ORIGINAL ARTICLE

Vitamin D status, dose-differentiated cholecalciferol and the structural response of the skin in women with premature skin ageing: a prospective single-centre study

Gulnara S. Babakulova*, Ulugbek Yu. Sabirov[1]

[1]*Republican Specialized Scientific and Practical Medical Centre of Dermatovenereology and Cosmetology, Tashkent, Republic of Uzbekistan*

*Corresponding author: Gulnara S. Babakulova; e-mail: nagaeva.gulnora@mail.ru

Abstract

Aim. To establish how often women aged 20–45 years with clinical signs of premature skin ageing have low serum 25-hydroxyvitamin D [25(OH)D], whether dose-differentiated oral cholecalciferol restores it, which laboratory measure tracks the histological response of the skin, and whether baseline vitamin D status is associated with the clinical response to radiofrequency microneedling.

Methods. In a prospective single-centre study, 61 women (24–44 years) with at least three signs of premature facial skin ageing and 16 healthy controls were examined. Serum 25(OH)D was measured by chemiluminescent immunoassay. Cholecalciferol was dosed by baseline category (7000, 5000, 4000 or 2000 IU/day) and 25(OH)D was re-measured after 84–118 days (n = 53). Paired abdominal skin biopsies (n = 20) were scored for three structural features; a full structural response required all three. In a separate cohort of 46 women treated with radiofrequency microneedling, changes in six instrumental skin parameters were compared between normal (n = 23) and low (n = 23) baseline status against a prespecified minimal detectable change of 1 percentage point.

Results. 25(OH)D was below 30 ng/mL in 46 of 61 women (75.4%; 95% confidence interval 63.3–84.5) and below 20 ng/mL in 57.4%. Mean 25(OH)D rose from 18.77 ± 1.54 to 40.12 ± 1.23 ng/mL ($p < 0.001$); 47 of 53 women (88.7%) reached 30 ng/mL, and every failure occurred at a baseline below 10 ng/mL. The number of structural features correlated with the achieved concentration ($\rho = 0.553$; Bonferroni-adjusted $p = 0.044$) and with its increment ($\rho = 0.505$; unadjusted $p = 0.023$), but not with the baseline concentration ($\rho = 0.081$; $p = 0.733$). An increment of 24.4 ng/mL or more identified a full structural response with 75.0% sensitivity and 91.7% specificity in the development sample. After radiofrequency microneedling, the advantage of normal status exceeded the 1-point threshold for hydration (2.67 points), wrinkle severity (1.27) and pigmentation (1.04), all $p < 0.001$, but not for porphyrins, sensitivity or cumulative damage.

Conclusion. Low vitamin D status was the rule in this phenotype. The structural response of the skin tracked the concentration reached and its increment rather than the starting level, which argues for re-measuring 25(OH)D after correction and before energy-based procedures. The increment threshold was derived and tested in the same 20 women and needs external validation.

Keywords: *25-Hydroxyvitamin D; Cholecalciferol; Dermis; Radiofrequency microneedling; Skin ageing; Vitamin D deficiency*

MAIN TEXT

Introduction

Premature skin ageing in women of reproductive age presents as fine wrinkles, uneven pigmentation, dryness and loss of turgor, and it rests on two tissue processes: slower epidermal renewal and remodelling of the dermal extracellular matrix, with fragmentation of collagen and disorganisation of elastic fibres[1-3]. Ultraviolet radiation is the best-studied external driver of these changes, yet the same radiation is required for cutaneous synthesis of vitamin D[4,12,13]. The skin expresses the enzymes that activate vitamin D and the receptor through which its active metabolite regulates keratinocyte proliferation and differentiation, barrier maintenance, DNA repair and resistance to oxidative

damage[5-8]. Vitamin D status is therefore a plausible, measurable co-variable of skin ageing, not merely a marker of sun exposure.

The clinical evidence that links serum 25-hydroxyvitamin D [25(OH)D] to facial ageing is cross-sectional and inconsistent. A higher 25(OH)D concentration accompanied more photoageing in middle-aged white women, which is expected when sun exposure drives both[9], while an Egyptian case–control study found no association with facial ageing scores[10], and a Mendelian randomisation analysis found no causal effect of 25(OH)D on wrinkling or pigmented spots[11]. These designs answer one question: whether the concentration measured once predicts the appearance of the skin. They do not answer the clinical question that arises when a woman with low 25(OH)D requests an aesthetic procedure: does restoring her status change the tissue, and does her status matter for the outcome of the procedure?

Two gaps make that question hard to settle. First, studies that measure 25(OH)D, correct it and then re-examine the same skin histologically are rare, so the laboratory variable that best reflects a tissue response (baseline level, level reached or the individual increment) has not been identified. Second, data on energy-based rejuvenation are almost never stratified by vitamin D status, although radiofrequency microneedling relies on a wound-healing response of the dermis[20,21] that vitamin D signalling modulates[8]. The deficiency itself is common even at low latitudes, where clothing, heat avoidance and indoor life limit effective exposure, as data from the Middle East show[14,15], so the question is relevant to populations with abundant sunshine.

We addressed these gaps in a single-centre prospective study of women aged 20–45 years with clinical signs of premature skin ageing in Tashkent (41° N). The study set out to (i) establish the frequency and depth of low 25(OH)D in this phenotype against healthy controls, (ii) quantify the response to oral cholecalciferol dosed by baseline category, (iii) determine which laboratory measure tracks the structural response in paired skin biopsies, (iv) derive a simple post-course index of correction sufficiency, and (v) test, in a separate cohort, whether baseline vitamin D status is associated with the instrumental response to radiofrequency microneedling. Throughout, statistical significance was interpreted alongside the prespecified minimal detectable change of each method, so that differences too small to be measured were not read as effects.

Material and Methods

Study design and setting

This was a prospective, single-centre, clinico-laboratory study conducted at the Republican Specialized Scientific and Practical Medical Centre of Dermatovenereology and Cosmetology, Tashkent, Uzbekistan. It comprised a paired before–after cohort with a healthy reference group, and a separate two-group comparison of radiofrequency microneedling outcomes. Reporting follows the STROBE recommendations for observational studies[22]. Recruitment ran for one calendar year so that baseline examinations covered all four seasons.

