ABSTRACT

Background: Hypovitaminosis D following burn injury has been reported in patients who develop scars. This retrospective study examined associations between vitamin D levels and abnormal scar formation in patients supplemented with different vitamin D regimens.

Materials and Methods: In a previous prospective study, fifty children with >30% total body surface area burns were randomized to daily inpatient supplementation with ergocalciferol (vitamin D$_{2}$), cholecalciferol (vitamin D$_{3}$), or placebo, which was administered in addition to a standardized nutritional protocol for acute care of burned children. The current retrospective study compared demographic and inpatient data from these patients, including biomarkers of vitamin D status, with outcomes related to abnormal scar development. Keloid or hypertrophic scar formation was determined from chart review, physician diagnosis, and photographic documentation.

Results: A trend was observed for reduced blood levels of cholecalciferol and calcidiol at the time of hospital discharge in patients who developed keloids or hypertrophic scars. There was no association between PTH and calcitonin levels and abnormal scar formation.

Conclusion: Reduced vitamin D levels in pediatric burn patients may be a contributing factor in development of keloids and hypertrophic scars. Further investigation is needed to confirm these findings, establish the mechanism of this relationship, and develop clinical guidelines for vitamin D supplementation in pediatric burn patients.

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KEY POINTS
Abnormal wound healing after burn injuries may result in keloids or hypertrophic scars. Burn patients are at risk for decreased vitamin D levels. This retrospective study makes clinicians aware of a possible association between reduced vitamin D levels and keloid or hypertrophic scarring, supporting the need for dietary vitamin D supplementation in pediatric burn patients.

INTRODUCTION
Keloids are benign overgrowths of scar tissue beyond the margins of an original wound with characteristic extracellular matrix overproduction, stiffness, and a raised, erythematous, pruritic presentation. In contrast, in hypertrophic scarring the erythematous and pruritic scar does not progress past the boundaries of the original wound. The timelines of scar formation differ between hypertrophic scars (HTS), which tend to regress after a multi-year maturation process, and keloid scars, which continue to proliferate in all dimensions beyond the injury site and rarely regress over time. Clinically, it may be difficult to distinguish between keloids and HTS, and although histopathological analysis is used to differentiate the two types of scars, this can be challenging due to morphological changes over time and shared histological features.

Risk factors for keloid and HTS development are wide-ranging and include time required for the wound to heal, dark skin color, young age, and burn severity. In particular, the risk of keloids is significantly elevated in Blacks and individuals of African descent. Second- and third-degree burns are more likely to lead to scarring, especially in pediatric patients.

Methods to prevent or reduce keloid and HTS formation are still evolving. Current prevention and treatment strategies for burn-related scarring target the inflammatory response in wound healing in order to reduce abnormal collagen production and fibrosis. Keloid and HTS are both treated with topical and oral immunomodulatory drugs, pressure therapy garments and intradermal injections of steroids or other agents. However, treatment with corticosteroid injection, often used as a first-line therapy, showed no significant reduction in incidence of burn-related keloids in a recent study of pediatric patients.

Antihistamines have been used to reduce symptoms including pruritus and pain associated with keloid scars. Surgical strategies include excision with skin grafts, which may present additional risk for keloid development at the donor site, or primary closure. Surgical excision of keloids is associated with high rates of recurrence, although surgical management of HTS can be used to release skin tension and decrease hypertrophy. Recent treatment approaches also include pulsed CO₂ laser, radiation, and combination therapies. All current therapies have variable efficacy and some, such as radiation treatment, may have undesirable side effects.

