Original Article

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Serum 25-Hydroxyvitamin D Status among Vegetarian, Partial Vegetarian, and Non Vegetarian Medical Students: A Cross-Sectional Study

Biochemistry Section

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ABSTRACT

Introduction: Beyond calcium homeostasis, vitamin D's impact on health is becoming more significant. Although dietary habits may influence vitamin D status, studies comparing vitamin D levels among vegetarian, partially vegetarian, and non vegetarian in medical students are scarce.

Aim: To evaluate the association between dietary patterns and vitamin D status among healthy Indian and Malaysian medical students.

Materials and Methods: This was a cross-sectional study which was conducted in the Department of Biochemistry, Jawaharlal Nehru Medical College, Belagavi, Karnataka, India. Apparently, healthy medical students of Indian and Malaysian ethnicity studying in medical colleges of Belagavi were included in the study. Data on age, height, weight, socio-economic status, and exposure to sunlight were collected. After analysis of the diet questionnaire, study participants were divided into three groups. Group 1: Vegetarian, Group 2: Partial vegetarian, and Group 3: Non vegetarian. Vitamin D, calcium, phosphorus, and alkaline phosphatase levels were estimated. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 20.0.

Results: A total of 200, 100 Indian and 100 Malaysian medical students were included in the study. In Malaysian group, 8 (8%) were vegetarian,12 (12%) were partial vegetarian, and 80 (80%) were non vegetarian. In Indian group 22 (22%) were vegetarian, 46 (46%) were partial vegetarians, and 32 (32%) were non vegetarians. The study shows Indian students were more vitamin D deficient, 78%, compared to Malaysian students, 66%. In both Indian and Malaysian medical students, mean levels of vitamin D and calcium were lower in the vegetarian and partial vegetarian groups as compared to in the non vegetarian group (p-value <0.05). Alkaline phosphatase was found to be normal in both Indian and Malaysian medical students in all three dietary habit groups. A statistically significant correlation of vitamin D with sunlight exposure was found in both Indian and Malaysian medical students (p-value <0.05).

Conclusion: The markedly higher prevalence of low vitamin D in medical students of India (78%) and Malaysia (66%) shows that vitamin D level depends on dietary habits in addition to ethnicity and sun exposure. In both Indian and Malaysian medical students, the mean level of vitamin D was lower in vegetarian and partial vegetarian groups compared to the non vegetarian group. The best way to prevent deficiencies and insufficiencies in vegetarians and partial vegetarians is to take a vitamin D supplement in the recommended dosage.

Keywords: Diet, Nutritional status, Vitamin D

INTRODUCTION

Vegetarianism has become more popular due to several factors, including cultural beliefs, ethical concerns, and health issues. Vegetarian diets have been demonstrated to lower the risk of numerous chronic illnesses; including diabetes, cardiovascular diseases, and cancer, but there are concerns regarding vegetarian diets' suitability for maintaining bone health [1].

Vitamin D plays an essential role in bone health by maintaining adequate serum calcium and phosphate concentrations. Vitamin D status is typically assessed as 25-hydroxy vitamin D {25(OH) D} in plasma or serum (S), as it reflects the sum of vitamin D from sunlight exposure and dietary sources [2]. Because hypovitaminosis D has been linked to non calcemic conditions such as metabolic syndrome, obesity, diabetes mellitus, cancer, cardiovascular illnesses, and depression, interest in vitamin D has increased recently. The cause-and-effect connection between vitamin D and chronic illnesses is still being studied [3]. The status of vitamin D is influenced by skin pigmentation, dietary habits, and sunlight exposure, all of which are determined by ethnicity [4]. Vitamin D deficiency is widespread among various regions and demographic groups. A high prevalence of vitamin D deficiency was found in countries with plenty of sunlight, such as Saudi Arabia, the United Arab Emirates, Australia, and India [5].