Participants

The main group consisted of 61 women aged 24–44 years (mean 32.6 ± 0.7) who attended the dermatology and cosmetology departments with at least three of six clinical signs of premature facial skin ageing: reduced light reflectance, altered skin tone, wrinkles, dyschromia, dryness and reduced turgor. Exclusion criteria, fixed before recruitment, were menopause, pregnancy or lactation, obesity, endocrine disease, intestinal disease with malabsorption, inflammatory dermatoses that could alter the involutional picture, and previous aesthetic correction that changes perceived age. Sixteen healthy female volunteers of comparable age (mean 32.4 years) with no clinical signs of premature ageing, no exclusion conditions and 25(OH)D ≥ 30 ng/mL formed the reference group and were examined once. Twenty women of the main group gave separate written consent to two skin biopsies. Reasons for loss to follow-up were failure to attend the control visit, withdrawal, vitamin D intake outside the protocol or an incomplete control set of tests.

Laboratory measurement

Serum 25(OH)D was measured in fasting venous blood by chemiluminescent immunoassay (LIAISON XL, DiaSorin S.p.A., Italy). Status was classified by the Endocrine Society thresholds[16,17]: severe deficiency < 10 ng/mL, deficiency 10 to < 20 ng/mL, insufficiency 20 to < 30 ng/mL and normal status ≥ 30 ng/mL. The clinical picture played no part in assigning a category. The minimal detectable change of the assay was set at 1.0 ng/mL from the manufacturer's documentation before any analysis.

Cholecalciferol dosing and follow-up

Oral cholecalciferol was dosed by baseline category: 7000 IU/day for severe deficiency (n = 9), 5000 IU/day for deficiency (n = 26), 4000 IU/day for insufficiency (n = 11) and a maintenance dose of 2000 IU/day for normal status (n = 15). Doses were chosen to lie above prophylactic regimens and below single high-dose loading, in line with current practice guidance for adults[17,24]; intramuscular administration was not used. Adherence was assessed by interview at the control visit. The control measurement was taken at the second visit, which took place 84–118 days (median 107 days; 12–17 weeks) after baseline. Fifty-three women completed the paired measurement; eight women, all with normal baseline status on the maintenance dose, did not return.

Histological examination

Twenty women underwent punch biopsy of abdominal skin at baseline and at the control visit, a site chosen to limit local photodamage. Specimens were fixed in neutral buffered formalin, paraffin-embedded and stained with haematoxylin–eosin, van Gieson and Mallory stains; sections were examined by light microscopy (BX53, Olympus Corporation, Japan). One pathologist, unaware of the sampling time point, compared each control specimen with the baseline specimen from the same woman and recorded three dichotomous changes: (a) increased epidermal thickness with more distinct rete ridges and a more active basal layer, (b) more orderly collagen and elastic fibres with less fragmentation, and (c) reduced basophilia of the dermal ground substance. Vascular congestion and perivascular oedema were described but excluded from the dynamic score because they depend on biopsy conditions. A full structural response was defined a priori as the presence of all three changes. Inter-observer agreement was not assessed.

Radiofrequency microneedling cohort

A separate cohort of 46 women aged 26–43 years scheduled for fractional radiofrequency microneedling of the face was recruited; none belonged to the main group, had taken vitamin D in the preceding three months or had inflammatory skin changes in the treatment area. Women were allocated by baseline 25(OH)D to a normal-status group (≥ 30 ng/mL; n = 23) or a low-status group (< 30 ng/mL; n = 23). No vitamin D correction was given. Needle depth, energy and number of passes were identical in both groups. Six skin parameters (hydration, wrinkle severity, porphyrins, pigmentation, sensitivity and cumulative damage) were recorded as percentages by one operator with a facial imaging system (JANUS Pro, PIE Co., Ltd., Republic of Korea) before the first session and after the course, under standardised lighting, distance and head position. The minimal detectable change was set at 1 percentage point before analysis.

Statistical analysis

Analyses were performed in IBM SPSS Statistics 29.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 365. Normality was tested with the Shapiro–Wilk test separately in each of the seven comparison groups; variables with normal distribution are reported as mean ± standard error (SE), others as median with interquartile range (Q1–Q3). Proportions are given with Wilson 95% confidence intervals (CI). Independent groups were compared with Student's t-test or the Mann–Whitney U test, more than two groups with the Kruskal–Wallis test, and paired measurements with the Wilcoxon signed-rank test. Associations were examined with Spearman's ρ and Fisher-transformed 95% CIs; the four correlations run on the biopsy subgroup were Bonferroni-adjusted. Contingency tables were analysed with Fisher's exact test. The increment threshold for a full structural response was set as the midpoint between the median increments of full and incomplete responders, and its performance was explored across seven candidate thresholds; no receiver operating characteristic curve was fitted because of the sample size. Post hoc power for $\rho = 0.505$ at $n = 20$ was 63%. Missing values were not imputed. Two-sided $p < 0.05$ was considered statistically significant. By a rule fixed in advance, a between-group difference smaller than the minimal detectable change of the method was not interpreted as an effect regardless of its p value[23].

Ethical considerations

All participants gave written informed consent; a separate consent was obtained for the paired biopsies. Withdrawal did not affect subsequent care. The protocol was approved by the Ethics Committee of the Republican Specialized Scientific and Practical Medical Centre of Dermatovenereology and Cosmetology.

Results

Baseline vitamin D status

Three in four women with premature skin ageing had 25(OH)D below 30 ng/mL: 46 of 61 (75.4%; 95% CI 63.3–84.5), and 35 (57.4 ± 6.3%) were below 20 ng/mL. Deficiency (10 to < 20 ng/mL) was the single largest category (Table 1). The median for the whole group, 18.58 ng/mL, lay inside the deficiency range and the third quartile, 29.72 ng/mL, fell just short of the target; individual values spanned 0.99 to 95.80 ng/mL. Each of the three low categories differed from controls ($p < 0.001$), whereas the normal category did not ($p = 0.441$): about a quarter of women presented with the same clinical phenotype at a 25(OH)D concentration indistinguishable from that of healthy volunteers. The four categories did not differ in age (Kruskal–Wallis $p = 0.605$), and the proportion with low status did not differ between age bands of 20–29, 30–39 and 40–45 years (85.0%, 66.7% and 87.5%; $p = 0.234$ for concentrations).

