Original Article

Vitamin B12 and D status in long-term vegetarians: Impact of diet duration and subtypes in Beijing, China

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Background and Objectives: This study aimed to evaluate vitamin B12 and D levels among long-term vegetarians and to assess the influence of their diet duration and diet subtypes on nutritional status in Beijing, China. **Methods and Study Design:** A cross-sectional analysis was conducted on 104 vegetarians (84 vegans, 20 lacto-ovo vegetarians). Serum vitamin B12 and D levels were measured, and demographic, dietary, and supplement use data were collected. Statistical analyses included ANOVA, Kruskal-Wallis tests, chi-square tests, and multivariate regressions. **Results:** The prevalence of vitamin B12 deficiency (<200 pg/mL) was 38.5%, with higher rates in vegans (44.1%) than lacto-ovo vegetarians (15.0%, $p = 0.021$). Vitamin D deficiency (<20 ng/mL) affected 84.6% of participants, worsening with longer diet duration ($p < 0.001$), yet showed no significant difference between vegan and lacto-ovo subtypes (85.7% vs 80.0%, $p = 0.524$). Multivariate analysis revealed negative correlations between diet duration (6–10 years and >10 years) and vitamin B12 and D levels ($p < 0.05$). Lacto-ovo vegetarians showed significantly higher vitamin B12 levels than vegans ($p = 0.029$), and supplement use improved both vitamins' status ($p < 0.05$). **Conclusions:** This study reveals a dual challenge among Beijing long-term vegetarians: vitamin B12 deficiency was strongly associated with the degree of exclusion of animal products from the diet (veganism), while vitamin D deficiency was highly prevalent and worsened with longer diet duration. The near-universal vitamin D deficiency observed in this study suggests that, in the Beijing context, the risk may extend beyond dietary choice, potentially reflecting regional environmental factors; however, this requires confirmation through comparative studies with omnivores controls.

Key Words: long-term vegetarians, vitamin B12, vitamin D, duration, subtypes

INTRODUCTION

The global shift toward plant-based diets, driven by health, environmental, and ethical motivations, has positioned vegetarian (plant-based plus dairy products and/or eggs) and vegan (100% plant-based) lifestyles as prominent dietary choices.[1-3] Extensive evidence links these diets to reduced risks of obesity, cardiovascular diseases, and type 2 diabetes, largely due to lower saturated fat intake and higher consumption of fiber, antioxidants, and phytochemicals.[4] However, long-term adherence to vegetarianism, particularly veganism, poses significant risks of micronutrient deficiencies. Among these, vitamin B12 and vitamin D deficiencies are most critical. Vitamin B12, essential for neurological function and red blood cell formation, is exclusively found in animal-derived foods,[5] while vitamin D, vital for bone health and immune regulation, primarily relies on sunlight exposure and fortified animal products.[6-7] Deficiencies in these nutrients may lead to irreversible neurological damage, osteoporosis, and compromised immunity, underscoring the need for targeted interventions in vegetarian populations.[8-10]

International studies, such as the EPIC-Oxford cohort, have documented alarmingly high deficiency rates: 52% of vegans and 7% of lacto-ovo vegetarians exhibit vitamin B12 deficiency,[11] while up to 70% of vegetarians in temperate climates suffer from vitamin D insufficiency.[10] However, most research lacks stratification by diet duration and diet subtypes, two factors that critically modulate deficiency risks. For instance, hepatic B12 stores deplete progressively over decades of veganism, while lacto-ovo

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diets may mitigate risks through dairy and egg consumption. In China, vegetarianism is culturally rooted in Buddhist traditions or emerging health trends, yet research remains fragmented.[12] Studies in Shanghai and southern China report deficiency rates of 41% (B12) and 78% (D) among vegetarians,[13] but these predominantly focus on short-term practitioners (<5 years) or fail to differentiate between vegan and lacto-ovo subgroups. Furthermore, northern regions like Beijing, characterized by limited fortified food availability and seasonal sunlight variability, remain understudied,[14] despite their unique dietary and environmental contexts.

This study addresses critical research voids by investigating vitamin B12 and D status in 104 long-term vegetarians (≥3 years) from Beijing, China—a population with distinct dietary habits and environmental exposures. Participants are stratified into subgroups based on: diet duration: 3–5 years, 6–10 years, >10 years (to assess cumulative deficiency risks); diet subtypes: vegan (100% plant-based) vs. lacto-ovo vegetarian (plant-based plus consuming dairy/eggs). By integrating demographic, lifestyle, and supplementation data, this study aims to quantify deficiency prevalence of B12 and D in a northern Chinese cohort; analyze correlations between deficiency risks and diet duration or diet subtypes and provide evidence-based recommendations for nutrient monitoring and intervention strategies.

METHODS

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Cancer Hospital, Chinese Academy of Medical Sciences (No.2023060515335102).

Study population

This cross-sectional study was designed to simultaneously observe and analyze the nutrition status of the long-term vegetarians. Participants were recruited from vegetarian social groups in November 2024. The inclusion criteria were adults aged 18 years or older who had adhered to a vegetarian diet for at least 3 years, encompassing both vegan and lacto-ovo vegetarian diets.

