ISSN

Journal of Medical Advances and Education

Vol. 1 No. 1 (2025): 1-12

Journal homepage : https://journals.gesociety.org/index.php/jmae

Predictors of low serum 25-hydroxyvitamin D levels in young female adults from an equatorial city in Indonesia

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Abstract. *Vitamin D has pleiotropic effects on women's health. Recent studies point out the alarming rate of vitamin D deficiency/insufficiency in women of various ethnicities and geographical locations. Our objective was to identify lifestyle and dietary factors related to serum 25-hydroxyvitamin D (25(OH)D) levels in young women of Minangkabau ethnicity residing in an equatorial city in Indonesia. A cross-sectional study was performed on 80 healthy females (17-25 years). Their lifestyle and dietary characteristics were assessed by a structured interview. A 24-hour food recall was performed to assess dietary intake. The skin pigmentation type was identified according to Fitzpatrick's scale. Sleep quality was examined by the Pittsburgh Sleep Quality Index (PSQI). Serum 25(OH)D concentration was measured by the ELISA method. Predictors of serum 25(OH)D level were analyzed using multiple linear regressions. Nearly all subjects (97.5%) were deficient/insufficient (≤20 ng/ml) of vitamin D (median 25(OH)D of 10.5 ng/ml). The regression model (adjusted R^2=0.782) showed that predictors of serum 25(OH)D levels were PSQI score (β=-0.436, p<0.001), estimated dietary vitamin D intake (β=0.327, p<0.001), and sunscreen application (β=-0.229, p<0.001). Modification of sleep habits and dietary intake is recommended to improve serum 25(OH)D levels in young women of an equatorial city in Indonesia.*

Keywords: *25(OH)D, dietary intake, PSQI, women, sunscreen, young adults*

Type of the Paper: Regular Article

1. Introduction

Vitamin D plays a vital function in skeletal maintenance and extra-skeletal regulation, with various roles in women's health [1]. Vitamin D deficiency/insufficiency is related to problems in the female reproductive cycle from pregnancy [2] to birth for both mother and fetus [3].

The primary source of vitamin D comes from ultraviolet-B (UV-B). In temperate countries, 80% of vitamin D comes from sunlight, whereas in tropical countries such as Indonesia, sun exposure has a contribution of around 90% as a source of vitamin D [1]. Vitamin D deficiency is most often found in four-season countries with limited sun exposure [4]. However, recent studies

Received June 16, 2025; Received in revised form June 28, 2025; Accepted June 28 2025, Published 30 June 2025
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reported vitamin D deficiency in subtropical and tropical countries such as Singapore, India, Malaysia, and Japan [5, 6, 7, 8, 9]. Indonesia is a tropical country that is crossed by the equator with a high intensity of sun exposure. A study conducted in North Sumatra found that 95% of 156 healthy women aged 20-50 years had vitamin D deficiency [10].

Vitamin D levels are not only influenced by sun exposure [11] alone but also influenced by dietary intake [12], body mass index (BMI) [13], clothing used and sunscreen application [14], skin pigmentation [15] and ethnicity [5]. Moreover, recent research stated that circadian behavior is associated with vitamin D deficiency [16].

Vitamin D deficiency is asymptomatic most of the time [17]. Vitamin D's complex role in reproductive-aged women makes vitamin D status essential to note [18]. Given the complex role of vitamin D in the female reproductive cycle and many factors associated with vitamin D levels and the limited data regarding the status of women's vitamin D in Indonesia, the authors felt the need to analyze factors associated with serum 25-hydroxyvitamin D (25(OH)D) levels in young adult women. Young female adults have more years to live and may suffer longer from the consequences of vitamin D deficiency/insufficiency; therefore, identifying modifiable risk factors is essential for early intervention.

2. Materials and Methods

2.1. Study subjects

The Ethics Committee of the Faculty of Medicine of Universitas Andalas approved this study, and we followed the amended Declaration of Helsinki. Subjects were 80 healthy female college students aged 17-25 years. They were randomly recruited, and all subjects gave informed consent. All subjects were of Minangkabau ethnicity, had no history of pregnancy, kidney disease, cardiovascular disease, diabetes mellitus, thyroid, liver, and lung disease and had no history of living in a non-tropical region within the last 30 days before joining the study.

