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Crosstalk among adipose tissue, vitamin D level, and biomechanical properties of hypertrophic burn scars

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ABSTRACT

Purpose: This cross-sectional study aimed to investigate whether adipose tissue loss and reduced vitamin D levels following severe burn injury are associated with pathologic scar formation and biomechanical scar properties.

Methods: A total of 492 male subjects with hypertrophic burn scars were enrolled from January 2014 to July 2018 and analyzed. Body fat content was measured using dual-energy X-ray absorptiometry. Values of melanin, erythema, and trans-epidermal water loss (TEWL) and the distensibility and elasticity of hypertrophic scars were examined using pigment- and TEWL-measuring devices and a suction skin elasticity meter.

Results: Burn patients with higher fat percentage tended to have higher 25(OH) vitamin D levels ($P < 0.001$). As body fat percentage increased, hypertrophic scars showed higher mean value of Uf (distensibility, $P < 0.001$) and lower mean value of Uv/Ue (viscoelasticity or interstitial fluid shifting, $P < 0.001$). Burn patients with higher 25(OH) vitamin D levels tended to have higher mean values of Uf ($P < 0.001$) and Ua/Uf (gross elasticity, $P = 0.013$) and lower mean value of Uv/Ue ($P = 0.008$).

Conclusion: Adipose tissue loss and decreased 25(OH) vitamin D levels following burn injury were related to scar rigidity and slow interstitial fluid shifting in hypertrophic scars.

1. Introduction

Hypertrophic scar formation is one of the common complications following burn injury, with a reported prevalence rate of 32–72% [1]. In addition to pain and itching, the rigid, non-pliable, raised hypertrophic scars can lead to cosmetic, psychological, and functional problems [2]. The frequency of pathologic and excessive scar formation is associated with prolonged wound healing, burn severity, occurrence of burn neck and upper limbs, meshed skin graft, dark skin, and young age [3]. Growing evidence suggests the participation of keratinocytes, mast cells, and T lymphocytes in the excessive deposition of extracellular matrix via direct or indirect activation of fibroblasts [4]. Interestingly, these cells, which are associated with hypertrophic scar formation, express vitamin D receptors [5], upon which vitamin D activities are dependent [6].

Abbreviations: BMI, body mass index; BSA, body surface area; DXA, dual-energy X-ray absorptiometry; TEWL, trans-epidermal water loss; TGF, transforming growth factor.

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Aside from regulating calcium homeostasis and bone metabolism, vitamin D plays pleiotropic roles in epidermal permeability barrier maintenance, cancer progression, and cell apoptosis [7,8]. Furthermore, 1,25(OH) vitamin D is involved in fibroblast and keratinocyte proliferation in the human dermis and epidermis [9,10], and low vitamin D levels following burn injury contribute to pathologic scar formation, resulting in prolonged wound healing in the early inflammatory phase [7,11].

Vitamin D receptor is interestingly expressed in adipose tissues as well [12,13], which have been acknowledged as the major storage and releasing site for vitamin D_3 [14,15]. Vitamin D_3 concentrations in adipose tissues are positively correlated with serum vitamin D level [16]. Intradermal adipocytes affect skin wound healing by mediating fibroblast recruitment and dermal reconstruction [17]. Furthermore, adipocytes suppress the proliferation of fibroblasts in dermis but promote the keratinocytes proliferation and differentiation in epidermis [18]. The hypermetabolic response after burn injury can result in the loss of adipose tissues and muscles, altering lipid, amino acid, and glucose metabolism [19]. To our knowledge, the effects of adipose tissues and vitamin D on biomechanical hypertrophic scar properties have scarcely been elucidated. Therefore, we hypothesized that adipose tissue loss and reduced vitamin D levels following severe burn injury may influence pathologic scar formation and biomechanical scar properties. To evaluate the crosstalk among adipose tissue, vitamin D level, and biomechanical hypertrophic scar properties, we investigated the total body fat percentage; 25(OH) vitamin D plasma levels; values of melanin, erythema, and trans-epidermal water loss (TEWL); and distensibility and elasticity of hypertrophic scars in burn patients.

2. Materials and methods

2.1. Subjects with hypertrophic burn scars

The present study was a cross-sectional study involving burn inpatients who were transferred from the burn center to the rehabilitation unit without returning home. A total of 1634 subjects (1418 men, 216 women) were initially enrolled from January 2014 to July 2018. However, 1106 subjects were excluded because they had at least one of the following criteria: (1) age <19 years or >50 years ($n = 472$); (2) burned body surface area (BSA) <20% ($n = 524$); (3) chronic disease involving the lungs, heart, parathyroid glands, and kidneys ($n = 34$); (4) intake of vitamin D supplements ($n = 26$); (5) intake of medications for osteoporosis ($n = 5$); and (6) missing information on one or more clinical values ($n = 45$). Additionally, 36 women were removed from the analysis because of small sample size. Finally, 492 men with hypertrophic burn scars were analyzed in this study.

