Original Article

Improved Healing of Diabetic Foot Ulcers After High-dose Vitamin D: A Randomized Double-blinded Clinical Trial

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Peter M. Halschou-Jensen, MD[1], Jannie Sauer[2], Pierre Bouchelouche, MD[2], Jesper Fabrin, MD[2], Stig Brorson, MD[2], and Søren Ohrt-Nissen, MD[2]

Abstract

Background. Chronic foot ulcers are a major cause of morbidity in people with diabetes with a lifetime risk of 25%. Treatment is challenging and the recurrence rates of foot ulcers are >50% after 3 years. Vitamin D deficiency is more common in people with diabetes with chronic foot ulcers, compared to both people without diabetes as well as people with diabetes but without foot ulcers. *Purpose/aim of study*. To assess the efficacy of high-dose compared to low-dose Cholecalciferol vitamin D3 on healing of chronic diabetic foot ulcers. *Materials and methods*. We included people with diabetes with one or more foot ulcers lasting for more than 6 weeks. Patients were randomly allocated to either a daily oral intake of high-dose (170 μg) or low-dose (20 μg) vitamin D3 (Cholecalciferol). We saw patients in the outpatient clinic after 4, 12, 24, 36, and 48 weeks. At each visit, we measured the ulcer with a specialized camera, and associated software and the area (cm^2) was calculated. Patients and assessors were blinded to treatment allocation. We followed all patients for 48 weeks or until wound healing or surgical treatment. *Findings/results*. We included 48 patients in the analysis (24 in each group), with a total of 64 ulcers. Among them 41 ulcers were followed until healing or 48-week follow-up and 20 ulcers were surgically treated during the study period. Three patients were lost for follow-up. The intention-to-treat analysis showed a significantly higher rate of ulcer healing in the high-dose group with 21 of 30 (70%) healed ulcers compared to 12 of 34 (35%) in the low-dose group ($P = .012$). Median ulcer reduction at final follow-up was 100% (interquartile range [IQR]: 72-100) in the high-dose group and 57% (IQR: −28 to 100) in the low-dose group. Furthermore, we found a significant effect of high-dose vitamin D on ulcer reduction in the repeated measures analysis of variance. *Conclusions*. We found high-dose vitamin D3 to be efficient, compared to low-dose vitamin D3, in promoting healing in chronic diabetic foot ulcers.

Keywords

vitamin D supplementation, chronic foot ulcer, diabetes, wound, camera

Introduction

Chronic foot ulcers are a major cause of morbidity in types 1 and 2 diabetic patients. The lifetime risk of chronic ulcers on the lower extremities is around 25% with a prevalence of 7%.[1,2] Treatment is often prolonged and the recurrence rates of foot ulcers are >50% after 3 years.[1] Eighty percent of non-traumatic lower leg amputations are due to complications of diabetes and the 1-year mortality following amputation is between 13% and 40%.[3] One study found that the combined cost of treating the average diabetic lower leg ulcer, including amputation, was US$27.000.[4] As such, the treatment of diabetic ulcer represents a major socioeconomic burden and a severe reduction in quality of life for the patients.

Vitamin D deficiency has been associated with various disorders, including osteoporosis, delayed wound healing, and diabetes.[5-7] Vitamin D is a fat-soluble vitamin that is absorbed through diet or sunlight.[8] Vitamin D regulates more than 200 genes directly or indirectly and plays an important role in the regulation of the immune system, glucose metabolism, and calcium metabolism.[1,6,9] In the skin, vitamin D is responsible for the regulation of cellular proliferation, differentiation, apoptosis, and angiogenesis.[8] In addition, vitamin D increases the ability of keratinocytes to recognize and control microbes, thereby helping to protect wounds from possible infections.[6,9]

[1]Herlev and Gentofte University Hospital, Herlev, Denmark
[2]Zealand University Hospital, Køge, Sjælland, Denmark

Corresponding Author:
Peter M. Halschou-Jensen, Department of Orthopaedic Surgery, Herlev University Hospital, Borgmester Ib Juuls Vej 1, 2730 Herlev, Copenhagen, Denmark.
Email: petermax@dadlnet.dk

A Danish study from 2012 reported vitamin D deficiency in 52.2% of adults aged 30 to 60 years, with the lowest levels measured in February and the highest in August.[10] Thomas et al[11] reported similar results in hospitalized patients in mixed specialities where vitamin D deficiency was measured in ~57%. Another study reported 78% of patients with diabetes to have vitamin D deficiency or insufficiency.[6]

Deficiency of vitamin D is associated with increased insulin resistance and increased risk of developing metabolic syndrome, type 2 diabetes and development of osteoporosis.[12] It is estimated that 1 billion people worldwide have a vitamin D deficiency (serum 25-hydroxy vitamin D [25(OH)D] <50 nmol/L).[6,13] Vitamin D deficiency is more common in patients with diabetes with chronic foot or leg ulcers, compared to patients without diabetes and patients with diabetes but without foot ulcers.[2,6,7,14,15] Greenhagen et al[6] reported diabetic foot ulcers to be associated with low serum vitamin D.