2.2. Serum 25(OH)D analysis

All enrolled subjects had blood samples taken from their antecubital vein. Blood samples were directly transferred and stored in the UPTD Health Laboratory Office of West Sumatra, for serum 25(OH)D assay. The serum samples were separated by centrifugation at 3,500 rpm at 4℃ for 10 min, then stored in aliquots at -80 ℃. Serum 25(OH)D level was measured by enzyme-linked immunosorbent assay (ELISA) method by using 25(OH)D ELISA Kit (Can-VD-510) produced by Diagnostic Biochem Canada (DBC®). Vitamin D status in this study was defined using the cut-off points suggested by the Institute of Medicine (deficient=0-11 ng/ml; insufficient=12-20 ng/ml; sufficient=>20 ng/ml) [4].

2.3 Estimated dietary vitamin D intake assessment

Subjects were interviewed by using a structured questionnaire, and 24-hour food recall for the last two days (one weekday and one weekend) was employed to assess vitamin D intake. The 24-hour food recall method was performed as a direct interview by two trained interviewers and was facilitated with photographs of various serving sizes of foods. Dietary vitamin D intake (μg) was calculated by using the 2007 Nutrisurvey software with the Indonesian food database. The intake of vitamin D was normalized to the estimated daily energy requirement to avoid under-/over-reported bias. Then, the energy requirement-adjusted values (/1000 kcal) were used for the statistical analyses.

2.4 Sunlight exposure, sunscreen use, and skin pigmentation assessment

The questionnaire-guided interview was performed to obtain data on duration of sunlight exposure during the last two days (minute/day), on the habitual dressing when going outdoor (percentage of body surface area (BSA) exposed to sunlight). We also assessed the skin pigmentation (Fitzpatrick's scale), and sunscreen application (regular, irregular, non-user) [19].

The estimated duration of sun exposure (minutes) was assessed by interviewing research subjects regarding the length of time each subject spent outdoor for two consecutive days before blood collection. In order to avoid recall bias, subjects were provided a diary and were explained how to fill it so they can record their activities. One of the researchers also kept in contact with all subjects to remind them to fill in the activity diary.

2.5 Other covariates

Participants' weight (kg) and height (cm) were measured by using a digital bathroom scale and a microtoise, respectively, and their body mass index (BMI; kg/m^2) was calculated. Sleep quality (good, bad) was assessed using the Pittsburgh Sleep Quality Index (PSQI) [20]. Each subject's circadian diet was assessed using a 24-hour dietary recall for two days (one weekday and one weekend). The diet was then classified into predominantly day-time (pDT), when caloric intakes mostly (>50%) happened from sunrise to sunset time, and predominantly night-time (pNT), when caloric intake mostly took place from sunset to sunrise time [16]. Sunrise and sunset times were set according to the information from the local meteorology agency at the time of data collection.

2.6 Statistical analyses

Data with normal distribution were expressed in mean and standard deviation. Data with non-normal distribution were expressed in median and range. Serum 25(OH)D, estimated dietary vitamin D intake, and estimated sunlight exposure time were logarithmically transformed (*log*10) before analysis to approximate a normal distribution. Correlation analysis was performed by Pearson's correlation or Spearman's rank test. The mean value of groups was compared by

Student's t-test or one-way analysis of variance (ANOVA). Multivariable analysis by linear regression with enter method was performed to identify predictors of serum 25(OH)D. Statistical significance was considered at p-value <0.05. IBM SPSS statistical software version 25.0 (IBM, US) was used for all analyses.

3. Results and Discussion

RESULTS

Characteristics of subjects

Subjects characteristics are presented in **Table 1**. Nearly all subjects were deficient/insufficient of serum 25(OH)D with a median level of 10.5 ng/ml. The median estimated intake of dietary vitamin D in our subjects was 2.9 µg/1000 kcal/day. The proportion of overweight/obese was 40%. Subjects showed a wide range of estimated sunlight exposure time, with the median of sunlight-exposed body surface area 7.8%. Most subjects had type IV skin pigmentation and used sunscreen on a regular or irregular basis. Regarding sleep quality, most reported having poor sleep quality based on PSQI score (66.2%) and a circadian pDT diet (85%).

