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Longitudinal study of vitamin D status among Thai individuals in a sun-abundant country

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ABSTRACT

Background: Vitamin D deficiency is a major public health problem worldwide, even in countries with abundant sunshine. Understanding the risk factors for vitamin D deficiency is important to inform public health recommendations. We conducted a longitudinal analysis of vitamin D status in Thai individuals to assess changes in vitamin D status over time and identify potential determinants.
Study design: This study is a long term prospective cohort study.
Methods: Of the 1239 participants who were employees of the Electricity Generating Authority of Thailand, serum 25-hydroxyvitamin D (25(OH)D) levels were measured by liquid chromatography/tandem mass spectrometry from samples collected in 2009 and 2019.
Results: There was a significant 14.8% increase in serum total 25(OH)D ($P < 0.001$) from 2009 to 2019, which resulted from significant increases in both $25(OH)D_3$ and $25(OH)D_2$. The epimeric form of $25(OH)D_2$ also increased significantly, while there was no increase in the epimeric form of $25(OH)D_3$. A univariate analysis showed significant associations between increased total 25(OH)D and increasing age, male sex, and lower body mass index. After controlling for baseline vitamin D status, multivariate regression analyses found that the direction of association and significance from univariate analyses persisted for total 25(OH)D and $25(OH)D_3$. However, a univariate association found between female sex and an increase in $25(OH)D_2$ was not significant in multivariate regression analysis.
Conclusions: A long-term trend of improved vitamin D status was found among Thai adult individuals over a 10-year period; however, improvements were less noticeable in women.

1. What this study adds

- This long-term prospective cohort study advanced understanding of the dynamics of vitamin D insufficiency by tracking the same individuals over an extended period to identify risk factors and inform the selection of effective intervention strategies
- Inadequate vitamin D status in the younger age groups is likely to become an increasingly important issue.

2. Introduction

Vitamin D, an essential fat-soluble nutrient, plays a critical role in maintaining optimal bone health and other physiological functions. Thailand has a significant prevalence of vitamin D deficiency and insufficiency despite being a sun-abundant country [1], which may be related to the tendency to avoid sun exposure among the population. Previous studies have shown that factors such as urbanisation and lifestyle changes also contribute to deficiency in a number of populations [2].

Although periodic health surveys have been conducted in Thailand to assess the population's health status as well as vitamin D levels, these cross-sectional studies do not provide sufficient data to evaluate trends and changes in individual vitamin D status over time [3,4]. By contrast, longitudinal studies offer a more comprehensive understanding of the dynamics of vitamin D insufficiency by tracking the same individuals over an extended period. Such research is essential in identifying risk

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factors, evaluating intervention strategies, and informing public health policies.

We conducted a longitudinal analysis of vitamin D insufficiency in Thai individuals to assess changes in their vitamin D status over time and identify potential determinants. By focusing on the same participants, our research aimed to provide valuable insights into the temporal patterns and factors associated with vitamin D insufficiency, thereby informing targeted interventions and policy recommendations for the improvement of public health in Thailand.

3. Materials and methods

3.1. Participants

Participants were recruited from the population studied in the Electricity Generating Authority of Thailand (EGAT). Participants in the cohort were employees of EGAT who volunteered to participate in a health survey. All participants completed a medical evaluation and had routine laboratory investigations including urinalysis. Blood was drawn after a 12 h fast. In 2009, 1239 participants were recruited to the cohort and the same individuals were resurveyed in 2019 along with having blood drawn. Each time, the same individuals were contacted by telephone and an invitation letter to attend the follow-up examination. Information about the cause of death was sought for individuals known to have died during the interim period. At each follow-up visit, participants underwent similar medical evaluations and had routine laboratory investigations as at the baseline visit. All serum samples were obtained and kept at −80 °C until analysis.