Table 1. Baseline serum 25(OH)D by vitamin D status category in women with premature skin ageing (n = 61) and in healthy controls (n = 16)

Group	n	Share of main group, % (95% CI)	25(OH)D, mean ± SE, ng/mL	25(OH)D, median (Q1–Q3), ng/mL	Age, years	p vs controls
Healthy controls	16	—	42.14 ± 1.76	40.83 (37.26–47.46)	32.4	—
Severe deficiency (< 10 ng/mL)	9	14.8 (8.0–25.7)	6.65 ± 1.13	7.99 (5.78–8.82)	30.3 ± 1.8	< 0.001
Deficiency (10 to < 20 ng/mL)	26	42.6 (31.0–55.1)	14.61 ± 0.61	14.61 (12.19–16.84)	33.2 ± 1.1	< 0.001
Insufficiency (20 to < 30 ng/mL)	11	18.0 (10.4–29.5)	24.79 ± 1.00	26.00 (21.95–27.23)	33.0 ± 1.6	< 0.001
Normal status (≥ 30 ng/mL)	15	24.6 (15.5–36.7)	43.68 ± 4.53	34.73 (32.83–47.71)	32.7 ± 1.4	0.441
All low categories (< 30 ng/mL)	46	75.4 (63.3–84.5)	—	—	—	—

CI, confidence interval; SE, standard error. Wilson 95% CI. Mann–Whitney U test for severe deficiency and normal status (Shapiro–Wilk p = 0.01 and < 0.001), Student's t-test otherwise. All six pairwise comparisons between categories: p < 0.001. One outlying value of 95.80 ng/mL in the normal category was retained; the median is therefore the descriptive measure for that group.

The season of the baseline visit was the strongest correlate of concentration (Kruskal–Wallis $p < 0.001$; Figure 1B). Every woman examined in spring (n = 16) or summer (n = 15) was in a low category, compared with 4 of 15 (26.7%) in winter and 11 of 15 (73.3%) in autumn. The expected summer rise did not occur in this clinical sample, an

observation we return to in the Discussion. Women who agreed to paired biopsies had a lower baseline concentration than the remaining participants (16.20 ± 2.01 versus 25.45 ± 2.79 ng/mL; p = 0.035) but did not differ in age (33.1 versus 32.4 years; p = 0.655), so the histological subgroup over-represents deeper deficiency.

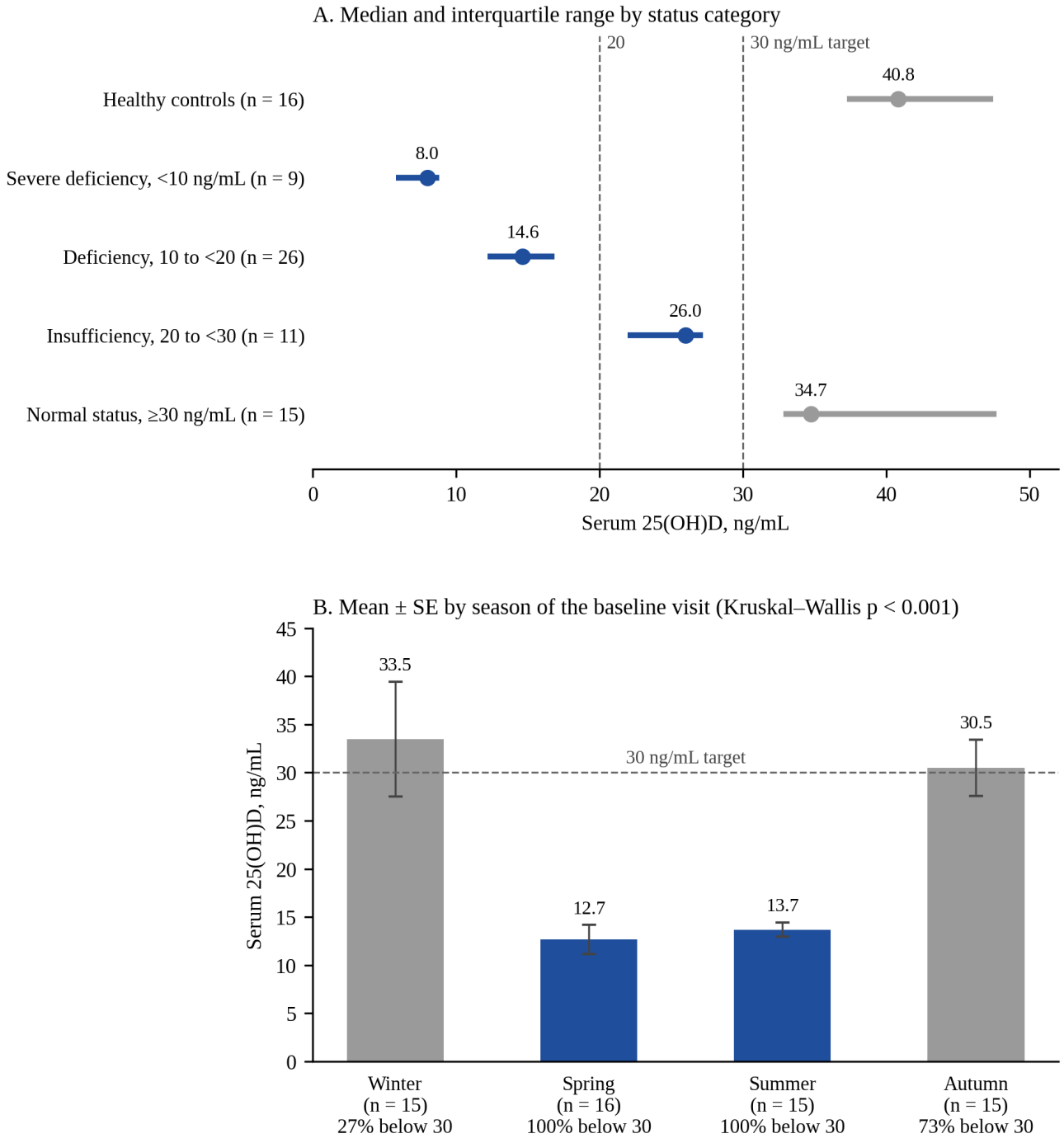


Figure 1. Baseline serum 25(OH)D in women with premature skin ageing. (A) Median and interquartile range by status category and in healthy controls; dashed lines mark the 20 ng/mL and 30 ng/mL thresholds. (B) Mean ± SE by season of the baseline visit with the proportion of women below 30 ng/mL; Kruskal–Wallis p < 0.001.