Table 1. Characteristics of female young adults of Minangkabau ethnicity (n=80).

Characteristics	n (%)	Mean ± SD	Median	Range
Serum 25(OH)D (ng/ml)	80 (100)	11.4 ± 4.1	10.5	5.3 - 26
Classification of serum 25(OH)D level according to the Institute of Medicine (ng/ml)				
Deficient (0-11)	48 (60)			
Insufficient (12-20)	30 (37.5)			
Sufficient (>20)	2 (2.5)			
Estimated intake of vitamin D (µg/1000 kcal/day)	77 (96.2)		2.9	0.5 - 9.4
BMI (kg/m^2)	80 (100)	23.2 ± 3.8		
BMI classification for Asians (kg/m^2)				
Underweight (<18.5)	5 (6.2)			
Normal (18.5-22.9)	43 (53.8)			
Overweight (23.0-24.9)	14 (17.5)			
Obese (>25.0)	18 (22.5)			
Estimated sunlight exposure (minute)	80 (100)		50	5 - 173
Estimated sunlight exposure (minute)				
<30	23 (28.8)			
30-60	28 (35)			
60-120	22 (27.5)			
>120	7 (8.8)			
Estimated sunlight exposed body area (%)	80 (100)		7.8	7.3 - 22
Skin pigmentation type				
Type IV	55 (68.8)			
Type V	25 (31.2)			
Sunscreen application				
Regular	27 (33.8)			
Irregular	29 (36.2)			
None-user	24 (30)			
Sleep quality				
Good	27 (33.8)			
Bad	53 (66.2)			
Circadian meal pattern				
pDT (predominantly day-time)	68 (85)			
pNT (predominantly night-time)	12 (15)			

Predictors of serum 25(OH)D level

We performed the bivariate correlation analyses (**not shown**) to identify the nutritional and lifestyle variables associated with the serum 25(OH)D level. The estimated intake of vitamin D had a strong positive correlation with serum 25(OH)D level (Pearson's $r=0.757$; $p<0.001$), while the estimated duration of sunlight exposure was moderately correlated with serum 25(OH)D level (Pearson's $r=0.442$; $p<0.001$). There was a negatively weak correlation between BMI and serum 25(OH)D level (Spearman's $\rho=-0.268$; $p=0.016$). The correlation between the estimated sunlight-exposed body surface area and serum 25(OH)D level was not statistically significant (Spearman's $\rho=-0.073$; $p=0.523$).

In our subjects, only two types of skin pigmentation were identified, type IV and V. The correlation of skin pigmentation type, sleep quality, and circadian eating pattern with serum 25(OH)D levels were analyzed by using unpaired Student's t-test. There were statistically significant differences in serum 25(OH)D levels according to skin pigmentation type ($p<0.001$) and sleep quality ($p<0.001$). Serum 25(OH)D level was higher in type IV compared to type V and higher in subjects with good sleep quality than those with poor sleep quality. There was a significant difference in serum 25(OH)D level according to the circadian diet pattern ($p=0.022$), with a higher level in the pDT group.

The difference in serum 25(OH)D level according to sunscreen use was analyzed using one-way ANOVA followed by Bonferroni post-hoc test. Serum 25(OH)D was higher in subjects who did not use sunscreen compared to subjects who used sunscreen irregularly ($p<0.01$) or regularly ($p<0.001$).

Predictors of serum 25(OH)D level were analyzed using multiple linear regressions, and the results were presented in **Table 2**. Our model accounted for 78.2% variability in serum 25(OH)D. Predictors of serum 25(OH)D levels in this study were PSQI score ($\beta=-0.436$, $p<0.001$), estimated dietary vitamin D intake ($\beta=0.327$, $p<0.001$), and sunscreen application ($\beta=-0.229$, $p<0.001$). The estimated sunlight exposure time was a marginally significant predictor of serum 25(OH)D ($\beta=0.107$; $p = 0.07$).

