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Original article

Upward changes in serum 25-hydroxyvitamin D levels in South Korea: the Korea National Health and Nutrition Examination Survey studies IX (2022–2023) versus VI (2013–2014)

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ABSTRACT

Objectives: To compare serum 25-hydroxyvitamin D (25[OH]D) concentrations and the prevalence of vitamin D deficiency between the Korea National Health and Nutrition Examination Survey (KNHANES) VI and IX, and to evaluate demographic, seasonal, and lifestyle factors associated with vitamin D status in South Korea.
Methods: Data from KNHANES IX (2022–2023), in which serum 25(OH)D concentrations were measured by liquid chromatography–tandem mass spectrometry, were compared with data from KNHANES VI (2013–2014), in which radioimmunoassay was used. Information on dietary supplement intake (≥2 weeks per year) was obtained through a standardized questionnaire. Vitamin D status was defined according to serum 25(OH)D concentrations as deficiency (<20 ng/mL), adequacy (≥20 and <50 ng/mL), and excess (≥50 ng/mL).
Results: Serum 25(OH)D levels significantly increased across both sexes and all age groups. The odds of vitamin D deficiency were significantly lower in KNHANES IX compared to VI, with adjusted odds ratios of 0.358 for men and 0.214 for women. The proportion of subjects taking dietary supplements increased significantly from 43.2% to 66.3%. Body mass index was significantly associated with higher odds of vitamin D deficiency exclusively among women in KNHANES IX. Winter remained persistently associated with higher odds of deficiency.
Conclusions: Over the past decade, South Korea has experienced an upward shift in population-level vitamin D concentrations. These changes may be associated with increased intake of dietary supplements, but because direct standardization of analytical methods has not been achieved, the relative contributions of behavioral changes and analytical method-related differences cannot be quantified.

1. Introduction

Vitamin D plays a central role in calcium-phosphate homeostasis and skeletal integrity, and its deficiency has long been associated with rickets, osteomalacia, and osteoporosis. Beyond classical skeletal effects, evidence accumulated over the past two decades has linked vitamin D status to a wide range of extraskeletal conditions, including immune dysfunction, cardiometabolic disorders, and certain malignancies, although causality remains debated. Circulating 25-hydroxyvitamin D (25[OH]D) is widely accepted as the best biomarker of vitamin D status, reflecting both cutaneous synthesis and intake from diet and supplements.

Population-based studies conducted in the early 2000s consistently reported a high prevalence of vitamin D insufficiency across diverse geographic regions and age groups. For example, analyses of the National Health and Nutrition Examination Survey (NHANES) demonstrated that a substantial proportion of the U.S. population had serum 25(OH)D concentrations below commonly accepted sufficiency thresholds [1]. Similar findings were observed in Europe and Asia, including South Korea, where limited sunlight exposure, urban lifestyles, and low dietary vitamin D intake contributed to widespread deficiency [2–4]. These observations led to increased clinical awareness and a marked rise in

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vitamin D testing and supplementation practices globally.

In response, many countries have implemented public health strategies to improve vitamin D status, including food fortification policies, updated dietary recommendations, and broader availability of over-the-counter supplements [5]. Concurrently, prescription vitamin D preparations have been increasingly used in clinical practice to prevent and treat deficiency, particularly in high-risk populations such as older adults and individuals with chronic diseases [6]. These shifts suggest that contemporary population vitamin D status may differ substantially from that observed a decade earlier.

Another important consideration in evaluating temporal trends in serum 25(OH)D concentrations is the evolution of laboratory measurement techniques. Historically, radioimmunoassay (RIA) and other automated immunoassays were widely used in large-scale epidemiologic studies. However, these methods are susceptible to analytical variability, including cross-reactivity with other vitamin D metabolites and differences in assay calibration [7]. More recently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as the reference method due to its superior specificity and ability to distinguish between 25(OH)D_2 and 25(OH)D_3 [7,8]. The transition between assay platforms may therefore influence observed population trends, complicating direct comparisons across time periods.

The Korea National Health and Nutrition Examination Survey (KNHANES) provides a valuable framework for assessing changes in vitamin D status at the population level. Such datasets enable the evaluation of population-level changes over time, the identification of at-risk subgroups, and the assessment of the impact of public health interventions. However, interpretation of these trends requires careful consideration of both behavioral factors, such as supplement use and lifestyle changes, and methodological factors, including assay standardization and calibration.

In this context, the present study aims to investigate changes in serum 25(OH)D concentrations over time in a nationally representative population. By leveraging data from repeated cross-sectional surveys, we sought to evaluate inter-survey differences in serum 25(OH)D concentration and vitamin D deficiency and to explore potential contributing factors, including increased use of vitamin D supplements and changes in laboratory measurement methods.

2. Methods

2.1. Study participants and data collection

KNHANES is a continuous surveillance system conducted by the Korea Disease Control and Prevention Agency to evaluate the health and nutritional status of South Korea's noninstitutionalized civilian population. To ensure national representativeness, KNHANES employs a stratified multi-stage cluster sampling design and provides integrated weights calculated using the probability of selection, non-response rates, and the age-sex structure of the population for analyses. The present study used data from KNHANES VI (2013–2014) and KNHANES IX (2022–2023) to investigate between-survey changes in vitamin D status. The numbers of participants were 8018 in year 2013, 7550 in year 2014, 6265 in year 2022, and 6929 in year 2023, yielding a total sample size of 28 762. Data were collected through household interviews and standardized physical examinations conducted at mobile examination centers.

