



Vitamin B-12 Deficiency Is Prevalent in 35- to 64-Year-Old Chinese Adults^{1,2}

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Abstract

Low vitamin B-12 status alone, or in combination with low folate status, is related to neural tube defects (NTD) and hyperhomocysteinemia, a risk factor for atherosclerotic disease, but little is known about vitamin B-12 status in Chinese adults. In a cross-sectional study, we measured plasma vitamin B-12 in 2407 apparently healthy Chinese men and women, 35–64 y old, living in the south and the north of China. Plasma vitamin B-12 concentrations were lower among the northerners than the southerners (geometric means, 209 vs. 309 pmol/L, $P < 0.001$). Controlling for gender, age, season (spring and fall), and area (urban and rural) had little impact on the difference. We estimated that 11% of the southerners and 39% of the northerners had plasma vitamin B-12 concentrations <185 pmol/L, a level to define vitamin B-12 deficiency. Within each region, men had lower plasma vitamin B-12 concentrations and higher prevalence of vitamin B-12 deficiency than women (279 vs. 333 pmol/L and 15 vs. 8% in the south; 192 vs. 233 pmol/L and 47 vs. 34% in the north; $P < 0.001$ for all the differences). Low intakes of animal-based food, especially fish and dairy products, were significantly associated with vitamin B-12 deficiency. In the north, 59% of the participants were deficient in either folate (<6.8 nmol/L) or vitamin B-12, and 17% had deficiency in both. The corresponding rates were 16 and 1% in the southerners. To our knowledge, our findings provide the first evidence that vitamin B-12 deficiency is common in 35- to 64-y-old Chinese adults, especially in the north. Further studies are needed to evaluate the health effects and possible intervention strategies in areas where B-12 vitamin deficiency is common. *J. Nutr.* 137: 1278–1285, 2007.

Introduction

Vitamin B-12 deficiency is associated with megaloblastic anemia and neurologic manifestations, including sensory and motor disturbances and cognitive impairment (1,2). There is also evidence that low vitamin B-12 status alone, or in combination with low folate status, is related to neural tube defects (NTD) and hyperhomocysteinemia, which is an independent risk factor for atherosclerotic disease (3–6). Previous investigations conducted in the developed countries suggested that low vitamin B-12 concentrations were common only in elderly adults (7–12). Because the ability to absorb food-bound dietary vitamin B-12 is significantly reduced after age 50 y, those investigations were predominantly conducted in the populations older than 60 y. In the United States, dietary vitamin B-12 is thought to be adequate in the general population, as the mean intake was estimated to exceed the Recommended Dietary Allowance of 2.4 $\mu\text{g}/\text{d}$ (13). However, recent data from the Framingham Offspring Study have shown that vitamin B-12 deficiency (vitamin B-12 concen-

tration <185 pmol/L) is common in the US population and it does not differ significantly by age (16% among 26–64 y olds, and 17% among 65–83 y olds) (14).

Animal-based food products and vitamin B-12 supplements are the major dietary source of vitamin B-12, whereas vegetables, which are a good source of folate, are devoid of vitamin B-12. Recent studies in developing countries revealed that vitamin B-12 deficiency is common even in younger populations. In India, 47% of men and women 27–55 y old who adhered to a vegetarian diet had serum vitamin B-12 concentrations <150 pmol/L (15). In Guatemala, 61% of predominantly breastfed infants and 50% of their lactating mothers were deficient (<148 pmol/L) (16). In rural Kenya, 70–80% of school age children were either severely (<125 pmol/L) or moderately (125–221 pmol/L) deficient (17). These data suggest that vitamin B-12 deficiency could present at a very young age, possibly due to low vitamin B-12 concentrations in maternal breast milk, and also be common in older children and adults, due to low intake of animal products, which are the major food source of vitamin B-12 for children and adults.