Burn wounds should completely be healed to examine burn scar using pigment- and TEWL-measuring devices and a suction skin elasticity meter. Complete wound healing was defined as assessed and agreed upon by one rehabilitation specialist and one surgeon. After burn wound completely healed, the patients were directly sent from the burn center to the rehabilitation unit for blood sampling and scar evaluation without returning home. Data on 25(OH) vitamin D plasma levels, percentage of burned BSA, duration from burn injury to examination, burn type, body mass index (BMI), body fat content, and biomechanical scar properties were collected.

The study was approved by the institutional review board, and the requirement for acquisition of written informed consent from the study subjects was waived owing to the nature of our study.

2.2. 25(OH) vitamin D plasma levels

Blood sampling was performed in the morning following fasting for >8 h. In clinical practice, 25(OH) vitamin D level is routinely measured to evaluate vitamin D level because 25(OH) vitamin D is the prominent circulating type [12,20]. In this study, 25(OH) vitamin D levels were confirmed using a radioimmunoassay analyzer (ADVIA Centaur XPT; Siemens, Munich, Germany) within 24 h of refrigerated storage. Any values less than the lowest standard, 5.0 ng/mL, are reported as "<4.2" and the lowest value was determined as 4.2 ng/mL.

2.3. Assessment of body fat content

Dual-energy X-ray absorptiometry (DXA) (Lunar®; GE Medical Systems Monterrey Mexico SA de CV, Apodaca, Mexico) was used in all subjects to measure body composition. DXA examinations were conducted in supine position following removal of any item that could interfere with the results. The report provided BMI (kg/m^2), total body lean mass and fat mas, and body fat percentage. Compared with computed tomography scan and magnetic resonance imaging, DXA is the appropriate approach for measuring lean, fat, and bone mass with respect to radiation exposure and cost [21].

2.4. Melanin, erythema, and TEWL

Mexameter® MX18 (Courage + Khazaka electronic GmbH, Cologne, Germany) was used to examine melanin and erythema levels in hypertrophic scars. Measurement was obtained as soon as the scar contacted with the sensor. Skin barrier function was evaluated using Tewameter® (Courage + Khazaka electronic GmbH, Cologne, Germany), which assesses TEWL in the burn scar. The probe was placed on the scar for 30 s [22,23].

2.5. Distensibility and elasticity of hypertrophic scars

Cutometer SEM 5801® (Courage + Khazaka electronic GmbH, Cologne, Germany) with an 8-mm-diameter probe was used to examine scar vertical deformation. Following pulling the skin upward with negative pressure for 5 s, this probe relaxes the skin for 3 s [8]. Fig. 1 shows a skin deformation curve with its corresponding absolute parameters. The distensibility, elasticity, and viscoelasticity of burn scar were investigated using two absolute parameters (Uf and H) and three relative parameters (Ua/Uf, Uv/Ue, and Ur/Uf) [8]. Final distensibility (Uf) is related to the ability to stretch collagen and elastin fibers, which indicates the firmness of the skin. Gross elasticity (Ua/Uf) refers to the capacity of the skin to revert to its previous form. Viscoelasticity (Uv/Ue) pertains to the deformation of the

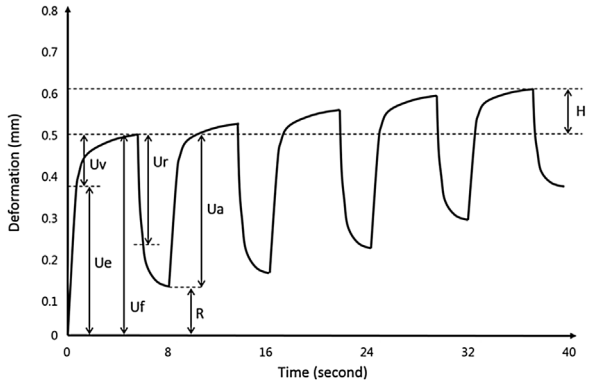


Fig. 1 – Skin deformation curve displays skin distensibility and elasticity with its corresponding absolute parameters. Uv, delayed distension; Ue, immediate distension; Uf, final distension; Ur, immediate retraction; R, residual deformation; Ua, final retraction; H, hysteresis [24].

Table 1 – Baseline demographics of the 492 subjects.

Age (years)	38.2 ± 8.0
Height (cm)	171.4 ± 7.3
Weight (kg)	68.0 ± 11.0
Burned BSA (%)	36.4 ± 15.0
Duration from injury to examination (days)	65.2 ± 25.8
25(OH) vitamin D (ng/mL)	13.5 ± 6.1
Body mass index (kg/m^2)	23.0 ± 2.8
Lean mass (kg)	47.3 ± 6.5
Fat mass (kg)	17.9 ± 5.9
Fat percentage (%)	26.5 ± 7.0
FB:EB:SB:CoB:friction burn (number)	289 : 118 : 51 : 25 : 9

Data for continuous variables are expressed as mean ± standard deviation. Median numbers of burned BSA and duration from burn injury to examination are 33 and 63, respectively. BSA, body surface area; FB, flame burn; EB, electrical burn; SB, scalding burn; CoB, contact burn.

viscoelastic component and the displacement of interstitial fluid containing viscous glycosaminoglycan. Higher viscoelasticity indicates attenuated interstitial fluid movement throughout the burn scar network. Biological elasticity (Ur/Uf) is frequently selected for elastin fiber function in the clinical environment. Hysteresis (H) is the length variance in skin expansion between the first and last suction, which implies a tiring effect [24].

