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Healing Diabetic Foot Ulcers with Topical Timolol Improves Healed Epithelial Integrity

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Keywords
Transepidermal water loss · Diabetic foot ulcer · Wound healing · Timolol · Beta-adrenergic receptor antagonism

Abstract

Introduction: Diabetic foot ulcers (DFUs) are a common complication in diabetes, leading to high amputation risk and significant healthcare costs. Given topical timolol's emergence as a potential wound-healing agent, our study explored its impact on epidermal integrity. ***Methods:*** This study was a post hoc analysis conducted as part of a randomized controlled trial at the Veterans Affairs Northern California Health Care System. Twenty patients, who had DFUs healed in the original trial, 10 in the timolol arm, and 10 in the placebo arm, were enrolled in the study. The primary outcome was transepidermal water loss, measured monthly for 3 months of post-healing using a closed-chamber device. The secondary outcome was re-ulceration rates over 1 year. ***Results:*** Transepidermal water loss at 1, 2, and 3 months of post-healing was significantly lower in the timolol group than in the placebo group ($p < 0.01$). Linear mixed models identified contralateral foot transepidermal water loss as a significant predictor of healed diabetic foot ulcer site transepidermal water loss (estimate = 0.76, $p < 0.001$). The interaction between timolol treatment and months since healing significantly reduced transepidermal water loss over time (estimate = −2.2, $p = 0.002$). The use of a wheelchair was also associated with a significant decrease in transepidermal water loss (estimate = −7.7, $p = 0.01$). Initial transepidermal water loss values were higher in patients who re-ulcerated, but the difference was not statistically significant ($p = 0.42$). There was no difference in re-ulceration rates in this small pilot study. ***Conclusion:*** Topical timolol significantly improved skin barrier function in healed DFUs, reducing transepidermal water loss. Although re-ulceration rates were not significantly different, the trend suggests potential benefits. Further studies with larger sample sizes and longer follow-up are needed to confirm these findings and explore transepidermal water loss's predictive value for re-ulceration.

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Introduction

Diabetic foot ulcers (DFUs) are a severe complication affecting approximately 25% of individuals with diabetes, posing a significant risk of amputation due to poor wound healing, recurrent infections, osteomyelitis, and sepsis [1]. In the USA, the economic burden of DFUs is substantial, with costs estimated between USD 9–13 billion, in addition to the healthcare expenses associated with diabetes management [2].

Standard care for DFUs involves a multifaceted approach including surgical debridement, wound dressings, offloading devices, vascular assessment, glycemic control, and infection control. Adjunctive therapies, such as nonsurgical debridement agents, specialized dressings, and topical agents, aim to enhance healing outcomes [3]. Despite these efforts, DFUs have a re-ulceration rate of nearly 40% within 1 year of healing, likely due to persistent precipitating factors and impaired skin barrier restoration [4, 5]. Presently, there is no reliable method available to predict DFU recurrence [6].

Over the past 3 decades, extensive bench research clinical trials and observational studies have highlighted the benefits of repurposing the drug timolol, as topically applied, to improve various aspects of wound healing (reviewed in [7]). Beta-adrenergic antagonism facilitates wound healing across the inflammatory, proliferative, and maturation phases, enhancing cellular communication among keratinocytes, fibroblasts, and other resident cells [8]. Additionally, other β2-adrenergic receptor antagonists have been found to accelerate epidermal barrier recovery, in acetone-damaged murine epidermis, whereas β2-agonists delay it, suggesting that timolol, a β1/2-adrenergic receptor antagonist, may likewise improve epidermal barrier function in the reforming epidermis of healed wounds [9].

TEWL is a noninvasive, objective measure of skin barrier function and re-epithelialization, reflecting the diffusion of water through the stratum corneum. Increased TEWL has long been evaluated as a marker of compromised barrier function [10]. TEWL can be measured with open or closed-chamber devices, both of which assess temperature and relative humidity, demonstrating similar sensitivity in assessing barrier disruption [11]. TEWL is a valuable metric in evaluating skin barrier integrity across various dermatological conditions such as acne vulgaris, psoriasis, atopic eczema, keloid and burn scars, and contact dermatitis [12–14]. Of interest, there has been a recent report with preliminary evidence suggesting that TEWL may have a role in predicting ulcer recurrence in DFUs [6]. Furthermore, timolol's capacity to enhance epithelial maturation has been shown to indirectly influence TEWL measurements, reinforcing its relevance as a marker of barrier restoration [9].