Data collection

A structured questionnaire was designed and self-administered through an online questionnaire platform to collect demographic information such as age, gender, educational level, diet duration and diet subtypes. Information was gathered regarding whether the participants had chronic diseases, such as hypertension or diabetes, and whether they used vitamin supplements. If supplements were used, further details were solicited, including the types (with a particular focus on vitamin B12 and vitamin D supplements), brand, dosage, and frequency of use. Subsequently, all questionnaire responses were verified by trained nutrition professionals through one-on-one interviews during the on-site hospital visit. Professional medical staff used calibrated height and weight measuring instruments to measure the participants' height and weight according to standard measurement methods. Body mass index (BMI) was then calculated using the formula: weight (kg) divided by the square of height (m). In the fasting state (8-12 hours of fasting), professional medical staff drew 5-10 mL of venous blood from the participants at the hospital, and the chemiluminescent immunoassay method was used to measure the levels of vitamin B12 and vitamin D in the blood within the hospital laboratory. The testing procedures were strictly carried out in accordance with the operation manuals of the instruments and kits to ensure the reliability of the test results.

Assessment of vitamin B12 and D

Serum vitamin B12 and vitamin D levels were categorized based on the thresholds widely adopted in international literature. Vitamin B12 deficiency was defined as a serum concentration <200 pg/mL, while levels ≥200 pg/mL were considered non-deficient. Similarly, vitamin D status was assessed via serum 25-hydroxyvitamin D [25(OH)D] levels. Vitamin D deficiency was defined as <20 μg/L (50 nmol/L), and concentrations ≥20 μg/L (≥50 nmol/L) indicated non-deficient.[16]

Grouping standards

We divided participants into three education level groups, i.e., high school/below, college degree, graduate/above. The intake of vitamin supplements were categorized into three groups: none, 1–2 times per week, and ≥ 3 times per week.

Data analysis

We used SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) and R version 4.5.0 for data cleaning and analysis. Continuous variables were assessed for normality using the Shapiro-Wilk test. Normally distributed variables are presented as mean ± standard deviation (SD), and between-group differences were analyzed using one-way analysis of variance (ANOVA). Non-normally distributed variables are expressed as median (interquartile range [IQR]), and between-group differences were evaluated using non-parametric Kruskal-Wallis tests. Categorical variables were compared using chi-square tests or Fisher's exact test when expected cell counts were <5.

For non-normally distributed vitamin B12 and D levels, Spearman's rank correlation tests were calculated to evaluate monotonic relationships with diet duration. The Cochran-Armitage trend test was employed to examine ordered trends in deficiency prevalence of vitamin B12 and D across diet duration categories.

Due to right-skewed distributions (Shapiro-Wilk p <0.05), serum vitamin B12 and 25(OH)D concentrations were natural log-transformed prior to linear regression analyses. Multivariable linear regression models were constructed to assess associations between diet duration/subtypes and ln-transformed vitamin levels. Regression coefficients (β) were exponentiated to represent percentage changes in original units per unit increase in predictors, with 95% confidence intervals (CIs). Logistic regression models were used to evaluate associations between dietary factors and vitamin deficiency status (binary outcome), expressed as odds ratios (ORs) with 95% CIs. Three hierarchical models were employed: Model 1 was a simple univariate analysis without adjustment; Model 2 adjusted for age, sex, education, chronic diseases, and BMI; In addition to the variables adjusted in Model 2, Model 3 included additional covariates related to nutrition, such as nutrition supply intake over the past 3 months, type of nutrition supply, and frequency of nutrition intake.

The significance level was set at $p < 0.05$.

RESULTS

Characteristics of the study population

Table 1 displays study population characteristics. A total of 104 long-term vegetarians were enrolled in this study, among whom 84 (80.8%) were vegans and 20 (19.2%) were lacto-ovo vegetarians. The mean age of the participants was 42.4 ± 9.3 years, with females accounting for 72.1% (n = 75). In terms of educational attainment, 88.5% of the participants had a college degree or above, indicating a relatively high educational level of the sample. The average body mass index (BMI) of the participants was 21.7 ± 2.7, falling within the normal range. Additionally, 8.7% of the participants had a history of chronic diseases, and 37.5% of them were using vitamin supplements; the proportion of supplement users was higher among lacto-ovo vegetarians than among vegans (55.0% vs. 33.3%). Specifically, in terms of the types of supplementation, 29.8% (31 out of 104) of the participants reported taking B12 supplements, while 18.3% (19 out of 104) reported taking vitamin D supplements, which indicated that the proportions of participants supplementing with both B12 and D were relatively low.

Vitamin B12 and D levels and their deficiency risks

The results for vitamin B12 and D levels as well as their deficiency risks among participants with different demographic and lifestyle characteristics were presented in Table 2, Figure 1 and Figure 2.

Vitamin B12

Overall, the average level of vitamin B12 among the 104 vegetarians was 234 pg/mL (IQR:163.6-361.0). Alarmingly, 38.5% of the participants were deficient in vitamin B12 (<200 pg/mL), and 61.5% was not deficient (≥200 pg/mL).

Males exhibited significantly lower median B12 levels (139 pg/mL, IQR: 103–212) compared to females (267 pg/mL, IQR: 179–406; p <0.001). Consequently, males had a higher B12 deficiency prevalence (69.0% vs. 26.7%, p <0.001).

Participants who used supplements ≥3 times/week had higher median B12 levels (299.0 pg/mL, IQR: 218–483) and lower deficiency risk (17.4%) than non-users (212 pg/mL, IQR: 128–315; 46.2% deficiency; p = 0.005 for levels, p = 0.040 for deficiency).

Lacto-ovo vegetarians had higher B12 levels (318 pg/mL, IQR: 227–417) and lower deficiency risk (15.0%) than vegans (215 pg/mL, IQR: 147–328; 44.1% deficiency; p = 0.028 for levels, p = 0.016 for deficiency).

Vitamin D

Among the 104 vegetarians, the average level of vitamin D was 7.7 ng/mL (IQR:4.4-13.8). A staggering 88 participants (84.6%) were deficient in vitamin D (< 20 ng/mL), and only 16 individuals (15.4%) were not deficient (≥ 20 ng/mL).