2.6. *Statistical analysis*

Data for continuous and categorical variables are presented as mean ± standard deviations and as numbers, respectively. Burn patients were divided into four groups based on quartiles of fat percentage and 25(OH) vitamin D levels. One-way analysis of variance (ANOVA) was used to investigate 25(OH) vitamin D levels and biomechanical properties of hypertrophic burn scar according to fat percentage quartiles and to also compare biomechanical scar properties according to quartiles of 25(OH) vitamin D levels. Multiple linear regression analysis was performed to demonstrate the effect of body fat content and 25(OH) vitamin D levels on biomechanical scar properties in burn patients. All analyses were conducted with SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA), and significance was established at $P < 0.05$.

3. Results

3.1. *Demographic characteristics of subjects*

The demographic characteristics of the study subjects are summarized in Table 1. In 492 male subjects analyzed (mean age, 38.2 ± 8.0 years; mean height, 171.4 ± 7.3 cm; mean weight, 68.0 ± 11.0 kg), the mean value of burned BSA percentage and duration from burn injury to examination were 36.4 ± 15.0% and 65.2 ± 25.8 days. Furthermore, the mean 25(OH) vitamin D level, BMI, lean mass, fat mass, and fat percentage were 13.5 ± 6.1 ng/mL, 23.0 ± 2.8 kg/m^2, 47.3 ± 6.5 kg, 17.9 ± 5.9 kg, and 26.5 ± 7.0%, respectively.

3.2. *Clinical characteristics of subjects according to quartiles of fat percentage*

With respect to the clinical characteristics of burn patients according to quartiles of body fat percentage, subjects were likely to be taller ($P < 0.001$), to be weightier ($P < 0.001$), and to have less lean body mass (all $P < 0.001$) as fat percentage increased. Those with a higher percentage of fat mass tended to have higher 25(OH) vitamin D levels ($P < 0.001$) (Table 2). Any significant differences in age, percentage of burned BSA, duration from burn injury to examination, and BMI according to quartiles of fat percentage were not observed (all $P > 0.05$).

3.3. *Biomechanical scar properties according to burn types*

Differences in biomechanical hypertrophic scar properties were examined among burn types, which were divided into flame burn, electrical burn, and other burns. The parameters of biomechanical properties were similar among the three burn groups with no significant differences after post hoc Tukey's test (all $P > 0.05$) (Table 3).

3.4. *Biomechanical hypertrophic scar properties according to quartiles of body fat percentage and 25(OH) vitamin D levels*

Firstly, we compared the mean values of melanin, erythema, and TEWL according to quartiles of fat percentage and 25(OH) vitamin D levels to investigate the relationship among fat percentage, vitamin D level, and biomechanical scar properties. There were no significant differences in the mean values of melanin, erythema, and TEWL according to increased fat percentage ($P = 0.141$, $P = 0.701$, and $P = 0.072$) and 25(OH) vitamin D levels ($P = 0.341$, $P = 0.104$, and $P = 0.490$) (Table 4). Therefore, the degree of scar pigmentation, erythema, and dryness was similar irrespective of an increase in quartiles of fat percentage and vitamin D levels.

Secondly, two absolute parameters (Uf and H) and three relative parameters (Ua/Uf, Uv/Ue, and Ur/Uf) were compared according to quartiles of body fat percentage (Fig. 2) and 25(OH) vitamin D levels (Fig. 3). As body fat percentage increased,

Table 2 – Clinical characteristics of the subjects according to quartiles of body fat percentage.

	Quartiles of fat percentage (%)				
N = 492	<21.9 (117)	21.9–26.7 (129)	26.7–31.2 (122)	>31.2 (124)	P-value
Age (years)	38.7 ± 8.9	37.1 ± 8.1	37.8 ± 8.4	39.2 ± 8.4	0.799
Burned BSA (%)	35.7 ± 15.1	37.9 ± 14.7	36.6 ± 16.7	35.6 ± 13.3	0.621
Duration to examination (days)	66.2 ± 26.3	64.7 ± 27.9	64.4 ± 22.1	65.4 ± 26.8	0.951
Height (cm)	168.5 ± 10.9	174.1 ± 6.0	171.1 ± 5.1	171.4 ± 5.3	<0.001*
Weight (kg)	66.0 ± 14.0	71.4 ± 10.5	67.1 ± 10.1	67.3 ± 7.7	<0.001*
Body mass index (kg/m^2)	22.9 ± 3.2	23.5 ± 2.8	23.2 ± 3.1	22.9 ± 2.2	0.215
Lean mass (kg)	49.0 ± 5.9	49.3 ± 4.7	48.0 ± 6.6	42.8 ± 6.4	<0.001*
Fat mass (kg)	12.3 ± 6.0	15.9 ± 2.2	19.5 ± 3.1	23.5 ± 4.6	<0.001*
25(OH) vitamin D (ng/mL)	12.2 ± 6.7	13.0 ± 5.1	14.7 ± 5.6	16.1 ± 6.1	<0.001*

