Multidimensional Voice Profiles of Children With and Without Vitamin D Deficiency: A Pilot Cross-Sectional Comparative Study

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SUMMARY: Objectives. This study aims to compare multidimensional voice profiles of children aged 3–12 years with and without vitamin D deficiency in a pilot pediatric sample and to examine whether low vitamin D status is associated with measurable differences in perceptual, parent-reported, acoustic, and aerodynamic voice characteristics.

Methods. This cross-sectional comparative study included 47 children who presented for routine pediatric evaluations at a private pediatric clinic in Adana, Türkiye. Participants were classified according to serum 25-hydroxyvitamin D [25(OH)D] levels as having vitamin D deficiency (< 20 ng/mL; n = 23) or normal vitamin D levels (≥ 20 ng/mL; n = 24). Voice assessment included the Turkish Pediatric Voice Handicap Index (pVHI), GRBAS scale, acoustic analysis of sustained /a/ including fundamental frequency, jitter, shimmer, harmonics-to-noise ratio (HNR), and cepstral peak prominence smoothed (CPPS), as well as maximum phonation time (MPT) and s/z ratio. Between-group comparisons were performed using independent-samples *t* tests or Mann–Whitney *U* tests, as appropriate. Age- and sex-adjusted sensitivity analyses were also conducted for parent-reported, acoustic, and aerodynamic outcomes.

Results. In the primary unadjusted analyses, no statistically significant differences were found between children with vitamin D deficiency and those with normal vitamin D levels for any GRBAS parameter, including Grade, Roughness, Breathiness, Asthenia, and Strain (all $P > 0.05$). Similarly, pVHI functional, physical, emotional, and total scores did not differ significantly between groups (all $P > 0.05$). No significant between-group differences were observed for any acoustic or aerodynamic measure, including fundamental frequency, jitter, shimmer, HNR, CPPS /a/, connected speech CPPS, MPT, or s/z ratio (all $P > 0.05$). In age- and sex-adjusted sensitivity analyses, acoustic and aerodynamic outcomes remained nonsignificant, although an isolated adjusted difference was observed for the pVHI Emotional subscale.

Conclusion. In this pediatric pilot sample, vitamin D deficiency was not associated with significant differences in primary multidimensional voice measures. These findings suggest that low vitamin D status may not produce overt voice impairment detectable by conventional perceptual, parent-reported, acoustic, and aerodynamic assessment in children, although subtle or domain-specific effects cannot be excluded. Larger age-stratified studies using more sensitive voice measures are needed.

Key Words: Vitamin D Deficiency–Dysphonia–Pediatric voice–Voice quality–Child.

INTRODUCTION

Vitamin D is a fat-soluble vitamin obtained primarily through cutaneous synthesis after sunlight exposure and, to a lesser extent, through dietary intake and supplementation.[1,2] Vitamin D is metabolized in the liver to 25-hydroxyvitamin D [25(OH)D], the major circulating form used to assess vitamin D status, and is subsequently converted in the kidneys to its biologically active form, 1,25-dihydroxyvitamin D.[2] Beyond its established role in calcium homeostasis and bone metabolism, vitamin D has broad extraskeletal effects mediated through vitamin D receptor signaling in multiple tissues.[3,4]

Vitamin D receptors have also been reported in skeletal muscle, supporting the potential relevance of vitamin D signaling to muscle function.[5] Accumulating evidence suggests that low 25(OH)D levels are associated with muscle weakness, impaired muscle function, and reduced functional mobility.[6–8] Mechanistically, vitamin D may influence muscle tissue through genomic and nongenomic pathways involving protein synthesis, calcium handling, phosphate metabolism, phospholipid composition, and muscle cell differentiation and proliferation.[5,9] Early experimental studies also support direct effects of vitamin D metabolites on muscle calcium uptake, phosphate accumulation, ATP content, and protein synthesis.[10,11] Clinically, low serum 25(OH)D status has been

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associated with poorer lower-extremity function, poorer physical performance, greater functional decline over time, and increased risk of loss of muscle strength and muscle mass.[6,12,13]

Given the documented effects of vitamin D deficiency on skeletal muscle, it is reasonable to consider whether low vitamin D status may also influence phonation.[14,15] Hamdan et al argued that the larynx, and specifically the vocal folds, consists of striated musculoskeletal structures that may be susceptible to functional and histologic changes associated with vitamin D deficiency.[14] Because phonation requires coordinated neuromuscular control of the laryngeal system, reduced laryngeal muscle function may plausibly affect vocal fold vibration, glottal closure, pitch regulation, and phonatory endurance. On theoretical grounds, vitamin D deficiency could contribute to altered thyroarytenoid muscle performance, vocal fatigue, increased phonatory effort, and glottal insufficiency.[14] This hypothesis has also been raised in subsequent studies, which noted that the laryngopharyngeal complex may be susceptible to vitamin D deficiency–related muscle dysfunction in a manner broadly analogous to other musculoskeletal structures.[14,15]