Three cutoff points (148 pmol/L, 185 pmol/L, and 258 pmol/L) have been commonly used in the literature for plasma vitamin B-12 deficiency (7–9,11,18–21). Although 148 pmol/L is most commonly used clinically for low vitamin B-12 status, there is evidence that the prevalence of vitamin B-12 deficiency is underestimated when using <148 pmol/L as the cutoff point, because

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many individuals above the level exhibit clinical symptoms, such as neuropsychiatric disorders (2,22). In addition, methylmalonic acid, a metabolite more sensitive and highly specific to vitamin B-12 deficiency, was commonly elevated among individuals with vitamin B-12 concentrations ≤ 185 pmol/L (11). It is therefore suggested that in the absence of additional metabolic or clinical indicators, a concentration < 185 pmol/L might be the best cutoff to define vitamin B-12 deficiency (11,12,19).

Our previous study indicated that a large proportion of Chinese adults had a low folate status (23). However, there are no data on vitamin B-12 status among healthy Chinese adult men and women. In this study we report, to our knowledge for the first time, the distribution of plasma vitamin B-12 concentrations and the estimated prevalence of deficiency (based on the 185 pmol/L cutoff) in 2407 35- to 64-y-old Chinese adults, who represent the Chinese population living in the north near Beijing and the south near Shanghai. We specifically evaluated the vitamin B-12 status by region (south vs. north), gender, area of residence (urban vs. rural), and season (spring vs. fall) and explored the associations of lifestyle and dietary factors with vitamin B-12 status.

Subjects and Methods

Study population. This study was conducted in March and September, 2001, in 2 rural counties, and 2 cities in China as described in detail previously (23). The subjects were selected using multistage, stratified cluster sampling by region (north and south), area (urban and rural), gender (male and female), and age (35–44, 45–54, and 55–64 y). We contacted 3840 age-eligible people, among these, 2545 (66%) agreed to participate and were recruited to attain enrollment of 50 men and 50 women in each of the 3 age groups, in each of the 2 areas, in each of the 2 seasons, and in each of the 2 regions. Subjects who were pregnant or had a severe illness (renal, heart, liver disease, or cancer) were excluded.

Data collection. We collected the information on birth date, personal history, diet, and lifestyle by face-to-face interviews. Body height and weight were measured by the health workers. Current smoking status was recorded in 3 categories: nonsmoker, smoking 1–20 cigarettes/d, or smoking ≥ 21 cigarettes/d. Alcohol consumption (including liquor, beer, and wine) was classified in 3 categories according to the frequency of alcohol consumption per day: none, < 1 drink/d, or ≥ 1 drinks/d. Multivitamin (the content of vitamin B-12 varied from 0.5 to 9.0 $\mu\text{g}/\text{pill}$, depending on the brand of supplements) use in the past 3 mo was categorized as user and nonuser. Dietary data were collected as part of the study, using a semiquantitative FFQ, which had been validated (24). Animal-based food intake was divided into dairy product (g/d milk, yogurt), egg, animal meat (g/d poultry, beef, lamb, and pork), and fish (g/d sea foods, river fish) intakes. The subjects were classified into 3 groups by dairy product intake in g/d (never consume and 2 groups of similar size of those who consumed dairy products in the past 3 mo). Egg, animal meat, and fish intakes were categorized in quintiles by g/d. BMI was calculated as weight (kg)/height (m)². Due to concerns about literacy, especially in the rural areas, all invited participants were asked to provide oral informed consent after hearing the informed consent script read by the investigators. The study was approved by the Institutional Review Boards of Peking University Health Science Center.

Biochemistry analyses. Fasting blood samples were drawn and collected in K₃EDTA-containing Vacutainer tubes (Becton Dickinson), and centrifuged at $800 \times g$ for 15 min at 4°C to separate plasma. Plasma vitamin B-12 was measured in duplicate samples using the Quantaphase II radioassay (Bio-Rad Laboratories). In the laboratory at the Institute of Reproductive and Child Health, Peking University, the intra- and inter-assay CV% were $< 7\%$ across the full range of vitamin B-12 concentrations.