Given the therapeutic potential of repurposing timolol as a topical therapeutic to improve wound healing, and the noted enhancement of epidermal barrier restoration through β2-adrenergic receptor antagonism, as well as the utility of TEWL as a measure of skin barrier integrity, this study hypothesized that DFUs treated with topical timolol would exhibit improved skin barrier function and reduced re-ulceration rates, as evidenced by decreased TEWL compared to placebo controls. The primary objective was to evaluate and compare re-epithelialization integrity after healing, measured by TEWL, between patients who had been healed with either topical timolol gel or a placebo hydrogel. The secondary objective was to assess the impact of topical timolol on re-ulceration rates over a 1-year follow-up period.

Methods

Study Design

This study was a post hoc analysis conducted as part of a phase two, double-blinded, randomized controlled trial at the Veterans Affairs Northern California Health Care System. The trial was approved by the institutional review board in 2018 (IRBNet ID 1581979), registered (NCT03282981), and details of the protocol were previously published [8, 15]. The inclusion and exclusion criteria have been detailed in Table 1. In the trial, participants were originally randomized into two groups: one received topical timolol plus standard of care, and the other a placebo gel plus standard of care. Treatment involved daily application of the study drug for up to 12 weeks or until ulcer healing. Patients who had healed in the previous RCT (full epithelization of the skin without drainage and dressing requirements) were eligible for immediate enrollment within this post hoc study (shown in Fig. 1a). The primary outcome of the study was the change in TEWL over time, with a secondary outcome of re-ulceration rates over the period of 1 year after healing.

Enrolled patients had TEWL measured using the VapoMeter™ after the wound was confirmed to have stayed healed for at least 1 week. TEWL was measured every 1–2 months at the healed DFU site and on the same location of the contralateral foot to serve as a patient-specific control. At each visit, measurements were repeated on each site a total of 5 times, allowing approximately 1 min between each measurement for the device

Table 1. Inclusion and exclusion criteria

Inclusion criteria
1. Male or female subject of any race 18 years old or older
2. Lower extremity ulcer located anywhere on the foot up to the ankle: a. Of more than 30 days duration and less than 2 years duration (medically documented) b. Surface area between 0.5 cm^2 and 20 cm^2 (as measured with the Silhouette imaging system at randomization). The ulcer with largest surface area meeting inclusion criteria will be selected as index ulcer c. If two ulcers present with the same surface area, the ulcer of the longest duration will be selected as index ulcer
3. Documented ankle-brachial index (ABI) between 0.8 and 1.2 on the study limb or toe pressure over 65 mm Hg within 3 months of screening phase
4. Documented biopsy report to rule out malignancy of ulcer of >6 months duration
Exclusion criteria
1. Ulcer of nondiabetic etiology, such as venous, arterial, and burn wounds
2. Index ulcer is less than 3 cm in distance from any other ulcer on the same extremity
3. There are greater than 3 ulcers on the study foot
4. Index ulcer presents with any of the following: cellulitis, osteomyelitis, exposed bone, tendon or fascia, purulent exudate or gangrene
5. Index ulcer shows evidence of infection (defined as a moderate or severe rating of all of the following clinical signs/symptoms: (1) increased warmth, (2) increased pain, (3) erythema, and (4) malodorous exudate at screening or at randomization (visit 1), OR total organism count > 1×10^5 colony forming units (CFUs) from the screening visit study ulcer culture sample)
6. Index ulcer surface area has decreased or increased > 40% between screening and at randomization (visit 1) as assessed by the Silhouette imaging system
7. Has medically documented history of human immunodeficiency virus (HIV)
8. Has active malignancy on the study limb
9. Has uncontrolled diabetes mellitus as defined by glycosylated hemoglobin A1C >12% within 3 months of screening
10. Has immunodeficiency as defined by serum IgG, IgA, and IgM less than one-half the lower limit of normal
11. Has severe protein malnutrition as defined by serum albumin < 2.5 g/dL
12. Has serum aspartate aminotransferase (AST, SGOT, GOT) or serum alanine aminotransferase (ALT, SGPT, GPT) levels greater than twice the upper limit of normal
13. Has fatigue, palpitations, dyspnea, and/or angina at rest
14. Has a medically documented or self-reported history, within the previous 12 months from date of screening visit, of alcohol or drug abuse, particularly methadone or heroin
15. Has received previous treatment with the following during the 60 days prior to screening: immunosuppressive agents, radiation, chemotherapy, growth factors (epidermal growth factor, tumor necrosis factor, transforming growth factor, platelet derived growth factor, etc.)
16. Has received previous treatment with the following during the 30 days prior to screening: the site of the study ulcer, split- or full-thickness skin graft at the site of the study ulcer, biologically-active (or engineered) cellular or acellular product(s) at the site of the study ulcer, investigational drug or device
17. Has been hospitalized for treatment of a diabetic foot ulcer within the previous 30 days from screening
18. Has history of bradycardia (heart rate less than 60)
19. Has ESR >70 mm/h and CRP >100 mg/L at time of screening
20. Has medically documented history of hypotension/orthostatic hypotension and/or symptomatic hypotension (systolic blood pressure below 90 and diastolic blood pressure less than 60). (Note: there is no standard testing regimen protocol for orthostatic hypotension, even for patients starting on oral timolol, per the consultant cardiologist, Dr. Schaefer)
21. Currently taking asthma or COPD medications (as documented in chart)