To ensure comparability with the previous study, we retained the same definitions for categorical covariates expected to influence serum 25(OH)D concentrations. Age in years was categorized into five groups: 10–19, 20–34, 35–49, 50–64, and 65 years or more. Regions of residence were classified as urban or rural areas. The urban category included nine administrative regions (Seoul, Busan, Daegu, Incheon, Gwangju, Daejeon, Ulsan, Sejong, and Gyeonggi), whereas the rural category included the remaining eight regions (Gangwon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, and Jeju). To evaluate seasonal variations, the timing of blood collection was categorized into four periods: spring (March to May), summer (June to August), fall (September to November), and winter (January, February, and December). In the present study, we additionally incorporated dietary supplement intake and body mass index (BMI) as covariates. Dietary supplement intake was defined as a binary variable (yes/no) indicating intake for more than two weeks in the past year. Vitamin D intake was not assessed in KNHANES VI (although it was assessed in KNHANES IX). BMI was categorized into four groups: underweight (<18.5 kg/m^2), normal weight (≥ 18.5 and <23 kg/m^2), overweight (≥ 23 and <25 kg/m^2), and obese (≥ 25 kg/m^2). Based on serum 25(OH)D concentrations, vitamin D status was classified into three categories: deficiency (<20 ng/mL), adequacy (≥ 20 and <50 ng/mL), and excess (≥ 50 ng/mL).

2.2. Measurement of serum 25(OH)D

The detailed procedures for blood sample collection, cold-chain transportation, and the analytical validation of the earlier RIA method have been described in a previous publication. Due to changes in the central testing institutes, the assay method for measuring serum vitamin D shifted from RIA in KNHANES VI to LC-MS/MS in KNHANES IX. For KNHANES VI, serum 25(OH)D concentrations were determined using a 1470 WIZARD gamma-counter (PerkinElmer, Turku, Finland) with a 25(OH)D^{125}I RIA kit (DiaSorin, Stillwater, MN, USA). In KNHANES IX, serum concentrations were analyzed via LC-MS/MS using a Triple Quad 4500MD system (SCIEX, Framingham, MA, USA) and the MSMS Vitamin D kit (PerkinElmer, Turku, Finland).

Unlike the RIA method, which reported a single total 25(OH)D value, the LC-MS/MS analysis provided separate measurements of 25(OH)D_2, 25(OH)D_3, and 3-epi-25(OH)D_3. Consequently, the total serum 25(OH)D concentration in KNHANES IX was calculated as the sum of these three measurements to facilitate comparison with KNHANES VI. Both testing institutes involved in the measurement process maintained high analytical standards by consistently participating in the international Vitamin D External Quality Assessment Scheme (DEQAS). Notably, serum vitamin D concentrations were measured in a random subsample of 2400 participants aged ≥ 10 years in KNHANES VI, whereas measurements were performed for all participants aged ≥ 10 years in KNHANES IX.

2.3. Statistical analyses

Statistical analyses were performed to evaluate the effects of participant characteristics, including age, BMI, season, dietary supplement intake, and region of residence, on serum 25(OH)D concentrations, as well as to compare the independent risk factors associated with vitamin D deficiency between KNHANES VI and IX. Participants with missing values for serum vitamin D were excluded from the analysis. All analyses were conducted separately for males and females to account for established gender-specific differences in vitamin D metabolism. To accommodate the complex sampling design of KNHANES, sampling weights, primary sampling units, and strata were applied to all analyses to ensure results were representative of the national population. Differences in mean serum concentrations by participant characteristics were tested using design-adjusted t-tests or ANOVA. Because KNHANES VI (2013–2014) and IX (2022–2023) were separated by a substantial time interval, we focused on between-wave differences in vitamin D status rather than continuous trend analysis.

Furthermore, as our previous findings indicated a complete absence of the vitamin D excess group during KNHANES VI, logistic regression models were employed to analyze differences in the prevalence of vitamin D deficiency (<20 ng/mL) between the waves, adjusting for the aforementioned potential confounding factors. For the logistic regression models, results were presented as odds ratios (ORs) with 95% confidence intervals (CIs). The statistical significance of each covariate (variable group) was determined using the Rao-Scott Likelihood Ratio

Test (LRT) to evaluate its overall contribution to the model. All statistical analyses were performed using R version 4.5.2 [9] with the survey package [10], and statistical significance was defined as $P < 0.05$.

3. Results

3.1. Demographic characteristics of the study samples and target population

The demographic characteristics of the study participants and the weighted estimates for the target population are summarized in Table 1 and Supplementary Table 1, respectively. A total of 16 448 individuals were included in the final analysis, comprising 4704 participants in KNHANES VI (2355 in year 2013 and 2349 in year 2014) and 11 744 participants in KNHANES IX (5560 in year 2022 and 6184 in year 2023). In the unweighted sample, the proportion of female participants increased from 50.6% in KNHANES VI to 55.8% in KNHANES IX. However, when accounting for sampling weights, the sex distribution remained nearly equal at approximately 49.9% for men and 50.1% for women across both waves, reflecting the actual South Korean population structure.