According to Tucker KL, vegetarians and vegans are more likely to fracture due to their often poor dietary intakes of calcium, vitamin D, zinc, PUFAs, and protein- all dietary elements that have been demonstrated to be significantly and positively correlated with Bone Mineral Density (BMD) [6]. Animal-derived foods like fatty fish, egg yolk, animal fat, cod liver oil, and shellfish are rich in vitamin D. The only naturally occurring plant sources of vitamin D are several varieties of mushrooms, which contain trace levels of the vitamin. There are restrictions on the quantity and distribution of food fortification with vitamin D [6]. Since only a few numbers of foods are now fortified with vitamin D, and the foods that naturally provide the largest amount of vitamin D are from animal sources, vitamin D deficiency may be more common in vegetarians and partial vegetarians compared to non vegetarian people. Data categorising prevalence by vegetarian and non vegetarian dietary groups are scarce [7]. There are very few studies on vitamin D levels of medical students [8-10]. There are no studies on vegetarian vs non vegetarian vitamin D status of

medical students. Thus, the present study was undertaken with the following objectives.

1. To assess the prevalence of vitamin D deficiency among vegetarian, partially vegetarian, and non vegetarian Malaysian and Indian medical students.
2. To compare levels of vitamin D, calcium, phosphate, and alkaline phosphatase among vegetarian, partially vegetarian, and non vegetarian Malaysian and Indian medical students.
3. To determine whether vitamin D levels are correlated with calcium, phosphate, alkaline phosphatase, Body Mass Index (BMI), socio-economic status, and sun exposure.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Biochemistry, Jawaharlal Nehru Medical College, Belagavi, Karnataka, India, from January 2024 to January 2025. Ethical clearance was obtained from the Institutional Ethics Committee (EC/KLECCH/03-2023). Written informed consent was taken from all participants.

Inclusion criteria: Apparently healthy age group 18-25 years, willing to consent, medical students of Indian and Malaysian ethnicity studying in medical colleges of Belagavi were included in the study.

Exclusion criteria: Chronic renal disease, chronic liver disease, osteoporosis, drugs affecting vitamin D (phenobarbital, orlistat, steroids), use of vitamin D and calcium supplementation, malabsorption, use of oral contraceptives, pancreatitis, cholecystectomy, metabolic bone disease, malignancy, and pregnancy were excluded from the study.

Sample size calculation: The calculation was predicated on an 80% power and a hypovitaminosis prevalence of 78.9% [11]. The minimum required sample size was 63.95. A sample size of 200 has been included to improve the power of this study.

Prevalence of vitamin D deficiency is 78.9% [11]
P=78.9%, q=100-78.9=21.1%
Absolute error (precision)=d=10%
95% confidence=z=1.96

$$N = \frac{Z^2 Pq}{d^2} = \frac{(1.96 \times 1.96)(7.89)(21.1)}{10 \times 10} = 63.95$$

Study Procedure

Approximately, 5 mL of venous blood drawn from antecubital vein and collected in plain vacutainer. The serum was separated and stored at -20°C until analysis. Vitamin D analysis was performed using a commercially available kit based on fluorescent immunoassay technique. Bone markers, including calcium, phosphorus, and alkaline phosphatase, were measured using a commercially available kit with an autoanalyser.

Vitamin D levels were categorised according to Endocrine Society guidelines as deficiency, insufficiency, and sufficiency based on serum vitamin D levels below 20 ng/mL, 20-29 ng/mL, and >30 ng/mL, respectively [12].

Study participants were divided into three groups.

- Group 1: Vegetarian (8 Malaysians, 22 Indians);
- Group 2: Partial vegetarian (12 Malaysians, 46 Indians);
- Group 3: Non vegetarian (80 Malaysians, 32 Indians).

The frequency of consumption of specific food items was evaluated by inquiring, 'How often do you personally consume the following food items: daily, weekly, occasionally, or never?' pertaining to the intake of milk or yogurt, legumes, dark green leafy vegetables, fruits, eggs, fish, poultry, or meat. Based on the frequency of consumption, vegetarian status was categorised by defining vegans as subjects who reported never consuming animal products (chicken or meat, fish, eggs, milk, or curd) and lacto-ovo vegetarians as those who reported consuming fruits, vegetables, pulses or beans, milk or curd, and/or eggs either daily, weekly, or occasionally but no fish or chicken or meat [13]. Pesco-vegetarians: who reported consuming fruits, vegetables, pulses or beans, milk or curd, and/or eggs or fish either daily, weekly, or occasionally but no chicken or meat [13]. Semi-vegetarian: individuals who reported the consumption of fruits, vegetables, pulses or beans, or animal products (chicken or meat, eggs, milk, or curd) on a daily, weekly, or occasional basis but excluded fish; non vegetarian: individuals who reported the consumption of fruits, vegetables, pulses or beans, and animal products (chicken or meat, fish, eggs, milk, or curd) on a daily, weekly, or occasional basis [13].