Statistical analyses. Because the distribution of plasma vitamin B-12 was positively skewed, natural logarithmic transformations were used to

normalize the distribution, and the geometric means, as well as the 95% CI, were determined. Student's *t* tests were used for comparisons between groups when using continuous variables, if the variables were normally distributed. The multivariate ANOVA was conducted to compare means with Bonferroni corrections, controlling for differences in region, age, gender, area, and season. In addition, polynomial contrasts were used to test for differences in vitamin B-12 concentrations across the 3 age groups. The significance of categorical variables was assessed by the chi-square test. The Spearman correlation coefficients between age and vitamin B-12 in men and women, and between plasma folate and vitamin B-12, were examined. We defined folate deficiency as plasma folate < 6.8 nmol/L (23) and vitamin B-12 deficiency as vitamin B-12 < 185 pmol/L (11,12,19). For comparison, we also presented the other 2 cutoff levels in Table 1. We used multiple logistic regression to examine the OR of plasma vitamin B-12 deficiency in association with BMI, alcohol use, cigarette smoking status, multivitamin use, and intakes of dairy product, egg, meat, and fish, controlling for region, area, age, gender, and season. The data were analyzed with SPSS 11.0. All *P*-values were 2-sided at the α -level 0.05.

Results

Of the 2545 participants, 2407 (95%) had available data for analysis; among these 1191 were from the south and 1216 from the north. Compared with the southerners, the northerners had higher BMI, more current cigarette smoking, less multivitamin use, less ever alcohol use (but among drinkers, more who consumed > 1 drinks/d), and more egg consumption but less intake of animal meat and fish. Dairy product intakes were similar in the southerners and the northerners (Table 1).

The curves of plasma vitamin B-12 distribution for both regions were skewed positively (Fig. 1), with a significantly lower median plasma vitamin B-12 concentration in the north than the south (Table 1). Using < 185 pmol/L as the cutoff of vitamin B-12 deficiency, 11% of the southerners and 39% of the northerners were deficient; using < 148 pmol/L as a cutoff, 4% of the southerners and 21% of the northerners were deficient; and when using < 258 pmol/L as the cutoff, 30% of the southerners and 66% of the northerners were vitamin B-12 deficient. In general, the prevalence of vitamin B-12 deficiency was 3- to 5-fold higher in the north than in the south, depending on the cutoff used (Table 1).

In the south, the overall vitamin B-12 concentrations were similar in the urban and rural areas after adjustment for gender, age, and season; however, in the north, those living in the urban area had lower vitamin B-12 (geometric mean = 196 pmol/L) than those in the rural area (230 pmol/L; $P < 0.001$) (Table 2). Within each area, the geometric mean concentrations were significantly lower in the spring than in the fall, except for the north rural area. The mean concentration of vitamin B-12 for those living in the south remained significantly higher than those living in the north after adjusting for gender, age, season, and area (Table 2).

In this study population of 35- to 64 y-old men and women, ages were not correlated with plasma vitamin B-12 concentrations (Spearman correlation coefficient, $r = -0.03$ in men and $r = -0.01$ in women). In general, men had lower vitamin B-12 concentrations than women after controlling for region, area, season, and age (233 vs. 277 pmol/L; $P < 0.001$) (Table 3). The results persisted when stratified by region, by area (data not shown), and after further controlling for other lifestyle and dietary factors (data not shown).

Next, we calculated the adjusted geometric means of vitamin B-12 (indicating the average vitamin B-12 status) and the odds of vitamin B-12 deficiency (indicating the more extreme deficient state) by BMI, multivitamin use, alcohol consumption, cigarette smoking status (Table 4), and intakes of major animal-based