Clinical studies have reported vitamin D supplementation to have an inhibitory effect on the development of osteoporosis, insulin resistance, biomarkers of inflammation, and oxidative stress.[5,8] A randomized trial reported supplementation of vitamin D to have a positive effect on wound healing in diabetic foot ulcers.[5] In this study, ulcer size was measured by hand, and by multiplying the largest by the second largest diameter of the skin lesion, which may be associated with reduced measurement reliability.

The purpose of our study was to assess the efficacy of high-dose oral vitamin D on chronic diabetic foot ulcers healing.

Materials and Methods

Trial Design and Registration

The study was a randomized, parallel, double-blinded, and controlled clinical trial. The study was approved by the regional ethics committee and the local data protection agency.

Participants

We included patients with diabetes and with 1 or more ulcers of the foot or lower leg without complete healing for more than 6 weeks. The patients were recruited in the outpatient wound clinic between April 2016 and July 2018. All exclusion criteria are listed in Table 1. The study did not include patients with critical ischemia, sepsis or ulcers that required surgical debridement upon initial visit. Furthermore, we excluded patients if the camera and software were unable to measure an exact area of the wound, that is, irregular wounds between the toes.

Table 1. Exclusion Criteria.

Pregnancy/breastfeeding
Vitamin D supplements >20 µg daily or a history of diseases influencing vitamin D metabolism
Granulomatous diseases such as tuberculosis, sarcoidosis, or silicosis
Hypercalcemia
Renal impairment (creatinine >150 µmol/L and/or eGFR <40 mL/min), liver failure (ALAT >70 U/L)
Bone disorders, ex. osteogenic sarcoma
Skin tumors
Treatment with epilepsy medication
Uncontrolled hypertension (>150/100 mm Hg)
Need for surgical debridement or vascular surgery

Abbreviations: eGFR, estimated glomerular filtration rate; ALAT, alanine (amino) transaminase.

Study Design

All participants were randomly assigned to either a high-dose or low-dose group and were followed for 48 weeks or until wound healing or surgical intervention.

Intervention

In the high-dose group, patients received oral vitamin D3 (Cholecalciferol) 170 µg (6800 IU/day) for 48 weeks or until wound healing. In the low-dose group, the participants received 20 µg/day oral vitamin D3. Initially, it was considered to establish a placebo group, however, the scientific ethics committee would not approve a dose of 0 µg/day oral vitamin D3 considering the well-known high rate of vitamin D deficiency in this specific patient group. Hence, it was decided not to use placebo. The Danish National Board of Health recommends a daily supplement of 20 µg (800 IU/day) of vitamin D for anyone with vitamin D deficiency, as well as anyone over 70 years of age and anyone at risk of developing osteoporosis.

Apart from the intervention, all participants underwent the usual care regarding ulcer treatment in the outpatient clinic and were advised not to change their ordinary physical activity and not to take any nutritional supplements during the trial.

Treatment Adherence

Demographics and baseline data on medical history, type and dosages of medication, duration of diabetes mellitus, presence of neuropathy, and duration of the foot ulcer were obtained in the outpatient clinic when the patients were included in the study. The participants were given enough supplements to last until the next visit and were instructed to return all unused supplements at each visit. We scheduled patients for follow-up visits at week 4, 12,

24, 36, and 48 for evaluation, blood samples, standard ulcer treatment, and registration.

All patients were treated in the outpatient clinic by specialized wound care nurses and/or physicians. Treatment followed the principles established by the Diabetic foot study group.[16] This included standard offloading either through removable cast walkers or specialized footwear. Total non-weight bearing was only used in 1 patient with poor compliance. Wound care typically involved superficial wound debridement, removal of callus and fibrin but patients had a series of outpatient visits and we did not record specific outpatient wound management for each visit. We recorded all wound treatments that occurred in the operating theatre. Patients who needed wound debridement in the operating theatre at the initial visit were not included in the study.