Table 1 (continued)

22. Has a medically documented diagnosis of myasthenia gravis, untreated hyperthyroidism, type 2, type 3 heart block, cardiogenic shock, overt cardiac failure
23. Female who is pregnant or refuses to use adequate contraceptive methods and is of childbearing age during the trial
24. Prisoners, institutionalized individuals or vulnerable population

ABI, ankle-brachial index; CFU, colony forming units; HIV, human immunodeficiency virus; AST, SGOT, GOT: aspartate aminotransferase; ALT, SGPT, GPT, alanine aminotransferase; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; COPD, chronic obstructive pulmonary disease.

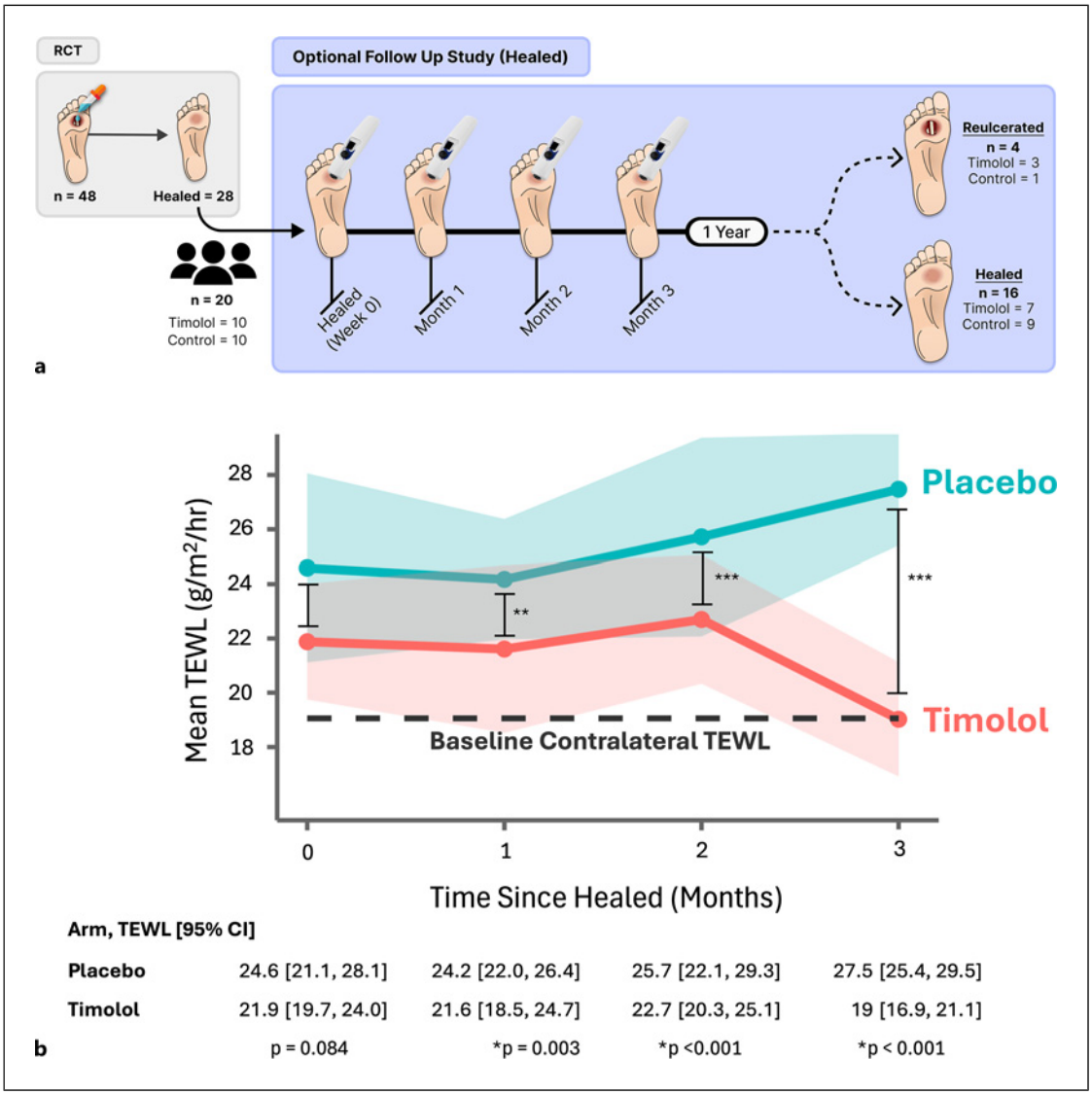

Fig. 1. a Flow diagram of study methods. A total of 20 patients were included in the post hoc. TEWL study from the previous RCT. TEWL measured monthly for 3 months after healing, and patients were followed for 1 year to assess re-ulceration status. **b** TEWL over time in the placebo and timolol arms. The dashed line represents the mean TEWL of the contralateral foot for all patients (n = 20). Patient numbers varied from 5 to 10 at each timepoint. Significant differences in mean TEWL between arms were observed at 1, 2, and 3 months after healing (p < 0.01). TEWL, transepidermal water loss.