3.2. Vitamin D measurement

Serum samples collected in the years 2009 and 2019 were analyzed in 2015 and 2022, respectively. Serum vitamin D levels, including 25-hydroxyvitamin-D_2 (25(OH)D_2), 25(OH)D_3, C-3 epimer of 25(OH)D_2 (3-epi-25(OH)D_2) and 3-epi-25(OH)D_3, were measured in the same testing laboratory. Standard analytical methods were used involving liquid chromatography/tandem mass spectrometry (Agilent 1260 Infinity liquid chromatograph, Agilent Technologies, Waldbronn, Germany) coupled to a QTRAP® 5500 tandem mass spectrometer (AB SCIEX, Foster City, CA, USA), and a MassChrom® 25-OH-Vitamin D_3/D_2 in serum/plasma reagent kit including a 3-epi-25-OH-Vitamin D_3/D_2 upgrade diagnostics kit (Chromsystems, Munich, Germany). All analyte values of the calibrator (Chromsystems 3PLUS1® Multilevel Serum Calibrator Set 25-OH-Vitamin D_3/D_2 and 3-epi-25-OH-vitamin D_3/D_2) and control (MassCheck® 25-OH-Vitamin D3/D2 and 3-epi-25-OH-vitamin D3/D2) used in this study were traceable to certified substances and standard reference materials of the National Institute of Standards and Technology ([NIST] 972 and NIST 972a, respectively). The summation of serum 25(OH)D_3, 25(OH)D_2, 3-epi-25(OH)D_3, and 3-epi-25(OH)D_2 was used to reflect vitamin D status. The inter-assay and intra-assay coefficients of variation of total serum 25(OH)D levels were 7.2% and 5.3%, respectively. In addition, traceability tests using a certified standard reference material (SRM 972a) from NIST was also carried out and showed concordant results with the assigned values of NIST SRM 972a in both studies (Table 1).

Table 1
The SRM 972a analyzed in both 2015 and 2022 compared with NIST-values.

	SMR972a		
	NIST-values (mean ± 95% confidence)	Analyzed in	
		2015	2022
Level 1			
25(OH)D3	28.8 ± 1.1	31.1	30.8
3-epi-25(OH)D3	1.81 ± 0.10	2.55	1.80
Level 2			
25(OH)D2	0.81 ± 0.06	0.58	1.33
25(OH)D3	18.1 ± 0.4	17.9	19.7
3-epi-25(OH)D3	1.28 ± 0.09	1.65	0.85
Level 3			
25(OH)D2	13.3 ± 0.3	14.8	14.0
25(OH)D3	19.8 ± 0.4	21.0	22.4
Level 4			
25(OH)D3	29.4 ± 0.9	31.9	32.6
3-epi-25(OH)D3	26.0 ± 2.2	26.3	23.4

Table 2A
Age distribution in 2009 and 2019 in all subjects and by sex.

Age (year)	2009			2019		
	All subjects (n = 1239)	Men (n = 818)	Women (n = 421)	All subjects (n = 1239)	Men (n = 818)	Women (n = 421)
20–30	108 (8.7%)	76 (9.3%)	32 (7.6%)	0	0	0
30–40	467 (37.7%)	280 (34.2%)	187 (44.4%)	108 (8.7%)	76 (9.3%)	32 (7.6%)
40–50	556 (44.9%)	390 (47.7%)	166 (39.4%)	467 (37.7%)	280 (34.2%)	187 (44.4%)
50–60	108 (8.7%)	72 (8.8%)	36 (8.6%)	556 (44.9%)	390 (47.7%)	166 (39.4%)
≥60	0	0	0	108 (8.7%)	72 (8.8%)	36 (8.6%)

3.3. Statistical analysis

Data were analyzed using descriptive and inferential statistics. Continuous variables are reported as means and standard deviations, while categorical variables are presented as frequencies and percentages. Paired t-tests were employed to compare the vitamin D levels between the two time points. Univariate and multivariate regression analyses were performed to identify potential determinants of the changes in vitamin D status. A P-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using Rstudio version 1.0.136 and R version 3.3.2 (RStudio Inc., Boston, MA, USA).

Table 2B
Body mass index and vitamin D levels in 2009 and 2019.