TABLE 1 Selected characteristics of the Chinese participants in the study¹

Characteristic	South	North	P-value
<i>n</i>	1191	1216	
Age, <i>y</i>	49.4 ± 8.8	49.2 ± 8.9	0.547
BMI, <i>kg/m</i> ²	24 ± 3	25 ± 3	<0.001
	%		
Multivitamin use	4	2	0.001
Smoking, <i>cigarettes/d</i>			
None	74	66	<0.001
1–20	22	26	
≥21	4	8	
Alcohol consumption, ² <i>drinks/d</i>			
None	59	62	<0.001
<1	33	23	
≥1	8	15	
Animal-based food intake			
Dairy product intake, ³ <i>g/d</i>			
None	56	53	0.314
<114	22	24	
≥114	22	23	
Egg intake, <i>g/d</i>			
<14	22	18	<0.001
14–27.9	23	17	
28–49.9	22	18	
50–62.9	17	23	
≥63	16	24	
Animal meat intake, ⁴ <i>g/d</i>			
<33	9	31	<0.001
33–62.9	16	24	
63–89.9	22	18	
90–137.9	24	17	
≥138	30	11	
Fish intake, ⁵ <i>g/d</i>			
<9	7	32	<0.001
9–16.9	13	28	
17–26.9	20	20	
27–46.9	29	11	
≥47	31	9	
Plasma vitamin B-12, <i>pmol/L</i>	309 (239–403)	209 (155–289)	<0.001
Vitamin B-12 deficient, ⁶ <i>pmol/L</i>			
<148	4	21	<0.001
<185	11	39	<0.001
<258	30	66	<0.001

¹ Values are means ± SD, medians (25th–75th percentile) or percentages.
² Alcohol drinking (including liquor, beer, and wine) is classified according to the frequency of alcohol consumption per day.
³ Includes milk and yogurt.
⁴ Includes poultry, beef, lamb, and pork.
⁵ Includes sea foods and river fish.
⁶ Defined as plasma vitamin B-12 concentrations <148, or <185 or 258 pmol/L.

foods (Table 5). BMI and cigarette smoking status (among men only, because very few women smoked in this population) were not related to vitamin B-12 concentrations (Table 4). Individuals who consumed ≥1 drinks of alcohol per day had significantly higher plasma vitamin B-12 concentration than never drinkers or those who consumed <1 drink/d. As expected, multivitamin users had significantly higher vitamin B-12 concentrations than never users. We further assessed these lifestyle factors in association with vitamin B-12 deficiency (<185 pmol/L), using logistic regression models (Table 4). BMI was positively associated with higher odds of vitamin B-12 deficiency in the univariate analysis,

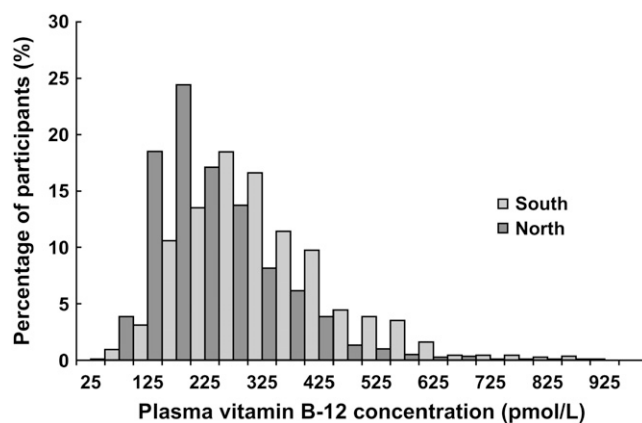


FIGURE 1 Distribution of plasma vitamin B-12 concentrations among middle-aged Chinese men and women aged 35 to 64 y in the south (*n* = 1191) and in the north (*n* = 1216) of China.

but the association was attenuated in the multivariate model controlling for region, area, gender, season, and age. Alcohol consumption was positively associated with the odds of vitamin B-12 deficiency in the univariate model. However, the direction of the association was altered in the multivariate model; individuals who consumed ≥1 drinks/d had lower odds of vitamin B-12 deficiency, which is more consistent with the geometric mean observations (Table 4). These findings suggest that region, area, gender, season, and age significantly confounded the associations of BMI and alcohol intake with vitamin B-12 deficiency. Multivitamin use and cigarette smoking were not associated with the odds of vitamin B-12 deficiency in either the univariate or the multivariate models. These associations remained unchanged after further controlling for animal-based food intakes (data not shown).