In cases of surgical intervention or drop-out, the patient was categorized as "not healed" for the primary analysis. For the secondary analysis, the last ulcer measurement prior to surgery or drop-out was noted and carried forward.

Patients were not followed up for ulcer measurements after surgery and so whether they were primarily closed at the surgery did not impact our results. The surgical indication was made by the treating physician but typically involved wound breakdown or infection.

Assessment of Outcomes

The primary outcome was ulcer healing within the study period (48 weeks). Ulcer healing was defined as an ulcer area of 0.0 cm^2. Secondary outcomes included absolute ulcer reduction as well as an assessment of ulcer reduction accounting for repeated measures.

Blood levels of hemoglobin A1C (HbA1C) were measured for evaluations of blood glucose control during the trial; and parathyroid hormone (PTH), 25-OH-D_3, and ionized Ca were measured to monitor vitamin D status and to evaluate the risk of intoxication. All blood samples were measured on a routine basis by the same standardized methods at the laboratory of the local department of clinical biochemistry. The laboratory and laboratory analyses were accredited each year by the Danish Accreditation Fund (DANAK).

For ulcer measurements, we used a Silhouette Star three-dimensional camera from Aranz Medical (Figure 1A). The borders of each ulcer were digitally defined by an observer and the software then calculated the ulcer area (Figure 1B). Prior to the study we performed a pilot study to assess the reliability of the camera/software. Four independent raters, assessing the dimensions of 5 ulcers, 5 times giving 100 measurement's, performed validation of the camera. The variances and coefficients of variation within raters (intrarater) and between raters (interrater) over the 5 ulcers surface area were 2.28% and 4.33%, respectively.

Sample Size

We calculated an adequate sample size of 16 patients in each group, based on the level of significance (α) of 0.05, power (β) of 0.8, and standard deviation (SD) of 5% (ulcer reduction). This allowed for detection of a 10% difference in ulcer reduction. Assuming a substantial dropout rate at 50%, the final sample size was planned to be 24 patients in each group.

Randomization

The participants were randomized using block randomization, each block containing 4 participants. Randomization occurred upon oral acceptance of inclusion in the study and written informed consent.

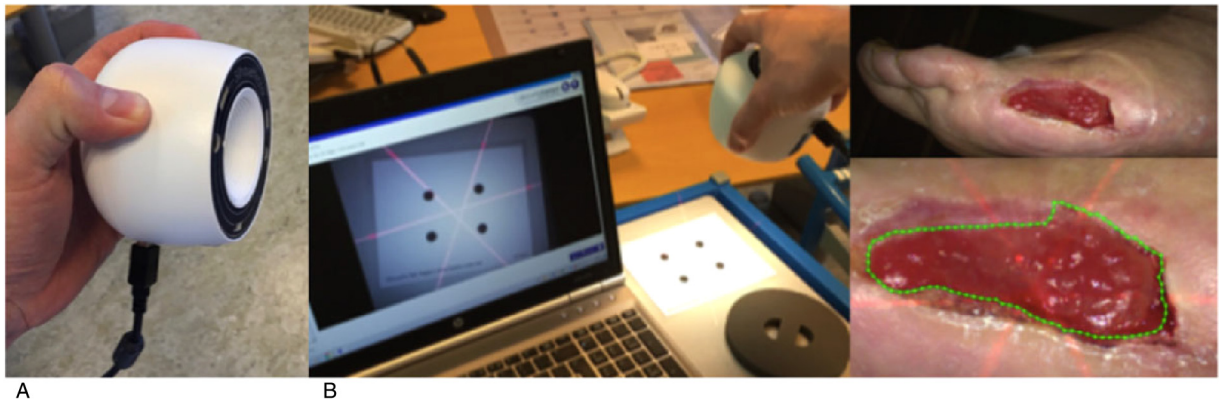

Figure 1. (A) Three-dimensional camera and (B) ulcer measurement with two-dimensional camera.

The randomized allocation sequence, enrolling participants, and allocating them to interventions were conducted by a trained staff at the clinic.

The allocation was concealed from the researchers and participants until all patients had reached the endpoint (48-week follow-up, complete healing or surgical intervention).