However, empirical findings on the relationship between vitamin D status and voice remain limited and somewhat inconsistent.[16] In their narrative review, Hamdan et al identified only four eligible studies, with a total pooled sample of 466 participants.[16] Across those studies, the most commonly used voice outcomes were self-reported phonatory symptoms, the Voice Handicap Index-10, GRBAS ratings, fundamental frequency, jitter, shimmer, HNR, MPT, and dysphonia severity index (DSI).[16] Overall, that review concluded that there was no statistically significant difference in self-reported dysphonia, perceptual voice ratings, or most acoustic measures between participants with and without vitamin D deficiency.[16] Similarly, earlier primary studies reported nonsignificant group differences in VHI-10 scores, phonatory symptoms, GRBAS ratings, and conventional acoustic variables.[14,17] However, these studies also emphasized that small sample sizes and limited vocal tasks may have obscured subtle effects.[14,17]

At the same time, more recent work suggests that the association may be more nuanced than initially thought.[15,18] In subacute stroke patients with dysphonia, Park and Yoo found that serum vitamin D levels were associated with DSI values.[15] Park and Yoo also reported that participants with vitamin D deficiency had significantly lower DSI values for at least one corner vowel, more negative DSI values indicating more severe dysphonia, although MPT did not differ significantly between groups.[15] More recently, Ongphiphadhanakul et al reported associations between 25(OH)D levels and multiple acoustic features in a large adult population sample, particularly spectral parameters.[18] These findings suggest that vitamin D status may influence vocal function in ways that are not fully captured by traditional measures alone.[18] Therefore, the potential contribution of vitamin D to voice may depend on the population studied, the acoustic features selected, age-related factors, and the comprehensiveness of the voice assessment protocol.[15,16,18]

Importantly, the available literature has been derived almost entirely from adult samples, including endocrinology clinic populations, patients with dysphagia or stroke, and community-based adult cohorts.[14–19] Pediatric data on the relationship between vitamin D status and multidimensional voice characteristics appear to be lacking in the studies identified in the available review and primary papers.[16] This is a meaningful gap because children are in an active period of anatomical growth, neuromuscular maturation, and vocal development, and subtle changes in laryngeal function may not be adequately captured by symptom reporting alone.

A multidimensional pediatric evaluation that combines parent-reported voice handicap, perceptual analysis, acoustic measures, and aerodynamic performance may therefore provide a more sensitive framework for examining possible voice-related correlates of low vitamin D status. Therefore, the present study aimed to compare multidimensional voice profiles of children aged 3 to 12 years with and without vitamin D deficiency. By extending the literature to a pediatric sample and by using a broader multidimensional voice protocol than most earlier studies, this study sought to clarify whether low vitamin D status is associated with measurable differences in children's voice characteristics.

METHODS

Study design and participants

This study employed a cross-sectional comparative design and was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. Participants were recruited from children who presented for routine pediatric evaluations at a private pediatric clinic in Adana, Türkiye, between April 2025 and February 2026. As part of the routine clinical evaluation process, serum 25-hydroxyvitamin D [25(OH)D] levels were assessed and used for group classification.

Children were eligible for inclusion if they were between 3 and 12 years of age and had available serum 25(OH)D measurements obtained during the routine clinical workup. Children were excluded if they had an acute upper respiratory tract infection at the time of assessment, a known neurologic or neurodevelopmental disorder that could affect voice or swallowing, hearing loss, or another condition likely to affect speech or voice performance, a history of nasal, pharyngeal, or laryngeal surgery, previously documented structural laryngeal pathology, or diagnosed gastroesophageal reflux or laryngopharyngeal reflux.

All participants had available serum 25-hydroxyvitamin D [25(OH)D] measurements and were classified into two groups according to serum vitamin D status: children with vitamin D deficiency (25[OH]D < 20 ng/mL) and children with normal vitamin D levels (25[OH]D ≥ 20 ng/mL). The

20 ng/mL (50 nmol/L) threshold was selected because it corresponds to the lower limit of sufficiency proposed in a pediatric position paper.[20] The vitamin D deficiency group consisted of 23 children (14 girls, 9 boys; mean age: 7.85 ± 2.74 years), whereas the normal vitamin D group consisted of 24 children (10 girls, 14 boys; mean age: 6.33 ± 2.33 years). Children with vitamin D deficiency were significantly older than children with normal vitamin D levels ($t(45) = 2.06$, $P = 0.046$, $d = 0.60$). Sex distribution did not differ significantly between groups ($\chi^2(1) = 1.73$, $P = 0.188$).

