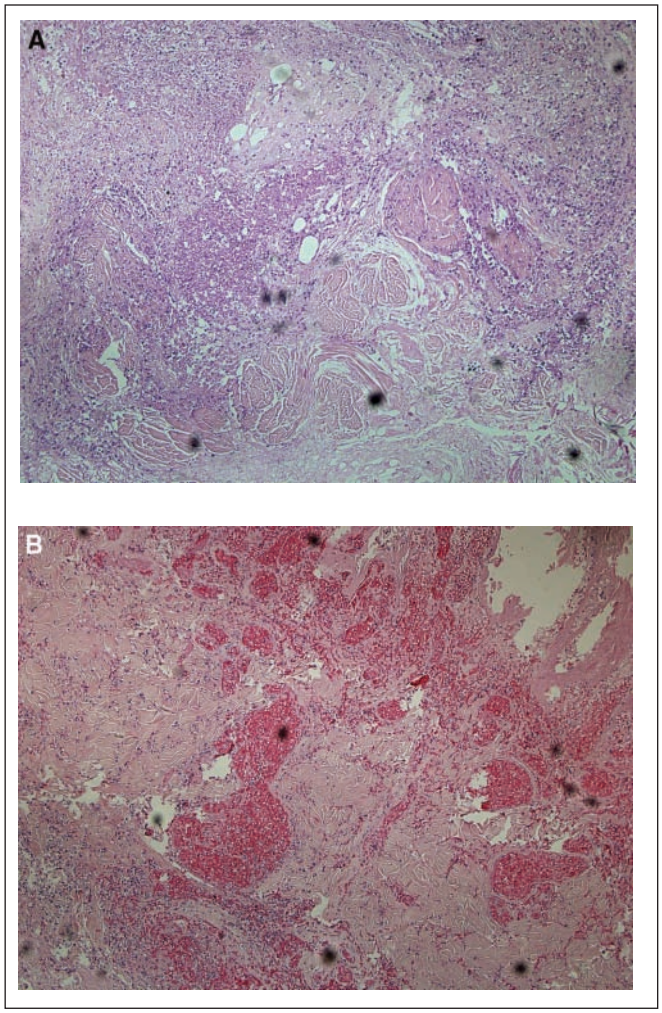

Figure 5. A histology sample of a lesion (hematoxylin–eosin staining, 200×) before (A) and after (B) 4 weeks of application of Vulnamin® gel. Whereas in "A" chronic inflammation elements are clearly identifiable, with a monocellular infiltration in all the layers of the lesions, in "B" new vessels and fibroblasts are conspicuously present suggesting a shift toward a healing pattern of the ulcer.

evolution of the lesion and the lesion vice versa may worsen the metabolic control of the patient.[27,28]

The amino acids present in the Vulnamin® gel formulation are reported to be regulators of the inflammatory process in different in vitro and in vivo models: they can modulate the secretion of proinflammatory cytokines by leukocytes, macrophages, and fibroblasts and by inhibiting the expression of many genes involved in the maintenance of inflammation, both directly and via the modification of the redox state of the host.[19,22] Their activity in the NLU model produced a shift of the lesions toward a less pronounced inflammatory pattern, with both an amelioration of the clinical score and faster healing as a consequence of its application.

The significant reduction in ulcer areas after 4 weeks of treatment, beyond being the clinical correlate of the activity of Vulnamin® gel, is also a positive prognostic sign, because

it has been demonstrated that a reduction of the area of the lesions during the first 4 weeks of treatment may have prognostic value in diabetic foot patients[29]; the observations in this study suggest that Vulnamin® gel and 4-layer bandages may be beneficial and encourage control studies with bigger sample sizes.

A histological sample from one of the patients confirmed our interpretation of the results from the study. A biopsy taken at baseline, involving both the edge and the bottom of the lesion, shows an intense inflammatory infiltrate, with characteristics of mononuclear cells and scarcity of fiblobblasts and vessels throughout the lesion (Figure 1A). After 4 weeks of treatment with Vulnamin®, the same lesion shows a completely different pattern: a marked reduction in the inflammatory infiltrate is associated with a colonization of the lesions by fibroblasts and newly formed vessels, which constitute the histological markers of the granulating tissue.

This is consistent with the findings of some recent studies that positively correlated the local concentration of endothelial nitric oxide (e-NOS) and ulcer healing, due to its neoangiogenic activity.[30]

The e-NOS concentration is reduced in chronic ulceration, especially in diabetic patients, and the supplementation with essential amino acids has demonstrated to induce the increase of e-NOS both in vitro and in vivo.[31]

Our data demonstrate that Vulnamin® gel, together with elastocompression, is as safe and more effective than the standard gel in inducing a faster and durable healing in NLU.

Declaration of Conflicting Interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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