A significant aging trend was observed between the two waves. In the unweighted sample, the proportion of participants aged 65 years or more rose substantially from 9.1% in KNHANES VI to 28.6% in KNHANES IX. The weighted estimates also confirmed this trend, with the elderly population increasing from 13.7% to 19.2% during the same period. Regarding BMI, the prevalence of obesity ($\geq$25.0 kg/m^2) in the sample increased from 29.5% in KNHANES VI to 33.7% in KNHANES IX. This shift was even more pronounced in the weighted population analysis, where obesity prevalence rose from 29.6% to 35.2%.

Dietary supplement intake showed the most dramatic increase between the periods. The percentage of participants reporting supplement use $\geq$2 weeks per year rose from 38.2% in KNHANES VI to 64.7% in KNHANES IX in the unweighted sample. The weighted prevalence similarly climbed from 43.2% in KNHANES VI to 66.3% in KNHANES IX. Residential distribution remained relatively stable, with urban residents accounting for approximately 70% of the population in both waves. The seasonal distribution of participants was also relatively uniform across both waves, with approximately 25% of the data collected in each season.

3.2. Serum 25(OH)D concentrations and their distributions

The mean serum vitamin D concentrations and their distributions across participant characteristics for KNHANES VI and IX are presented in Table 2 and Fig. 1, respectively. A significant shift in vitamin D status was observed between the two periods. In KNHANES VI, the overall mean serum 25(OH)D concentrations were 17.14 (standard error [SE] 0.22) ng/mL for men and 15.55 (SE 0.19) ng/mL for women, with mean values in both groups falling below the deficiency threshold of 20 ng/mL. However, in KNHANES IX, these concentrations increased significantly to 23.00 (SE 0.23) ng/mL for men and 25.24 (SE 0.22) ng/mL for women, marking an increase and a reversal of the sex difference, with women exhibiting higher mean concentrations than men. Notably, sex-specific comparisons between the two waves demonstrated that mean serum 25(OH)D concentrations increased significantly from KNHANES VI to IX across all participant characteristics—including age groups, BMI categories, seasons, dietary supplement intake, and residential areas (all $P < 0.001$).

Fig. 1 illustrates this temporal shift in distribution. While the majority of participants in KNHANES VI were concentrated below the 20 ng/mL deficiency line (red dashed line), the boxplots for KNHANES IX shifted upward, with mean concentrations (represented by black dots) surpassing the deficiency threshold for both sexes. Furthermore, the emergence of the excess group ($\geq$50 ng/mL, blue dashed line) was clearly visible in KNHANES IX through the presence of extended upper whiskers and outliers, which were nearly absent in the KNHANES VI period.

The analysis by participant characteristics revealed that age remained a significant factor ($P < 0.001$), with the highest concentrations observed in the elderly group ($\geq$65 years). Specifically, women in this age group during KNHANES IX reached a mean of 31.03 (SE 0.39) ng/mL. While BMI did not significantly affect vitamin D concentrations in KNHANES VI, it became a significant factor in KNHANES IX for both

Table 1
Demographic characteristics of the study participants in KNHANES VI (2013–2014) and IX (2022–2023).

		KNHANES VI			KNHANES IX		
Year		2013	2014	Total	2022	2023	Total
Number of participants		2355	2349	4704	5560	6184	11744
Sex	Male	1177 (50.0)	1145 (48.7)	2322 (49.4)	2456 (44.2)	2737 (44.3)	5193 (44.2)
	Female	1178 (50.0)	1204 (51.3)	2382 (50.6)	3104 (55.8)	3447 (55.7)	6551 (55.8)
Age group (yr)	10-19	387 (16.4)	372 (15.8)	759 (16.1)	452 (8.1)	512 (8.3)	964 (8.2)
	20-34	557 (23.7)	548 (23.3)	1105 (23.5)	848 (15.3)	863 (14.0)	1711 (14.6)
	35-49	620 (26.3)	619 (26.4)	1239 (26.3)	1213 (21.8)	1305 (21.1)	2518 (21.4)
	50-64	577 (24.5)	598 (25.5)	1175 (25.0)	1450 (26.1)	1745 (28.2)	3195 (27.2)
	$\geq$65	214 (9.1)	212 (9.0)	426 (9.1)	1597 (28.7)	1759 (28.4)	3356 (28.6)
BMI	underweight	167 (7.1)	177 (7.5)	344 (7.3)	326 (5.9)	380 (6.1)	706 (6.0)
	normal	981 (41.7)	956 (40.7)	1937 (41.2)	2073 (37.3)	2307 (37.3)	4380 (37.3)
	overweight	534 (22.7)	494 (21.0)	1028 (21.9)	1196 (21.5)	1315 (21.3)	2511 (21.4)
	obese	670 (28.5)	720 (30.7)	1390 (29.5)	1871 (33.7)	2082 (33.7)	3953 (33.7)
	NA	3 (0.1)	2 (0.1)	5 (0.1)	94 (1.7)	100 (1.6)	194 (1.7)
Season	Spring	584 (24.8)	608 (25.9)	1192 (25.3)	1376 (24.7)	1620 (26.2)	2996 (25.5)
	Summer	606 (25.7)	627 (26.7)	1233 (26.2)	1399 (25.2)	1675 (27.1)	3074 (26.2)
	Fall	570 (24.2)	606 (25.8)	1176 (25.0)	1535 (27.6)	1515 (24.5)	3050 (26.0)
	Winter	595 (25.3)	508 (21.6)	1103 (23.4)	1250 (22.5)	1374 (22.2)	2624 (22.3)
Dietary supplement intake	Yes	899 (38.2)	896 (38.1)	1795 (38.2)	3569 (64.2)	4029 (65.2)	7598 (64.7)
	No	1159 (49.2)	1177 (50.1)	2336 (49.7)	1639 (29.5)	2054 (33.2)	3693 (31.4)
	NA	297 (12.6)	276 (11.7)	573 (12.2)	352 (6.3)	101 (1.6)	453 (3.9)
Residential area	Urban	1698 (72.1)	1698 (72.3)	3396 (72.2)	3843 (69.1)	4255 (68.8)	8098 (69.0)
	Rural	657 (27.9)	651 (27.7)	1308 (27.8)	1717 (30.9)	1929 (31.2)	3646 (31.0)