Prior to participation, written parental informed consent was obtained from the parents or legal guardians of all children, and age-appropriate assent was obtained from the children. The study was conducted in accordance with the Declaration of Helsinki (1975, revised 2024)[21] and was approved by the Tarsus University Non-Interventional Clinical Research Ethics Committee (decision no. 2025/36, dated March 26, 2025).

Voice recording procedure

All voice samples were collected by the same researcher in a sound-insulated room under constant recording conditions, with the ambient noise level maintained below 30 dB. Recordings were made using a Samson Go Mic Portable USB microphone. The microphone was positioned approximately 15 cm from the participant's mouth, with its point of maximum sensitivity oriented perpendicular to the participant and at an angle of approximately 45°. All recordings were obtained at 16-bit resolution and saved in .wav format. Sustained vowel productions were recorded at a sampling rate of 44,100 Hz, whereas sentence recordings were obtained at 25,000 Hz.

Each child was instructed to produce a sustained vowel /a/ at a comfortable pitch and loudness following a deep inhalation, count numbers at a habitual speaking rate and intensity, and repeat the phonetically balanced sentence from the Turkish Pinocchio Passage *("Serüvenim, resimde gördüğünüz doğa harikası şu dağ köyünde başladı" ["My adventure began in that mountain village, a natural wonder you see in the picture"])*.[22,23] This sentence repetition task was used as the CPPS sample for CPPS analysis. For children who had difficulty repeating the full sentence, a shortened utterance derived from the same passage was elicited. In addition, an age-appropriate speech sample was obtained when needed. For sustained phonation, participants were asked to maintain steady phonation for as long as possible. When necessary, recordings were repeated to obtain at least one stable and analyzable sample. Acoustic analyses of sustained phonation were conducted on the mid-portion of the sustained vowel sample (minimum 3 seconds), excluding onset and offset segments to minimize variability related to phonation initiation and termination.

Voice assessment measures

A multidimensional voice assessment protocol was used. The evaluated parameters included perceptual voice quality using the GRBAS scale, parent-reported voice handicap using the Turkish version of the pVHI, acoustic measures including fundamental frequency, jitter, shimmer, HNR, and CPPS, and aerodynamic measures including MPT and the s/z ratio.

GRBAS Scale

Perceptual voice quality was evaluated using the GRBAS scale described by Hirano.[24] The GRBAS scale includes five perceptual parameters: Grade, Roughness, Breathiness, Asthenia, and Strain. Grade reflects the overall severity of dysphonia; Roughness refers to the perceived irregularity or fluctuation in vocal fold vibration; Breathiness indicates the audible escape of air during phonation, usually associated with incomplete glottal closure; Asthenia reflects weakness or lack of vocal power; and Strain refers to the perception of excessive vocal effort or hyperfunctional phonation. Each parameter is rated on a 4-point ordinal scale ranging from 0 to 3, where 0 indicates normal voice quality, 1 slight deviance, 2 moderate deviance, and 3 severe deviance.[24] In the present study, GRBAS ratings were performed on the basis of the recorded voice samples obtained from each participant.

GRBAS evaluations were performed independently by two speech-language therapists who were doctoral students, one with seven years and the other with five years of clinical experience. Inter-rater agreement for the GRBAS evaluations was assessed using quadratic weighted kappa coefficients, given the ordinal 4-point structure of the GRBAS scale. The quadratic weighted kappa values were 0.883 for Grade, 0.866 for Roughness, 0.781 for Breathiness, 0.801 for Asthenia, and 0.867 for Strain. After the independent ratings, the two raters reviewed samples with discrepant ratings, repeated the evaluation together, and reached consensus scores, which were used in the final analyses. Initial disagreement between the two raters was observed in 28 of 47 voice samples for at least one GRBAS parameter. Across the 235 parameter-level rater-pair comparisons, 28 discrepant ratings were identified. When examined by parameter, discrepancies were observed for Grade in five samples, Roughness in five samples, Breathiness in five samples, Asthenia in six samples, and Strain in six samples.

Turkish Pediatric Voice Handicap Index

The pVHI is a parent-completed patient-reported outcome measure designed to assess the functional, physical, and emotional impact of a child's voice disorder on daily life. The original pVHI was developed by Zur et al as an adaptation of the adult Voice Handicap Index for pediatric use.[25] The Turkish version was adapted and validated by Tadıhan Özkan et al and has been shown to be a valid and reliable instrument for use in Turkish-speaking pediatric populations.[26] It consists of 23 items distributed across three subdomains: functional (7 items), physical (9 items),

and emotional (7 items). Each item is rated on a 5-point Likert scale from 0 (never) to 4 (always). The total score ranges from 0 to 92, with higher scores indicating greater perceived voice-related handicap. Reported cut-off values for the Turkish version are 5.5 for the functional domain, 6.5 for the physical domain, 5.5 for the emotional domain, and 13 for the total score.