Statistical Methods

All statistical analyses were performed using R version 3.4.0 (R Core Team). Data are reported as proportions (%), means ± SDs, or medians with interquartile ranges (IQRs). Data distribution was assessed by histograms. Intention-to-treat analysis was carried out on all included 48 patients (64 ulcers) with a last-value-carried-forward principle. Primary analyses compared the 2 groups using Pearson's χ^2 test to assess the rate of ulcer healing within the study period. Absolute ulcer size at baseline and final follow-up were compared using Wilcoxon rank-sum test. Furthermore, ulcer size was assessed using repeated measures analysis of variance (rmANOVA) to account for the correlation between repeated measurements. Ulcer measurements were square root transformed to ensure that the assumptions of a multivariate normal distribution were met. For the univariate comparative analysis, continuous data were compared between groups using unpaired, 2-tailed *t* test or Wilcoxon rank-sum test. Multiple logistic regression analysis was performed to adjust for potentially confounding factors, including baseline ulcer size and HbA1C. Results are given as odds ratio (OR) with 95% confidence interval (CI). For all analyses, $P < .05$ was considered significant.

Results

Sixty-four ulcers in 48 patients (24 in each group) were included in the analysis (Figure 2). In total, 6 and 5 patients had more than one ulcer in the low-dose and high-dose groups, respectively. All patients but 1 were noted to have neuropathy diagnosed either by clinical examination or standardized monofilament examination. We did not record the grade of neuropathy. Forty-one ulcers were followed until healing or 48-week follow-up. Fourteen patients (20 ulcers) were surgically treated during the study period. Surgical treatment included lower leg amputation (0 patients in the high-dose and 2 in the low-dose group), metatarsal amputation (2 vs 5 patients), toe amputation (2 vs 0 patients) and extensive but bone preserving debridement (1 vs 2 patients).

Two patients were lost to follow-up. Of these, 1 patient (low-dose group) had 2 ulcers of which the first healed quickly. The second ulcer did not heal within the available follow-up period (9 weeks) and was lost to follow-up. The second patient (low-dose group) was lost to follow-up after 14 weeks with a slowly decreasing ulcer area. One

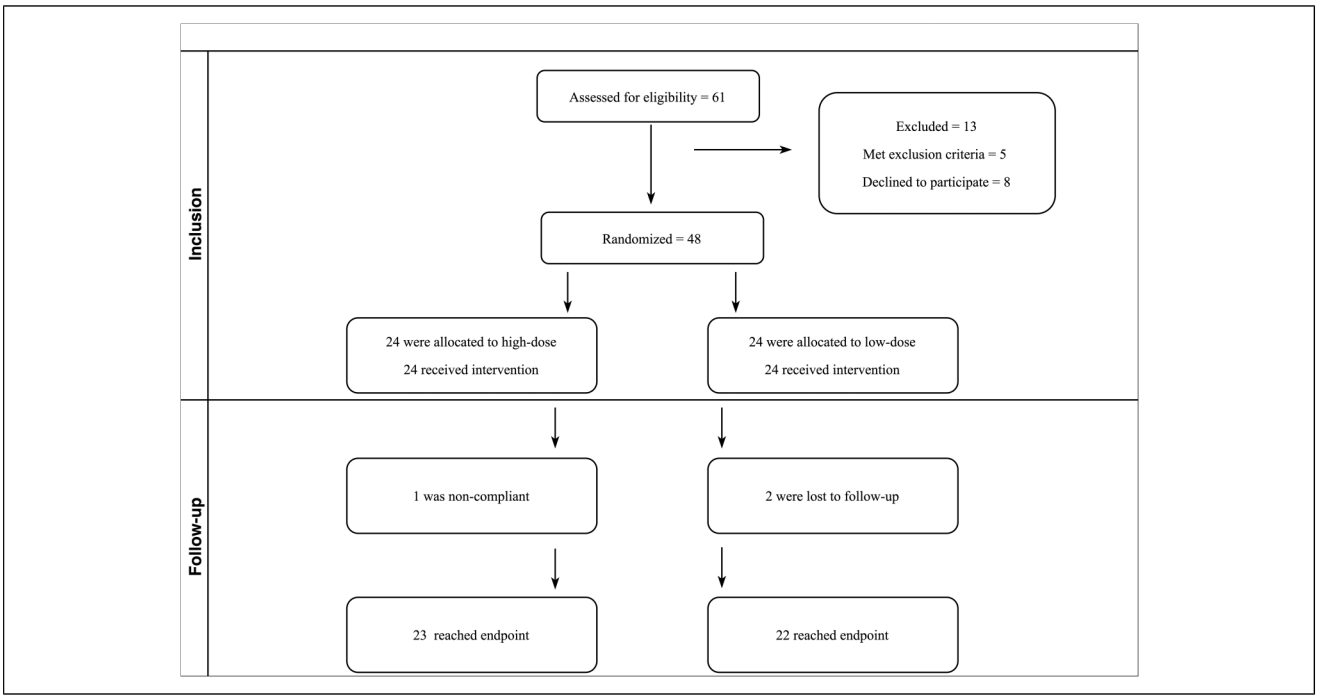

Figure 2. Summary of the patient flow diagram.

patient (high-dose, 2 ulcers) was noncompliant as the patient only took half the prescribed dose of vitamin D. This meant that 61 ulcers in 45 patients were available for the per-protocol analysis.