Intakes of dairy products, egg, animal meat, and fish were each positively related to vitamin B-12 concentrations with significant trends, suggesting that all of these foods are contributors to vitamin B-12 intake (Table 5). In the univariate logistic regression analysis, intakes of dairy (ever vs. never), animal meat (≥33 g/d vs. <33 g/d), and fish (≥9 g/d vs. <9 g/d), but not eggs, was each inversely associated with the odds of vitamin B-12 deficiency. In the multivariate analysis controlling for age, gender, season, area, and region, intakes of dairy products and higher fish consumption remained strongly protective for vitamin B-12 deficiency ($P_{\text{trend}} < 0.001$ for both), whereas higher animal meat intake was no longer associated with vitamin B-12 deficiency, suggesting that dairy products and fish are the major contributors to vitamin B-12 adequacy (Table 5). Additional control for BMI, multivitamin use, alcohol drinking, and cigarette smoking status had little influence on these findings (data not shown).

In our study population, plasma vitamin B-12 concentrations were positively correlated with folate concentrations (Spearman's correlation coefficient, $r = 0.34$; $P < 0.001$). However, among women in the south who had the highest concentrations and the lowest deficiency rates of both, the 2 B vitamins were no longer correlated. The patterns of geographical and gender differences in vitamin B-12 status were similar to that of folate (23) (Table 3). The prevalence of folate or vitamin B-12 deficiency alone or together was much higher in the north than that in the south; 59% of participants in the north and 16% in the south were deficient in at least 1 of the 2 B vitamins. In the north, 17% had both folate and vitamin B-12 depletion, which was much higher compared with 1% in the south (Fig. 2).

TABLE 2 Plasma vitamin B-12 concentrations in Chinese adults by region, area, and season¹

Area	Season	South			North		
		<i>n</i>	Vitamin B-12, pmol/L	Vitamin B-12 deficient, ² %	<i>n</i>	Vitamin B-12, pmol/L	Vitamin B-12 deficient, ² %
Urban	Overall ³	609	308 (299–317) [‡]	8	605	196 (189–204) [†]	46
	Spring	305	294 (282–306)**	11	291	184 (176–193)**	51
	Fall	304	323 (311–336)	5	314	208 (197–220)	41
Rural	Overall ³	582	304 (293–315) [‡]	15	611	230 (222–238)	32
	Spring	297	255 (244–267)***	22	307	235 (224–247)	29
	Fall	285	365 (347–383)	7	304	224 (213–236)	34
Overall	Overall ⁴	1191	306 (299–313) [‡]	11	1216	212 (207–218)	39
	Spring	602	274 (265–283)***	16	598	209 (202–216)	40
	Fall	589	343 (332–354)	6	618	216 (208–224)	38

¹ Values are geometric means (95% CI) or percentages. *Different from fall: *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$; [†]different from the rural area, $P < 0.001$; [‡]different from the north, $P < 0.001$.

² Defined as plasma vitamin B-12 concentrations <185 pmol/L.

³ Adjusted for gender, age, and season.

⁴ Adjusted for gender, age, season, and area.

Discussion

Our study was conducted in 2 rural counties and 2 cities, in the north around Beijing and in the south around Shanghai, that are currently part of the US-China Collaborative Project on Neural Tube Defects Prevention in China (25). Although our study subjects were not a random sample of all Chinese, they have typical lifestyles representative of the population in the north around Beijing and in the south around Shanghai, which together cover a population of 21 million. And 98% of the study participants are of Han nationality, which is the largest proportion of Chinese population, and most are omnivores. To our knowledge, this is the first study to report data on vitamin B-12 status in a large sample of apparently healthy middle-aged Chinese adult men and women. The most notable finding in this study was that, in addition to our previous report of high prevalence of folate deficiency, vitamin B-12 deficiency was also common, especially in the north of China. In this study, ~25% of the Chinese adults aged 35 to 64 y had vitamin B-12 concentrations <185 pmol/L and 48% had vitamin B-12 concentrations <258 pmol/L. Using 148 pmol/L as the cutoff, in the south of China, the prevalence of vitamin B-12 deficiency (4%) was lower than that of the 26- to 83-y-old subjects in the Framingham Offspring Study (9%, with little