Acoustic and aerodynamic analyses

Acoustic analyses were performed using *Praat* (Version 6.4.63).[27] The analyzed acoustic variables included fundamental frequency, jitter, shimmer, HNR, and CPPS. For the s/z ratio, participants were asked to produce /s/ and /z/ three times each for as long as possible. The longest durations were measured in seconds and the ratio was calculated by dividing the longest /s/ duration by the longest /z/ duration. For MPT, participants were instructed to sustain the vowel /a/ three times for as long as possible. The longest duration across the three trials was recorded in seconds and used in the analysis. Because two children were unable to sustain /s/ and /z/ in the required manner, s/z ratio data could not be obtained for those participants. In such cases, those children were excluded only from analyses involving the relevant parameter, while remaining eligible for all other completed assessments. No missing-data imputation was performed.

Statistical analysis

Statistical analyses were conducted using *jamovi* (Version 2.6.17)[28] and *IBM SPSS Statistics* (Version 27.0). Descriptive statistics included mean, standard deviation, median, minimum, maximum, frequency, and percentage. The distribution of continuous variables was assessed using the Shapiro–Wilk test. For between-group comparisons, the independent-samples *t* test was used for normally distributed variables. For non-normally distributed variables, the Mann–Whitney *U* test was used. To examine whether the between-group comparisons were influenced by age and sex, age- and sex-adjusted sensitivity analyses were performed for parent-reported, acoustic, and aerodynamic voice outcomes using univariate general linear models. In each model, group was entered as the fixed factor of interest, age as a covariate, and sex as an additional fixed factor. Because of the modest sample size, interaction terms were not included in these models. Effect sizes were reported as Cohen's d (*d*) for the independent-samples *t* test, and as the rank-biserial correlation coefficient (r_{rb}) for the Mann–Whitney *U* test, and as partial eta squared for the age- and sex-adjusted general linear models. Cohen's d values were interpreted as small (0.20–0.49), medium (0.50–0.79), and large (≥0.80).[29] The magnitude of r_{rb} was interpreted as very small (< 0.10), small (0.10–0.29), medium (0.30–0.49), or large (≥0.50).[30] Sex distribution was compared between groups using the Pearson chi-square test. All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant.

RESULTS

As shown in Table 1, no statistically significant differences were found between children with vitamin D deficiency and those with normal vitamin D levels for any of the GRBAS parameters, including Grade, Roughness, Breathiness, Asthenia, and Strain (all $P > 0.05$). The corresponding effect sizes ranged from very small to small. The distributional patterns of GRBAS scores in both groups are further visualized in the raincloud plots presented in Figure 1.

As presented in Table 2, Turkish pVHI scores did not differ significantly between the vitamin D deficiency and normal vitamin D groups for the functional subscale ($P = 0.589$), physical subscale ($P = 0.312$), emotional subscale ($P = 0.076$), or total score ($P = 0.726$). The corresponding effect sizes ranged from very small to small in magnitude.

As shown in Table 3, no statistically significant differences were observed between children with vitamin D deficiency and those with normal vitamin D levels for any of the acoustic or aerodynamic voice measures (all $P > 0.05$).

TABLE 1.
Comparison of GRBAS Parameter Scores Between Children With Vitamin D Deficiency and Normal Vitamin D Levels

GRBAS Scale	Group	N	Mean	SD	Med	Min	Max	*U*	*P*	Effect size (r_{rb})
G	Vitamin D deficiency	23	1.08	0.51	1.00	0.00	2.00	219	0.176	-0.206
	Normal vitamin D	24	0.83	0.76	1.00	0.00	2.00			
R	Vitamin D deficiency	23	1.17	0.65	1.00	0.00	2.00	228	0.250	-0.173
	Normal vitamin D	24	0.95	0.62	1.00	0.00	2.00			
B	Vitamin D deficiency	23	0.95	0.36	1.00	0.00	2.00	232	0.243	-0.161
	Normal vitamin D	24	0.79	0.65	1.00	0.00	2.00			
A	Vitamin D deficiency	23	0.34	0.57	0.00	0.00	2.00	258	0.644	0.067
	Normal vitamin D	24	0.41	0.58	0.00	0.00	2.00			
S	Vitamin D deficiency	23	0.61	0.78	0.00	0.00	2.00	271	0.905	-0.020
	Normal vitamin D	24	0.54	0.65	0.00	0.00	2.00			

Note: Test statistic is reported as the Mann–Whitney *U* statistic (U). Effect size is reported as the rank-biserial correlation coefficient (r_{rb}) for Mann–Whitney *U* tests.
Abbreviations: G, grade; R, roughness; B, breathiness; A, asthenia; S, strain; N, sample size; SD, standard deviation; Med, median; Min, minimum; Max, maximum.