Vitamin D treatment of keloid fibroblasts reduced fibrotic responses to growth factor stimulation, and vitamin D receptor (VDR) expression was reported to be reduced in keloid scar epidermis, suggesting involvement of vitamin D in keloid pathology. Vitamin D is produced in skin keratinocytes in response to sunlight exposure; thus, it has been hypothesized that reduced vitamin D production in individuals with darkly pigmented skin might contribute to a higher risk of abnormal scar formation. Vitamin D has antiproliferative, immunosuppressive, and antiﬁbrotic properties, and was shown to inhibit proliferation of keloid fibroblasts, suggesting mechanisms whereby hypovitaminosis D may play a role in development of keloids and HTS. The prevalence of hypovitaminosis D among burn patients is well-documented. Contributors to suboptimal vitamin D status can include lack of exposure to sunlight due to long periods of hospitalization, inadequate vitamin D intake, metabolic derangement, and malabsorption. Vitamin D deﬁciency in burn patients may extend beyond the period of hospitalization, lasting many years after injury. In one study, vitamin D deﬁciency and reduced vitamin D production were reported in children with burn scars at a mean of 14 months after injury. That study reported reduced vitamin D production in biopsies of burn scars, as well as adjacent normal skin, suggesting that skin scarring may contribute to vitamin D deﬁciency, although no cause-and-effect relationship was demonstrated.

Vitamin D is involved in calcium homeostasis and proper bone formation; thus, bone loss is a common outcome in patients with large burn injuries. There is mounting evidence that vitamin D also has a biological role in wound repair; vitamin D deﬁciency is linked to hyperproliferative diseases and progressive tissue ﬁbrosis, and vitamin D was shown to regulate the inﬂammatory response to cutaneous wounding. A previous prospective study at our institution, a pediatric burn hospital, investigated the potential impact of supplementation with different forms of vitamin D on vitamin D status and clinical outcomes. In that study, supplementation with either ergocalciferol (vitamin D2)
or cholecalciferol (vitamin D3), in addition to a standard nutritional regimen that included vitamin D, had no significant effects on clinical outcome or biomarkers of vitamin D status. Patients in all groups had low serum vitamin D levels one year following hospital discharge. The quantitative data collected in that study provided a patient cohort with known biomarker levels, enabling further investigations of vitamin D status and scar formation. The current retrospective study utilized that patient cohort to examine the possible relationship between hypovitaminosis D and abnormal scarring in burn patients.

**Methods**

A retrospective secondary analysis was conducted with the approval of the University of Cincinnati Institutional Review Board (IRB) using medical records for patients who had previously participated in a prospective trial of vitamin D supplementation at the Shriners Hospitals for Children-Cincinnati. Complete details of the prospective study design and results have been published previously. Briefly, the prospective study was designed to investigate the effects of supplementation with ergocalciferol or cholecalciferol on vitamin D status and clinical outcomes in pediatric burn patients. A cohort of 50 patients with a mean age of 7.5 ± 0.8 years and a mean total body surface area (TBSA) burns of 55.7 ± 2.5% was enrolled in the prospective trial during their acute burn injury treatment period. Exclusion criteria included liver disease, chronic renal disease, gastric or bowel resection, or a history of either anticonvulsant or pharmacological vitamin D use. All patients received a standard multivitamin supplement containing 800 IU cholecalciferol per liter of tube feeding, as per standard of care at our institution. The patients were randomized to one of three groups, with daily supplementation of an additional 100 IU/kg/day of ergocalciferol, cholecalciferol, or placebo. Vitamin D biomarkers, including serum cholecalciferol, calcidiol, calcitriol, parathyroid hormone (PTH), and calcitonin, were obtained at 4 time points post-burn: baseline (at time of admission to Shriners Hospital, 2-7 days after initial injury), midpoint of hospital stay (based on TBSA), discharge, and 1 year follow-up. Data are only reported here for cholecalciferol, calcidiol, PTH, and calcitonin at the time of hospital discharge.

Charts of patients involved in the prospective vitamin D supplementation trial were reviewed for documentation of scar development. Patient demographics (age, race, and gender) were recorded. Latitude of home state was determined, and locations ≥ 36 degrees latitude were classified as “Northern.” Classification or initial diagnosis of scar recorded by a physician was recorded and confirmed with evaluation of patient photographs. Physician diagnosis of scarring included documentation of any of the following: keloid, cheloid, hypertrophic scar, or the ICD-9 code 701.4. Any distinguishing factors in photographs such as hyperemia, hypertrophy, contracture, or margin overgrowth were noted.