difference between age groups) (14). The prevalence of low vitamin B-12 concentrations in the south of China were consistently lower than the results from the Framingham Offspring Study, when we defined vitamin B-12 deficiency as <185 pmol/L (11 vs. 17%) or 258 pmol/L (30 vs. 39%) (14). However, the prevalence of vitamin B-12 deficiency in the north was much higher than that of Americans and 3- to 5-fold higher than the southerners, depending on the cutoff levels. Because individuals with vitamin B-12 concentrations as high as 258 pmol/L have been shown to be at risk for neurologic signs and symptoms of vitamin B-12 deficiency and for hyperhomocysteinemia (3,11,22), these prevalence rates, even in the south of China, raise concerns about the need for improvement of vitamin B-12 status in the general Chinese population.

Published data have demonstrated that older age is a major determinant of vitamin B-12 deficiency, because dietary vitamin B-12 absorption was significantly lower in healthy adults aged 55–75 y than in young adults (19–35 y old), with a further reduction in those >75 y (26). Beside reduced intakes of animal products, elderly people have a high prevalence of atrophic gastritis, which results in a low acid-pepsin secretion by the gastric mucosa, leading to a reduced release of free vitamin B-12

TABLE 3 Plasma vitamin B-12 and folate concentrations and their correlations by gender and region among Chinese adults¹

	<i>n</i>	Vitamin B-12		Folate		Correlation between vitamin B-12 and folate	
		Geometric mean (95%CI)	Deficient ²	Geometric mean (95%CI)	Deficient ³	<i>r</i> ⁴	<i>P</i> -value
Men							
Overall ⁵	1162	233 (227–238)*	31	9.7 (9.4–10.0)	31	0.336	<0.001
South	573	279 (269–289)*	15	14.6 (13.9–15.3)	10	0.116	0.006
North	589	192 (184–201)*	47	7.0 (6.6–7.3)	52	0.168	<0.001
Women							
Overall ⁵	1236	277 (270–283)	20	14.3 (13.9–14.7)	12	0.296	<0.001
South	613	333 (322–346)	8	19.6 (18.8–20.6)	2	–0.029	0.469
North	623	233 (223–243)	34	9.9 (9.4–10.3)	23	0.116	0.004

¹ Values are geometric means (95% CI), percentages, or Spearman's correlation coefficients. *Different from women controlling for age, season, and area (urban and rural); $P < 0.001$.

² Defined as plasma vitamin B-12 <185 pmol/L.

³ Defined as plasma folate <6.8 nmol/L.

⁴ Spearman's correlation coefficient between vitamin B-12 and folate.

⁵ Controlling for region (south and north), area (urban and rural), age, and season.

TABLE 4 Plasma vitamin B-12 concentrations and OR of vitamin B-12 deficiency by BMI, multivitamin use, alcohol consumption, and cigarette smoking among Chinese adults¹