The data are displayed as mean ± standard deviations. Statistical analysis of data was performed using one-way analysis of variance (ANOVA).
* $p < 0.001$. BSA, body surface area.

Table 3 – Biomechanical scar properties according to burn types.

N = 492	Flame burn N = 289	Electrical burn N = 118	Others N = 85	P-value
Melanin value (AU)	176.76 ± 122.4	184.87 ± 111.3	182.96 ± 83.5	0.645
Erythema value (AU)	435.05 ± 102.6	399.32 ± 101.5	402.52 ± 133.1	0.219
Trans-epidermal water loss (g/hr/m^2)	22.61 ± 11.6	20.52 ± 10.2	23.31 ± 13.4	0.067
Distensibility (Uf)	0.84 ± 0.6	0.81 ± 0.5	0.96 ± 0.5	0.083
Gross elasticity (Ua/Uf)	0.21 ± 0.3	0.23 ± 0.3	0.23 ± 0.4	0.674
Viscoelasticity (Uv/Ue)	0.32 ± 0.5	0.36 ± 0.5	0.31 ± 0.4	0.458
Biological elasticity (Ur/Uf),	0.23 ± 0.4	0.21 ± 0.5	0.24 ± 0.8	0.842
Hysteresis (H)	0.08 ± 0.1	0.07 ± 0.1	0.08 ± 0.1	0.361

The data are displayed as mean ± standard deviations. The differences in the biomechanical scar properties among burn types were examined using the one-way analysis of variance (ANOVA). Burn types were divided into flame burn, electrical burn, and other burns (chemical burn, scalding burn, contact burn and friction burn). BSA, body surface area. N, number; AU, arbitrary unit.

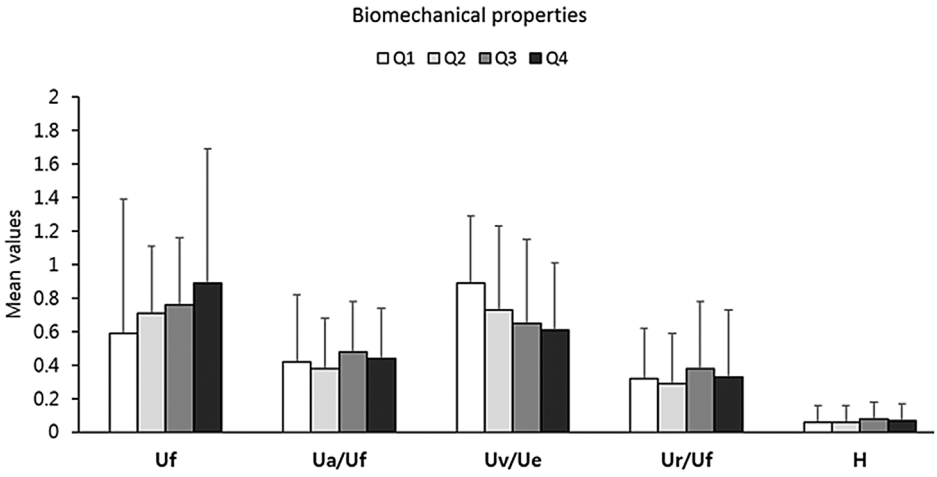
Table 4 – Mean values of melanin, erythema and TEWL according to quartiles of body fat percentage and 25(OH) vitamin D levels.

Quartiles	Biomechanical properties		
	Melanin (AU)	Erythema (AU)	TEWL (g/hr/m^2)
Fat percentage (%)			
Quartile 1 (<21.9)	179.53 ± 112.6	487.38 ± 115.4	21.45 ± 9.5
Quartile 2 (21.9–26.7)	191.12 ± 132.0	484.41 ± 114.8	22.93 ± 11.2
Quartile 3 (26.7–31.2)	170.37 ± 94.9	489.86 ± 135.3	23.62 ± 12.3
Quartile 4 (>31.2)	181.15 ± 101.1	495.53 ± 111.3	23.09 ± 10.4
P for trend	0.141	0.701	0.072
25(OH) vitamin D (ng/mL)			
Quartile 1 (<9.2)	190.71 ± 153.1	492.78 ± 104.8	21.65 ± 8.9
Quartile 2 (9.2–12.8)	170.93 ± 89.1	469.32 ± 103.7	21.49 ± 10.6
Quartile 3 (12.8–17.4)	188.72 ± 94.7	472.52 ± 135.3	24.21 ± 12.8
Quartile 4 (>17.4)	179.48 ± 77.6	481.50 ± 123.8	23.52 ± 11.5
P for trend	0.341	0.104	0.490

The data are displayed as mean ± standard deviations. One-way analysis of variance (ANOVA) was used to compare the mean values of melanin, erythema, and TEWL according to quartiles of fat percentage and 25(OH) vitamin D levels. TEWL; trans-epidermal water loss; AU, arbitrary unit.