to equilibrate. Patients were instructed to not apply any emolliating agent 24 h before TEWL measurement collection.

All measurements were taken indoors in a temperature-controlled room and all patients had their measurements taken when all sites were dry with no visible moisture or sweat at the time of measurement. After removal of the patient's shoes, the patients' feet were left to acclimate for 10–15 min prior to TEWL measurement. Patient demographics and DFU recurrence were collected from medical records.

Statistical Analysis

The statistical analysis was conducted using R software (version 4.3.2), and the code has been made available in a GitHub repository [16]. Data visualization and distribution analysis included generating histograms and density plots to assess the distribution of TEWL values across study arms and over time. To address the variability within visit TEWL measurements, we computed summary statistics, including the mean, standard deviation, minimum, maximum, range, and the coefficient of variation to measure relative variability.

Outliers were identified and excluded using the interquartile range method to ensure the robustness of the analysis. A total of 7 outliers were removed, resulting in a dataset comprising 313 observations from the initial 320. Data were binned into 0, 1, 2, and 3 months after healing, with the contralateral foot serving as a control to help normalize data and mitigate the impact of any missing values.

Analysis of covariance models were fitted at each time point (0, 1, 2, and 3 months) to assess the effect of the treatment arm, time since healing (days), and TEWL at the contralateral site as covariates. Additionally, a linear mixed model (LMM) was employed to evaluate the effect of these variables on TEWL, accounting for both fixed and random effects to address intra-patient variability. Covariates evaluated in the models included various patient and wound characteristics such as age, wound duration, and diabetes duration. Model selection was based on optimization using Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).

Results

Participant Characteristics

A total of 20 patients from the original 28 healers in the randomized controlled trial agreed to enroll in the post hoc TEWL study. After blinding was broken, 10 patients were found to be from the timolol arm and 10 from the placebo arm (Table 2). Consistent with demographics of the population in the VA, the majority were male (95%, $n = 19$), and the average age was similar across groups at 70 years (SD: 7). The average body mass index (BMI) was 36.7 (SD: 9.2), with the placebo group showing a higher average BMI (39.1–34.5, respectively), though not statistically significant ($p = 0.27$). Most patients were white (85%, $n = 17$), with a small representation of black (10%, $n = 2$) and Asian (5%, $n = 1$) patients. Overall, 55% ($n = 11$) required the use of durable medical equipment, with 25% ($n = 5$) requiring a cane for ambulation, 15% ($n = 3$) a walker, and another 15% ($n = 3$) using a wheelchair daily. No significant baseline characteristics differences in the demographics were found between groups, regarding sex, age, ethnicity, BMI, wound duration, diabetes history, offloading, or use of durable medical equipment.

Wound Characteristics

Ulcer site distribution was similar across groups, with the most common sites being the hallux (30% $n = 6$) and submetatarsals 1–2 (25%, $n = 5$) (Table 2). The time to healing averaged 84 days (SD: 48) in the placebo group and 81 days (SD: 65) in the timolol group of those included in the study. The ankle-brachial index (ABI) values and toe pressures were also similar between groups (timolol ABI: 1.21, SD: 0.16; placebo ABI: 1.25, SD: 0.33). Sensory neuropathy, as measured by monofilament score, was higher in the timolol group (3.1–1.1, respectively), though was not significant ($p = 0.17$).

Healed DFU TEWL Scores

TEWL values from healed DFUs were analyzed over time. Analysis of covariance models indicated a significant reduction in TEWL at 1, 2, and 3 months of post-healing in the timolol treatment group compared to the placebo group, with the difference between the two being 2.6, 3.0, and 8.5 g/m^2/h respectively ($p < 0.01$). Initial TEWL values followed a similar trend, with timolol-treated DFUs showing a mean TEWL of 21.9 compared to a mean of 24.6 in the placebo group, however, the difference was not significant ($p = 0.08$). DFU sites treated with timolol showed improved TEWL values over time, approaching the same TEWL values of the uninvolved contralateral foot sites 3 months after healing (shown in Fig. 1b). In contrast, the placebo-treated sites continued to diverge from the baseline contralateral values.