Participants aged ≥45 years exhibited higher median vitamin D levels (8.4 ng/mL, IQR: 5.4–14.8) compared to younger participants (6.7 ng/mL, IQR: 4.2–13.2), although this difference did not reach statistical significance (p = 0.072). Similarly, males had marginally higher vitamin D levels (9.4 ng/mL, IQR: 6.0–14.1) than females (6.6 ng/mL, IQR: 4.2–13.4), but this difference was also not statistically significant (p = 0.124).

Vegetarian duration showed an inverse association with vitamin D status. Those with >10 years of vegetarianism had significantly lower vitamin D levels (6.5 ng/mL, IQR: 4.5-12.7) and higher deficiency prevalence (93.2%) compared to those with 3-5 years duration (11.3 ng/mL, IQR: 6.2-24.5; 59.2% deficiency; p = 0.007 for both level and deficiency comparisons). A declining trend in D levels with longer vegetarian duration was observed (p = 0.03 for trend test), accompanied by increasing deficiency risk (p = 0.004).

Lacto-ovo vegetarians had significantly higher D levels (12.4 ng/mL, IQR: 9.4–15.9) than vegans (6.1 ng/mL, IQR: 4.0–13.2; p = 0.001), though deficiency rates were comparable (80.0% vs. 85.7%, p = 0.524).

Multivariate linear associations between diet duration/subtypes and vitamin B12/D levels

The results from the multivariate linear regression models for vitamin B12 and D were presented in Table 3.

Vitamin B12

Multivariable linear regression analyses demonstrated progressive associations between vegetarian duration and vitamin B12 levels across model specifications. In unadjusted analyses (Model 1), neither 6-10 years duration (β = 0.9, 95% CI: 0.7-1.2, p = 0.085) nor >10 years duration (β = 0.8, 95% CI: 0.6-1.0, p = 0.282) showed significant reductions. Adjustment for demographic factors (Model 2) marginally strengthened these associations (>10 years: β = 0.8, p = 0.055). The fully adjusted model (Model 3) revealed significant 20% lower levels for 6-10 years (exponentiated β = 0.8, 95% CI: 0.6-0.99, p = 0.048) and a borderline reduction for >10 years (β = 0.8, 95% CI: 0.6-1.0, p = 0.053), with a significant duration-response trend (p-trend = 0.042).

Dietary subtype analyses showed consistent protective effects of lacto-ovo vegetarianism across all models, with fully adjusted results demonstrating 40% higher B12 levels versus vegans (Model 1: β = 1.4, p = 0.012; Model 2: β = 1.4, p = 0.008; Model 3: β = 1.4, p = 0.003).

Vitamin D

Vitamin D levels showed stronger duration-dependent reductions across model specifications. Unadjusted models (Model 1) already indicated significant 40% lower levels for >10 years vegetarians (β = 0.6, 95% CI: 0.4-0.9, p = 0.010), which became more pronounced after demographic and BMI adjustment (Model 2: β = 0.5, 95% CI: 0.4-0.8, p = 0.004). Fully adjusted analyses (Model 3) confirmed significant 40% reductions for both 6-10 years (β = 0.6, 95% CI: 0.4-0.95, p = 0.006) and >10 years durations (β=0.6, 95% CI: 0.4-0.8, p = 0.032), with robust trend significance (p-trend = 0.005).

Table 1. Characteristics of the study participants by diet duration and vegetarian structure†

Characteristics	Total (n=104)	Diet duration				Vegetarian structure		
		3–5 years (n=26)	6–10 years (n=34)	>10 years (n=44)	p	Vegan (n=84)	Lacto-ovo vegetarian (n=20)	p
Age (years)	42.4 ± 9.3	42.5 ± 9.6	39.9 ± 7.7	44.2 ± 10.0	0.126	42.5 ± 8.8	41.9 ± 11.4	0.782
18-44	66 (63.5%)	16 (61.5%)	25 (73.5%)	25 (56.8%)	0.306	53 (63.1%)	13 (65.0%)	0.874
45-	38 (36.5%)	10 (38.5%)	9 (26.5%)	19 (43.2%)		31 (36.9%)	7 (35.0%)	
Sex								
Male	29 (27.9%)	7 (26.9%)	8 (23.5%)	14 (31.8%)	0.715	23 (27.4%)	6 (30.0%)	0.814
Female	75 (72.1%)	19 (73.1%)	26 (76.5%)	30 (68.2%)		61 (72.6%)	14 (70.0%)	
Education								
High school or below	12 (11.5%)	5 (19.2%)	2 (5.9%)	5 (11.4%)	0.228	11 (13.1%)	1 (5.0%)	0.563
College	70 (67.3%)	17 (65.4%)	21 (61.8%)	32 (72.7%)		55 (65.5%)	15 (75.0%)	
Graduate or above	22 (21.2%)	4 (15.4%)	11 (32.4%)	7 (15.9%)		18 (21.4%)	4 (20.0%)	
BMI (kg/m^2)	21.7 ± 2.7	21.8 ± 2.5	21.1 ± 2.4	22.1 ± 3.0	0.287	21.5 ± 2.7	22.6 ± 2.4	0.105
<18.5	11 (10.6%)	3 (11.5%)	3 (8.8%)	5 (11.4%)	0.409‡	9 (10.7%)	2 (10.0%)	0.340‡
18.5-23.9	74 (71.2%)	18 (69.2%)	28 (82.4%)	28 (63.6%)		62 (73.8%)	12 (60.0%)	
≥24	19 (18.3%)	5 (19.2%)	3 (8.8%)	11 (25.0%)		13 (15.5%)	6 (30.0%)	
Chronic disease	9 (8.7%)	4 (15.4%)	3 (8.8%)	2 (4.6%)	0.297	7 (8.3%)	2 (10.0%)	0.812
Supplement use								
None	65 (62.5%)	18 (69.2%)	20 (58.8%)	27 (61.4%)	0.094‡	56 (66.7%)	9 (45.0%)	0.032‡
1–2 times/week	16 (15.4%)	4 (15.4%)	2 (5.9%)	10 (22.7%)		9 (10.7%)	7 (35.0%)	
≥3 times/week	23 (22.1%)	4 (15.4%)	12 (35.3%)	7 (15.9%)		19 (22.6%)	4 (20.0%)	
Vitamin types								
Had B12 supply	31 (29.8%)	7 (26.9%)	11 (32.3%)	13 (29.5%)	0.900	24 (28.6%)	7 (35.0%)	0.572
Had D supply	19 (18.3%)	7 (26.9%)	7 (20.6%)	5 (11.4%)	0.243	14 (16.7%)	5 (25.0%)	0.386