We further explored the importance of baseline vitamin D deficiency. Twenty-three patients (48%) showed vitamin deficiency at baseline (<50 nmol/L) (Figure 3A). These patients were evenly distributed between the low dose and high dose group (11 vs 12 patients). We did not find any association between initial vitamin D deficiency and healing rate with 15 healed ulcers in the deficiency group and 17 in the nondeficiency group ($P = .951$).

Primary Analysis

The intention-to-treat analysis showed a significantly higher rate of ulcer healing in the high-dose group with 21 of 30 (70%) healed ulcers compared to 12 of 34 (35%) in the low-dose group ($P = .012$) (Table 2). Median ulcer reduction at final follow-up was 100% (IQR: 72-100) compared to 57% (IQR: −28 to 100). Furthermore, we found a significant effect of high-dose vitamin D3 in the rmANOVA ($P = .014$).

The per-protocol analysis also showed a significantly higher rate of ulcer healing in the high-dose group with 20 of 28 (71%) compared to 11 of 29 (37%) healed ulcers in the low-dose group ($P = .023$) (Table 2).

Biochemical Assessment

Figure 3A to D shows the results of the biochemical analyses. Serum 25(OH)D levels at baseline were 54.8 ± 23.8 in the high-dose group and 55.2 ± 28.4 in the low-dose group ($P = .947$) (Figure 3A). The high-dose group showed higher levels of HbA1C at baseline (11.6 ± 2.5 vs 9.8 ± 2.1, $P = .006$) but at the final follow-up, the measurements were similar (Figure 3B). Serum calcium and