Table 2. Predictors of serum 25(OH)D level (ng/ml) in female young adults of Minangkabau ethnicity (n=80), results of multiple linear regression analysis by enter method.

Adjusted R^2	Predictors	Unstandardized coefficients		Standardized β coefficient	95% CI for β		p-value
		B	Standard error		Lower bound	Upper bound	
0.782	Constant	0.969	0.103				0.000
	Body mass index (kg/m^2)	0.001	0.002	0.036	-0.078	0.151	0.531
	Estimated dietary vit. D intake (µg/1000 kcal/day)	0.185	0.044	0.327	0.171	0.484	**0.000**
	Estimated sunlight exposure time (min/day)	0.050	0.027	0.107	-0.010	0.224	0.072
	Sunscreen application (non-user=1, irregular=2, regular=3)	-0.043	0.011	-0.229	-0.351	-0.106	**0.000**
	Circadian meal pattern (pNT=1, pDT=2)	0.021	0.023	0.051	-0.058	0.160	0.355
	PSQI score	-0.036	0.006	-0.436	-0.573	-0.299	**0.000**
	Skin pigmentation type (type IV=1, type V=2)	-0.021	0.019	-0.066	-0.184	0.052	0.267

Serum 25(OH)D, estimated dietary vitamin D intake, and estimated sunlight exposure time were log 10 transformed. pNT=predominantly night-time, pDT=predominantly day-time, PSQI=Pittsburgh Sleep Quality Index.

Identification of dietary sources and estimated intake of vitamin D

Nutrisurvey's analysis identified 25 food items containing vitamin D consumed by our subjects. The food items and their estimated vitamin D content, the consumption frequency, and the estimated range of vitamin D intake from each item were depicted in **Figure 1**. Analysis of dietary intake showed that there was a tendency for a high consumption rate of food items with low vitamin content per weight, such as poultry (83.1%). Consumption of chub mackerel, mackerel tuna, and milk contributed to a relatively high intake of vitamin D per day.