†Data are presented as n (%)
‡Fisher exact test was used.

Table 2. Levels of vitamin B12 and D and their deficiency risks across the study participants with varied characteristics

Characteristics	Vitamin B12 (pg/mL)					Vitamin D† (ng/mL)				
	Median (Q25, Q75)	*p*	Deficiency risk (<200)	Non-deficiency (≥200)	*p*	Median (Q25, Q75)	*p*	Deficiency risk (<20)	Non-deficiency (≥20)	*p*
Total	234 (164, 361)	-	40 (38.5%)	64 (61.5%)	-	7.7 (4.4, 13.8)	-	88 (84.6%)	16 (15.4%)	-
Age (years)										
18-44	213 (139, 338)	0.099	30 (45.5%)	36 (54.5%)	0.053	6.7 (4.2, 13.2)	0.072	59 (89.4%)	7 (10.6%)	0.075
45 -	279 (195, 415)		10 (26.3%)	28 (73.7%)		8.4 (5.4, 14.8)		29 (76.3%)	9 (23.7%)	
Sex										
Male	139 (103, 212)	<0.001	20 (69.0%)	9 (31.0%)	<0.001	9.4 (6.0, 14.1)	0.124	25 (86.2%)	4 (13.8%)	0.780
Female	267 (179, 406)		20 (26.7%)	55 (73.3%)		6.6 (4.2, 13.4)		63 (84.0%)	12 (16.0%)	
Education										
High school/below	170 (100, 365)	0.772	6 (50.0%)	6 (50.0%)	0.581	4.8 (2.8, 13.3)	0.329	11 (91.7%)	1 (8.30%)	0.167‡
College	232 (161, 367)		27 (38.6%)	43 (61.4%)		7.5 (5.4, 14.8)		55 (80.0%)	14 (20.0%)	
Graduate or above	254 (164, 347)		7 (31.8%)	15 (68.2%)		9.4 (6.0, 12.5)		21 (95.5%)	1 (4.60%)	
BMI(kg/m^2)										
<18.5	279 (153, 402)	0.890	4 (36.4%)	7 (63.6%)	0.760	6.0 (2.2, 9.9)	0.231	11 (100%)	0	0.313‡
18.5-23.9	228 (160, 367)		30 (40.6%)	44 (59.5%)		7.7 (4.3, 14.1)		62 (83.8%)	12 (16.2%)	
≥24	231 (152, 285)		6 (31.6%)	13 (68.4%)		9.3 (5.3, 14.0)		15 (79.0%)	4 (21.1%)	
Chronic disease										
yes	321±140	0.403	2 (22.2%)	7 (77.8%)	0.295‡	11.0±6.9	0.902	7 (77.8%)	2 (22.2%)	0.552
no	272±171		38 (40.0%)	57 (60.0%)		11.4±11.3		81 (85.3%)	14 (14.7%)	
Supplement use										
None	212 (128, 315)	0.005	30 (46.2%)	35 (53.8%)	0.040	6.2 (4.2, 12.0)	0.093	58 (89.2%)	7 (10.8%)	0.076‡
1 - 2 times/week	215 (162, 342)		37 (57.5%)	10 (62.5%)		9.4 (6.0, 13.0)		14 (87.5%)	2 (12.5%)	
≥3 times/week	299 (218, 483)		4 (17.4%)	19 (82.6%)		14.0 (7.4, 24.5)		16 (69.6%)	7 (30.4%)	
Trend test	0.005		0.016			0.093		0.034		
Vitamin types										
Had B12/D supply	337 (163, 382)	0.075	5 (12.5%)	15 (23.4%)	0.169	14.7 (6.9, 27.8)	0.014	12 (13.6%)	8 (50.0%)	<0.001‡
No B12/D supply	227 (149, 330)					6.5 (4.3, 12.2)				
Diet duration										
3 - 5 years	257 (179, 482)	0.313	8 (30.8%)	18 (69.2%)	0.648	11.3 (6.2, 24.5)	0.007	18 (59.2%)	8 (30.8%)	0.007
6 - 10 years	245 (149, 355)		14 (41.2%)	20 (58.8%)		6.3 (3.7, 12.7)		29 (85.3%)	5 (14.7%)	
>10 years	217 (129, 298)		18 (40.9%)	26 (59.1%)		6.5 (4.5, 12.7)		41 (93.2%)	3 (6.80)	
Trend test	0.067		0.489			0.03		0.004		
Vegetarian type										
Vegan	215 (147, 328)	0.028	37 (44.1%)	47 (56.0%)	0.016	6.1 (4.0, 13.2)	0.001	72 (85.7%)	12 (14.3%)	0.524
Lacto-ovo vegetarian	318 (227, 417)		3 (15.0%)	17 (85.0%)		12.4 (9.4, 15.9)		16 (80.0%)	4 (20.0%)	