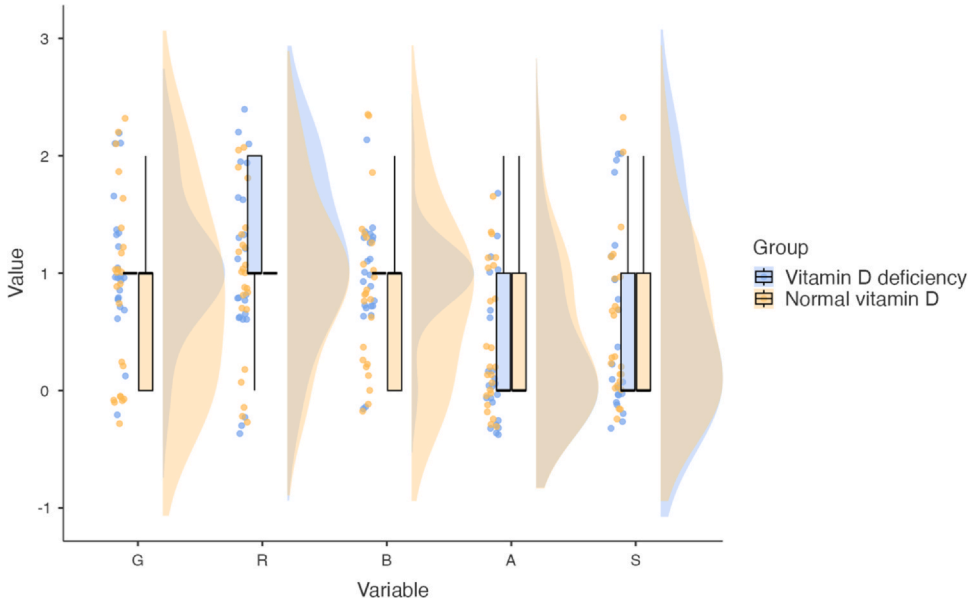

FIGURE 1. Distribution of GRBAS scale scores in children with vitamin D deficiency and normal vitamin D levels.

Specifically, between-group differences were not significant for fundamental frequency /a/ (P = 0.058), jitter (P = 0.551), shimmer (P = 0.093), harmonic-to-noise ratio (P = 0.700), CPPS /a/ (P = 0.147), CPPS (P = 0.661), MPT (P = 0.322), or s/z ratio (P = 0.666). Effect sizes were mostly small to moderate in magnitude, with the largest values observed for fundamental frequency (d = −0.567), shimmer (d = 0.501), and CPPS /a/ (d = −0.430).

As shown in Table 4, age- and sex-adjusted sensitivity analyses were performed for parent-reported, acoustic, and aerodynamic outcomes to examine whether the between-group comparisons were influenced by age and sex. After adjustment for age and sex, there were no statistically significant differences between children with vitamin D deficiency and those with normal vitamin D levels for any acoustic or aerodynamic outcome. For the pVHI outcomes, no significant between-group differences were observed for the Functional, Physical, or Total scores. However, the adjusted between-group difference for the Emotional subscale reached statistical significance (F(1, 43) = 4.768, P = 0.034, partial η^2 = 0.100).

DISCUSSION

To our knowledge, this is the first study to examine the relationship between vitamin D status and multidimensional voice characteristics in a pediatric sample. The main finding based on the primary unadjusted analyses was that children with vitamin D deficiency did not differ significantly from children with normal vitamin D levels on perceptual, parent-reported, acoustic, or aerodynamic voice measures. Specifically, no between-group differences were found for GRBAS parameters, Turkish pVHI subscale or total scores, or conventional acoustic and aerodynamic measures, including fundamental frequency, jitter, shimmer, HNR, CPPS, MPT, and s/z ratio. In this respect,

TABLE 2.
Comparison of Turkish Pediatric Voice Handicap Index Subscale and Total Scores between Children With Vitamin D Deficiency and Normal Vitamin D Levels