	All subjects (n = 1239)		Male (n = 818)		Female (n = 421)	
	Year 2009	Year 2019	Year 2009	Year 2019	Year 2009	Year 2019
BMI (mg/m^2)	23.7 ± 0.1	24.8 ± 0.1	24.4 ± 0.1	25.4 ± 0.1	22.3 ± 0.2	23.8 ± 0.2
25(OH)D_3 (ng/mL)	22.3 ± 0.2	25.1 ± 0.2	23.6 ± 0.2	26.9 ± 0.2	19.8 ± 0.2	21.7 ± 0.3
25(OH)D_2 (ng/mL)	0.63 ± 0.07	1.34 ± 0.14	0.55 ± 0.02	0.90 ± 0.10	0.79 ± 0.19	2.25 ± 0.35
3-epi-25(OH)D_3 (ng/mL)	1.47 ± 0.05	1.49 ± 0.02	1.62 ± 0.06	1.65 ± 0.03	1.19 ± 0.09	1.18 ± 0.02
3-epi-25(OH)D_2 (ng/mL)	0.002 ± 0.001	0.024 ± 0.005	0.002 ± 0.001	0.009 ± 0.003	0.001 ± 0.001	0.053 ± 0.012
Total 25(OH)D (ng/mL)	24.4 ± 0.2	28.0 ± 0.2	25.7 ± 0.2	29.4 ± 0.2	21.8 ± 0.3	25.2 ± 0.4

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin-D; 3-epi-25(OH)D_2, C-3 epimer of 25(OH)D_2; 3-epi-25(OH)D_3, C-3 epimer of 25(OH)D_3.

Table 3
Paired changes in BMI and vitamin D metabolites between 2009 and 2019 in all subjects and by sex.

Characteristic	All subjects (n = 1239)	P-value	Men (n = 818)	P-value	Women (n = 421)	P-value
BMI (mg/m^2)	2.0 ± 7.6	<0.0001	0.99 ± 1.83	<0.0001	1.6 ± 2.3	<0.0001
25(OH)D_3 (ng/mL)	5.9 ± 30.2	<0.0001	3.3 ± 5.6	<0.0001	1.9 ± 6.4	<0.0001
25(OH)D_2 (ng/mL)	4.9 ± 40.5	<0.0001	0.34 ± 2.9	<0.001	1.5 ± 7.3	<0.0001
3-epi-25(OH)D_3 (ng/mL)	1.8 ± 34.5	0.71	0.03 ± 1.8	0.62	−0.01 ± 1.8	0.94
3-epi-25(OH)D_2 (ng/mL)	0.16 ± 0.93	<0.0001	0.01 ± 0.09	<0.05	0.05 ± 0.24	<0.0001
Total 25(OH)D (ng/mL)	6.7 ± 36.6	<0.0001	3.7 ± 6.1	<0.0001	3.4 ± 7.8	<0.0001

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin-D; 3-epi-25(OH)D_2, C-3 epimer of 25(OH)D_2; 3-epi-25(OH)D_3, C-3 epimer of 25(OH)D_3. Data are shown as mean ± SD. The P-values are for paired *t*-test.

Table 4
Changes in vitamin D status between baseline and 10 years later in all subjects and by sex.

Total 25(OH)D	All subjects (n = 1239)		Men (n = 818)		Women (n = 421)	
	2009	2019	2009	2019	2009	2019
<10 ng/dL	1 (0.1%)	2 (0.2%)	0 (0.0%)	0 (0.0%)	1 (0.2%)	2 (0.5%)
10–20 ng/dL	287 (23.2%)	159 (12.8%)	118 (14.4%)	58 (7.1%)	169 (40.1%)	101 (24.0%)
≥20 ng/dL	951 (76.8%)	1078 (87.0%)	700 (85.6%)	760 (92.9%)	251 (59.6%)	318 (75.5%)

25(OH)D, 25-hydroxyvitamin-D.

Table 5
Factors associated with the observed changes in vitamin D metabolite levels over the study period.