BMI, body mass index; KNHANES, Korea National Health and Nutrition Examination Survey; NA, not available.
Data are presented as n (%) unless otherwise indicated.

Table 2
Weighted mean serum 25(OH)D concentrations by participant characteristics in KNHANES VI (2013–2014) and IX (2022–2023).

		KNHANES VI				KNHANES IX			
		Male		Female		Male		Female	
		Mean (SE)	P-value	Mean (SE)	P-value	Mean (SE)	P-value	Mean (SE)	P-value
All participants		17.14 (0.22)		15.55 (0.19)		23.00 (0.23)		25.24 (0.22)	
Age group (yr)	10-19	17.13 (0.35)	<0.001	15.38 (0.35)	<0.001	20.67 (0.47)	<0.001	17.56 (0.45)	<0.001
	20-34	15.55 (0.32)		14.25 (0.26)		19.10 (0.41)		19.96 (0.43)	
	35-49	16.98 (0.29)		15.01 (0.27)		22.87 (0.39)		23.31 (0.35)	
	50-64	19.43 (0.37)		17.53 (0.34)		24.88 (0.37)		28.71 (0.35)	
	≥65	18.83 (0.50)		18.09 (0.59)		26.69 (0.39)		31.03 (0.39)	
BMI	underweight	17.23 (0.68)	0.312	15.03 (0.43)	0.118	23.73 (0.89)	<0.001	23.28 (0.67)	<0.001
	normal	17.24 (0.30)		15.39 (0.23)		23.63 (0.35)		25.67 (0.32)	
	overweight	17.52 (0.36)		16.11 (0.35)		23.77 (0.39)		26.33 (0.43)	
	obese	16.79 (0.28)		15.65 (0.32)		22.06 (0.30)		24.35 (0.33)	
Season	Spring	15.94 (0.27)	<0.001	14.27 (0.28)	<0.001	21.70 (0.36)	<0.001	24.49 (0.47)	<0.001
	Summer	18.99 (0.47)		17.19 (0.37)		25.45 (0.41)		26.42 (0.36)	
	Fall	19.01 (0.49)		17.06 (0.39)		24.89 (0.36)		26.17 (0.39)	
	Winter	14.40 (0.28)		13.46 (0.24)		19.77 (0.39)		23.66 (0.45)	
Dietary supplement	Yes	18.36 (0.29)	<0.001	16.75 (0.27)	<0.001	25.48 (0.26)	<0.001	27.66 (0.26)	<0.001
	No	16.37 (0.26)		14.63 (0.23)		18.90 (0.31)		19.00 (0.28)	
Residential area	Urban	16.73 (0.26)	0.003	15.47 (0.22)	0.441	22.56 (0.27)	0.005	25.25 (0.26)	0.972
	Rural	18.19 (0.42)		15.80 (0.37)		24.07 (0.46)		25.23 (0.36)	

25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; KNHANES, Korea National Health and Nutrition Examination Survey; SE, standard error.
P-values were determined using design-adjusted *t*-test or ANOVA.