†Vitamin D was serum 25(OH)D (ng/mL)
‡Fisher's exact test was used for comparisons

Table 3. Multivariable Llinear regression models for vitamin B12 and D†

Variable	Model 1		Model 2		Model 3	
	$e^{\beta \pm 1.96 \times SE}$	*p*	$e^{\beta \pm 1.96 \times SE}$	*p*	$e^{\beta \pm 1.96 \times SE}$	*p*
Vitamin B12 (logB12)						
Diet duration (Ref: 3-5 years)						
6-10 years	0.9 (0.7-1.2)	0.085	0.9 (0.7, 1.1)	0.252	0.8 (0.6, 0.99)	0.048
>10 years	0.8 (0.6, 1.0)	0.282	0.8 (0.6, 1.0)	0.055	0.8 (0.6, 1.0)	0.053
Trend test	0.96 (0.92, 1.0)	0.083	0.96 (0.92, 1.0)	0.053	0.96 (0.92, 0.998)	0.042
Vegetarian type (Ref: vegan)						
Lacto-ovo vegetarian	1.4 (1.1, 1.9)	0.012	1.4 (1.1, 1.8)	0.008	1.4 (1.1, 1.8)	0.003
Vitamin D (logD)						
Diet duration (Ref: 3-5 years)						
6-10 years	0.6 (0.4, 0.998)	0.049	0.6 (0.4, 0.998)	0.042	0.6 (0.4, 0.95)	0.006
>10 years	0.6 (0.4, 0.9)	0.010	0.5 (0.4, 0.8)	0.004	0.6 (0.4, 0.8)	0.032
Trend test	0.9 (0.86, 0.98)	0.009	0.9 (0.8, 0.97)	0.004	0.9 (0.8, 0.97)	0.005
Vegetarian type (Ref: vegan)						
Lacto-ovo vegetarian	1.8 (1.2, 2.7)	0.006	1.7 (1.1, 2.6)	0.012	1.7 (1.1, 2.5)	0.015

†The dependent variables B12 and D were log-transformed to log(B12) and log(D) to achieve a normal distribution. Subsequently, multiple linear regression was performed. The regression coefficients (β) were then exponentiated (i.e., transformed to e^β) ,which reflected the multiplicative changes in vitamin B12 and vitamin D levels associated with diet duration and type. Model 1 was a simple univariate analysis without adjustment; Model 2 adjusted for age, sex, education, chronic diseases, and BMI; In addition to the variables adjusted in Model 2, Model 3 included additional covariates related to nutrition, such as nutrition supply intake over the past 3 months, type of nutrition supply, and frequency of nutrition intake

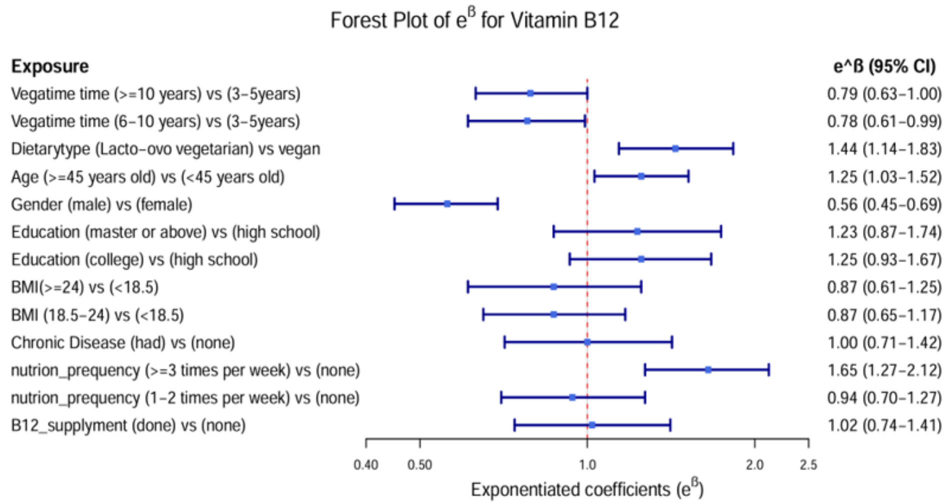

Figure 1. Forest plot of exponentiated coefficients (e^β) for factors associated with Vitamin B12 levels. Each horizontal line represents the 95% confidence interval (CI) for the e^βof a given exposure variable, with the square marker indicating the point estimate. An e^β> 1 suggests a positive association with Vitamin B12 levels, while e^β< 1 suggests a negative association. Variables crossing the vertical dashed line (e^β= 1) indicate non - significant associations at the 95% CI level.