Questionnaire	Group	N	Mean	SD	Med	Min	Max	*U*	*P*	Effect size (r_{rb})
pVHI Functional	Vitamin D deficiency	23	2.21	4.88	0.00	0.00	20.00	255	0.589	-0.078
	Normal vitamin D	24	0.79	1.56	0.00	0.00	6.00			
pVHI-Physical	Vitamin D deficiency	23	1.00	2.65	0.00	0.00	8.00	242	0.312	0.125
	Normal vitamin D	24	0.75	1.73	0.00	0.00	7.00			
pVHI Emotional	Vitamin D deficiency	23	1.95	3.13	0.00	0.00	10.00	207	0.076	-0.252
	Normal vitamin D	24	0.54	1.14	0.00	0.00	4.00			
pVHI-Total	Vitamin D deficiency	23	5.17	9.69	0.00	0.00	38.00	261	0.726	-0.056
	Normal vitamin D	24	2.08	3.11	0.00	0.00	10.00			

Note: Test statistic is reported as the Mann–Whitney *U* statistic (U). Effect size is reported as the rank-biserial correlation coefficient (r_{rb}) for Mann–Whitney *U* tests.
Abbreviations: pVHI, Pediatric Voice Handicap Index; N, sample size; SD, standard deviation; Med, median; Min, minimum; Max, maximum.

TABLE 3.
Comparison of Acoustic and Aerodynamic Voice Measures Between Children With Vitamin D Deficiency and Normal Vitamin D Levels

Variables	Group	N	Mean	SD	Med	Min	Max	U^a/t^b	*P*	Effect size ($r_{rb}{}^a/d^b$)
Fundamental Frequency /a/	Vitamin D deficiency	23	246.75	69.38	263.74	105.00	354.80	-1.946[b]	0.058	-0.567[b]
	Normal vitamin D	24	286.86	71.82	272.74	98.10	479.31			
Jitter (local) /a/	Vitamin D deficiency	23	0.50	0.30	0.41	0.16	1.57	248[a]	0.551	-0.103[a]
	Normal vitamin D	24	0.44	0.21	0.39	0.15	1.11			
Shimmer (local) /a/	Vitamin D deficiency	23	6.17	2.34	6.05	2.28	11.78	1.717[b]	0.093	0.501[b]
	Normal vitamin D	24	5.15	1.68	4.82	3.17	9.94			
Harmonic-to-Noise Ratio /a/	Vitamin D deficiency	23	18.68	7.43	19.93	4.40	28.45	0.388[b]	0.700	0.113[b]
	Normal vitamin D	24	17.91	6.24	18.23	5.86	28.04			
CPPS /a/	Vitamin D deficiency	23	12.47	2.00	12.44	7.99	17.34	-1.475[b]	0.147	-0.430[b]
	Normal vitamin D	24	13.41	2.34	13.26	8.35	17.78			
CPPS (Connected Speech)	Vitamin D deficiency	23	9.65	1.81	9.92	4.99	13.17	-0.441[b]	0.661	-0.128[b]
	Normal vitamin D	24	9.87	1.51	10.05	7.30	12.31			
Maximum Phonation Time (s)	Vitamin D deficiency	23	6.70	4.93	5.49	1.76	24.42	208[a]	0.322	-0.174[a]
	Normal vitamin D	24	7.02	4.56	5.64	2.03	23.05			
s/z ratio	Vitamin D deficiency	21	1.02	0.48	0.94	0.28	2.16	255[a]	0.666	0.076[a]
	Normal vitamin D	24	0.92	0.44	0.82	0.43	2.29			

Note: Superscript a indicates that the reported test statistic is the Mann–Whitney *U* statistic (U) and the corresponding effect size is the rank-biserial correlation coefficient (r_{rb}). Superscript b indicates that the reported test statistic is the t statistic and the corresponding effect size is Cohen's d.
Abbreviations: CPPS, cepstral peak prominence smoothed; N, sample size; SD, standard deviation; Med, median; Min, minimum; Max, maximum.

TABLE 4.
Age- and Sex-adjusted Group Comparisons for Parent-Reported, Acoustic, and Aerodynamic Voice Outcomes

Variables	df	*F*	*P*	Partial η^2
Fundamental Frequency /a/	1, 43	2.870	0.097	0.063
Jitter (local) /a/	1, 43	0.684	0.413	0.016
Shimmer (local) /a/	1, 43	1.344	0.253	0.030
Harmonic-to-Noise Ratio /a/	1, 43	0.489	0.488	0.011
CPPS /a/	1, 43	0.257	0.615	0.006
CPPS (Connected Speech)	1, 43	0.009	0.924	0.000
Maximum Phonation Time (s)	1, 43	1.785	0.189	0.040
s/z ratio	1, 41	0.035	0.853	0.001
pVHI Functional	1, 43	2.326	0.135	0.051
pVHI-Physical	1, 43	1.127	0.294	0.026
pVHI Emotional	1, 43	4.768	0.034*	0.100
pVHI-Total	1, 43	3.415	0.072	0.074