Response to dose-differentiated cholecalciferol

After 12–17 weeks, mean 25(OH)D in the 53 re-measured women rose from 18.77 ± 1.54 to 40.12 ± 1.23 ng/mL, a mean individual increment of 21.35 ± 1.48 ng/mL (Wilcoxon $p < 0.001$). No paired value fell and none remained below 20 ng/mL. Forty-seven women (88.7%) reached the 30 ng/mL target; their mean concentration, 42.09 ± 1.09 ng/mL, matched that of healthy controls (42.14 ± 1.76 ng/mL; $p = 0.980$). The six women who did not reach the target (11.3%; mean 24.74 ± 1.14 ng/mL) all started below 10 ng/mL, so residual insufficiency was confined to the severe-deficiency category (Table 2).

Table 2. Dosing scheme and attainment of the 30 ng/mL target by baseline category

Baseline category	Cholecalciferol, IU/day	Started, n	Re-measured, n	Reached ≥ 30 ng/mL, n (%)	Status after the course
Severe deficiency	7000	9	9	3 (33.3)	6 insufficiency, 3 normal
Deficiency	5000	26	26	26 (100)	all normal
Insufficiency	4000	11	11	11 (100)	all normal
Normal status	2000 (maintenance)	15	7	7 (100)	all normal
Total	—	61	53	47 (88.7)	6 insufficiency, 47 normal

Interval between measurements 84–118 days (median 107). Eight women with normal baseline status did not return for the control visit.

The increment did not scale with dose (Figure 2). The largest rise, 26.13 ± 1.67 ng/mL, occurred in the deficiency category on 5000 IU/day, whereas severe deficiency on 7000 IU/day gained 23.28 ± 3.30 ng/mL from a median that lay more than 22 ng/mL below the target; the insufficiency category on 4000 IU/day gained 17.15 ± 2.66 ng/mL, about four times the distance it had to cover. Category means that differed more than six-fold at baseline converged to 29.93–48.09 ng/mL. The increment did not differ between age bands (19.96–22.93 ng/mL; $p = 0.768$). No woman in the severe-deficiency group exceeded 50 ng/mL, and no adverse event led to dose change or withdrawal. The eight women lost to follow-up had a higher baseline concentration than completers (46.58 ± 7.91 versus 18.77 ± 1.54 ng/mL; $p < 0.001$) and similar age (30.8 versus 32.6 years; $p = 0.341$).

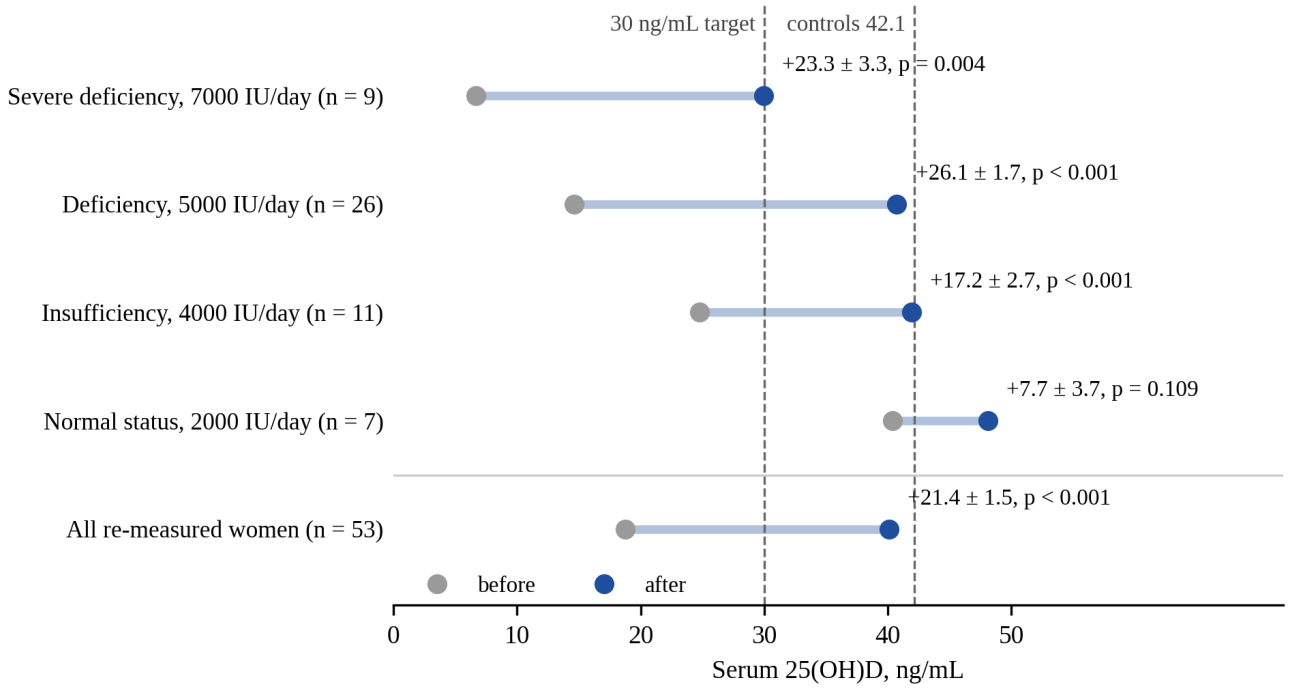


Figure 2. Mean serum 25(OH)D before and after dose-differentiated oral cholecalciferol by baseline category (n = 53 paired measurements). Labels give the mean increment ± SE and the p value of the Wilcoxon signed-rank test. Dashed lines mark the 30 ng/mL target and the mean of healthy controls (42.14 ng/mL).