For statistical analysis, the Kruskal-Wallis test was used to compare vitamin D biomarkers between groups due to non-Gaussian distribution and sample size; where the overall test was statistically significant, pairwise Wilcoxon rank sum tests were run, using a Bonferroni correction for multiple testing. The median values with maxima and minima are reported, unless otherwise specified. For the categorical variables, differences in proportion were determined using Fisher’s exact test, due to the sample size. Where the 3-group test was significant, pairwise tests were run using a Bonferroni correction for multiple testing. P-values <0.05 were considered statistically significant, both unadjusted and adjusted.

**Results**

Patient demographic data is presented in Table 1. Patients in the original prospective study were randomized into one of three groups to receive supplementation with ergocalciferol, cholecalciferol, or placebo, in addition to the standard of care nutritional formulation. Subjects in the study were identified with keloid scarring, hypertrophic scarring, or no keloid or HTS, as shown in Table 1. Because no differences in clinical outcomes or vitamin D biomarker levels were observed among the three treatment groups, the patients were considered as a single cohort in the current analysis. Of the 50 patients, four subjects (8%) developed keloid scars and 29 (58%) developed HTS. None of the subjects who developed keloids were in the group supplemented with ergocalciferol, and only 1 (25%) was in the group supplemented with cholecalciferol. 75% were in the placebo group. However, the distribution of patients with keloid or HTS compared to those without abnormal scarring in each supplementation group was not statistically significant (p= 0.15).

Demographics were comparable between subjects that developed keloids or HTS and those that had no known abnormal scars; no significant differences were observed in age, race, or sex (Table 1). No significant differences in percent TBSA burns was observed between
patients with keloids, HTS, or no abnormal scarring, although patients with keloids or HTS had significantly lower %TBSA full thickness burns (P = 0.006). There was no significant difference in the number of patients with keloids, HTS, or no abnormal scarring who required post-pyloric enteral feeding (“tube feeding”). However, significant differences were observed among scar groups for the number of tube feeding days (P = 0.004): patients who had keloid scarring had a median of 21 days, compared with 35 days for HTS patients and 61 days for patients with no keloids or HTS (Table 1). There was a trend for Northern geographic locations for the homes of keloid patients (100%) compared with HTS patients (72%) and patients with no keloids or HTS (53%), but the differences were not statistically significant (Table 1).

Patients in the keloid group had a median of 7 areas of scarring compared to 3 in the hypertrophic scar group (data not shown). Body sites of keloid and hypertrophic scarring observed in this cohort are listed in Table 2. The most common location for keloids and HTS was the head and neck region (52% of patients, 24% of all scars), followed by the upper torso and chest region (44% of patients, 17% of all scars).

Circulating levels of cholecalciferol at the time of hospital discharge were lower in patients with keloid or hypertrophic scarring, compared with patients with no keloids or HTS, but the differences were not statistically significant (Table 3). Similarly, there was a higher frequency of patients with very low cholecalciferol levels (<25 ng/ml) in keloid scar patients (67%) compared with the HTS group (48%) or no abnormal scar group (19%), but again the differences were not statistically significant (Table 3). Similar results were observed for calcidiol, with the lowest median levels observed in keloid scar patients (25.7 ng/ml), followed by HTS patients (37.8 ng/ml) and then patients with no keloid or HTS (44.4 ng/ml), but the differences did not reach statistical significance.

PTH and calcitonin levels were measured because these hormones are involved in calcium homeostasis and can affect vitamin D levels (Figure 1). PTH stimulates
calcium release from bones to maintain serum calcium levels, and also upregulates conversion of 25-vitamin D to the biologically active form 1,25-vitamin D. Calcitonin opposes the effects of PTH, lowering serum calcium levels. No significant differences in PTH were observed among patients with keloids, HTS, or no abnormal scarring, although median levels at discharge were below the normal range of 15-56 pg/ml (Table 3). Median calcitonin levels at discharge were below the normal range of 3-19 pg/ml for patients with keloids or HTS (2.4 and 2.0 pg/ml, respectively), but the median level for patients with no abnormal scarring was at the lower limit of the normal range, 3.0 pg/ml (Table 3). However, there was a wide range of values among patients in each group and the differences were not statistically significant.