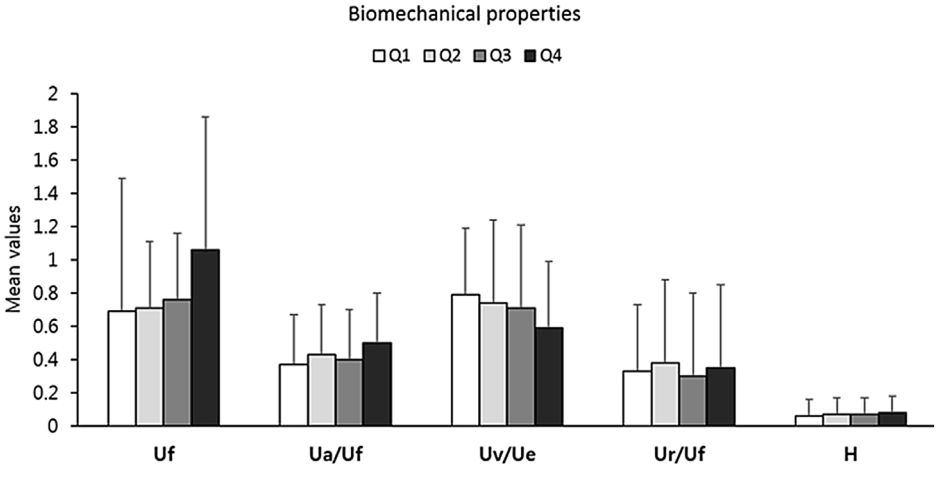
hypertrophic scars showed higher mean value of distensibility (Uf, $P < 0.001$) and lower mean value of viscoelasticity (Uv/Ue, $P < 0.001$). Fat percentage quartiles were not significantly associated with the mean values of gross elasticity (Ua/Uf, $P = 0.085$), biological elasticity (Ur/Uf, $P = 0.269$), and hysteresis (H, $P = 0.378$) (Fig. 2).

Burn patients with higher 25(OH) vitamin D levels tended to have higher mean values of distensibility (Uf,



Q1	0.59 ± 0.8	0.42 ± 0.4	0.89 ± 0.4	0.32 ± 0.3	0.06 ± 0.1
Q2	0.71 ± 0.4	0.38 ± 0.3	0.73 ± 0.5	0.29 ± 0.3	0.06 ± 0.1
Q3	0.76 ± 0.4	0.48 ± 0.3	0.65 ± 0.5	0.38 ± 0.4	0.08 ± 0.1
Q4	0.89 ± 0.8	0.44 ± 0.3	0.61 ± 0.4	0.33 ± 0.4	0.07 ± 0.1
***p*-value**	0.029*	0.085	<0.001**	0.269	0.378

Fig. 2 – Biomechanical hypertrophic scar properties according to quartiles of body fat percentage. As body fat percentage increased, hypertrophic scars showed higher mean value of distensibility (Uf, $P < 0.029$) and lower mean value of viscoelasticity (Uv/Ue, $P < 0.001$). Fat percentage quartiles were not significantly associated with mean values of gross elasticity (Ua/Uf, $P = 0.085$), biological elasticity (Ur/Uf, $P = 0.269$), and hysteresis (H, $P = 0.378$). *$P < 0.05$ and **$P < 0.001$.



Q1	0.69 ± 0.8	0.37 ± 0.3	0.79 ± 0.4	0.33 ± 0.4	0.06 ± 0.1
Q2	0.71 ± 0.4	0.43 ± 0.3	0.74 ± 0.5	0.38 ± 0.5	0.07 ± 0.1
Q3	0.76 ± 0.4	0.40 ± 0.3	0.71 ± 0.5	0.30 ± 0.5	0.07 ± 0.1
Q4	1.06 ± 0.8	0.50 ± 0.3	0.59 ± 0.4	0.35 ± 0.5	0.08 ± 0.1
***p*-value**	<0.001**	0.013*	0.008*	0.540	0.237

Fig. 3 – Biomechanical hypertrophic scar properties according to quartiles of 25(OH) vitamin D levels. Burn patients with higher 25(OH) vitamin D levels tended to have higher mean values of distensibility (Uf, $P < 0.001$) and gross elasticity (Ua/Uf, $P = 0.013$) and lower mean value of viscoelasticity (Uv/Ue, $P = 0.008$). Biological elasticity and hysteresis were not significantly associated with quartiles of 25(OH) vitamin D levels (Ur/Uf, $P = 0.540$; H, $P = 0.237$). *$P < 0.05$ and **$P < 0.001$.