Structural response of the skin and its relation to 25(OH)D

Every one of the 20 paired biopsies showed at least one of the three structural changes, and 8 (40.0%) showed all three (Table 3). Epidermal thickening with more distinct rete ridges was the most frequent change (85.0%), followed by more orderly collagen and elastic fibres (80.0%) and reduced basophilia of the ground substance (70.0%); the confidence intervals of these frequencies overlap widely at this sample size. In no specimen was the elastic network fully restored after 12–17 weeks, so the response was partial. In the 19 women whose concentration exceeded 30 ng/mL at the control visit, a full structural response was present in 8, which shows that reaching the laboratory target and reaching a full tissue response are not the same event.

Table 3. Structural changes in paired abdominal skin biopsies after cholecalciferol (n = 20) and their association with 25(OH)D measures

Structural change (control versus baseline biopsy)	n (%)	95% CI, %
Increased epidermal thickness, distinct rete ridges, active basal layer	17 (85.0)	64.0–94.8
More orderly collagen and elastic fibres, less fragmentation	16 (80.0)	58.4–91.9
Reduced basophilia of dermal ground substance	14 (70.0)	48.1–85.5
All three changes (full structural response)	8 (40.0)	21.9–61.3
Two changes	11 (55.0)	34.2–74.2
One change	1 (5.0)	0.9–23.6

Correlate of the number of changes (0–3)	Spearman ρ (95% CI)	p	Bonferroni-adjusted p
25(OH)D reached at control visit	0.553 (0.147 to 0.800)	0.011	0.044
Individual increment in 25(OH)D	0.505 (0.081 to 0.774)	0.023	0.092
Baseline 25(OH)D	0.081 (−0.375 to 0.506)	0.733	1.000
Age	−0.399 (−0.715 to 0.053)	0.081	0.324

CI, confidence interval (Wilson for proportions, Fisher transformation for ρ). Post hoc power for ρ = 0.505 at n = 20 was 63%; 29 pairs would be needed for 80%.

The number of structural changes correlated with the concentration reached at the control visit (ρ = 0.553; 95% CI 0.147–0.800; p = 0.011) and with the individual increment (ρ = 0.505; 95% CI 0.081–0.774; p = 0.023), but not with the baseline concentration (ρ = 0.081; p = 0.733) or age (ρ = −0.399; p = 0.081). After Bonferroni adjustment for four comparisons, only the association with the achieved concentration remained significant (p = 0.044); the increment fell to p = 0.092. Women with a full response had gained 29.78 ± 3.12 ng/mL against 18.35 ± 1.73 ng/mL in incomplete responders (medians 28.88 and 19.85; Mann–Whitney p = 0.012). Leaving out each woman in turn kept ρ for the increment between 0.447 and 0.641, so no single observation drove the association. For each individual feature the increment was numerically higher when the feature was present (fibre reordering 24.72 versus 15.74 ng/mL, p = 0.080; epidermal thickening 24.06 versus 16.47, p = 0.305; basophilia 23.46 versus 21.67, p = 0.968), without reaching significance. By baseline category, a full response occurred in 6 of 10 women with deficiency and 2 of 4 with insufficiency, but in none of the 4 with severe deficiency (mean increment 21.35 ng/mL) and none of the 2 with normal baseline status.

A two-component index of correction sufficiency

Because the achieved concentration and the increment, but not the baseline, tracked the tissue response, we combined the two post-course measures into an unweighted 0–4 score (Table 4). The score separated the biopsy subgroup into three levels whose frequency of full structural response fell from 75.0% at full correction to 25.0% at partial correction and 0% at insufficient correction (Kruskal–Wallis p = 0.019); the score correlated inversely with the number of structural changes (ρ = −0.717; p < 0.001). Of four candidate compositions, the increment alone (ρ = −0.734; between-level p = 0.003) and the two-component score (ρ = −0.717; p = 0.005) separated the sample best; the achieved concentration alone (ρ = −0.494; p = 0.076) and a three-component score that added the baseline concentration (ρ = −0.533; p = 0.088) did worse. The two-component form was retained because a large increment can coexist with persisting insufficiency, which the achieved concentration records.

Table 4. Composition of the correction-sufficiency score and the structural response at each level (n = 20)

Component	0 points	1 point	2 points
25(OH)D reached at control visit, ng/mL	≥ 40	30 to < 40	< 30
Individual increment in 25(OH)D, ng/mL	≥ 25	15 to < 25	< 15

Sufficiency level	Score	n (%)	Full structural response, n (%)	Structural changes per woman, mean
Full correction	0–1	8 (40.0)	6 (75.0)	2.75
Partial correction	2	8 (40.0)	2 (25.0)	2.25
Insufficient correction	3–4	4 (20.0)	0 (0.0)	1.75

The 30 and 40 ng/mL boundaries are literature-based; the 15 and 25 ng/mL increment boundaries were derived from this sample. Between-level difference: Kruskal–Wallis $p = 0.019$; Spearman ρ between score and number of changes -0.717, $p < 0.001$.

As a single working cut-off, an increment of 24.4 ng/mL (the midpoint between the median increments of full and incomplete responders) identified a full structural response with a sensitivity of 75.0% (6 of 8; 95% CI 40.9–92.9) and a specificity of 91.7% (11 of 12; 95% CI 64.6–98.5), Fisher's exact p = 0.004 (Figure 3B). Across seven cut-offs from 18.0 to 30.0 ng/mL, sensitivity fell from 87.5% to 37.5% while specificity rose from 25.0% to 100%; overall accuracy peaked at 90.0% at 26.0 ng/mL. Shifting the scoring boundaries for the increment by 5 ng/mL moved two women between levels and left the full-response rate above 70% at the top level and at 0% at the bottom level. All of these estimates come from the 20 women in whom the thresholds were derived and have not been tested in an independent sample.