Variables	Changes in vitamin D levels[a]		
	Total 25(OH)D (ng/mL)	25(OH)D_3 (ng/mL)	25(OH)D_2 (ng/mL)
Age (years)	0.07	0.04	0.03
	P < 0.05	P = 0.16	P = 0.27
Female	−0.02	−0.12	0.11
	P = 0.41	P < 0.0001	P < 0.0001
BMI (kg/m^2)	−0.05	−0.03	−0.02
	P = 0.07	P = 0.25	P = 0.47

[a] Changes from the year of 2009–2019.25(OH)D, 25-hydroxyvitamin-D; BMI, body mass index.

4. Results

The mean age of participants in 2009 was 39.9 ± 6.9 years. Most participants were men (818; 66.0%) because of the demographics of the employees of EGAT. For all subjects in 2009, the mean body mass index (BMI) was 23.7 ± 3.7 kg/m^2 and 340 participants (27.4%) were obese according to the Asian cut off BMI of 25 kg/m^2. The age distribution by year and sex is shown in Table 2A while Table 2B shows the BMI and vitamin D metabolites by year and sex.

The paired changes in BMI and vitamin D metabolites between 2009 and 2019 are shown in Table 3. There was a significant 14.8% increase in serum total 25(OH)D (P < 0.001) over time, resulting from a significance increase in both 25(OH)D_3 and 25(OH)D_2. Furthermore, 3-epi-25(OH)D_2 increased significantly while there was no increase in 3-epi-25(OH)D_3. Over the study period, there was also a slight increase in the BMI of the study population (P < 0.0001).

When examining the changes in vitamin D status at baseline and 10 years later, there were improvements in vitamin D status as categorized into 3 categories: total 25(OH)D < 10 ng/dL, 10–20 ng/dL and >20 ng/dL all subjects as well as in both men and women (Table 4).

A univariate analysis was performed to identify factors associated with the observed increases in vitamin D metabolite levels. The results demonstrated significant associations between increased total 25(OH)D and increasing age, male sex, and lower BMI. It is of note that the decrease in 25(OH)D_3 was associated with female sex. By contrast, the increase in 25(OH)D_2 was related to female sex (Table 5).

In multivariate regression analysis, the direction of association and significance from univariate analyses after controlling for baseline vitamin D status persisted for total 25(OH)D and 25(OH)D_3 (Tables 6A and 6B). However, the association between female sex and the increase in 25(OH)D_2 was not significant in multivariate analysis (Table 6C).

Table 6A
Multivariate regression analyses for the change in total 25(OH)D.

Independent variables	Coefficient	SE	Beta	P-value
Age (years)	0.13	0.03	0.14	<0.001
Female	−2.29	0.39	−0.16	<0.001
BMI (kg/m^2)	−0.12	0.05	−0.06	<0.05
Baseline total 25(OH)D (ng/mL)	−0.45	0.03	−0.44	<0.001

25(OH)D, 25-hydroxyvitamin-D; BMI, body mass index; SE, standard error. Sex = 1 for female and 0 for male.

Table 6B
Multivariate regression analyses for the change in 25(OH)D_3.

Independent variables	Coefficient	SE	Beta	P-value
Age (years)	0.08	0.02	0.09	<0.001
Female	−3.00	0.36	−0.24	<0.001
BMI (kg/m^2)	−0.12	0.04	−0.08	<0.01
Baseline 25(OH)D_3 (ng/mL)	−0.35	0.03	−0.34	<0.001

25(OH)D_3, 25-hydroxyvitamin-D_3; BMI, body mass index; SE, standard error. Sex = 1 for female and 0 for male.

Table 6C
Multivariate regression analyses for the change in 25(OH)D_2.

Independent variables	Coefficient	SE	Beta	P-value
Age (years)	0.03	0.02	0.05	<0.001
Female	1.30	0.29	0.13	0.714
BMI (kg/m^2)	0.01	0.04	0.01	<0.001
Baseline 25(OH)D_2 (ng/mL)	−0.56	0.06	−0.27	0.065

25(OH)D_2, 25-hydroxyvitamin-D_2; BMI, body mass index; SE, standard error. Sex = 1 for female and 0 for male.