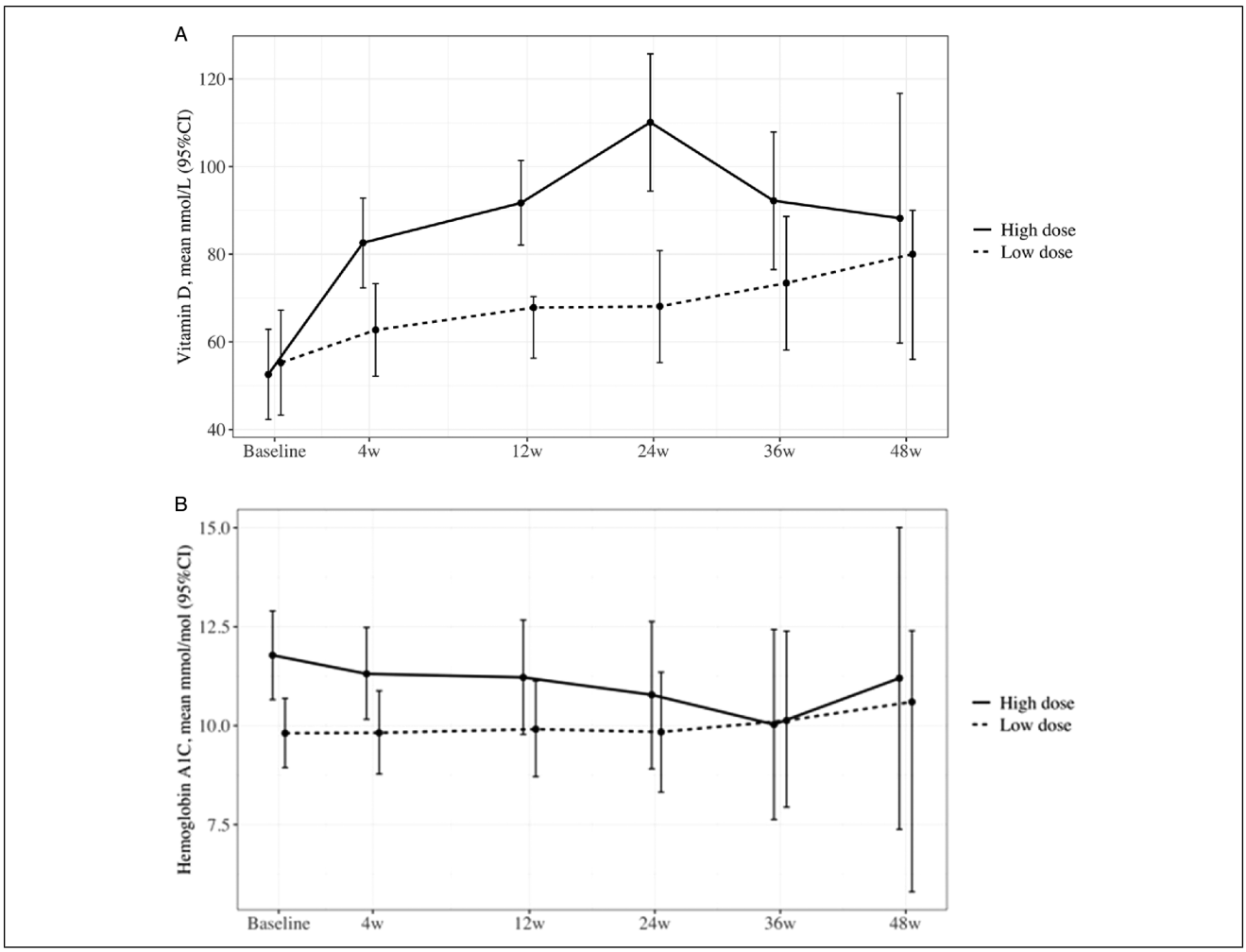

Figure 3. (A–D) Overview of blood samples throughout the study period. *(continued)*