$P < 0.001$) and gross elasticity (Ua/Uf, $P = 0.013$) and lower mean value of viscoelasticity (Uv/Ue, $P = 0.008$). Biological elasticity and hysteresis were not significantly associated with quartiles of 25(OH) vitamin D levels (Ur/Uf, $P = 0.540$; H, $P = 0.237$) (Fig. 3).

3.5. *Effect of body fat percentage and 25(OH) vitamin D levels on biomechanical hypertrophic scar properties*

Multiple linear regression analyses were conducted to investigate the effect of fat content and 25(OH) vitamin D levels on biomechanical scar properties in burn patients (Table 5). Scar distensibility and viscoelasticity significantly increased and decreased, respectively, according to body fat percentage (Uf, $P = 0.019$; Uv/Ue, $P = 0.002$). Furthermore, 25(OH) vitamin D levels positively affected the distensibility and gross elasticity of hypertrophic scars (Uf, $P < 0.001$; Ua/Uf, $P = 0.043$); in contrast, viscoelasticity was negatively affected (Uv/Ue, $P = 0.007$). Our results demonstrate that as body fat percentage and 25(OH) vitamin D levels decreased, the fibrous network became firmer. Age, percentage of burned BSA, and duration from burn injury to examination were not related to biomechanical parameters of dependent variables in burn subjects (all $P > 0.05$).

4. Discussion

Hypertrophic scar formation is one of the common complications following severe burn injury [1]. Epidermal keratinocytes and dermal fibroblasts play major role in the pathogenesis of excessive scarring [4] and conversion of 7-dehydrocholesterol to previtamin D_3 via vitamin D receptor expression [11]. Moreover, $125(OH)_2D_3$ causes growth inhibition in receptor-positive normal fibroblasts [9], and $1\alpha,25(OH)_2D_3$ inhibits epidermal keratinocyte proliferation [10]. As an endocrine organ, adipose tissue releases not only vitamin D_3 [14] but also diverse cytokines [25]. Adipocytes have been shown to suppress fibroblast proliferation and promote keratinocyte proliferation in a reconstructed rat skin model [18]. Therefore, we hypothesized that the biomechanical properties of burn hypertrophic scars may be associated with adipose tissue loss and reduced vitamin D levels following severe burn injury and examined the values of melanin, erythema, and TEWL; distensibility and elasticity of hypertrophic burn scars; total body fat percentage; and 25(OH) vitamin D levels to validate our hypothesis.

As body fat content increased in burn patients, there was a tendency to increase in 25(OH) vitamin D levels. This result was not consistent with previous findings in which body fat percentage was inversely associated with 25(OH) vitamin D levels in healthy women and the elderly [26,27]. Vitamin D can inhibit adipogenesis through the vitamin D receptor [28]. However, recent studies reported contradictory findings in human tissues and showed that 125(OH) vitamin D_3 increased the expression of adipogenic markers in subcutaneous preadipocytes [29] and that it stimulated the expression of adipogenic marker genes in mesenchymal progenitor cells from adipose tissues [30]. Overall, vitamin D exerts both promotive and inhibitory effects on adipogenesis [31]. Our results may be understood by the reduction in vitamin D storage and secretion owing to adipose tissue loss resulting from increased lipolysis in burned patients rather than by the contribution of decreased vitamin D levels to adipogenesis inhibition.

Melanin absorbs ultraviolet radiation competitively with 7-dehydrocholesterol in epidermal keratinocytes; consequently, high melanin levels in pigmented skin can cause a decrease in vitamin D production [32]. However, there was not significant difference in melanin values according to the quartiles of vitamin D level because our burn patients have scar characteristics of erythema but not those of hyperpigmentation.

This study provides a novel observation about the significant relationship between adipose tissues and 25 (OH) vitamin D levels and the biomechanical properties of hypertrophic burn scars. From the fourth quartile to the first quartile of fat percentage, Uf (distensibility) significantly tended to decrease, whereas Uv/Ue (viscoelasticity) significantly tended to increase. Additionally, we showed that Uf (distensibility) and Ua/Uf (gross elasticity) significantly increased and Uv/Ue (viscoelasticity) significantly decreased across the quartiles of 25(OH) vitamin D levels. These results suggest that decreased fat percentage and 25(OH) vitamin D

Table 5 – Multiple linear regression analysis of body fat percentage and 25(OH) vitamin D levels affecting biomechanical scar properties.