**Discussion**

Scar formation is a common secondary effect of acute burn injury. Keloid and hypertrophic scars can be cosmetically disfiguring and prevent normal joint movement and growth, both of which can be distressing particularly for the pediatric patient. Identification of risk factors that contribute to formation of keloids and hypertrophic scars can help guide prevention strategies and development of effective treatments. Consistent with their widespread injuries and increased metabolic demands, pediatric patients with acute burn injuries have compromised vitamin D status, with deficient levels of various forms of vitamin D throughout the course of acute hospitalization. This retrospective study was conducted to determine whether a relationship exists between vitamin D status and scar formation following burn injury in children.

In this patient cohort, no significant association was found between the incidence of keloid or hypertrophic scar formation and blood levels of cholecalciferol or calcidiol, although trends were observed that suggested lower levels in keloid scar patients. The lack of statistical significance was likely due to the relatively small number of patients developing keloid scarring in this patient cohort, and the high level of variability in vitamin D levels at discharge, particularly for patients with HTS and no abnormal scarring. Thus, the study likely was insufficiently powered to detect a significant difference among groups.

A surprising finding of this study was that patients with keloids and hypertrophic scars had lower % TBSA full thickness burns than patients with no abnormal scarring. In particular, patients who developed HTS had significantly lower % TBSA full thickness burns compared with patients with no keloids or HTS. This may be due to treatment of excised full thickness burns with skin autografting, which is considered the standard of care, whereas excised partial thickness burns may have been allowed to heal spontaneously without skin grafting, which could increase the risk of HTS formation.

### TABLE 2: Locations of keloids and hypertrophic scars.

Shown are total numbers of all keloid and hypertrophic scars identified in 50 patients enrolled in study.

<table>
<thead>
<tr>
<th>Body Site</th>
<th>Number of Patients N (%) of 50 patients</th>
<th>Number of Scars N (%) of 186 scars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head/neck</td>
<td>28 (52%)</td>
<td>44 (24%)</td>
</tr>
<tr>
<td>Upper torso/chest/axilla</td>
<td>22 (44%)</td>
<td>31 (17%)</td>
</tr>
<tr>
<td>Hand(s)</td>
<td>19 (38%)</td>
<td>37 (20%)</td>
</tr>
<tr>
<td>Leg(s)</td>
<td>19 (38%)</td>
<td>28 (15%)</td>
</tr>
<tr>
<td>Arm(s)</td>
<td>9 (18%)</td>
<td>9 (5%)</td>
</tr>
<tr>
<td>Back/shoulder</td>
<td>8 (16%)</td>
<td>10 (5%)</td>
</tr>
<tr>
<td>Foot(s)</td>
<td>7 (14%)</td>
<td>9 (5%)</td>
</tr>
<tr>
<td>Perineum</td>
<td>6 (12%)</td>
<td>7 (5%)</td>
</tr>
<tr>
<td>Abdomen/flank</td>
<td>6 (12%)</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>Buttock</td>
<td>4 (8%)</td>
<td>5 (3%)</td>
</tr>
</tbody>
</table>
Another surprising finding was that patients with keloid scarring had significantly fewer tube feeding days compared with patients who did not develop abnormal scars. This may be explained by differences in the ranges of burn severity among the different scar groups. Although the median %TBSA burn was similar among all groups, the range of burn sizes differed: the largest burn in the four patients who developed keloids was 61.5% TBSA (54.5% full thickness), whereas the largest burns in the HTS and no abnormal scar groups were 95.6% (94.0% full thickness) and 88.0% (88.0% full thickness), respectively. Thus, the median number of tube feeding days may have been greater in the HTS and no abnormal scar groups because these groups included patients with extremely large burns.

The most common locations for development of keloids and HTS in this patient group were the head, upper torso and chest, hands, and legs. Similar findings have been previously reported by others, and this is consistent with the concept that skin tension contributes to the formation of abnormal scars.