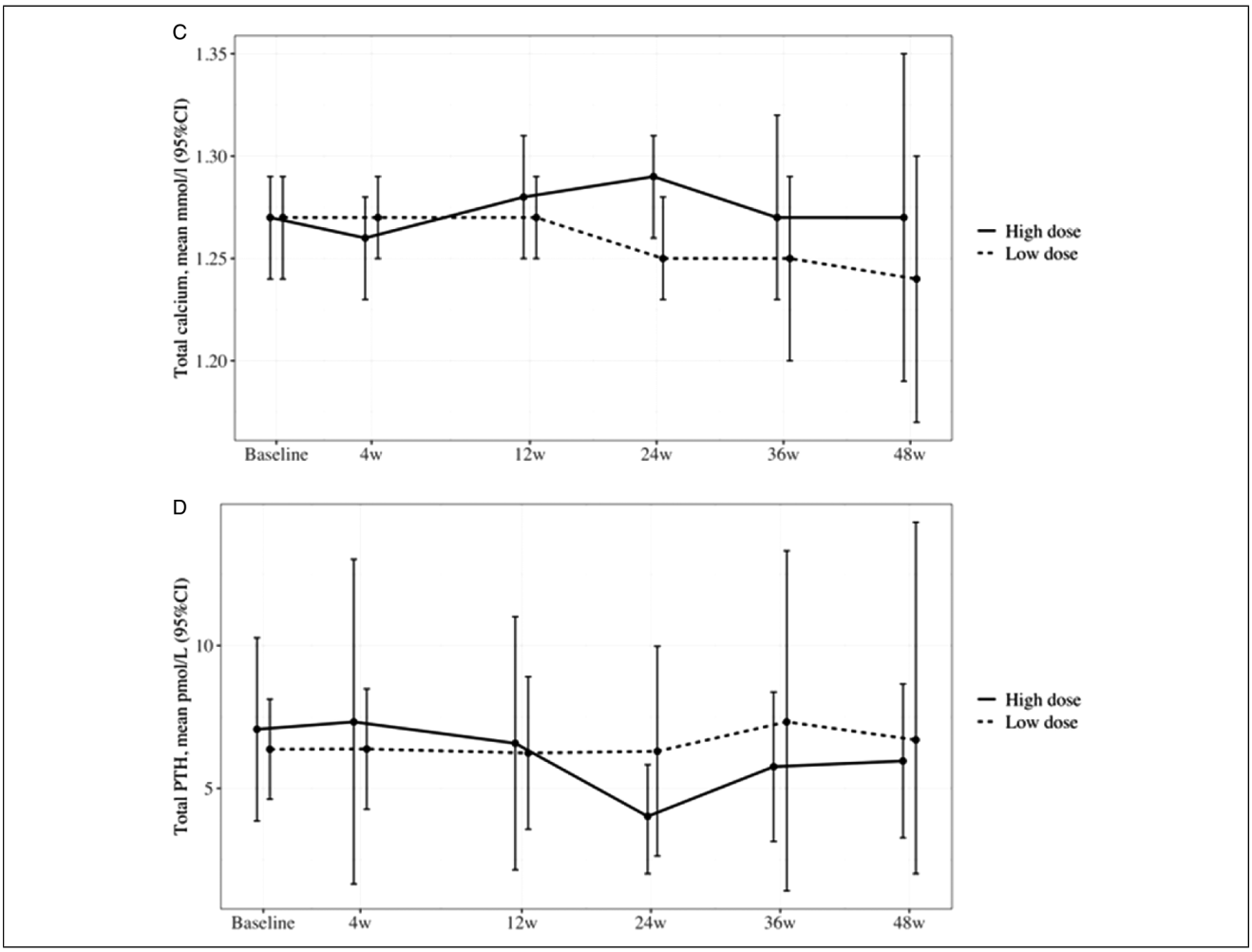

Figure 3. (continued)

PTH levels were similar throughout the study period (Figure 3C and D).

Post hoc Analysis

To estimate the effect of baseline vitamin D on ulcer healing, we compared baseline vitamin D between patients with complete healing at final follow-up and patients with incomplete healing (or surgical intervention). Mean 25(OH)D concentration was 54.75 ± 24.27 versus 56.96 ± 35.85 in the healed and nonhealed groups, respectively ($P = .738$). We observed a considerable difference in baseline ulcer size between the 2 groups as well as higher baseline HbA1C in the high-dose group (Table 2 + Figure 3). Thus, we found it relevant to examine the effect of the key parameters in a multiple logistic regression model with ulcer healing at final follow-up as the dependent variable. We found that a larger baseline ulcer area significantly decreased the odds of healing (adjusted OR = 0.74, 95% CI = 0.57-0.91). Also, high-dose vitamin D significantly increased the odds of healing (adjusted OR = 4.11, 95% CI = 1.11-17.29). Baseline HbAlC did not significantly affect the odds of healing when adjusting for baseline area and vitamin D supplementation (adjusted OR = 0.94, 95% CI = 0.72-1.21).

Discussion

We found a substantial effect of high-dose compared to low-dose vitamin D. The effect was both clinically significant (70% vs 35% healing rate) as well as statistically significant ($P = .012$).

This study mainly describes patients with smaller foot ulcers (mean ulcer size: 1.25 and 2.1 cm^2) although the range was substantial (0.1-20.8 cm^2). To ensure the external validity of our results we included all types and sizes of ulcer to ensure that our cohort represents the large range

Table 2. Demographic and Baseline Characteristics of Study Participants and Ulcer Area.

	High dose	Low dose	*P* value
Age, mean years ± SD	63.3 ± 9.3	64.5 ± 11.4	.688
Female sex, No.	6 (25%)	2 (8%)	.245
Distal toe pressure, mean in mm Hg (SD)	76.3 (37.8)	83.5 (47.3)	.600
Toe/arm pressure index, mean (SD)	0.6 (0.3)	0.6 (0.3)	.841
Diabetes type, No.			
Type 1	6 (25.0)	4 (17.4)	
Type 2, insulin dependent	12 (50.0)	10 (43.5)	
Type 2, noninsulin dependent	6 (25.0)	9 (39.1)	
Ischemic heart disease, No.	7 (29.2%)	9 (39.1%)	
Hypertension, No.	16 (66.7%)	21 (91.3%)	
Hypercholesterolemia, No.	9 (37.5%)	10 (43.5%)	
Psychiatric disorder, No.	0	1 (4.3%)	
Respiratory disease, No.	1 (4.3%)	1 (4.3%)	
Ulcer area at inclusion, median in cm^2 [IQR] (min, max)	1.25 [0.4-3.2] (0.1, 12.4)	2.1 [0.8-5.0] (0.2, 20.8)	.105
Ulcer area at the end of treatment[a], median in cm^2 [IQR] (min, max)	0 [0.0-0.8] (0.0, 11.7)	1.0 [0.0-3.1] (0.0, 18.6)	.018

IQR: interquartile range, SD: standard deviation.
[a]In case of surgical intervention or drop-out, the last ulcer measurement was noted and carried forward.

of diabetic foot ulcers seen in a standard wound clinic. However, due to the distribution of ulcers, we cannot conclude that vitamin D substitution will have the same positive effect on larger ulcers.