In this study vegans were combined with lacto-vegetarians and lacto-ovo-vegetarians to form the "vegetarian" group, and semi-vegetarians and pesco-vegetarians were combined to form the "partial vegetarian" group. Each individual was asked about the usual daily sun exposure in hours. The modified Kuppuswamy classification of socio-economic class was used for the assessment of the socio-economic status of Indians [14]. Malaysians' socio-economic status was categorised by household income [15].

STATISTICAL ANALYSIS

The statistical analysis was done using SPSS version 20.0. Data were entered as mean, standard deviation, and percentage. Student's t-test was used to calculate differences between the variables. The correlation between different parameters was calculated using the correlation coefficient. The Kolmogorov-Smirnov test was used to determine whether the dataset in a group follows a normal distribution. A p-value <0.05 were considered statistically significant.

RESULTS

There was a statistically significant difference among two groups regarding gender, age, and BMI (p-value <0.05). The Indian group has more vitamin D-deficient students (78, 78%) compared to the Malaysian group (66, 66%). There was no statistically significant difference in vitamin D level, sunlight exposure, and socio-economic status between the Malaysian group and the Indian group (p-value > 0.05) [Table/Fig-1].

Indian: The mean level of vitamin D was lowest in the vegetarian group (11.05±1.94), followed by the partial vegetarian (13.85±2.59) group as compared to the non vegetarian group (25.91±9.25). Statistically significant differences (p-value <0.05) were observed in serum vitamin D, calcium, and phosphorus levels among vegetarian, partial vegetarian and non vegetarian groups. A statistically significant difference (p-value <0.05) was observed in serum vitamin D, calcium, and phosphorus levels between the vegetarian vs non vegetarian groups and between the partial vegetarian vs non vegetarian groups [Table/Fig-2].

Malaysian: The mean level of vitamin D was lower in the vegetarian (8.74±1.22) and partial vegetarian (13.52±1.55) groups compared to the non vegetarian group (20.67±9.80). Calcium level was lower in the vegetarian (7.68±0.30) and partial vegetarian (8.02±0.46) groups. Statistically significant differences (p-value <0.05) were observed in mean levels of serum vitamin D, calcium, and phosphorus among vegetarian, partial vegetarian, and non vegetarian groups. Statistically significant differences were observed in serum vitamin D and calcium levels between vegetarian vs non vegetarian groups [Table/Fig-3].

In both Indian and Malaysian vegetarian groups, vitamin D showed a statistically significant correlation with alkaline phosphatase (r value 0.6012 and r value 0.9673). In the Indian non vegetarian group, vitamin D showed a statistically significant (p-value <0.05) correlation with calcium (r value 0.9245). In the Malaysian non vegetarian group, vitamin D showed a statistically significant (p-value

Variables		Malaysian group (100)		Indian group (100)		p-value
Demographic	Male	72 (72%)		46 (46%)		0.0080*
	Female	28 (28%)		54 (54%)		
	Age (years)	20.54±0.50		22.55±3.87		0.0010*
	BMI	20.22±0.42		19.40±2.92		0.0010*
Socio-economic status	B40-Bottom 40% low income earners		18 (18%)	Upper class	26 (26%)	0.7643
	M40-Middle 40% medium income earners		51 (51%)	Upper middle	33 (33%)	
	T20-Top 20% high income earners		31 (31%)	Lower middle	22 (22%)	
				Upper lower	15 (15%)	
				Lower	4 (4%)	
Exposure to sunlight in hours	Less than 30 min	35 (35%)		40 (40%)		0.6582
	30 min-1 h	29 (29%)		38 (38%)		
	1-2 h	19 (19%)		12 (12%)		
	>2 h	17 (17%)		10 (10%)		
Diet	Vegetarian	8 (8%)		22 (22%)		0.0019*
	Partial vegetarian	12 (12%)		46 (46%)		
	Non vegetarian	80 (80%)		32 (32%)		
Vitamin D (ng/mL)	Sufficient (>30 ng/mL)	16 (16%)		10 (10%)		0.6076
	Insufficient (20-29 ng/mL)	18 (18%)		12 (12%)		
	Deficiency (<20 ng/mL)	66 (66%)		78 (78%)		