Note: Values represent the adjusted group effect from univariate general linear models. In each model, group was entered as the fixed factor of interest, age as a covariate, and sex as an additional fixed factor. The denominator degrees of freedom differed for the s/z ratio because data were unavailable for two participants.
*$P < 0.05$ is indicated with an asterisk.
Abbreviations: df, degrees of freedom; F, F statistic; partial η^2, partial eta squared; CPPS, cepstral peak prominence smoothed; HNR, harmonics-to-noise ratio; pVHI, Pediatric Voice Handicap Index.

the present findings extend to children the general pattern reported in the predominantly adult literature, in which vitamin D deficiency has not been consistently associated with higher self-reported voice handicap, greater perceptual dysphonia, or poorer conventional acoustic outcomes.[14,16,17]

At the same time, the present results should not be interpreted as definitive evidence that vitamin D status is irrelevant to pediatric voice. Although none of the primary unadjusted comparisons reached statistical significance, some variables showed comparatively larger between-group differences than others, particularly fundamental frequency, shimmer, CPPS /a/, and the emotional subscale of the pVHI. These descriptive patterns should be interpreted cautiously because they were not supported by statistically significant primary comparisons; nevertheless, they may help generate hypotheses for future studies using larger samples and more sensitive outcome measures. This interpretation is broadly consistent with more recent adult work suggesting that vitamin D-related effects on voice may be parameter-specific. For example, Park and Yoo found that serum vitamin D levels were associated with DSI values in subacute stroke patients, whereas MPT did not differ significantly between groups.[15] Likewise, Ongphiphadhanakul et al reported associations between 25(OH)D levels and multiple acoustic features, particularly spectral parameters, in a large adult sample, with stronger associations among younger participants.[18] Taken together, these studies suggest that the relationship between vitamin D and voice may be more nuanced than a simple presence-or-absence effect detectable by traditional measures alone.[15,18]

One possible explanation for the absence of clear group differences is that the laryngeal system may not respond to vitamin D deficiency in the same way as limb or axial skeletal muscles. Prior discussions in the literature have emphasized the distinctive histological and functional properties of intrinsic laryngeal muscles, including their fiber-type composition and task-specific specialization for phonation, airway protection, and respiration, which may confer relative resistance to the kinds of overt weakness and fatigability more commonly reported in other skeletal muscle systems.[14,16,17] In addition, because the present sample consisted of children, it is plausible that the duration and cumulative burden of low vitamin D exposure were shorter or less biologically consequential than in adult clinical populations. Thus, even if low vitamin D status affects laryngeal neuromuscular function, its effects in childhood may be too mild to produce overt changes in conventional voice measures.

A second explanation relates to measurement sensitivity. Earlier adult studies and the recent review by Hamdan et al noted that small samples and limited vocal tasks may obscure subtle voice-related effects of vitamin D deficiency.[14,16] Although the present study used a broader pediatric voice protocol than most earlier studies by combining perceptual, parent-reported, acoustic, and aerodynamic assessments, the protocol still relied primarily on conventional measures derived from sustained phonation and a short CPPS task. It did not include composite indices such as the DSI, vocal loading paradigms, high-dimensional spectral feature sets such as eGeMAPS, or instrumental visualization of laryngeal behavior. This is relevant because the positive findings reported by Park and Yoo were observed for DSI rather than MPT, and Ongphiphadhanakul et al identified associations mainly in spectral and energy-related voice features rather than in the limited set of conventional parameters used in many earlier studies.[15,18] Accordingly, vitamin D-related voice differences, if present in children, may be better captured by more sensitive or integrative outcome measures than those used routinely in standard clinical acoustic analysis.

The parent-reported and perceptual findings in the present study also merit consideration. Median pVHI scores were 0 across all subscales and the total score in both groups, indicating very low parent-perceived voice handicap overall. Similarly, GRBAS medians suggested mostly absent to mild perceptual deviation in both groups. This pattern raises the possibility of a floor effect, whereby subtle phonatory alterations may not have been salient enough to be perceived by parents or reflected in mild perceptual ratings. In a pediatric sample recruited from routine clinical evaluations rather than from a voice clinic population, such a low symptom burden is not unexpected. This may partly explain why no statistically significant

between-group differences were detected in pVHI or GRBAS outcomes, despite small numerical differences favoring the normal vitamin D group in several parameters. In the age- and sex-adjusted sensitivity analysis, the pVHI Emotional subscale showed a significant adjusted between-group difference, whereas the Functional, Physical, and Total scores remained nonsignificant. This isolated finding should be interpreted cautiously because the overall pVHI scores were low, median scores were 0 across subscales in both groups, and the analysis was exploratory in the context of a modest sample size. Therefore, this result may indicate a possible signal in parent-perceived emotional impact, but it should not be interpreted as conclusive evidence of vitamin D-related voice handicap in children.