Our results are in line with a recently published randomized clinical trial comparing placebo to high-dose vitamin D (50,000 IU vitamin D every 2 weeks for 12 weeks).[5] The authors report a significant reduction of ulcer length and width in the vitamin D group. In this study, however, assessment of ulcer reduction relied on manual measurements of ulcer length, width and depth. Furthermore, the authors provide no description of any surgical interventions although the ulcers were all grade 3 (abscess or osteomyelitis) on inclusion. There are no standardized methods for measuring the size of diabetic foot ulcers although several medical devices have been proposed. For this study, we introduced a handheld camera and software to measure the ulcer area. The device was found to have low intrarater and interrater variations. The photographic record and measurements could be collected in ~2 min and in a noncontact fashion.

Directly related to this project was the question of how much vitamin D was needed to ensure target serum 25(OH)D concentrations. To ensure an optimal high and constant concentration of 25(OH)D vitamin in the blood we aimed at reaching the upper limit of the reference interval for 25(OH)D vitamin level which is 50 to 160 nmol/L. It was important to use a vitamin D dose which gives optimal but not excessive blood levels of vitamin D. A total vitamin D supply of 170 µg/day was therefore used in this study. There was no evidence of adverse effects with this vitamin D dose. The subjects were closely monitored with ionized calcium, PTH, and 25(OH)D serum levels. In our study, serum calcium and PTH levels were similar throughout the study period and no adverse clinical symptoms were experienced. Increases in serum 25(OH)D concentrations were significant and tolerated without developing hypercalcemia. The choice of oral vitamin D dose used in this study is supported by the recommended safe tolerable upper intake level for vitamin D value of 250 µg/day for the general population.[16]

Why does vitamin D improve wound healing? Several reports dating back to the 1970s described striking morphological changes in the skeletal muscle in patients with vitamin D deficiency.[17] Since then, a variety of mechanisms by which vitamin D impacts on muscle cells and fibers have been elucidated. Vitamin D regulates calcium-mediated functions of muscle, namely contraction, plasticity, mitochondrial function, and insulin signaling.[18] Furthermore, vitamin D deficiency is associated with insulin resistance,[19] intramuscular fatty deposition,[20] and muscle weakness.[21] These observational studies suggest broader implications for the pathogenesis of type 2 diabetes. Studies have shown a modest reduction of HbA1C, reduction in blood pressure, and fasting glucose concentration as well as improvements in insulin sensitivity after vitamin D treatment in adults with type 2 diabetes.[22,23]

One limitation of the study was the unexpected difference in baseline ulcer size. Although not statistically significant median ulcer area in the high-dose group was 1.25 cm^2 compared to 2.1 cm^2 in the low-dose group. We addressed this difference in the multiple logistic regression model. When accounting for differences in baseline we still found

4 times higher odds of healing in the high-dose group. Also, unexpectedly, we found higher baseline HbA1C in the high-dose group. This would potentially result in a higher risk of ulcer deterioration.[24] Still, we found a significantly higher rate of ulcer healing in the high-dose group when adjusting for this difference.

Patients with more than one ulcer presented a challenge in terms of analysis and interpretation. We chose to include all ulcers in every patient as the development of one ulcer may influence the outcome of the other (ie, progression to surgical indication). Patients with more than one wound were equally distributed between the 2 groups and the effect of vitamin D was also assessed in the rmANOVA accounting for correlated data.

The main strengths of the study include an objective and reproducible ulcer measurement method, a low drop-out rate and a double-blinded design. Vitamin D supplementation should be considered in diabetic foot ulcers with healing problems. Because of the limitations in our study, we would encourage a future study with a similar design to verify our findings. We cannot recommend only giving supplementation to patients with low vitamin D serum levels as our results indicate that the effect of vitamin D is not simply a matter of "replacing what was missing." There seems to be an additive effect of vitamin D that this study was not designed to elucidate. We would encourage future studies to further describe the mechanism by which vitamin D induces ulcer healing in diabetics, and also whether the effect of supplementation varies depending on ulcer size.

Conclusions

In this double-blinded randomized clinical trial, we found supplementation of high-dose vitamin D3 compared to low-dose vitamin D3, to have a substantial effect in promoting healing in chronic diabetic foot ulcers.

Declaration of Conflicting Interests

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

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Ethical Approval

The trial was conducted in accordance with the declaration of Helsinki and oral and written informed consent was received from all patients. The protocol was approved by the ethics committee and was registered in clinicaltrials.gov.

ORCID iDs

Peter M. Halschou-Jensen https://orcid.org/0000-0002-0009-353X
Stig Brorson https://orcid.org/0000-0001-5337-758X

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