LMM Analysis

To evaluate the factors influencing TEWL at the healed DFU sites across treatment arms, a LLM was employed. Predictor variables were selected based on

Table 2. Patient characteristics

	Placebo (*N* = 10)	Timolol (*N* = 10)	Overall (*N* = 20)	*p* value*
Sex, *n* (%)				1.000
Male	10 (100)	9 (90)	19 (95)	
Female	0 (0)	1 (10)	1 (5)	
Age, *n* (%)				0.904
Mean (SD)	70 (6)	70 (8)	70 (7)	
Median [min, max]	71 [63, 83]	74 [56, 80]	71 [56, 83]	
Race, *n* (%)				0.589
White	9 (90)	8 (80)	17 (85)	
Black	1 (10)	1 (10)	2 (10)	
Asian	0 (0)	1 (10)	1 (5)	
NA	1 (10)	0 (0)	1 (5)	
BMI, *n* (%)				0.273
Mean (SD)	39.1 (9.6)	34.5 (8.7)	36.8 (9.2)	
Median [min, max]	38.9 [25.3, 55.3]	31.7 [23.0, 50.3]	33.5 [23.0, 55.3]	
Duration of diabetes, *n* (%), years				0.728
Mean (SD)	21 (11)	20 (11)	20 (11)	
Median [min, max]	21 [2, 46]	21 [5, 42]	21 [2, 46]	
DFU Severity Score, *n* (%)				0.126
Mean (SD)	5.1 (2.9)	3.4 (1.7)	4.3 (2.5)	
Median [min, max]	3.5 [3.0, 11.0]	3.0 [0, 6.0]	3.0 [0, 11.0]	
Durable medical equipment, *n* (%)				0.807
Cane	3 (30)	2 (20)	5 (25)	
Walker	2 (20)	1 (10)	3 (15)	
Wheelchair	1 (10)	2 (20)	3 (15)	
None	4 (40)	5 (50)	9 (45)	
Home health status, *n* (%)				0.648
No	5 (50)	7 (70)	12 (60)	
Yes	5 (50)	3 (30)	8 (40)	
Permanently disabled, *n* (%)				1,000
No	8 (80)	9 (90)	17 (85)	
Yes	2 (20)	1 (10)	3 (15)	
History of homelessness, *n* (%)				1,000
No	10 (100)	9 (90)	19 (95.0)	
Yes	0 (0)	1 (10)	1 (5.0)	
Charlson Comorbidity Index, *n* (%)				0.650
Mean (SD)	5.2 (1.5)	5.5 (1.4)	5.4 (1.4)	
Median [min, max]	5.0 [4.0, 9.0]	5.5 [3.0, 8.0]	5.0 [3.0, 9.0]	
Smoking history, *n* (%)				0.177
Never	4 (40)	8 (80)	12 (60)	
Former	4 (40)	1 (10)	5 (25)	
Current	2 (20)	1 (10)	3 (15)	
Amputation history, *n* (%)				0.099
Left	3 (30)	0 (0)	3 (15)	
Right	0 (0)	2 (20)	2 (10)	
Bilateral	1 (10)	0 (0)	1 (5)	
None	6 (60)	8 (80)	14 (70)	

Table 2 (continued)

	Placebo (N = 10)	Timolol (N = 10)	Overall (N = 20)	p value*
Initial wound size, cm				0.413
Mean (SD)	2.0 (3.0)	1.1 (1.3)	1.6 (2.3)	
Median [min, max]	0.7 [0.3, 20.0]	0.6 [0.4, 4.5]	0.6 [0.3, 10.2]	
Ulcer age, weeks				0.158
Mean (SD)	8.1 (4.0)	5.8 (2.8)	6.9 (3.5)	
Median [min, max]	7.5 [4.0, 16.0]	4.50 [3.0, 12.0]	5.5 [3.0, 16.0]	
Ulcer site, n (%)				0.386
Arch	1 (10)	1 (10)	2 (10)	
Hallux	3 (30)	4 (40)	7 (35)	
Heel	2 (20)	0 (0)	2 (10)	
Met 1–2	2 (20)	3 (30)	5 (25)	
Met 5	2 (20)	0 (0)	2 (10)	
Digit 2 (plantar)	0 (0)	1 (10)	1 (5)	
Midfoot	0 (0)	1 (10)	1 (5)	
Days to heal				0.797
Mean (SD)	88 (47)	81 (65)	84 (56)	
Median [min, max]	66 [42, 182]	53 [28, 217]	60 [28, 217]	
Ankle-brachial index				0.696
Mean (SD)	1.25 (0.33)	1.21 (0.16)	1.22 (0.23)	
Median [min, max]	1.18 [0.98, 1.88]	1.20 [0.95, 1.59]	1.20 [0.95, 1.88]	
Non-compressible, n (%)	4 (40%)	0 (0%)	4 (20%)	
Toe pressure				0.317
Mean (SD)	99 (18)	112 (27)	106 (23)	
Median [min, max]	104 [63, 115]	111 [66, 158]	107 [63, 158]	
Amputation, n (%)	3 (30)	2 (20)	5 (25)	
Monofilament score				0.167
Mean (SD)	1.1 (1.9)	3.1 (4.0)	2.1 (3.2)	
Median [min, max]	0 [0, 6.0]	1.0 [0, 10.0]	0 [0, 10.0]	