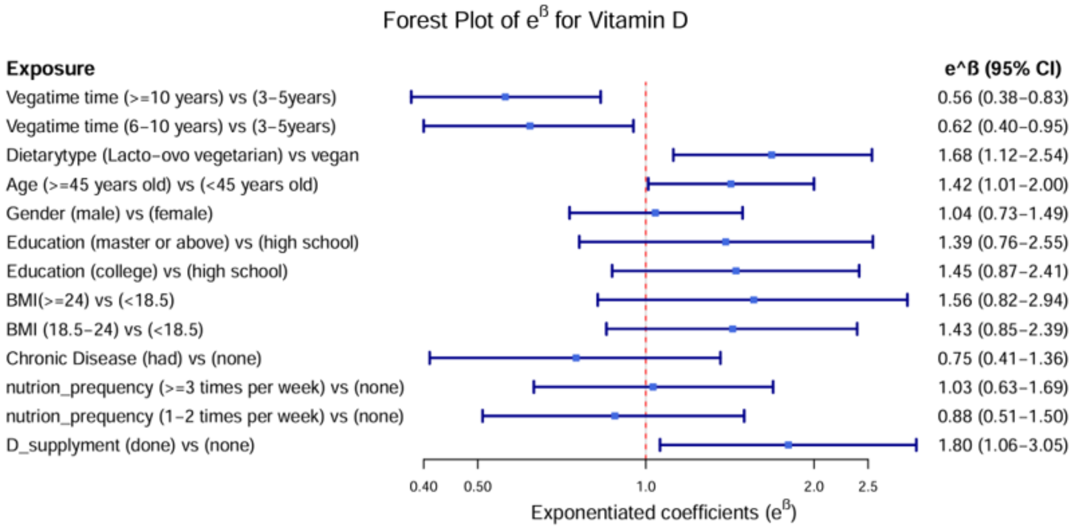

Figure 2. Forest plot of exponentiated coefficients (e^β) for factors associated with Vitamin D levels. Each horizontal line represents the 95% confidence interval (CI) for the e^βof a given exposure variable, with the square marker indicating the point estimate. An e^β> 1 suggests a positive association with Vitamin D levels, while e^β< 1 suggests a negative association. Variables crossing the vertical dashed line (e^β= 1) indicate non - significant associations at the 95% CI level

The lacto-ovo vegetarian advantage was evident in all models (Model 1: β=1.8, p = 0.006; Model 2: β = 1.7, p = 0.012; Model 3: β=1.7, p = 0.015), demonstrating 70% higher levels versus vegans in final analyses.

Multivariate regression analysis between diet duration/subtypes and vitamin B12/D deficiency

Table 4, Figure 3 and Figure 4 presented the results of multivariate logistic regression analyses examining the association between vegetarian duration, vegetarian type, and deficiencies in vitamin B12 and Vitamin D.

Vitamin B12

Multivariable logistic regression analyses revealed no significant association between vegetarian duration and vitamin B12 deficiency risk across all models. In the fully adjusted model (Model 3), neither 6-10 years (OR = 2.1, 95% CI: 0.5-8.3, p = 0.418) nor >10 years (OR = 1.9, 95% CI: 0.5-6.8, p = 0.515) of vegetarian duration showed significantly elevated risk compared to the reference group (3-5 years). No significant trend relationship was observed (p-trend = 0.359).

The protective effect of vegetarian subtypes showed remarkable consistency across models. Lacto-ovo vegetarians already exhibited an 80% lower risk of B12 deficiency compared to strict vegans in Model 1 (OR = 0.2, 95% CI: 0.06-0.8, p = 0.024). This protective association persisted in both Model 2 (OR = 0.2, p = 0.015) and Model 3 (OR = 0.2, p = 0.017), with narrowing confidence intervals indicating improved precision after covariate adjustment.

Vitamin D

Hierarchical regression analyses revealed significant associations between vegetarian duration and vitamin D deficiency risk. In the unadjusted Model 1, participants with >10 years of vegetarianism showed 6.0-fold higher odds of deficiency (95% CI: 1.4-25.5, p = 0.014) compared to the 3-5year reference group, with a significant duration-response trend (p-trend = 0.010). After adjusting for demographics and BMI (Model 2), this association strengthened to 9.7-fold increased odds (95% CI: 1.9-49.4, p = 0.009), while the trend test p-value decreased to 0.009. The association remained stable in the fully adjusted Model 3 (OR = 9.1, 95% CI: 1.5-53.3, p = 0.017).

Unlike vitamin B12, vegetarian type showed no significant association with vitamin D deficiency risk in all three models (lacto-ovo vs vegan: OR = 0.9, 95% CI: 0.1-7.5, p = 0.901 in Model 3).

DISCUSSION

This cross-sectional study provides important insights into the vitamin B12 and D status of long-term vegetarians in Beijing, China, revealing concerning deficiencies associated with dietary patterns and vegetarian duration. While our findings regarding vitamin B12 align with global evidence,[1,17] the interpretation of vitamin D deficiency requires careful consideration of contextual factors. Most importantly, the absence of a regional control group precludes attributing the observed vitamin D deficiency solely to vegetarian diets, as it likely reflects broader environmental factors affecting the general Beijing population. Our study thus contributes valuable evidence for understanding nutritional status in Chinese long-term vegetarians while highlighting the need for future controlled studies to disentangle dietary from regional influences.

The high prevalence of vitamin B12 deficiency (38.5%) corroborates Western studies reporting elevated risks in vegetarians, particularly vegans.[18-20] The stark disparity in B12 levels between vegans (257.5 pg/mL) and lacto-ovo vegetarians (353.8 pg/mL, p = 0.021) mirrors the findings from the EPIC-Oxford cohort, where vegans faced a 7-fold higher deficiency risk than lacto-ovo vegetarians.[19] This divergence likely stems from lacto-ovo diets incorporating B12-rich animal derivatives (e.g., dairy, eggs), whereas vegans rely solely on supplements or fortified foods—options less accessible in China due to limited fortification policies. A critical methodological consideration is the application of the international diagnostic threshold (200 pg/mL). While this facilitates