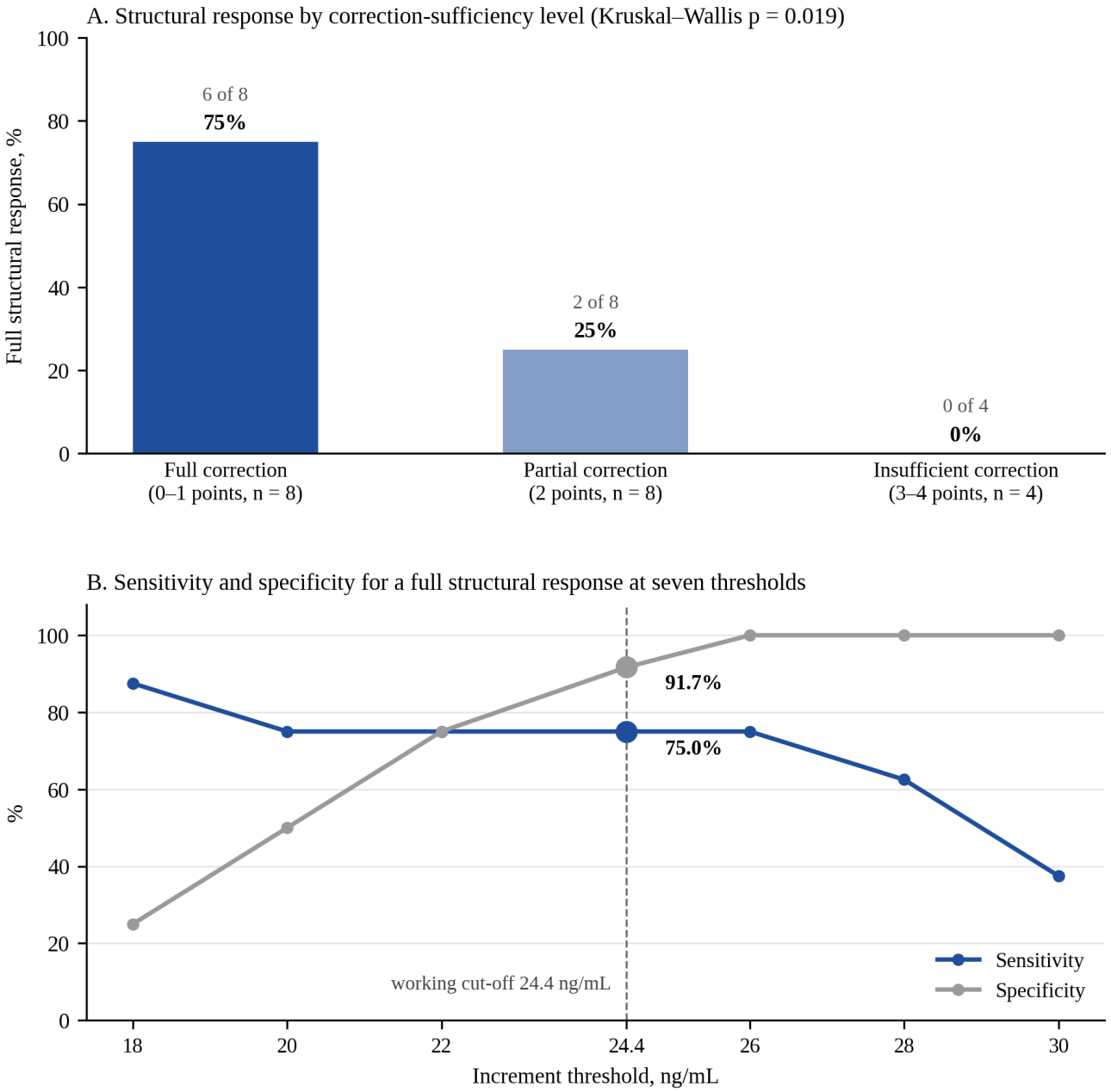


Figure 3. Performance of the correction-sufficiency index in the biopsy subgroup (n = 20). (A) Proportion of women with a full structural response at each level of the two-component score. (B) Sensitivity and specificity of a full structural response for seven candidate increment thresholds; the working cut-off of 24.4 ng/mL is marked. Both panels describe the development sample.

Vitamin D status and the response to radiofrequency microneedling

In the separate radiofrequency microneedling cohort, the normal-status and low-status groups had baseline 25(OH)D of 38.06 ± 0.97 and 18.66 ± 1.18 ng/mL ($p < 0.001$) and the same age range (26–43 years). All six parameters improved in both groups after the course (Wilcoxon $p < 0.001$ for each; Table 5): hydration increased, and wrinkle severity, porphyrins, pigmentation, sensitivity and cumulative damage decreased. The direction of change was the same in the two groups; the magnitude differed, and every between-group comparison was statistically significant at $p < 0.001$.

Table 5. Instrumental skin parameters (%) before and after fractional radiofrequency microneedling in women with normal and low baseline vitamin D status (mean ± SE)

Parameter	Normal status (n = 23), before	Normal status, after	Low status (n = 23), before	Low status, after	Change, normal	Change, low
Hydration	44.15 ± 1.02	50.63 ± 1.10	43.77 ± 0.99	47.59 ± 0.98	+6.48 ± 0.24	+3.82 ± 0.22

Parameter	Normal status (n = 23), before	Normal status, after	Low status (n = 23), before	Low status, after	Change, normal	Change, low
Wrinkle severity	32.96 ± 1.11	29.71 ± 1.13	31.31 ± 1.00	29.33 ± 0.97	−3.24 ± 0.19	−1.98 ± 0.12
Porphyrins	16.73 ± 0.89	14.86 ± 0.95	16.98 ± 0.76	15.97 ± 0.79	−1.87 ± 0.10	−1.01 ± 0.09
Pigmentation	27.43 ± 1.33	25.03 ± 1.33	26.33 ± 1.04	24.97 ± 1.07	−2.40 ± 0.17	−1.37 ± 0.12
Sensitivity	19.88 ± 0.85	18.09 ± 0.87	20.49 ± 1.00	19.33 ± 0.96	−1.80 ± 0.10	−1.16 ± 0.09
Cumulative damage	22.44 ± 1.05	20.55 ± 1.03	21.88 ± 1.05	20.65 ± 1.00	−1.89 ± 0.15	−1.23 ± 0.10

Within-group change: Wilcoxon signed-rank test $p < 0.001$ for every parameter in both groups. Between-group comparison of the change: Mann–Whitney U test $p < 0.001$ for every parameter. A decrease is an improvement for all parameters except hydration.