SD, standard deviation; BMI, body mass index; DFU, diabetic foot ulcer. *p value: t tests used for continuous variables, Fisher's exact test used for categorical variables.

clinical relevance, and the model was optimized using AIC and BIC. The final model included RCT arm, TEWL of the contralateral foot, time since healing (months), use of durable medical equipment, BMI, ABI, and DFU Severity Index Score. A forest plot presenting the estimates and 95% confidence intervals is shown in Figure 2.

Three variables significantly influenced TEWL at the healed DFU sites. First, the TEWL of the contralateral foot was a significant predictor ($p < 0.001$), demonstrating a positive correlation with healed DFU TEWL, where each 1 g/m^2/h increase in contralateral foot TEWL was associated with a 0.8 g/m^2/h increase at the healed site. Second, the use of a wheelchair had a significant negative association with healed DFU TEWL ($p = 0.01$), corresponding to a 7.8 g/m^2/h decrease. Finally, among participants treated with timolol, time since healing was negatively correlated with TEWL ($p = 0.002$), indicating a 2.2 g/m^2/h decrease in TEWL for each month post-healing. Seasonal variation in TEWL was also analyzed and found to have no significant impact on our outcomes or intra- and inter-patient variations.

Re-Ulceration Analysis

TEWL values taken within the first month of healing were evaluated as a potential marker for re-ulceration (shown in Fig. 3). Mean TEWL values for those that re-ulcerated ($n = 3$) were 28.5, whereas it was 22.2 for those who did not re-ulcerate. However, the difference was not found to be statistically significant ($p = 0.42$).