[Table/Fig-1]: Baseline characteristics of the participants and levels of Vitamin D.
*p-value <0.05 statistically significant

	Vitamin D (ng/mL)				Calcium (mg/dL)			
			95% CI for mean				95% CI for mean	
Dietary	Mean±SD	SE	Lower	Upper	Mean±SD	SE	Lower	Upper
Veg	11.05±1.94	0.59	9.75	12.36	7.67±0.50	0.15	7.33	8.01
Partial veg	13.85±2.59	0.54	12.73	14.97	7.75±0.47	0.10	7.54	7.95
Non veg	25.91±9.25	2.31	20.98	30.84	8.81±1.58	0.40	7.96	9.65
F-value	30.1570				6.6937			
p-value	0.0001*				0.0028*			
Veg vs Partial veg	p-value=0.3674				p-value=0.9767			
Veg vs Non veg	p-value=0.0001*				p-value=0.0132*			
Partial veg vs Non veg	p-value=0.0001*				p-value=0.0049*			
	Phosphorus (mg/dL)				Alkaline Phosphatase (IU/L)			
			95% CI for mean				95% CI for mean	
Dietary	Mean±SD	SE	Lower	Upper	Mean±SD	SE	Lower	Upper
Veg	3.58±0.78	0.23	3.06	4.11	116.27±30.09	9.07	96.06	136.49
Partial veg	3.78±0.43	0.09	3.60	3.97	109.00±19.58	4.08	100.53	117.47
Non veg	4.30±0.69	0.17	3.94	4.67	126.81±28.89	7.22	111.42	142.21
F-value	5.4671				2.3442			
p-value	0.0073*				0.1070			
Veg vs Partial veg	p-value=0.6494				p-value=0.7140			
Veg vs Non veg	p-value=0.0110*				p-value=0.5404			
Partial veg vs Non veg	p-value=0.0296*				p-value=0.0880			

[Table/Fig-2]: Comparison of Vitamin D (ng/mL), Calcium (mg/dL), Phosphorus (mg/dL) and Alkaline Phosphatase (IU/L) levels in different dietary groups among Indian medical students by One-way ANOVA.
*p-value <0.05 statistically significant

<0.05) correlation with calcium (r value 0.6632), phosphorus (r value 0.6262), and alkaline phosphatase (r value 0.7581) [Table/Fig-4].

[Table/Fig-5] shows correlations between Vitamin D with other parameters in two groups by Spearman's rank correlation. Vitamin D correlated with sun exposure in total (Spearman R=0.76), Malaysian (Spearman R=0.81), and Indian groups (Spearman R=0.70) significantly (p-value <0.05) [Table/Fig-5].

DISCUSSION

The current study revealed a high prevalence of vitamin D deficiency among Indian and Malaysian medical students enrolled in medical colleges in Belagavi. The present study showed Indian students were more vitamin D deficient (78%) as compared to Malaysian students (66%). A study by Al Sadat N and Moy FM and Shafinaz IS et al., reported findings similar to the present study [11,16]. The Fitzpatrick skin type chart and Mexameter (MX 18) were used to determine the skin type in Malaysia. The findings indicated that Indians predominantly fell within types V to VI (dark brown to black), followed by Malaysians in type III (light brown) and Chinese in type II (white skin). This could be the cause of the low vitamin D levels among Indians and Malaysians [17]. In both Indian and Malaysian medical students, the mean level of vitamin D was lower in vegetarian and partial vegetarian groups as compared to in the non vegetarian group.