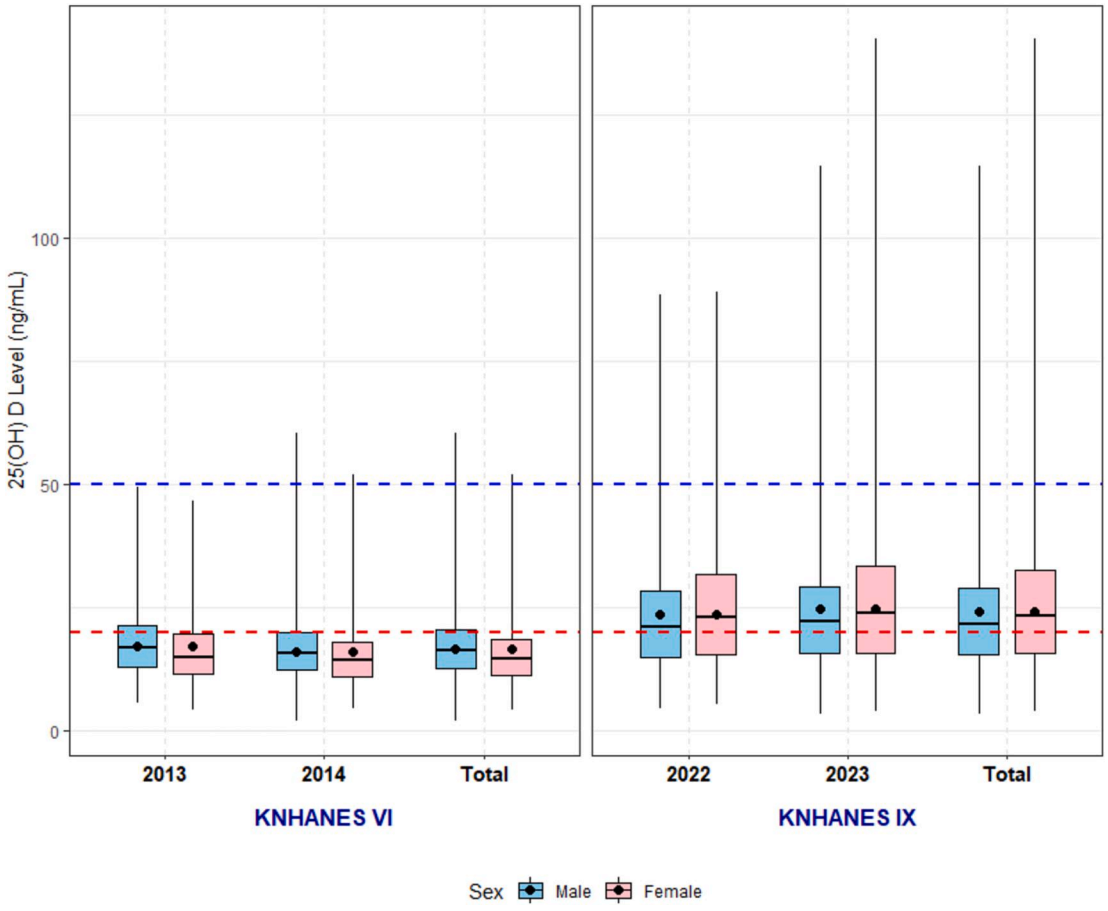

Fig. 1. Comparison of serum 25(OH)D concentration distributions between KNHANES VI and IX by sex. The black filled circles indicate the mean serum 25(OH)D concentrations. The red and blue dashed horizontal lines indicate the deficiency threshold 20 ng/mL and excess threshold 50 ng/mL, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sexes ($P < 0.001$). Seasonal variations were consistent across both waves, with the lowest concentrations recorded in winter and the

highest in summer or fall (P < 0.001). Dietary supplement intake was a major determinant of serum concentrations in both waves (P < 0.001). In KNHANES IX, mean serum concentrations were significantly higher among supplement users (25.48 [SE 0.26] ng/mL for men and 27.66 [SE 0.26] ng/mL for women) than among non-users (18.90 [SE 0.31] ng/mL and 19.00 [SE 0.28] ng/mL). Residential area significantly influenced male serum concentrations in both waves, whereas no significant difference was observed among women.

The overall shift in vitamin D status between the two waves is further illustrated by the prevalence of each category in Fig. 2. In KNHANES VI, the prevalence of vitamin D deficiency (<20 ng/mL) was 72.8% for men and 80.5% for women. During this period, the excess group (≥50 ng/mL) was practically non-existent in both sexes. By KNHANES IX, the prevalence of deficiency substantially decreased to 43.6% in men and 39.8% in women. In contrast, the proportion of individuals with adequate status (≥20 and <50 ng/mL) and excess status (≥50 ng/mL) expanded significantly. Specifically, the newly emerged excess group accounted for 1.9% of men and 4.1% of women in the ninth wave, reflecting a distinct upward shift in the population's vitamin D distribution.

3.3. Factors associated with vitamin D deficiency

The results of the sex-specific logistic regression analysis for factors associated with vitamin D deficiency (<20 ng/mL) are presented as odd ratios (ORs) in Table 3. A profound reduction in the risk of vitamin D deficiency was observed in KNHANES IX compared with KNHANES VI for both sexes. After adjusting for potential confounders, the odds of deficiency in the ninth wave significantly decreased to an OR of 0.358 (95% confidence interval [CI] 0.305–0.420) for men and an even more pronounced OR of 0.214 (95% CI 0.178–0.257) for women (P < 0.001 for wave, both men and women).