This retrospective study had several limitations, in addition to the relatively small number of subjects. As mentioned above, it is not known whether keloids or HTS occurred primarily in full-thickness or partial-thickness wounds, and we do not know what percentage of donor sites resulted in abnormal scars. Because this study was retrospective, data was limited to that previously collected during the prospective study or obtained from the patient medical records. Another limitation is that diagnosis of keloids and HTS was primarily based on physicians’ clinical assessments, without histopathological analysis for confirmation. Diagnostic criteria for distinguishing keloids from hypertrophic scars generally includes whether the scar spreads beyond the original wound margin. However, scars are dynamic and changes in size or appearance may result in changes in diagnoses over time. Patient photographs were available for retrospective review during the IRB-approved data review period for the study, and the initial diagnoses made by the treating physicians were used.

### Table 3: Vitamin D biomarker levels at discharge, stratified by scar diagnosis.

Data are shown for all patients included in the original prospective vitamin D study for whom measurements of biomarkers were available (N = 46 total). Median (minimum, maximum) values are shown unless otherwise specified.

<table>
<thead>
<tr>
<th></th>
<th>Keloid N=3</th>
<th>HTS N=27</th>
<th>No keloid or HTS N=16</th>
<th>P-value overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholecalciferol, ng/ml (range)</td>
<td>24.0 (21.0-29.0)</td>
<td>25.1 (10.0-74.6)</td>
<td>34.9 (18.4-58.0)</td>
<td>0.13</td>
</tr>
<tr>
<td>Cholecalciferol &lt;30 ng/ml, N (%)</td>
<td>3 (100)</td>
<td>16 (59)</td>
<td>6 (38)</td>
<td>0.11</td>
</tr>
<tr>
<td>Cholecalciferol &lt;25 ng/ml, N (%)</td>
<td>2 (67)</td>
<td>13 (48)</td>
<td>3 (19)</td>
<td>0.09</td>
</tr>
<tr>
<td>Calcidiol, ng/ml (range)</td>
<td>25.7 (12.6-30.0)</td>
<td>37.8 (12.6-75.4)</td>
<td>44.4 (22.0-59.0)</td>
<td>0.21</td>
</tr>
<tr>
<td>Calcidiol &lt;30 ng/mL, N (%)</td>
<td>2 (67)</td>
<td>7 (26)</td>
<td>4 (25)</td>
<td>0.37</td>
</tr>
<tr>
<td>PTH, pg/ml (range)</td>
<td>7.0 (3.0-12.0)</td>
<td>12.0 (1.8-56.0)</td>
<td>8.0 (1.4-31.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>PTH &lt;15 pg/mL, N (%)</td>
<td>3 (100)</td>
<td>18 (67)</td>
<td>11 (69)</td>
<td>0.68</td>
</tr>
<tr>
<td>Calcitonin, pg/ml (range)</td>
<td>2.4 (2.0-13.0)</td>
<td>2.0 (1.2-7.6)</td>
<td>3.0 (1.0-14.2)</td>
<td>0.53</td>
</tr>
<tr>
<td>Calcitonin &lt;3 pg/mL, N (%)</td>
<td>2 (67)</td>
<td>16 (59)</td>
<td>8 (50)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Abbreviations: HTS, Hypertrophic scar; PTH, parathyroid hormone.

Note: Vitamin D biomarker levels were not available for 1 Keloid, 2 HTS and 1 no Keloid or HTS subject.
physicians were confirmed by comparisons with chart record photographs to the best of our abilities. However, errors in diagnosis of keloids or HTS may have occurred, particularly because abnormal scar formation was not a focus of the initial prospective study. The absence of an established, validated set of criteria for differential diagnosis of keloids and HTS is a limitation for most if not all clinical studies involving abnormal scarring.