Dietary	Vitamin D (ng/mL)				Calcium (mg/dL)			
	Mean±SD	SE	95% CI for mean		Mean±SD	SE	95% CI for mean	
			Lower	Upper			Lower	Upper
Veg.	8.74±1.22	0.55	7.23	10.25	7.68±0.30	0.14	7.30	8.06
Partial veg	13.52±1.55	0.63	11.89	15.14	8.02±0.46	0.19	7.54	8.50
Non veg	20.67±9.80	1.57	17.49	23.84	9.06±1.15	0.18	8.69	9.44
F-value	5.1710				5.7983			
p-value	0.0093*				0.0056*			
Veg vs partial veg	p-value=0.6477				p-value=0.8562			
Veg vs Non veg	p-value=0.0180*				p-value=0.0206*			
Partial veg vs Non veg	p-value=0.1664				p-value=0.0680			
Dietary	Phosphorus (mg/dL)				Alkaline Phosphatase (IU/L)			
	Mean±SD	SE	95% CI for mean		Mean±SD	SE	95% CI for mean	
			Lower	Upper			Lower	Upper
Veg	3.70±0.48	0.22	3.10	4.30	100.20±19.02	8.51	76.59	123.81
Partial veg	3.72±0.35	0.14	3.34	4.09	104.17±19.07	7.79	84.15	124.18
Non veg	4.24±0.58	0.09	4.05	4.42	113.10±23.29	3.73	105.55	120.65
F-value	3.9424				1.0160			
p-value	0.0261*				0.3699			
Veg vs partial veg	p-value=0.9987				p-value=0.9546			
Veg vs Non veg	p-value=0.1111				p-value=0.4562			
Veg vs Non veg	p-value=0.0900				p-value=0.6406			

[Table/Fig-3]: Comparison of Vitamin D (ng/mL), Calcium (mg/dl), Phosphorus (mg/dl) and Alkaline Phosphatase (IU/L) levels with dietary patterns in Malaysian group by One-way ANOVA.
*p-value <0.05 statistically significant

Group	Dietary patter	Variables	r-value	p-value
Malaysian group	Veg	Calcium	0.5297	0.3585
		Phosphorus	0.3213	0.5981
		Alkaline Phosphatase	0.9673	0.0071*
	Partial veg	Calcium	0.1067	0.8405
		Phosphorus	0.5569	0.2511
		Alkaline Phosphatase	-0.6347	0.1758
	Non veg	Calcium	0.6632	0.0001*
		Phosphorus	0.6262	0.0001*
		Alkaline Phosphatase	0.7581	0.0001*
Indian group	Veg	Calcium	0.5237	0.0982
		Phosphorus	-0.2684	0.4248
		Alkaline Phosphatase	0.6012	0.0500*
	Partial veg	Calcium	0.3351	0.1180
		Phosphorus	0.1324	0.5471
		Alkaline Phosphatase	0.2203	0.3124
	Non veg	Calcium	0.9245	0.0001*
		Phosphorus	0.4741	0.0635
		Alkaline Phosphatase	0.4670	0.0682

[Table/Fig-4]: Correlation between Vitamin D with Calcium, Phosphorus and Alkaline Phosphatase levels by Karl Pearson's correlation coefficient.
*p-value <0.05 statistically significant

Samples	Variable	Spearman R	p-value
Total	BMI	0.0416	0.6810
	Sun exposure	0.7690	0.0001*
	Socio-economic status	-0.1174	0.2448
Malaysian group	BMI	0.0063	0.9656
	Sun exposure	0.8137	0.0001*
	Socio-economic status	-0.1193	0.4094
Indian group	BMI	0.0032	0.9823
	Sun exposure	0.7062	0.0001*
	Socio-economic status	-0.0422	0.7709

[Table/Fig-5]: Correlations between Vitamin D with other parameters in two groups by Spearman's rank correlation.
*p-value <0.05 statistically significant

The limited consumption of vitamin D-rich foods, the majority of which come from animal sources like fish and egg yolk, may be the cause of the higher vitamin D deficiency among vegetarians in this study. Since vitamin D fortification of food is still in its infancy in India, a vegetarian may find it challenging to achieve their daily vitamin D needs [18]. The results of the present study investigation are identical to those of Crowe FL et al., and Bakaloudi DR et al., [19,20]. A study by Bakaloudi DR et al., discovered that vegan diets were linked to lower vitamin D than omnivorous diets and non vegetarian diets [20]. In order to ascertain the degree to which the low vitamin D intake seen in vegetarians affects plasma 25-hydroxyvitamin D concentrations, Crowe FL et al., conducted a cross-sectional study [19]. The degree of animal-based food exclusion (meat eaters > fish eaters > vegetarians > vegans) was reflected in plasma 25-hydroxyvitamin D concentrations, according to results from a total of 2017 British individuals. Vegans had the lowest mean plasma 25-hydroxyvitamin D concentration (55.8 nmol/L), while meat eaters had the highest (77.0 nmol/L) [19].