Age was a highly significant factor influencing vitamin D status in both sexes (P < 0.001). Interestingly, young adult men (20–34 years) exhibited a significantly higher risk of deficiency compared to the reference group (10–19 years), with an OR of 1.825 (95% CI 1.439–2.314). In contrast, the elderly population showed the lowest risk, particularly among women aged ≥65 years, who had remarkably low odds of deficiency (OR 0.165, 95% CI 0.128–0.212). The impact of BMI showed a distinct gender difference. While BMI did not significantly influence the odds of deficiency in men (P = 0.331), it was a major determinant for women (P < 0.001). For women, the risk of deficiency increased progressively with BMI, reaching an OR of 1.725 (95% CI 1.381–2.155) in the obese group.

Environmental and lifestyle factors also played critical roles. Seasonal variation was significant for both sexes (P < 0.001). Notably, winter was a risk factor for men (OR 1.420, 95% CI 1.177–1.713) but showed a less pronounced effect for women (OR 1.089, 95% CI 0.918–1.291). Regional differences were significant only for men (P < 0.001), with rural residency acting as a protective factor (OR 0.667,

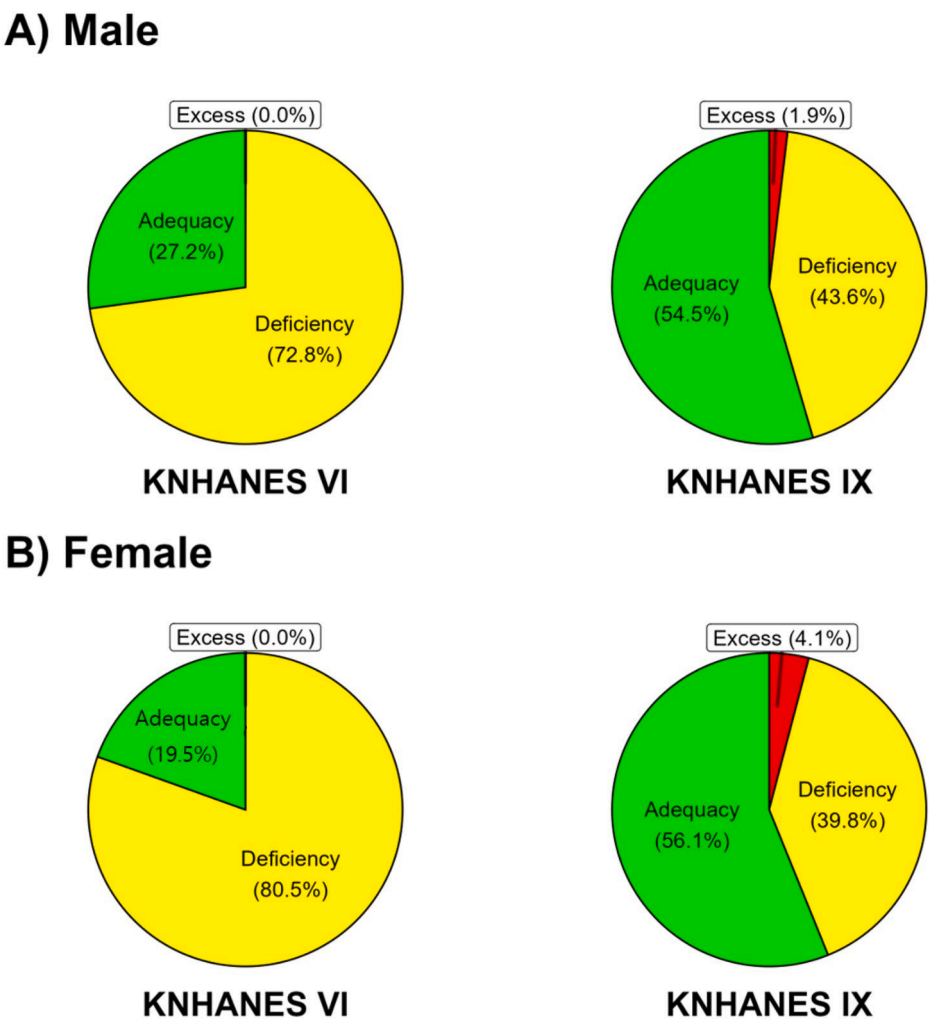

Fig. 2. Comparison of vitamin D status prevalence between KNHANES VI (2013–2014) and IX (2022–2023) by sex. Data for the (A) male and (B) female groups are presented separately. Vitamin D status is categorized into deficiency (<20 ng/mL, yellow), adequacy (≥20 and <50 ng/mL, green), and excess (≥50 ng/mL, red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Adjusted odds ratios for vitamin D deficiency (<20 ng/mL) from sex-specific logistic regression in KNHANES VI (2013–2014) and IX (2022–2023).