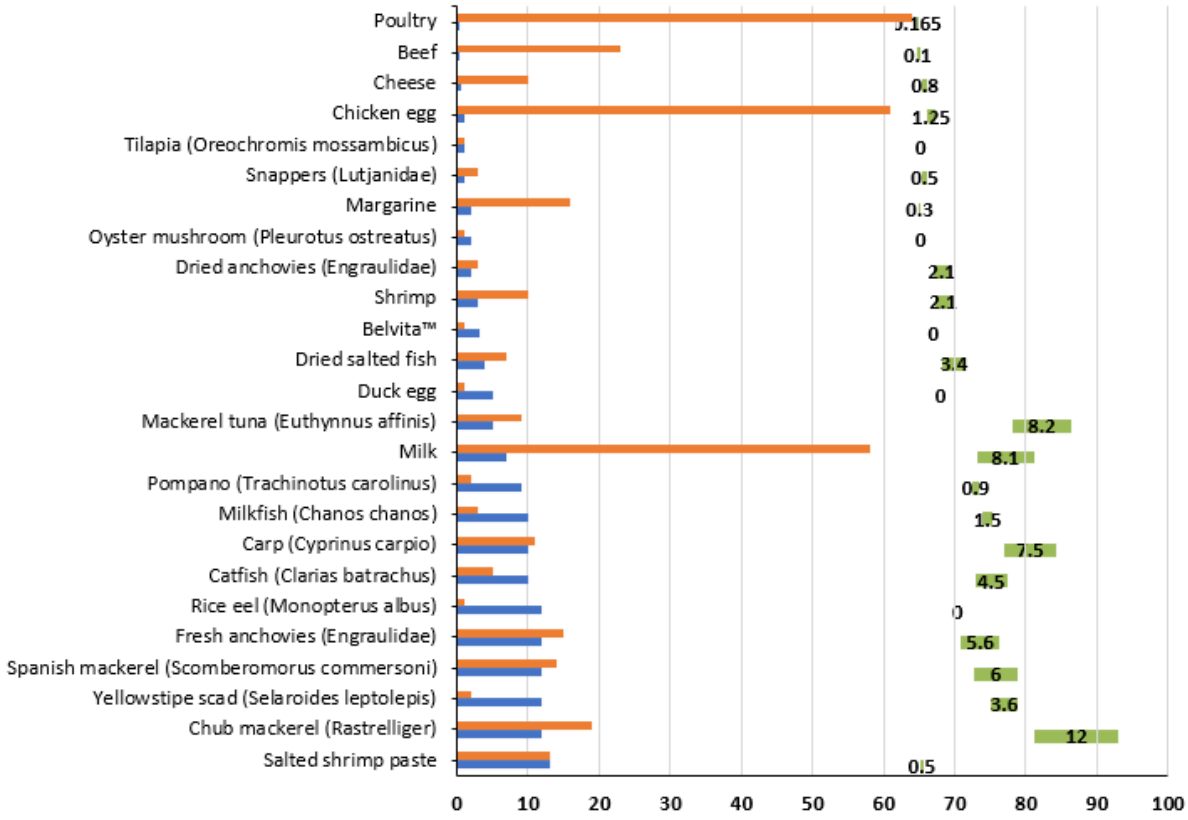

Figure 1. Dietary sources and range of estimated intake of vitamin D from each source in female young adults of Minangkabau ethnicity (n=80). Blue bars signify vitamin D content (µg) per 100 g of item sources. Red bars refer to the number of subjects consuming each item. Green bars refers to the range of estimated vitamin D intake from each source (µg/day).

DISCUSSION

In this study, the level of serum 25(OH)D in young female adults living in Padang, Indonesia (11.4±4.1 ng/ml; mean±SD) is somewhat similar to that of young girls in the same tropical geography, Malaysia (9.7±0.2 ng/ml; mean±SD) [5], but lower compared to young women in subtropical Hong Kong (16.2±4.8 ng/ml; mean±SD) [21] and Australia (27.2±10.8 ng/ml; mean±SD) [22]. It is striking that 97.5% of female college students in our study are deficient/insufficient of vitamin D as compared to 47.9% in female college students in UAE (18±8 ng/ml; mean±SD) [23]. Multiple linear regression analysis showed that the variables examined in this study contribute to 78.2% serum 25(OH)D variance, suggesting that our model has accounted for major 25(OH)D level determinants. Our findings showed that the PSQI score, estimated vitamin D intake, and sunscreen application, are predictors of 25(OH)D serum levels.

The PSQI score is the main predictor of serum 25(OH)D variability in this study. PSQI score assesses subjective sleep quality during the past month [20], while the concentration of 25(OH)D in the blood persists within 3-4 weeks [24]. Therefore, the PSQI score is reflecting the concurrent time as of serum 25(OH)D level. In this study, increasing score, indicating worse sleep quality, is related to a lower level of serum 25(OH)D. Our result is in line with a study of industrial workers in Korea, where there is a significant correlation between low vitamin D levels with poor sleep quality [25]. Vitamin D supplementation has been shown to reduce the PSQI score [26]. A recent study in Japanese adults found that subjects with the lowest quartile of serum 25(OH)D_3, are 1.68 times more likely to experience poor sleep quality than the highest quartile [27]. Despite finding no association between PSQI score with 25(OH), a study in German community sample detected association of higher 25(OH)D concentration with longer and earlier sleep [28]. The exact mechanism and relationship between sleep quality and vitamin D level are still not fully known, but plausible mechanisms have been proposed.

Sleep is a circadian behavior that involves activity and rest cycles. These include sleep/wakefulness and fasting/eating, which are related to the state of the environment, especially the 24-hour dark-light interval [29]. The circadian clock is affected by changes in light, temperature, and vitamin D. Daily 25(OH)D levels indicate circadian oscillations [30]. Vitamin D receptor has been demonstrated in the brain [31]. Since sunlight helps the synthesis of vitamin D [32] and regulates circadian rhythm [33], vitamin D could be transmitting light signals to regulate circadian rhythms [34] .