Table 4. Multivariable logistic regression for vitamin B12 and D deficiency

Variable	Model 1†		Model 2‡		Model 3§	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Vitamin B12 deficiency						
Diet duration (Ref: 3-5 years)						
6-10 years	1.5 (0.5, 4.4)	0.409	1.7 (0.5, 6.1)	0.349	2.1 (0.5, 8.3)	0.418
>10 years	1.5 (0.5, 4.5)	0.398	1.7 (0.5, 5.9)	0.839	1.9 (0.5, 6.8)	0.515
Trend test	1.1 (0.9, 1.3)	0.391	1.1 (0.9, 1.3)	0.440	1.1 (0.9, 1.4)	0.359
Vegetarian type (Ref: Strict vegan)						
Lacto-ovo vegetarian	0.2 (0.06, 0.8)	0.024	0.2 (0.04, 0.8)	0.015	0.2 (0.03, 0.8)	0.017
Vitamin D deficiency						
Diet duration (Ref: 3-5 years)						
6-10 years	2.5 (0.7, 8.9)	0.142	2.3 (0.5, 10.1)	0.370	3.7 (0.6, 22.9)	0.182
>10 years	6.0 (1.4, 25.5)	0.014	9.7 (1.9, 49.4)	0.009	9.1 (1.5, 53.3)	0.017
Trend test	1.3 (1.1, 1.6)	0.010	1.4 (1.1, 1.8)	0.009	1.4 (1.1, 1.9)	0.015
Vegetarian type (Ref: vegan)						
Lacto-ovo vegetarian	0.7 (0.2, 2.5)	0.526	0.8 (0.2, 3.4)	0.513	0.9 (0.1, 7.5)	0.901

†Model 1: a simple univariate analysis without adjustment
‡Model 2: adjusted for age, sex, education, chronic diseases, and BMI
§Model 3: In addition to the variables adjusted in Model 2, additional covariates related to nutrition, such as nutrition supply intake over the past 3 months, type of nutrition supply, and frequency of nutrition intake were included

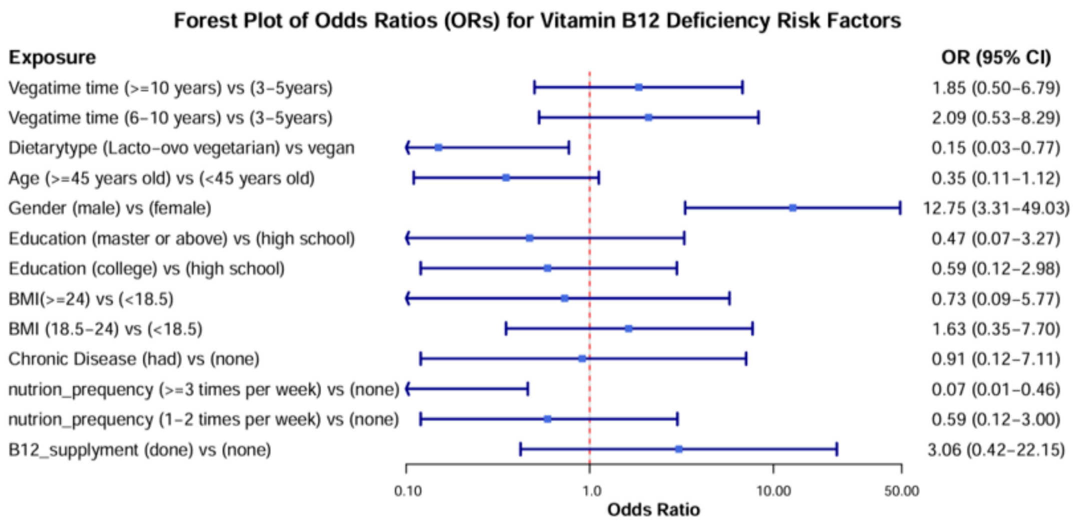

Figure 3. Forest plot of odds ratios (ORs) for Vitamin B12 deficiency risk factors. Horizontal lines represent 95% confidence intervals (CIs) for ORs of each exposure variable, with square markers denoting point estimates. OR > 1 indicates increased deficiency risk, OR < 1 indicates reduced risk. The vertical dashed line (OR = 1) marks no association; intervals crossing it suggest non - significance at the 95% CI level

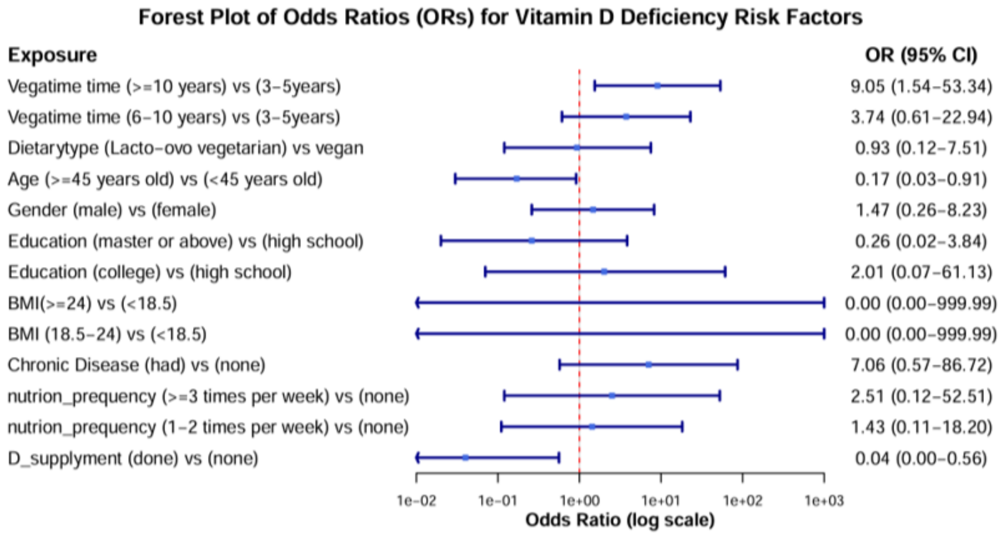

Figure 4. Forest plot of odds ratios (ORs) for Vitamin D deficiency risk factors. Horizontal lines represent 95% confidence intervals (CIs) for ORs of each exposure variable, with square markers denoting point estimates. OR > 1 indicates increased deficiency risk, OR < 1 indicates reduced risk. The vertical dashed line (OR = 1) marks no association; intervals crossing it suggest non - significance at the 95% CI level