	Uf		Ua/Uf		Uv/Ue		Ur/Uf	
	β	P-value	β	P-value	β	P-value	β	P-value
Fat percentage (%)	0.104	0.019*	0.032	0.484	−0.143	0.002*	0.002	0.967
25(OH) vitamin D (ng/mL)	0.272	<0.001**	0.027	0.043*	−0.122	0.007*	0.080	0.082
Age (years)	−0.008	0.856	0.057	0.210	−0.023	0.606	0.016	0.716
Burned BSA (%)	0.015	0.737	0.066	0.152	−0.036	0.430	0.071	0.122
Duration to examination (days)	−0.075	0.089	0.039	0.397	0.056	0.213	0.052	0.259
Adjusted R^2	0.13		0.02		0.17		0.06	

Multiple linear regression analyses were used to demonstrate the influence of body fat percentage and 25(OH) vitamin D levels on parameters of dependent variables in burn patients with hypertrophic scars. BSA, body surface area; Uf, distensibility; Ua/Uf, gross elasticity; Uv/Ue, viscoelasticity; Ur/Uf, biological elasticity.

* $P < 0.05$.

** $P < 0.001$.

levels were related to firmer texture and reduced interstitial fluid movement throughout the fibrous network in hypertrophic burn scars [24].

Adipose-derived stem cells express keratinocyte progenitor cell markers and differentiate into epidermal keratinocytes [33]. The proliferation and differentiation of adipocyte precursor cells are enhanced after skin wounding, and mature adipocytes are involved in fibroblast recruitment and extracellular matrix protein deposition during wound healing [17]. Furthermore, adipocytes suppress the dermal fibroblast proliferation but stimulate the epidermal keratinocytes proliferation in the reconstructed skin [18]. Vitamin D promotes the expression of connective tissue growth factor and transforming growth factor (TGF)-β1 genes, increases fibroblast migration and differentiation [34], and reduces the expression of profibrotic factor and collagen in multipotent mesenchymal cells [35]. TGF-β plays both stimulating and inhibitory roles in keratinocytes depending on wound closure [4]. Before wound closure, TGF-β-activated keratinocytes enhance collagen production in fibroblasts [20]. However, after wound closure, TGF-β contributes to attenuated collagen production in fibroblasts via inactivation of keratinocytes [36]. Therefore, both adipose tissue loss and reduced 25(OH) vitamin D levels can likely result in impaired communication between keratinocytes and fibroblasts and subsequent disturbance in collagen and elastin production.

The present study has several limitations. First, the duration from burn injury to examination was not constant for each patient, as the time taken to completely heal the wound varied in each subject. Second, considering the exclusion of female patients from the analysis, sex differences were not evaluated owing to the small sample size. Furthermore, we could not control the amount of subjects' sun exposure in the hospital. To eliminate the influence of sun exposure, we only enrolled burned inpatients in the rehabilitation unit who had never returned home. Therefore, the potential for sun exposure among subjects prior to blood sampling and scar evaluation was minimal. Finally, the cross-sectional design made causality inference difficult. Further investigations with continuous models that consider sex, sun exposure, and other important confounding factors are warranted.

To our best knowledge, this study provides the first demonstration on the crosstalk among adipose tissue, vitamin D level, and biomechanical properties of hypertrophic burn scars. Adipose tissue loss and decreased 25(OH) vitamin D levels following severe burn injury were associated with scar rigidity and slow interstitial fluid shifting in hypertrophic scars. We suggest that the maintenance of adipose tissue content and intake of vitamin D supplements may help prevent or treat pathologic excessive scar formation in burn patients. Moreover, further investigations using continuous study model are required to demonstrate causality among body fat content, 25(OH) vitamin D levels, and biomechanical scar properties.

Contributors

CH Seo and YS cho contributed to study concept and design; SY Joo collected and assembled the data; YS Cho drafted the manuscript; CH Seo revised the manuscript critically for important intellectual content; and J Lee and YS Cho performed statistical analysis. All authors approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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REFERENCES

[1] Lawrence JW, Mason ST, Schomer K, Klein MB. Epidemiology and impact of scarring after burn injury: a systematic review of the literature. J Burn Care Res 2012;33:136–46.
[2] Bock O, Schmid-Ott G, Malewski P, Mrowietz U. Quality of life of patients with keloid and hypertrophic scarring. Arch Dermatol Res 2006;297:433–8.
[3] Finnerty CC, Jeschke MG, Branski LK, Barret JP, Dziewulski P, Herndon DN. Hypertrophic scarring: the greatest unmet challenge after burn injury. Lancet 2016;388:1427–36.
[4] van der Veer WM, Bloemen MC, Ulrich MM, Molema G, van Zuijlen PP, Middelkoop E, et al. Potential cellular and molecular causes of hypertrophic scar formation. Burns 2009;35:15–29.
[5] Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004;338:143–56.
[6] Hahn JM, Supp DM. Abnormal expression of the vitamin D receptor in keloid scars. Burns 2017;43:1506–15.
[7] Piotrowska A, Wierzbicka J, Żmijewski MA. Vitamin D in the skin physiology and pathology. Acta Biochim Pol 2016;63:17–29.
[8] Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 2007;7:684–700.
[9] Clemens TL, Adams JS, Horiuchi N, Gilchrest BA, Cho H, Tsuchiya Y, et al. Interaction of 1,25-dihydroxyvitamin-D3 with keratinocytes and fibroblasts from skin of normal subjects and a subject with vitamin-D-dependent rickets, type II: a model for study of the mode of action of 1,25-dihydroxyvitamin D3. J Clin Endocrinol Metab 1983;56:824–30.
[10] Smith EL, Walworth NC, Holick MF. Effect of 1α,25-dihydroxyvitamin D3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. J Invest Dermatol 1986;86:709–14.
[11] Mostafa WZ, Hegazy RA. Vitamin D and the skin: focus on a complex relationship: a review. J Adv Res 2015;6:793–804.
[12] Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev 2015;96:365–408.
[13] Ding C, Gao D, Wilding J, Trayhurn P, Bing C. Vitamin D signalling in adipose tissue. Br J Nutr 2012;108:1915–23.