Measured against the prespecified minimal detectable change of 1 percentage point, however, only three of the six differences qualified as effects (Figure 4). Women with normal status gained 2.67 points more hydration (6.48 ± 0.24 versus 3.82 ± 0.22), lost 1.27 points more wrinkle severity (−3.24 ± 0.19 versus −1.98 ± 0.12) and 1.04 points more pigmentation (−2.40 ± 0.17 versus −1.37 ± 0.12). The differences for porphyrins (0.85), cumulative damage (0.66) and sensitivity (0.63) stayed below the instrument's resolution and were not interpreted as clinical advantages despite their p values. For hydration, the response of the low-status group was roughly three-fifths of that of the normal-status group, so low status attenuated the response rather than abolishing it. No adverse event required discontinuation.

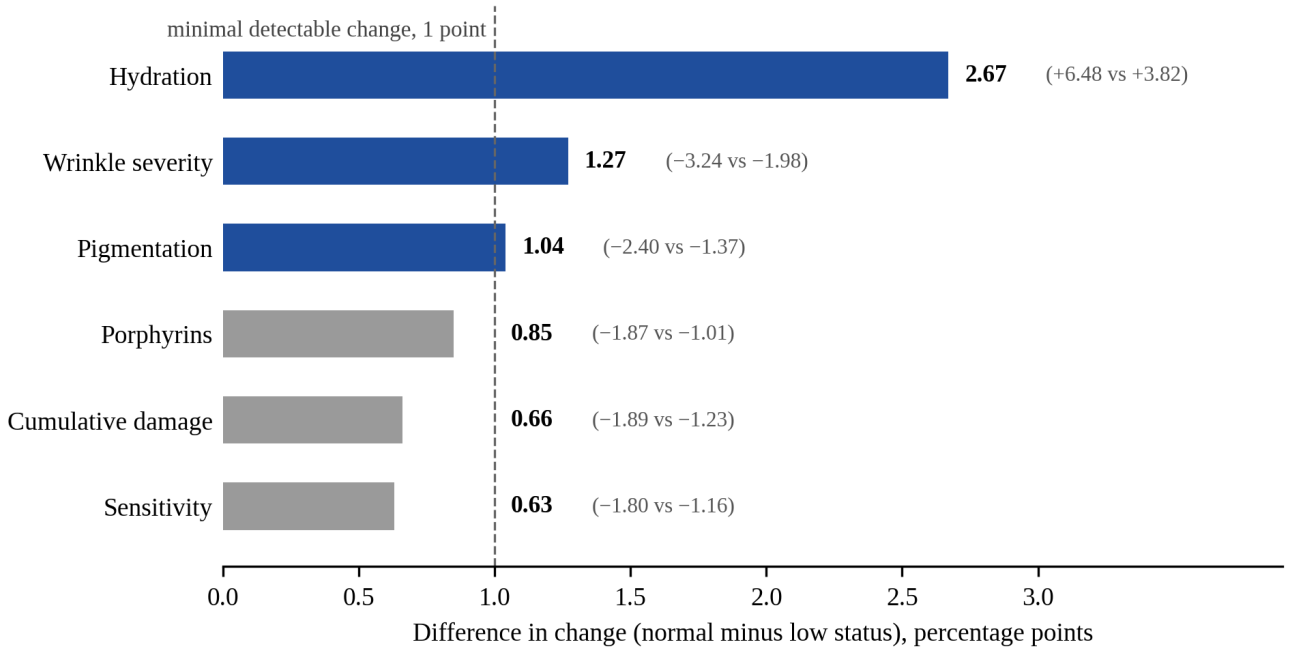


Figure 4. Advantage of normal over low baseline vitamin D status in the change of six instrumental skin parameters after fractional radiofrequency microneedling (n = 23 per group). Bars show the between-group difference in change, in percentage points; the dashed line marks the prespecified minimal detectable change of 1 point. All six differences had $p < 0.001$; only the three that exceed the line were interpreted as effects.

Discussion

This study followed the same women from a low 25(OH)D concentration through its correction to a second skin biopsy, and it compared radiofrequency microneedling outcomes by vitamin D status in a separate cohort. Four findings stand out. Low status affected three in four women aged 24–44 years who presented with premature skin ageing in a city at 41° N, with no case of normal status among those first seen in spring or summer. Oral cholecalciferol dosed by category normalised status in 88.7% within 12–17 weeks, and every failure occurred below a baseline of 10 ng/mL. The structural response of the skin tracked the concentration reached and the size of the individual rise, but not the starting concentration. Finally, in women treated with radiofrequency microneedling, low status was associated with a smaller gain in hydration and smaller reductions in wrinkles and pigmentation that

exceeded the resolution of the instrument, whereas three other statistically significant differences did not.

Deficiency in a sunny region

The 75.4% frequency of 25(OH)D below 30 ng/mL is in the range reported for adult women in the Middle East and the Gulf, where abundant sunshine coexists with high deficiency rates because of heat avoidance, covering clothing and indoor life[14,15]. The seasonal pattern in our sample, with the lowest values in spring and summer rather than the expected late-summer peak, fits that behavioural explanation but also admits a selection effect: photodamage becomes more visible after summer and may prompt consultation, so the women who present in summer may differ from those who present in winter. Neither mechanism was tested here. Either way, the frequency argues that a clinical phenotype of premature ageing is not a basis for assuming adequate status, and that a measured concentration is needed before a decision about correction[16,17].

Why the starting concentration did not predict the tissue response

Cross-sectional studies have not found a consistent link between a single 25(OH)D measurement and facial ageing[9-11], and in our biopsy subgroup the baseline concentration was equally uninformative ($\rho = 0.081$). The variables that did track the response were those that describe the correction itself: the concentration reached and the increment. This pattern is what a nutrient-effect framework predicts, in which a response depends on moving from a deficient to a replete state and is not expected to be visible in a cross-section of people at different points on the curve[19]. It is also consistent with the biology of the cutaneous vitamin D system, in which keratinocytes both activate the hormone and respond to it, and in which the receptor governs proliferation, differentiation and repair programmes that would be expected to change once ligand supply changes rather than to mirror a static serum value[5-8,28]. The association survived Bonferroni adjustment for the achieved concentration ($p = 0.044$) but not for the increment ($p = 0.092$), and the subgroup had 63% power; the increment result therefore needs confirmation in a larger series. The 40 ng/mL boundary built into the index reflects the view that extra-skeletal effects may require concentrations above the skeletal threshold of 30 ng/mL[18,30], but our data support that boundary only descriptively.