Estimated vitamin D intake is the second predictor that plays a role in influencing the variability in serum 25(OH)D level in this study. Our finding is in line with a previous study in North Sumatra, where 82.7% of women had low vitamin D intake with an average intake of 5.2 ±

6.9 µg/day [10]. Vitamin D intake data in this study has been normalized with daily energy per 1000 kcal. Assuming subjects consuming a 2,000-kcal diet, the estimated intake level of our subjects (2.9 µg/1000 kcal/day) is still far below the recommended dietary allowance (15 µg) adopted by the Indonesian Ministry of Health [35]. However, the dietary recommendation is based on a general guideline without consideration of ethnicity. Ethnic difference in dietary vitamin D exists [36] and considering the ubiquitous vitamin D deficiency in women of Minangkabau ethnicity [19, 37], with a particular lifestyle and genetic factors [38], this may call for personalized or ethnic-related dietary recommendation allowance.

This study identifies mackerel, tuna, carp, and dairy consumption as significant contributors of dietary vitamin D. Despite being protein sources with the highest vitamin D content per weight, only a small proportion of subjects consume fish. A large proportion of subjects choose poultry, which has the lowest vitamin D content, as their protein source. These results suggested that dietary choice plays a significant role in the low intake of vitamin D in our subjects. The complexity of the role of vitamin D in health and low vitamin D intake in most of the study subjects reflected the need for promotion to increase the consumption of vitamin D-rich foods.

In this study, sunscreen application is the third predictor that affects serum 25(OH)D variance. Our result is in line with a cross-sectional study in Korean students aged 18-29 years during summer, where nearly 60% of research subjects use sunscreen, and this lifestyle is associated with low levels of 25(OH)D [39]. Using sunscreen properly (2 mg/cm^2) can reduce vitamin D3 production by up to 99% [14]. Sunscreen protects from the cutaneous DNA damage by sunlight ultraviolet-A and UV-B. On the other hand, UV-B is essential for the dermal synthesis of vitamin D. The use of sunscreen is recommended by WHO to prevent skin cancer and premature aging [40]. Given the importance of skin health protection, improving vitamin D intake rather than increasing skin exposure to sunlight is recommended to achieve vitamin D sufficiency.

The current study comprehensively assesses relevant lifestyle and dietary factors influencing serum 25(OH)D, focusing on a subpopulation of young female adults from an equatorial country, addressing a profound vitamin D deficiency/insufficiency pandemic with multiple potential health implications. This study is limited by the cross-sectional design where causation cannot be concluded and the number of subjects is small.

4. Conclusions

In summary, poor sleep quality, low dietary vitamin D intake, and sunscreen application predict low serum 25(OH)D levels in young female adults of Minangkabau ethnic, Indonesia. Lifestyle and dietary modification should be recommended to improve their vitamin D status.

Abbreviations

ANOVA	analysis of variance
BMI	body mass index
BSA	body surface area
ELISA	enzyme-linked immunosorbent assay
pDT	predominantly day-time
pNT	predominantly night-time
PSQI	Pittsburgh Sleep Quality Index
UV-B	ultraviolet B
25(OH)D	25-hydroxyvitamin D

Data availability statement

Data from this study are available within the manuscript.

Credit authorship contribution statement

CI was responsible for obtaining the funding, designing the study, providing feedback on data analysis, writing the manuscript, and the overall management. ES carried out the data collection, data analysis, and wrote the initial draft of the paper. DD provided suggestions for the study design and data analysis. MR provided insightful feedback on data analysis and manuscript writing. All authors read and approved the final manuscript.

Declaration of Competing Interest

None of the authors had any personal or financial conflict of interest.

Acknowledgement

This study was supported by a grant from Universitas Andalas (Skim Klaster Riset Percepatan Guru Besar, No:10/UN.16.17/PP.PGB/LPPM/2018) to CI. The funder had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

The authors would like to appreciate all of the study participants. Special thanks to Ms. Indah Khoirunn Nisa and Ms. Rani Aulia Dwi Nanda for their contribution and assistance during data collection.

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