		Male		Female	
Variable	Value	OR (95% CI)	P-value	OR (95% CI)	P-value
Wave	9th	0.358 (0.305-0.420)	<0.001	0.214 (0.178-0.257)	<0.001
Age group (yr)	20-34	1.825 (1.439-2.314)	<0.001	0.847 (0.649-1.107)	<0.001
	35-49	0.936 (0.745-1.175)		0.528 (0.418-0.667)	
	50-64	0.557 (0.452-0.686)		0.238 (0.186-0.303)	
	≥65	0.439 (0.350-0.550)		0.165 (0.128-0.212)	
BMI	normal	1.105 (0.821-1.486)	0.331	1.242 (1.015-1.519)	<0.001
	overweight	1.080 (0.791-1.476)		1.446 (1.143-1.829)	
	obese	1.211 (0.891-1.647)		1.725 (1.381-2.155)	
Season	Summer	0.359 (0.296-0.436)	<0.001	0.492 (0.409-0.591)	<0.001
	Fall	0.411 (0.334-0.505)		0.557 (0.464-0.669)	
	Winter	1.420 (1.177-1.713)		1.089 (0.918-1.291)	
Region	Rural	0.667 (0.566-0.786)	<0.001	0.944 (0.813-1.095)	0.446
Dietary supplement	Yes	0.329 (0.287-0.377)	<0.001	0.294 (0.257-0.335)	<0.001

Reference categories for each variable were as follows: KNHANES VI, age 10–19 years, underweight BMI, spring, urban area, and no dietary supplement use.
P-values were determined using the Rao-Scott likelihood ratio test.
CI, confidence interval; KNHANES, Korea National Health and Nutrition Examination Survey; OR, odds ratio.

95% CI 0.566-0.786). Finally, dietary supplement intake for 2 weeks or more was the most consistent protective factor across both sexes ($P < 0.001$), reducing the odds of deficiency to 0.329 (95% CI 0.287–0.377) for men and 0.294 (95% CI 0.257–0.335) for women.

4. Discussion

In the present analysis, we observed an upward shift in serum 25(OH)D concentrations in the general population compared with those measured approximately a decade earlier. This finding may reflect a combination of true biological changes—principally increased vitamin D intake—and methodological factors related to assay evolution. However, the relative contribution of each factor cannot be quantified because direct standardization between the two analytical platforms has not been performed. Both aspects warrant careful consideration when interpreting temporal trends.

A major explanatory factor is the substantial rise in vitamin D supplementation over the past decade. Following early reports of widespread vitamin D deficiency and its potential links to multiple chronic diseases, public awareness increased, accompanied by expanded clinical testing [11] and supplementation practices [12,13]. Prescription vitamin D preparations, as well as over-the-counter cholecalciferol and ergocalciferol supplements, have become widely accessible and frequently used in both preventive and therapeutic contexts [14,15]. Importantly, serum 25(OH)D concentrations increase predictably in response to oral supplementation, reflecting replenishment of body vitamin D stores [12]. Thus, increased supplement use is biologically plausible as a contributor to higher circulating 25(OH)D concentrations, although its contribution cannot be separated from assay-related effects in the present analysis.

In parallel, dietary patterns may have contributed to higher vitamin D intake. Increased fortification of foods and greater consumption of vitamin D-enriched products, along with heightened clinical attention to at-risk populations, may further amplify this effect. Because direct measures of sunlight exposure and outdoor activity were not consistently available, the contribution of exogenous intake relative to other behavioral factors and assay-related differences remains uncertain.

However, methodological differences in 25(OH)D measurement represent a critical alternative explanation. Over the last decade, there has been a transition in many laboratories from RIA and other immunoassays toward LC-MS/MS, now widely regarded as the reference method for vitamin D metabolite quantification [12]. This shift has important analytical implications. Immunoassays, including RIA, are subject to cross-reactivity, matrix effects, and limited specificity, which can lead to systematic bias and variability in measured 25(OH)D concentrations [16]. In contrast, LC-MS/MS offers improved analytical specificity, enabling separate quantification of $25(OH)D_2$ and $25(OH)D_3$ and reducing interference from structurally related metabolites.

Several comparative studies have demonstrated substantial discrepancies between assay methods. For example, measurements obtained by immunoassays may differ significantly from LC-MS/MS values, with either positive or negative bias depending on the platform [17]. In some settings, RIA has been shown to yield higher 25(OH)D concentrations than LC-MS/MS, reflecting systematic overestimation [18], whereas other studies report the opposite direction of bias [17]. Moreover, large population-based analyses indicate that LC-MS/MS measurements can differ from RIA by clinically meaningful margins (eg, >10 ng/mL), emphasizing the challenge of comparing data across time periods when assay platforms have changed [19].

Although direct comparison of absolute serum 25(OH)D concentrations between the two KNHANES waves is limited by the change in assay platform, previous Korean data measured by LC-MS/MS provide useful context. In a 2013 study of 17 252 Korean health examinees [20], the mean total serum 25(OH)D concentrations measured by LC-MS/MS were 21.9 ng/mL in men, 19.2 ng/mL in women, and 20.6 ng/mL overall. These values were higher than the RIA-based mean concentrations observed in the sixth KNHANES, but remained lower than the LC-MS/MS-based concentrations observed in the ninth survey. Therefore, although assay-related differences may have contributed to the apparent increase between surveys, the higher serum 25(OH)D concentrations observed in the ninth survey may not be explained solely by the transition from RIA to LC-MS/MS.

These methodological inconsistencies are further compounded by issues of assay standardization. Lack of harmonization across laboratories and platforms has historically limited comparability of 25(OH)D data, potentially leading to misclassification of vitamin D status at both individual and population levels [16]. Although international standardization efforts (eg, alignment with reference measurement procedures and standard reference materials) have improved assay agreement, residual variability persists and may influence apparent between-survey changes.