Variables	Adjusted geometric mean of vitamin B-12 ² (95% CI), pmol/L	Vitamin B-12 deficient ³			
		Yes, <i>n</i>	No, <i>n</i>	Crude OR (95% CI)	Adjusted OR ² (95% CI)
BMI, kg/m ²					
<21	261 (250–273)	69	276	0.8 (0.6–1.1)	1.0 (0.7–1.4)
21–22.9	255 (246–266)	98	349	0.9 (0.7–1.2)	0.9 (0.7–1.3)
23–24.9	253 (244–262)	128	416	1 (ref)	1 (ref)
25–26.9	251 (242–260)	138	345	1.3 (1.0–1.7)	1.2 (0.9–1.6)
≥27	254 (245–263)	172	416	1.3 (1.0–1.8)	1.0 (0.8–1.4)
<i>P</i> for trend	0.720			0.003	0.821
Alcohol consumption, drinks/d					
Never	251 (245–257)	342	1097	1 (ref)	1 (ref)
<1	250 (242–259)	181	487	1.2 (1.0–1.5)	1.1 (0.8–1.4)
≥1	281 (266–296)	79	193	1.3 (1.0–1.8)	0.7 (0.5–1.0)
<i>P</i> for trend	0.001			0.085	0.044
Multivitamin use					
Nonuser	254 (249–258)	593	1749	1 (ref)	1 (ref)
User	287 (259–318)	12	53	0.7 (0.4–1.3)	0.9 (0.5–1.8)
<i>P</i> -value	0.020			0.212	0.790
Cigarette smoking (men only), cigarettes/d					
Never	232 (224–241)	153	340	1 (ref)	1 (ref)
1–20	234 (226–243)	150	381	0.9 (0.7–1.1)	0.9 (0.7–1.2)
≥21	228 (213–244)	53	87	1.4 (0.9–2.0)	1.1 (0.7–1.7)
<i>P</i> for trend	0.786			0.088	0.439

¹ Values are geometric means (95% CI) or OR (95% CI).

² Adjusted for region, area (urban and rural), gender, age, and season.

³ Defined as plasma vitamin B-12 <185 pmol/L.

from food proteins. Framingham elders 67–96 y old (median age 77 y) were twice as likely as younger adults (22–63 y old, median age 30 y) to have serum vitamin B-12 concentrations <258 pmol/L (11). In the Framingham Offspring Study (26–83 y old), there was a significant linear trend toward lower concentration with increasing age, but the categorical tests for plasma vitamin B-12 concentration differences across age groups were not significant (14). In our study population (35–64 y old), stratification by decade showed no age trend in plasma vitamin B-12 concentrations, nor did the concentrations correlate with age, which may reflect the lack of older individuals (>65 y) in our study. Our finding suggests that even among middle-aged Chinese, especially in the north, low vitamin B-12 status is very common.

In our study, men had higher prevalence of vitamin B-12 deficiency than women, consistent with previous reports among Americans, Canadians, and Chileans (9,12,27–29). Indian adults showed no significant gender difference in vitamin B-12 concentrations, but methylmalonic acid was higher in men than women (15). However, in the Framingham Offspring Study population, aged 26–83 y old, there was no statistically significant gender difference (14). The reasons for the gender difference in plasma vitamin B-12 concentration are unclear. In this study, the gender difference was attenuated but still remained highly significant after controlling for region, area, season, age, and the intakes of the major food sources of vitamin B-12, including animal meat, fish, eggs, and dairy products, suggesting that either these factors could not fully explain the gender difference, these dietary intakes were not perfectly measured, or both.

In searching for possible explanations for the significant geographical difference in vitamin B-12 status, we found that intakes of alcohol, multivitamins, and all animal-based foods assessed, including eggs, animal meat, dairy products, and fish, were significantly and positively related to the plasma vitamin B-12

concentrations with a linear relation, suggesting that all of these factors might be contributors of dietary vitamin B-12 intake. Unlike multivitamins and animal-based foods, the finding for alcohol consumption is somewhat unexpected. Because beer, some types of wine, and liquor consumed in China contain vitamin B-12 (0.3 or 0.4 μg/100 g of beverage) (30), intakes of these might contribute to the increased plasma vitamin B-12 concentrations. However, we did not ascertain the individual types of alcohol beverages in our study; thus we cannot assess their vitamin B-12 content. Whether alcoholic beverage consumption truly contributes to vitamin B-12 status deserves further investigation in other studies.

When evaluating which factors related to the odds of vitamin B-12 deficiency, we found that intakes of alcohol, dairy products, and fish were significantly associated with lower probability of vitamin B-12 deficiency, suggesting that they might be the major determinants for vitamin B-12 deficiency in this study population. Although intakes of meat and eggs were associated with blood concentrations of vitamin B-12, they did not significantly predict vitamin B-12 deficiency, probably because they were less strong determinants of vitamin B-12 status and the statistical power was reduced when the concentrations were dichotomized into deficient or not deficient.