The present study nevertheless has several strengths. First, it addresses a meaningful gap in the literature by extending this topic to children, whereas the existing evidence has been derived almost entirely from adult endocrinology, stroke, dysphagia, or community samples. Second, it used a multidimensional voice framework that incorporated parent-reported handicap, perceptual evaluation, acoustic analysis, and aerodynamic performance within a standardized recording protocol. Third, recordings were obtained under controlled acoustic conditions by the same researcher, and GRBAS ratings were based on independent evaluations with good inter-rater reliability before consensus scoring. These features increase the internal consistency of the assessment process and support the reliability of the voice assessment procedures.

Several limitations should also be acknowledged. Given its exploratory nature, the present study should be interpreted as a pilot cross-sectional comparative investigation rather than as definitive evidence regarding the absence of an association between vitamin D status and pediatric voice outcomes. The modest sample size may have reduced statistical power and increased the possibility of a Type II error, particularly for small or moderate effects. With 23 and 24 participants in the two groups, the study was powered primarily to detect relatively large between-group effects; therefore, the absence of statistically significant differences should not be interpreted as evidence that smaller but potentially clinically meaningful differences are absent. Moreover, because clinically meaningful difference thresholds are not well established for many pediatric acoustic, aerodynamic, and parent-reported voice outcomes in the context of vitamin D deficiency, the adequacy of statistical power to detect clinically meaningful effects remains uncertain. Therefore, the primary value of this study is to provide preliminary pediatric data and to inform the design of future adequately powered studies. The cross-sectional design precludes causal inference and does not allow evaluation of whether changes in vitamin D status over time are associated with changes in voice. Vitamin D status was classified on the basis of a single serum 25(OH)D measurement obtained during routine clinical care, and information on the duration of low vitamin D status was not available. The age range was broad (3–12 years), encompassing substantial developmental variability in anatomy, neuromuscular maturation, and task cooperation. Moreover, children with vitamin D deficiency were significantly older than children with normal vitamin D levels, indicating that age cannot be fully excluded as a potential confounding factor. This age imbalance may be particularly relevant for age-sensitive voice measures such as fundamental frequency and phonatory performance. Although age- and sex-adjusted sensitivity analyses were performed, the modest sample size limits the robustness of covariate-adjusted interpretations. Although sex distribution did not differ significantly between groups and sex was included in the adjusted sensitivity analyses, the modest sample size precluded reliable sex-stratified subgroup analyses. Potentially relevant covariates such as seasonal timing, dietary intake, pubertal status, physical activity, and habitual voice use were not modeled analytically. Finally, the study did not include laryngeal imaging, vocal loading tasks, or advanced spectral analyses, and s/z ratio data were unavailable for two participants. These limitations suggest that subtle or context-dependent associations may have gone undetected. Larger studies with age-stratified or developmentally narrower samples are needed to determine whether subtle vitamin D-related differences in voice characteristics may emerge in specific pediatric subgroups and whether such differences vary according to sex and developmental stage.

Taken together, the present findings suggest that low vitamin D status, as defined in this study, was not associated with overt differences in traditional multidimensional voice measures in children aged 3–12 years. However, the descriptive pattern of larger but nonsignificant between-group differences in selected variables, together with emerging adult evidence from DSI-based and high-dimensional acoustic studies, suggests that the issue may warrant further investigation. Future pediatric studies should include larger, developmentally stratified samples; consider the severity and duration of vitamin D deficiency; and incorporate more sensitive outcome measures, such as DSI, advanced spectral feature sets, vocal loading protocols, and laryngeal imaging. Longitudinal designs, including repeated voice assessment before and after correction of vitamin D deficiency, would be especially valuable in clarifying whether vitamin D status has a clinically meaningful effect on children's phonatory function.

CONCLUSION

In conclusion, the primary unadjusted analyses showed no significant differences in perceptual, parent-reported, acoustic, or aerodynamic voice measures between children with vitamin D deficiency and those with normal vitamin D levels. These findings suggest that, in this pediatric sample, low vitamin D status was not associated with overt voice impairment as captured by conventional multidimensional voice assessment. Nevertheless, given the pilot nature of this study, the limited pediatric literature, the modest sample size, and recent adult evidence pointing to more parameter-specific associations, further studies using larger cohorts, age-stratified designs, and more sensitive voice measures are needed before a definitive conclusion can be reached.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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