Taken together, the observed increase in serum 25(OH)D concentrations is likely multifactorial. These observations suggest that a decrease in vitamin D deficiency is more significantly associated with an increase in dietary vitamin D intake or supplement use than with changes in measurement methods. However, concurrent changes in assay methodology, particularly the transition from RIA-based techniques to LC-MS/MS, may have introduced systematic shifts in measured

concentrations that partially account for the observed differences. Consequently, caution is required when interpreting between-survey changes in vitamin D status, especially when historical and contemporary data are derived from different analytical platforms. This observation suggests that the observed reduction in vitamin D deficiency is more strongly attributable to the dramatic increase in dietary vitamin D intake or supplement use than to changes in measurement methods alone.

Future studies should prioritize assay standardization and, where possible, apply calibration or harmonization strategies when comparing datasets across time. Additionally, detailed information on supplement use, dietary intake, and assay methodology should be incorporated into epidemiologic analyses to disentangle biological changes from analytical artifacts.

In this analysis of nationally representative data from KNHANES, we observed an inverse association between adiposity and serum 25(OH)D concentrations in Korean women. Individuals with obesity had a significantly higher prevalence of vitamin D deficiency compared with normal or underweight individuals, and this relationship persisted after adjustment for age, sex, season, and lifestyle factors. These findings are consistent with prior population-based studies demonstrating a graded decline in circulating 25(OH)D with increasing BMI [21,22].

Several biological mechanisms plausibly explain this association. The volumetric dilution hypothesis suggests that vitamin D is distributed into a larger fat mass compartment in individuals with obesity, leading to lower circulating concentrations without necessarily indicating reduced total-body stores [23]. In parallel, adipose tissue sequestration may limit the bioavailability of vitamin D, as demonstrated by reduced increments in serum 25(OH)D following ultraviolet exposure or oral supplementation in individuals with obesity [24]. The sex-specific association between BMI and vitamin D deficiency may reflect differences in body composition and regional fat distribution that BMI does not fully capture. At the same BMI, women generally have a higher body fat percentage than men, which may provide a larger adipose reservoir for vitamin D distribution or sequestration [25]. Women also tend to have relatively greater subcutaneous adipose tissue, whereas men accumulate more visceral adipose tissue [26]. These sex-related differences in adiposity may partly explain why the inverse association between BMI and 25(OH)D was more apparent in women. However, because direct measures of body fat percentage or regional fat distribution were unavailable, this interpretation remains speculative.

Although this study has several strengths, including the use of a large, nationally representative KNHANES dataset and comprehensive adjustment for confounders, it also has several limitations. First, due to the cross-sectional design of KNHANES, we could not establish causal relationships between the evaluated factors and vitamin D status. Second, no cross-calibration analysis was performed between the RIA and LC-MS/MS methods used in KNHANES VI and IX, respectively. Thus, potential analytical bias cannot be ruled out when directly comparing the absolute values across the two waves. Third, we were unable to adjust for sunlight exposure in the present analysis. Sunlight exposure and outdoor activity are important determinants of serum 25(OH)D concentrations; however, according to the raw data use guide for KNHANES, items on outdoor activity experience and duration of sunlight exposure were not collected in both the sixth and ninth survey periods. As a result, these variables could not be consistently compared across the two survey waves or included as covariates. This limitation is relevant because temporal changes in outdoor activity, clothing behavior, sunscreen use, and ultraviolet exposure may have contributed to changes in serum 25(OH)D concentrations independently of dietary supplement intake or assay methodology. Fourth, the dietary supplement variable used in this study captured any use of dietary health supplements and did not specifically identify vitamin D-containing supplements. Therefore, the observed association between dietary supplement use and serum 25(OH)D concentrations cannot be attributed specifically to vitamin D supplementation. Nevertheless, the marked increase in overall supplement use may partly reflect broader changes in health-related behaviors, including increased use of vitamin D-containing products.

5. Conclusions

In conclusion, serum 25(OH)D concentrations in KNHANES IX were higher than in KNHANES VI, while the prevalence of vitamin D deficiency was lower. Given the significant increase in dietary supplement intake during the same period, changes in vitamin D intake may have influenced this apparent rise. Furthermore, previous Korean data measured by LC-MS/MS in 2013 showed concentrations higher than those measured by RIA in KNHANES VI but lower than those in KNHANES IX, suggesting a possible increase over time. However, since the analytical platforms used during the two survey periods differed and direct information on sun exposure was not consistently secured, the differences between the surveys should be interpreted with caution. Further research utilizing standardized analytical methods and detailed information on UV exposure, outdoor activities, dietary intake, and supplement use is necessary to clearly elucidate the true temporal changes in vitamin D status in South Korea.

CRediT author statement

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Declaration of AI or AI-assisted technology

During the preparation of this manuscript, the authors used ChatGPT (OpenAI, USA) to assist with language refinement and improve overall readability. Following this assistance, the authors carefully reviewed, verified, and revised the contents. The authors take full responsibility for all aspects of the manuscript.

Conflicts of interest

The authors declare no competing interests.

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Appendix A. Supplementary data

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