[14] Abbas MA. Physiological functions of vitamin D in adipose tissue. J Steroid Biochem 2017;165:369–81.
[15] Rosenstrein SJ, Rich C, Volwiler W. Deposition in and release of vitamin D3 from body fat: evidence for a storage site in the rat. J Clin Invest 1971;50:679–87.
[16] Blum M, Dolnikowski G, Seyoum E, Harris SS, Booth SL, Peterson J, et al. Vitamin D3 in fat tissue. Endocrine 2008;33:90–4.
[17] Schmidt BA, Horsley V. Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. Development 2013;140:1517–27.
[18] Sugihara H, Toda S, Yonemitsu N, Watanabe K. Effects of fat cells on keratinocytes and fibroblasts in a reconstructed rat skin model using collagen gel matrix culture. Br J Dermatol 2001;144:244–53.
[19] Jeschke MG. Post-burn hypermetabolism: past, present and future. J Burn Care Res 2016;37:86–96.
[20] Tsuprykov O, Chen X, Hocher CF, Skoblo R, Yin Lianghong, Hocher B. Why should we measure free 25(OH) vitamin D? J Steroid Biochem Mol Biol 2018;180:87–104.
[21] Wang ZM, Visser M, Ma R, Baumgartner RN, Kotler D, Gallagher D, et al. Skeletal muscle mass: evaluation of neutron activation and dual-energy X-ray absorptiometry methods. J Appl Physiol 1985;1996(80):824–31.
[22] van Zuijlen PP, Angeles AP, Kreis RW, Bos KE, Middelkoop E. Scar assessment tools: implications for current research. Plast Reconstr Surg 2002;109:1108–22.
[23] Oliveira GV, Chinkes D, Mitchell C, Oliveras G, Hawkins HK, Herndon DN. Objective assessment of burn scar vascularity, erythema, pliability, thickness, and planimetry. Dermatol Surg 2005;31:48–58.
[24] Dobrev H. In vivo study of skin mechanical properties in psoriasis vulgaris. Acta Derm Venereol 2000;80:263–6.
[25] Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci 2013;9:191–200.
[26] Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. J Clin Endocrinol Metab 2003;88:157–61.
[27] Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, et al. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. J Clin Endocrinol Metab 2005;90:4119–23.
[28] Wood RJ. Vitamin D and adipogenesis: new molecular insights. Nutr Rev 2008;66:40–6.
[29] Nimitphong H, Holick MF, Fried SK, Lee MJ. 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 promote the differentiation of human subcutaneous preadipocytes. PLoS One 2012;7:e52171.
[30] Narvaez CJ, Simmons KM, Brunton J, Salinero A, Chittur SV, Welsh JE. Induction of STEAP4 correlates with 1,25-dihydroxyvitamin D3 stimulation of adipogenesis in mesenchymal progenitor cells derived from human adipose tissue. J Cell Physiol 2013;228:2024–36.
[31] Mutt SJ, Hyppönen E, Saarnio J, Järvelin MR, Herzig KH. Vitamin D and adipose tissue-more than storage. Front Physiol 2014;5:228.
[32] Clemens TL, Henderson SL, Adams JS, Holick MF. Increased skin pigment reduces the capacity of skin to synthesize vitamin D3. Lancet 1982;1:74–6.
[33] Hasegawa T, Sakamoto A, Wada A, Fukai T, Iida H, Ikeda S. Keratinocyte progenitor cells reside in human subcutaneous adipose tissue. PLoS One 2015;10:e0118402.
[34] Ding J, Kwan P, Ma Z, Iwashina T, Wang J, Shankowsky HA, et al. Synergistic effect of vitamin D and low concentration of transforming growth factor beta 1, a potential role in dermal wound healing. Burns 2016;42:1277–86.
[35] Artaza JN, Norris KC. Vitamin D reduces the expression of collagen and key profibrotic factors by inducing an antifibrotic phenotype in mesenchymal multipotent cells. J Endocrinol 2009;200:207–21.
[36] Harrison CA, Gossiel F, Bullock AJ, Sun T, Blumsohn A, Mac Neil S. Investigation of keratinocyte regulation of collagen I synthesis by dermal fibroblasts in a simple in vitro model. Br J Dermatol 2006;154:401–10.