Dosing and the severe-deficiency category

The largest increment, 26.13 ng/mL, came from 5000 IU/day in the deficiency category, not from the highest dose. Women starting below 10 ng/mL gained 23.28 ng/mL on 7000 IU/day yet two-thirds remained insufficient, because their median lay more than 22 ng/mL below the target. The result is better explained by the distance to be covered within a fixed interval than by any ceiling on dose response, and it agrees with guidance that treatment of severe deficiency should be followed by re-measurement and, where necessary, continued therapy rather than judged by a single course[17,24]. The 88.7% normalisation rate compares with 73.5% after individualised dosing in a regional paediatric series[29], but differences in age, adiposity and baseline preclude a claim of superiority. Convergence of category means from a six-fold spread to 29.93–48.09 ng/mL is expected when women in each category receive the same dose and may also reflect the removal of behavioural variance in exposure during the course.

The histological response was partial

Epidermal thickening with restored rete ridges and reordering of collagen fibres were seen in most paired biopsies, but no specimen showed a reconstituted elastic network at 12–17 weeks. Elastic fibre turnover is slow and its repair incomplete in adult skin[25], and the loss of collagen synthesis in aged dermis is a long-standing process[1,3,26], so partial change at this interval is plausible. We sampled covered abdominal skin to minimise local photodamage; the biopsies therefore speak to intrinsic rather than photo-induced ageing, and extrapolation to the face, where the inclusion criteria were assessed, is not warranted without separate study. The 40% rate of full response against a 95% rate of laboratory normalisation in the same women is the central argument for assessing correction by a tissue-anchored index rather than by the 30 ng/mL threshold alone.

Vitamin D status and energy-based rejuvenation

Fractional radiofrequency microneedling produces its effect through a controlled dermal wound and the remodelling that follows, with new collagen and elastin[20,21]. Vitamin D signalling participates in epidermal repair and in the inflammatory phase of wound healing[8,27], which offers a mechanism for the attenuated response we observed in women with low status. The statistical finding is unambiguous ($p < 0.001$ for all six parameters), but with standard errors of about 0.1 point, differences of 0.6 point reach significance while lying below what the instrument can resolve. Interpreting every significant difference as an effect would have overstated the result; applying the prespecified minimal detectable change limited the claim to hydration, wrinkles and pigmentation[23]. Even for these, the comparison is between women who differed in status at baseline, not within women before and after correction. Whether correcting status before a procedure improves its outcome remains untested.

Clinical implications

The findings support a sequence that is simple to implement where a 25(OH)D assay and cholecalciferol are available: measure 25(OH)D at the first consultation for premature skin ageing; dose by category; re-measure after 12–17 weeks; and judge the correction by the concentration reached and the increment rather than by the category alone. A score of 0–1 would favour proceeding to an energy-based procedure, a score of 2 continued supplementation with re-testing, and a score of 3–4 a review of the regimen before the procedure. These actions are a proposal derived from a development sample; they have not been compared with usual care, and the index should be used as a research tool until its thresholds are validated.

Strengths and limitations

The strengths of the study are the paired design, blinded histological reading, prespecified thresholds and minimal detectable changes, and the separation of statistical significance from measurement resolution. The limitations are substantial. The study was conducted at one centre. Controls were examined once, so the natural change in 25(OH)D over the same months is unknown and a seasonal contribution to the increment cannot be excluded. The biopsy subgroup was self-selected, skewed to deeper deficiency, small and not powered in advance; histology was qualitative and read by one pathologist, so inter-observer agreement is unknown. The increment threshold and score were derived and evaluated in the same 20 women, and no receiver operating characteristic analysis was possible. Eight women with normal baseline status did not return, which enriches the paired sample for low baselines. Adherence was self-reported. The radiofrequency cohort did not receive correction, so the effect of correcting status on procedural outcome was not measured, and the minimal detectable changes were taken from manufacturers' documentation rather than measured in our hands. Vitamin D receptor polymorphisms, which could explain why women with similar increments showed one to three structural changes, were not genotyped. Validation in at least 60 women with at least 30 full responders, and a design in which radiofrequency microneedling follows documented correction in the same participants, are the next steps.

Conclusion

Among women aged 24–44 years with premature skin ageing in Tashkent, 75.4% had serum 25(OH)D below 30 ng/mL, and none of those first seen in spring or summer had normal status. Oral cholecalciferol dosed by baseline category restored status in 88.7% within 12–17 weeks; residual insufficiency was confined to women who started below 10 ng/mL and calls for re-measurement and continued treatment in that group. In paired biopsies, the structural response of the skin was associated with the concentration reached and with the individual increment, not with the baseline concentration, and a laboratory normalisation rate of 95% coexisted with a full histological response in 40%. A two-component score based on the achieved concentration and the increment separated full from incomplete responders in the development sample; an increment of about 24–26 ng/mL marked the boundary. In a separate cohort, low vitamin D status was associated with a measurably smaller improvement in hydration, wrinkles and pigmentation after radiofrequency microneedling. Measuring 25(OH)D before aesthetic procedures and re-measuring it after correction is justified by these data; the proposed thresholds require validation in an independent sample

before clinical use.

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Author contributions. GSB: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Visualization. UYS: Conceptualization, Supervision, Resources, Validation, Writing – review & editing. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Information about the authors

Gulnara S. Babakulova — MD, Republican Specialized Scientific and Practical Medical Centre of Dermatovenereology and Cosmetology, Tashkent, Republic of Uzbekistan

Ulugbek Yu. Sabirov — MD, DSc, Professor, Republican Specialized Scientific and Practical Medical Centre of Dermatovenereology and Cosmetology, Tashkent, Republic of Uzbekistan