Calcium correlates strongly with vitamin D in both non vegetarian groups of Indian and Malaysian medical students. The present study findings were the same as those of Neufingerl N and Eilander A and Harinarayan CV [21,22]. Calcium from plant sources is less bioavailable due to oxalates and phytates, requiring higher consumption to reach the same serum levels as non vegetarians. The modulation of calcium and phosphorus balance for bone mineralisation is the most well-known role of vitamin D. Dietary calcium cannot be absorbed if blood levels of vitamin D are insufficient. Vegetarians with low calcium levels should concentrate on including foods rich in calcium and high in bioavailability in their meals [23].

In the present study, sun exposure positively correlated with vitamin D in both groups, and it was statistically significant. The present study findings are the same as those of Harinarayan CV et al.,

and Goswami R et al., [24,25]. We as humans can get vitamin D from abundant sunshine by exposing 18% of our body surface area (without sunscreen) to midday sun for 30-45 min to cause one Minimal Erythemal Dose (MED), which is equivalent to taking about 600 to 1000 IU of vitamin D. This is about the recommended daily dose by the expert group on human nutrient requirements [26]. A study conducted by Farrar MD et al., revealed that 94% of participants attained concentrations exceeding 20 ng/mL through more precise sun exposure recommendations, specifically 45 minutes of unshaded midday exposure three times per week, with 35% of the skin surface area exposed, thereby establishing a correlation between sunlight exposure and vitamin D levels [27].

The present study showed a non significant correlation of vitamin D with BMI. These results are contradictory to Wortsman J et al., obesity-associated vitamin D insufficiency is likely due to the decreased bioavailability of vitamin D from cutaneous and dietary sources because of its deposition in body fat compartments [28].

The present study showed a non significant correlation of vitamin D with socio-economic status. Individuals with higher socio-economic status often lead more sedentary lives and may engage less in outdoor activities that expose them to sunlight. Conversely, adolescents from lower socio-economic backgrounds tend to participate in outdoor activities and have prolonged exposure to sunlight, possibly due to their involvement in work [29].

Maintaining a normal BMD and getting enough vitamin D may be possible with a vegetarian diet that includes the right foods and supplements [30]. Numerous lifestyle and environmental factors, such as the application of anti-UV filter creams, skin pigmentation, geographical latitude, obesity, and age, render sunlight exposure an inconsistent source of vitamin D. Therefore, to prevent vitamin D insufficiency and deficiency in individuals adhering to vegetarian and vegan diets, it is advisable to administer a vitamin D supplement at an appropriate dosage [31].

González-Padilla E et al., reported a high prevalence of hypovitaminosis D in medical students from Spain [32]. Multani SK et al., found that 87.5% of resident doctors from India had low vitamin D levels [33]. There is a necessity for heightened awareness regarding the significance of sufficient sun exposure and the consumption of a balanced diet rich in vitamin D to optimise vitamin D levels among medical students. Screening for vitamin D deficiency should be done once by all medical professionals. Once it is confirmed, vitamin D supplements must be provided for corrections and prevention of complications associated with vitamin D deficiency.

Limitation(s)

As this study was conducted in medical college students, the results of the study cannot be generalised. All the information regarding health or dietary patterns taken in the present study was based on self-administered questionnaires. Recall bias from participants may also influence the results. Lack of information on the medications and vitamin D supplements used among participants was another limitation.

Further multicentric studies with a large sample size are required to validate these findings.

CONCLUSION(S)

The present study showed that both Indian and Malaysian medical students are vitamin D-deficient. It raises a great concern about the future skeletal health of these young medical students. The significantly increased incidence of low vitamin D among medical students in India (78%) and Malaysia (66%), despite ample sunlight, indicates that vitamin D levels are influenced by dietary practices and food fortification, alongside ethnicity and sun exposure. In both Indian and Malaysian medical students, the mean level of vitamin D was low in vegetarian and partial vegetarian groups as compared to the non vegetarian group. The best way to prevent vitamin D deficiency and insufficiencies in vegetarians and vegans is to take a vitamin D supplement at the recommended dosage.

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