A further limitation of the current study is that patients were from multiple different regions with different levels of daily sun exposure prior to their burn injuries. The study was conducted at a single pediatric burn hospital; however, the patient population comes from a very wide geographic area. Further, vitamin D levels in the patients prior to burn injury were not known. Records of vitamin D status prior to injury could provide further characterization of subjects’ baseline cholecalciferol-synthesizing ability and may identify populations predisposed to abnormal wound healing. Longer-term follow up vitamin D levels and reliable assessment of post-

![Diagram of Vitamin D Metabolism](image)
discharge vitamin D supplementation could further clarify the likelihood of abnormal wound healing in various contexts of vitamin D status. Skin integrity and wound healing rates also change with overall nutritional status and age, which could be further examined to determine the individual impact of vitamin D deficiency on abnormal wound healing. Examination of a larger patient cohort undergoing randomized clinical intervention with vitamin D supplementation, with long-term assessment of vitamin D biomarkers and scar formation, is needed to confirm this study’s preliminary associations between hypovitaminosis D and scarring. Greater ethnic diversity in a larger population analysis would also provide insight into the relationship(s) among vitamin D status, ethnicity, skin pigmentation, and likelihood of abnormal scarring.

The pathogenesis of keloids and HTS is postulated to involve over-exuberant healing with excess deposition of connective tissue during the inflammatory and proliferative stages of wound healing. Results from preclinical studies suggest that, in addition to a role in bone loss, the clinical relevance of vitamin D following burns may include its potential extra-skeletal effects on cell proliferation and differentiation. Fibroblasts from keloid scars have been shown to express higher levels of transforming growth factor beta 1 (TGF-β1), which is involved in collagen deposition and extracellular matrix formation during the proliferative phase. Keloid fibroblasts are more sensitive to TGF-β1, requiring a lower dose to achieve the same responses as normal fibroblasts. Expression levels and nuclear localization of VDR were reported to be reduced in keloid scar epidermis. However, keloid fibroblasts in vitro express VDR, and treatment with calcitriol was shown to counteract the TGF-β1-induced expression of collagen I, fibronectin, and alpha smooth muscle actin. Cholecalciferol is an anti-inflammatory mediator that indirectly inhibits NF-κB expression, reducing levels of transcription factor binding to the promoter regions of the interleukin 6 (IL-6) and IL-8 genes, which encode proinflammatory cytokines that are overexpressed in keloid fibroblasts. Thus it is tempting to speculate that vitamin D might have therapeutic potential for suppression of keloid scarring by counteracting the profibrotic effects of TGF-β1 and reducing expression of proinflammatory mediators in keloid fibroblasts.

Vitamin D has a well-established role in regulation of epidermal proliferation and differentiation. Recent studies suggest keratinocytes may play a significant regulatory role in promoting the fibroblast proliferation characterizing keloid formation. This suggests a possible role for vitamin D in regulation of keloid fibroblasts both indirectly, via regulation of keratinocyte function, and directly, by controlling the fibrotic phenotype of keloid fibroblasts. Derangements of vitamin D signaling have been observed in psoriasis, an inflammatory skin disease characterized by epidermal hyperproliferation. Low vitamin D status has been associated with psoriasis, and decreased VDR expression was reported in psoriatic skin samples. Oral vitamin D supplementation and topical vitamin D application are commonly used to control psoriasis, and can be used without serious adverse side effects.

Burned human skin and adjacent normal skin both showed a 5-fold decrease in the conversion of 7-dehydrocholesterol to cholecalciferol. This deficit of active vitamin D production is exacerbated by the limited sun exposure of children recovering from burns, as they are indoors during lengthy hospital stays and often experience pruritus and pain when exposed to sunlight after discharge. Hypothetically, vitamin D deficiency after burns may increase the risk of keloid and HTS. Additional vitamin D supplementation during hospitalization and after discharge may play an important role in prevention of abnormal scarring.

Despite limitations due to sample size and retrospective study design, the current study supports the emerging body of literature regarding the relationship between vitamin D status and quality of wound healing. Improved therapeutic options and preventative strategies for keloids and HTS are greatly needed. The possibility that vitamin D might represent a relatively safe therapeutic approach for suppression of keloid or hypertrophic scarring warrants additional investigation. Further research is required to determine parameters for ideal vitamin D levels in children as well as appropriate clinical guidelines for vitamin D supplementation in both acutely hospitalized and post-discharge pediatric burn patients in order to prevent keloid and hypertrophic scar formation.

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Author Contributions
Each author contributed to review of data and writing and revision of manuscript.

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REFERENCES