cross-study comparability, emerging evidence suggests the lower limit for healthy Chinese adults may be higher (e.g., 250.8 pg/mL).[21] If a population-specific threshold were applied, the prevalence of deficiency we report (38.5%) would likely be substantially higher. This reclassification would notably alter the interpretation of our observation that many non-supplementing participants were not deficient, potentially indicating that our study underestimated the true burden of functional B12 inadequacy. Therefore, our reported deficiency rates should be viewed as a conservative estimate. Notably, only 29.8% of participants reported B12 supplement use, suggesting inadequate awareness or availability may exacerbate deficiencies in this population.

Although the median vitamin D level of lacto-ovo vegetarians (12.4 ng/mL) was statistically significantly higher than that of vegans (6.1 ng/mL, p = 0.001), the overwhelming majority of participants in both subgroups remained comparable deficient (80.0% vs. 85.7%, p = 0.524). This indicates that while a lacto-ovo diet may offer a modest protective advantage for vitamin D status, it is insufficient to prevent deficiency in most individuals. The key evidence lies in the observation that the 75th percentile values for both subgroups (lacto-ovo: 15.9 ng/mL; vegan: 13.2 ng/mL) were substantially below the 20 ng/mL deficiency threshold, indicating that the vast majority of individuals in both groups were in a deficient state. The critically high overall prevalence (84.6%) points to a regional public health challenge. We hypothesize that environmental factors common in Beijing, such as Beijing's urban environment, limited sunlight exposure due to lifestyle or cultural practices, and lack of widespread food fortification, pose a background risk potentially affecting the entire population. This hypothesis must be tested in future studies with matched omnivores controls to quantify the distinct contributions of diet and environment.

The negative correlation between the diet duration (6–10 and >10 years) and vitamin levels underscores cumulative dietary risks. For B12, marginal stores in new vegetarians may delay deficiency onset, but prolonged avoidance of animal sources depletes the reserves.[22-23] Similarly, chronic lack of fortified foods and insufficient sunlight exposure likely drive the progressive decline in D levels over time.[24] These temporal trends emphasize the need for early and sustained interventions in long-term vegetarians.

Supplement use significantly improved both vitamins' status, echoing global recommendations for vegetarians.25 However, low adherence (29.8% for B12, 18.3% for D) highlights gaps in education or access. Lacto-ovo diets partially mitigated B12 deficiency risks, supporting their adoption as a pragmatic approach for individuals hesitant to use supplements.[22,25] Nevertheless, the persistently high D deficiency across all subgroups suggests that dietary modifications alone are insufficient, necessitating combined strategies (e.g., supplementation, sunlight promotion).[26-28] A noteworthy finding was that 62.5% of the surveyed participants did not use nutritional supplements, yet only 38.5% were classified as vitamin B12 deficient. This may be attributed to several factors, First, as discussed previously, the use of an international B12 threshold (200ng/mL) may have led to an underestimation of deficiency prevalence, suggesting that the reported rate of 38.5% should be considered a conservative figure. Second, individual variations in B12 absorption or baseline stores, as well as the unintentional consumption of B12-containing fermented or fortified foods, may also contribute to this observation. Future studies should incorporate functional biomarkers, such as homocysteine (Hcy) levels, and combine them with more detailed food frequency questionnaires to more accurately assess vitamin B12 status and metabolic function, thereby advancing the understanding of the mechanisms underlying this observation.

This study has limitations. Firstly, its cross-sectional design precludes causal inferences. Secondly, the sample size of this study was relatively small, particularly in the lacto-ovo vegetarian subgroup, which may limit the statistical power of certain subgroup analyses and affect the generalizability of the research findings. Regional sampling in Beijing may not generalize to other Chinese populations with differing cultural or environmental contexts. Additionally, unmeasured confounders (e.g., detailed sunlight exposure, fortified food intake) could influence results. Future longitudinal studies with larger, nationally representative samples are warranted to explore causality and cultural-specific factors.

Conclusions

In conclusion, this study reveals a substantial burden of vitamin B12 and D deficiencies among long-term vegetarians in Beijing. Vitamin B12 status was predominantly determined by the degree of exclusion of animal products form the diet, with vegans at significantly higher risk. Vitamin D deficiency was pervasive and worsened with longer vegetarian duration. Our data highlight that lacto-ovo vegetarianism provided significant protection against B12 deficiency and a modest boost to Vitamin D levels, but was not sufficient to resolve the widespread D deficiency. This finding, combined with the extreme prevalence of D deficiency, points to the need for a dual approach: 1) individualized nutritional guidance for vegetarians, emphasizing B12 supplementation for all vegetarians, with particularly intensive support for vegans, and 2) population-level strategies to address the potential regional vitamin D deficiency crisis, which may include public education on safe sun exposure and advocacy for food fortification policies.

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DISCLOSURE ON THE USE OF AI AND AI-ASSISTED TECHNOLOGIES

The authors declare that no AI-assisted technologies were used for data collection, data analysis, or creation of images/graphical elements in this manuscript.

CONFLICT OF INTEREST AND FUNDING DISCLOSURES

The authors declare no conflict of interest.

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