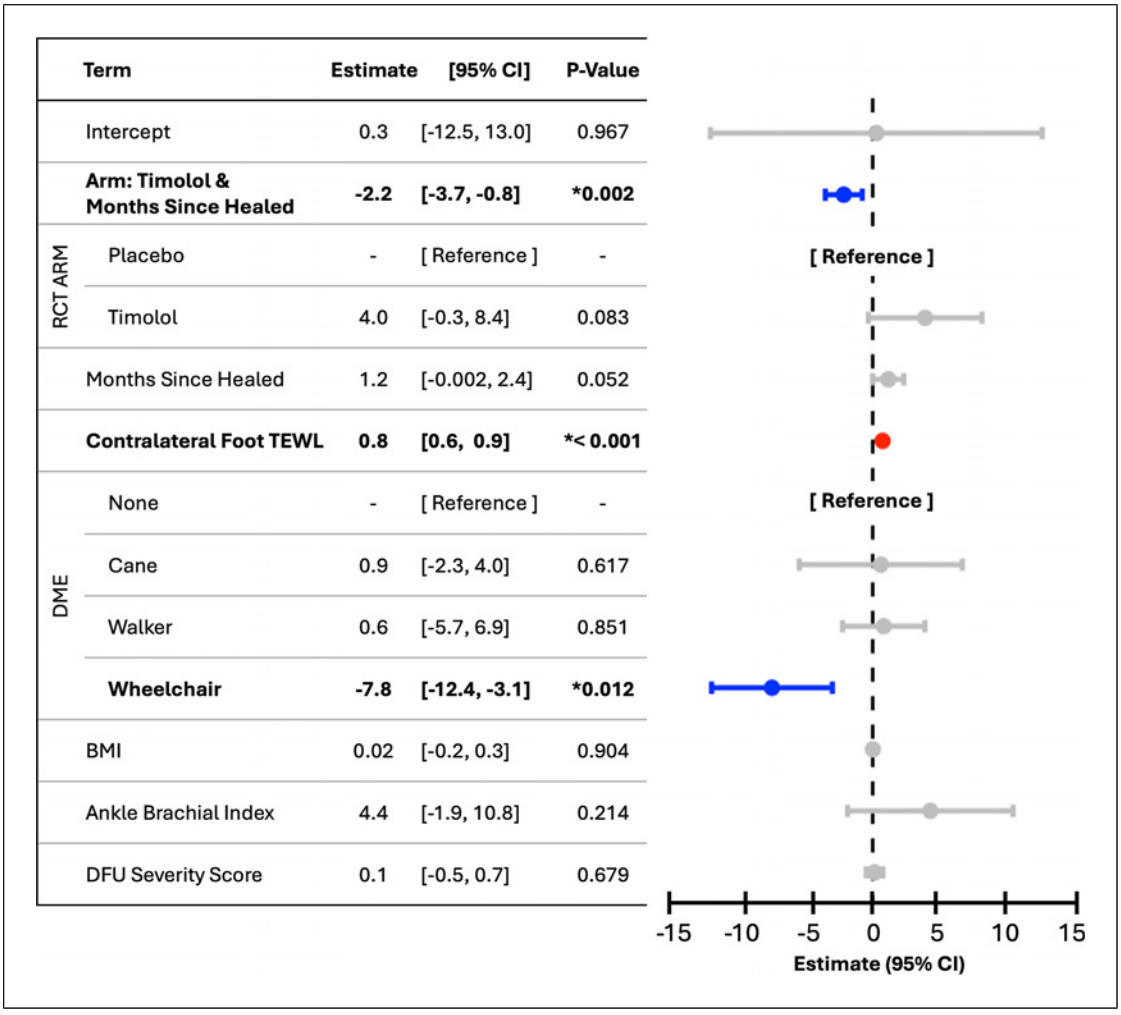

Fig. 2. Forest plot of the LLM showing the interaction between variables and the healed DFU site TEWL. Variables included the interaction between the RCT timolol arm and months since healing, the RCT arm and months since healing alone, contralateral foot TEWL, DME, BMI, ankle-brachial index, and DFU Severity Score. Significant variables included the timolol arm and months since healed interaction (p = 0.002), contralateral foot TEWL (p < 0.001), and the use of a wheelchair (p = 0.01). CI, confidence interval; TEWL, transepidermal water loss; BMI, body mass index; DFU, diabetic foot ulcer.

Discussion

This study aimed to evaluate the impact of topically applied timolol on epidermal integrity, as measured by TEWL, in healed DFUs and its potential as a predictor for re-ulceration. Our results indicate that ulcers treated with topical timolol exhibited significantly lower TEWL values once healed compared to those treated with placebo, with a complete return to baseline observed 3 months of post-healing (p < 0.001). Of note, the TEWL changes were sustained for at least 3 months after discontinuation of the topical drug at the time of full healing. Given that TEWL is a recognized quantitative measure of skin barrier function, we hypothesize that the lower TEWL values in the timolol group reflect a more robust re-epithelialization process and improved barrier function, which are essential for maintaining healed DFUs [17].

By the LLM, the most significant predictor of healed DFU site TEWL was the contralateral foot TEWL. Additionally, the model highlighted a significant interaction between timolol treatment and time since healing. Specifically, for those in the timolol arm, TEWL decreased by 2.2 g/m^2/h each month post-healing, suggesting that the use of timolol in healing the DFU facilitated ongoing improvements in

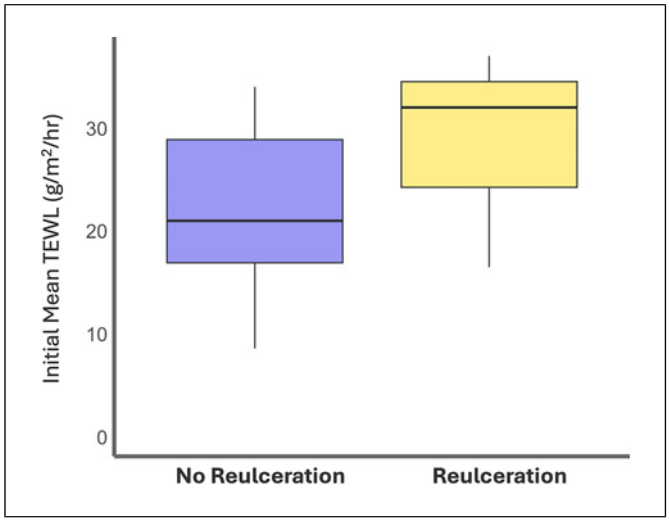

Fig. 3. Boxplot of DFU TEWL values within the first month of healing. Patients without re-ulceration had lower TEWL values than those with re-ulceration. Mean TEWL was 22.2 for patients without re-ulceration (n = 12) and 28.5 for patients with re-ulceration (n = 3), though the difference was not statistically significant (p = 0.42). TEWL, transepidermal water loss.

epithelial restructuring even after it has been discontinued (p = 0.001). Additionally, the use of a wheelchair was associated with lower TEWL, likely due to the benefits of offloading. These findings collectively support the notion that the application of timolol during the healing process enhances the long-term and persistent restoration of the skin barrier for months after healing, which could lead to reducing the risk of re-ulceration by promoting more effective and durable re-epithelialization.