5. Discussion

The present longitudinal study provides valuable insights into changes in vitamin D status among Thai individuals over a 10-year period, revealing significant improvements in vitamin D levels, predominantly driven by increases in vitamin D_3. During the time period of the study, there was increased awareness of vitamin D deficiency among the Thai population after dissemination of newly published evidence [1,5,6]. While such heightened awareness may have contributed to the observed improvements in vitamin D status, increased awareness does not necessarily translate into improved health outcomes. Several factors influence the effectiveness of awareness campaigns, such as the accuracy and accessibility of information, individual health literacy, and the

availability of interventions [7]. Further research and discussion are needed to evaluate the impact of public awareness campaigns on vitamin D status, and to identify best practices for translating increased awareness into meaningful health improvements.

Associations between increasing age and lower BMI with improvements in vitamin D status were also demonstrated in the present study. The positive relationship between age and vitamin D status could potentially be explained by an increased awareness of the importance of vitamin D among older adults, leading to lifestyle modifications or supplementation. By contrast, inadequate vitamin D status in younger age groups is a particular problem in Asian rather than Western countries [8,9]. Impactful public health measures targeted specifically at the younger generation are necessary to alleviate vitamin D deficiency. The inverse relationship between lower BMI and increased vitamin D levels found in the current study is in keeping with previous research [10,11].

Our study found that women were less likely to experience improvements in vitamin D status compared with men, potentially because of a higher tendency among women, particularly young women, to avoid sun exposure. This finding highlights the importance of understanding sex differences in behaviours and attitudes related to sun exposure and the impact these differences can have on vitamin D status. Cultural factors, social norms, and aesthetic preferences might influence women's sun avoidance behaviours, potentially contributing to low vitamin D levels [12,13]. For instance, concerns about skin ageing, hyperpigmentation, or a preference for lighter skin tones might lead some women to limit their sun exposure or use sunscreen with high sun protection factors, thereby reducing their skin's ability to synthesise vitamin D [14,15]. Given these findings, it is essential to develop targeted health promotion campaigns and effective interventions, particularly for women who are at a higher risk of vitamin D insufficiency [16]. Public health campaigns should consider addressing misconceptions about sun exposure and providing clear guidance on safe and responsible sun exposure practices, emphasising the importance of balancing sun protection with the need for adequate vitamin D synthesis. Additionally, interventions could focus on promoting alternative ways to increase vitamin D levels, such as through dietary sources or supplementation. Healthcare professionals should also be aware of these sex differences and consider screening women, especially young women, for vitamin D insufficiency as part of routine health assessments. By identifying individuals at risk, healthcare providers can offer personalised recommendations on sun exposure, diet, and supplementation to ensure optimal vitamin D levels and overall health.

Vitamin D has multiple derivatives including its C3 epimer. Despite its existence, its comprehensive biological significance is still a topic of ongoing research. Health surveys from various regions have started to variably adopt its measurement. For instance, NHANES in the US, unveiled that 86% of the US population exhibited detectable levels of 3-epi-25(OH)D although the levels are generally low [17]. Similarly, our present study as well as a national health survey from Thailand noted the epimer's presence in a substantial proportion of adults, but it made up less than 10% of the total 25(OH) [18]. National health surveys from other countries have rarely included vitamin D epimers in their assessments of vitamin D metabolites.

Although 3-epi-25(OH)D's full physiological roles await extensive research, its evident prevalence and potential skewing of vitamin D status estimations necessitate its meticulous measurement in health surveys.

The strength of our study was its longitudinal design, which enabled the examination of temporal trends and changes in the vitamin D status of individuals. Additionally, the use of a large and diverse cohort of participants increased the generalizability of our findings. However, there were some limitations. First, our study population consisted solely of employees of EGAT, which may not be fully representative of the general Thai population. Second, the study did not assess dietary intake or sun exposure, which are critical factors influencing vitamin D levels. Future research should consider these factors to provide a more comprehensive understanding of the determinants of vitamin D status.

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Ethical approval

The Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University approved the study, and it conformed with the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). All participants gave their written informed consent before participating in the study.

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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