Regarding the potential of initial TEWL as a marker for re-ulceration [6], our data showed higher mean TEWL values in patients who experienced re-ulceration (28.5 vs. 22.2). However, this difference was not statistically significant (p = 0.42), and our sample size for re-ulceration was limited to 3 patients. While these preliminary trends suggest that initial TEWL could be a potential marker for re-ulceration, further studies with larger sample sizes are necessary to validate this finding and determine its clinical utility.

Within the skin, β-adrenergic receptors are present on keratinocytes, fibroblasts, and melanocytes and may contribute to the pathophysiology of dermatologic conditions [18]. Timolol has been demonstrated to play a role in several phases of wound healing, notably inflammation, proliferation, and maturation [7]. Activation of β-adrenergic receptors inhibits keratinocyte migration by downregulating the phosphorylation of extracellular signal-related kinases necessary for migration [19]. Moreover, β-adrenergic receptor antagonists promote extracellular signal-related kinase activity and facilitate keratinocyte migration [18]. β2-adrenergic receptor antagonism has also been demonstrated to accelerate barrier recovery and reduce epidermal hyperproliferation in murine models and in human skin post-laser resurfacing [9, 20]. Taken together, the facilitation of keratinocyte migration followed by enhancement of epidermal maturation is one mechanism by which timolol may accelerate epidermal barrier recovery and promote a more structured and effective barrier in a healed wound. Further studies are needed to elucidate additional mechanisms by which β2-adrenergic receptor antagonism enhances the epidermal barrier.

This study's limitations, including the small sample size and the relatively short follow-up duration of 1 year for re-ulceration status, constrain the generalizability of the findings and the statistical power to detect significant differences in re-ulceration rates. Future studies with larger sample sizes and longer follow-up periods are warranted to validate these findings and further explore the relationship between TEWL and DFU recurrence. Additionally, this study was conducted in Sacramento, CA, where the average humidity is approximately 60% with minimal seasonal variation, a factor that could influence TEWL measurements. To address potential environmental inconsistencies in the clinical setting, such as door openings or air conditioning, a closed-chamber device was selected for its reliability. While this method has its own limitations, including reduced sensitivity at very low TEWL levels and a potential risk of saturation, standardized measurement conditions were implemented to minimize these effects and ensure consistency, further supporting the validity of the findings [12].

Despite these limitations, the findings of the present study contribute to the evolving body of research on how repurposing the drug timolol can improve skin wound healing. While prior work demonstrated that antagonism of the β2-adrenergic receptor by other drugs can improve epidermal integrity in murine skin barrier models, here we demonstrate that the FDA-approved topical formulation of timolol induces a sustained improvement in the epidermal integrity of a healed DFU wound. The current study provides yet another therapeutic functional role of timolol in the management of chronic wounds and also suggests that TEWL may be a valuable measure in assessing the effectiveness of such treatments in DFUs.

Conclusion

This study demonstrates that the use of topical timolol to heal DFUs leads to sustained, significantly reduced TEWL compared to placebo, suggesting improved skin barrier function and re-epithelialization. The result is sustained for at least 3 months of post-discontinuation of the topical drug application. TEWL in the healed DFU sites was significantly influenced by the interaction between timolol treatment and healing duration, with those healed by timolol treatment exhibiting significantly lower TEWL compared to those healed by placebo. Additionally, the use of a wheelchair was associated with lower TEWL likely due to off-loading benefits. Although initial TEWL values have been proposed as markers for re-ulceration, in this exploratory pilot study, there was no difference detected between re-ulceration rates in the timolol and control groups. Further research with larger sample sizes will be necessary to validate the potential of TEWL as a predictive marker for re-ulceration. These findings nevertheless expand the therapeutic potential of repurposed topically applied timolol in enhancing DFU healing, as well as reinforcing the possibility of TEWL as a valuable noninvasive measure for assessing treatment efficacy.

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Statement of Ethics

This study was conducted at the Veteran Affairs Northern California Healthcare System (VANCHS) and registered on ClinicalTrials.gov (NCT03282981). The VANCHS Institutional Review Board reviewed and approved the study protocol (IRBNet ID 1581979). All participants provided written informed consent prior to enrollment.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

R.E.L.: data curation (equal), formal analysis (equal), writing – original draft (equal), and visualization; M.E.D.: data curation (equal), formal analysis (equal), and writing – original draft (equal); P.L.: investigation, project administration, data curation, and writing – review and editing. A.B. and A.M.: data curation and writing – review and editing; and S.E.D. and R.R.I.: conceptualization, supervision, project administration, and writing – review and editing.

Data Availability Statement

The data supporting the findings of this study are not publicly available due to federal ownership but can be obtained from the corresponding author, R.R.I., upon reasonable request. The completed STROBE checklist is provided as supplementary material (for all suppl. material, see https://doi.org/10.1159/000545357).

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