The southern subjects had higher intakes of fish but the northern subjects had higher intakes of eggs and animal meat. Intakes of dairy products were similar in both regions. However, the significant difference of plasma vitamin B-12 concentration between the north and the south remained, although it was attenuated after further controlling for the major animal-based food intakes (data not shown). Thus the variations of animal-based food intakes may not fully explain the variation of vitamin B-12 concentrations by region, as well as by season or by urbanicity. In this study, we found that plasma vitamin B-12 concentrations

TABLE 5 Plasma vitamin B-12 concentrations and OR of vitamin B-12 deficient by milk, egg, animal meat, and fish intakes among Chinese adults¹

Variables	Adjusted geometric mean of vitamin B-12 ² (95% CI), pmol/L	Vitamin B-12 deficient ³			
		Yes, n	No, n	Crude OR (95%CI)	Adjusted OR ² (95%CI)
Dairy product, g/d					
Never	246 (240–252)	353	940	1 (ref)	1 (ref)
<114	255 (246–264)	140	413	0.9 (0.7–1.1)	0.7 (0.6–0.9)*
≥114	275 (264–282)	109	424	0.7 (0.5–0.9)**	0.5 (0.4–0.7)***
P for trend	<0.001			0.010	<0.001
Egg, g/d					
<14	243 (234–252)	110	365	1 (ref)	1 (ref)
14–27.9	247 (238–256)	124	353	1.2 (0.9–1.6)	1.2 (0.8–1.6)
28–49.9	252 (243–262)	113	362	1.0 (0.8–1.4)	1.0 (0.7–1.3)
50–62.9	260 (250–270)	136	340	1.3 (1.0–1.8)	1.0 (0.7–1.4)
≥63	270 (260–280)	119	357	1.1 (0.8–1.5)	0.8 (0.6–1.1)
P for trend	0.001			0.336	0.196
Animal meat, g/d					
<33	240 (231–252)	149	326	1 (ref)	1 (ref)
33–62.9	253 (244–263)	141	335	0.9 (0.7–1.2)	1.1 (0.8–1.5)
63–89.9	256 (247–266)	115	361	0.7 (0.5–0.9)*	1.0 (0.7–1.3)
90–137.9	266 (256–276)	93	383	0.5 (0.4–0.7)***	0.7 (0.5–1.0)
≥138	256 (246–266)	104	372	0.6 (0.5–0.8)**	1.0 (0.7–1.4)
P for trend	0.011			<0.001	0.163
Fish, g/d					
<9	218 (210–227)	202	260	1 (ref)	1 (ref)
9–16.9	239 (230–248)	154	335	0.6 (0.5–0.8)***	0.7 (0.5–0.9)*
17–26.9	254 (245–263)	108	367	0.4 (0.3–0.5)***	0.5 (0.4–0.7)***
27–46.9	280 (270–291)	70	407	0.2 (0.2–0.3)***	0.4 (0.3–0.5)***
≥47	284 (274–295)	68	408	0.2 (0.2–0.3)***	0.4 (0.3–0.5)***
P for trend	<0.001			<0.001	<0.001

¹ Values are geometric means (95% CI) or OR (95% CI); ****P* < 0.001, ***P* < 0.01, and **P* < 0.05.

² Adjusted for region, area (urban and rural), gender, age, and season.

³ Defined as plasma vitamin B-12 <185 pmol/L.

were generally lower in spring than in fall (Table 2). However, our preliminary data from the semiquantitative FFQ showed that intakes of animal-based food were significantly higher in spring than in fall (which is consistent with the Chinese habit of eating more meat during the spring festival). The intakes in the previous season may affect the seasonal changes in blood

concentrations, since the turnover of vitamin B-12 is slow; that is, the lower concentrations in the spring may partially reflect lower intakes in the winter, and the higher concentrations in the fall may reflect higher intakes in the summer. Also, our dietary data suggested that, in both the north and the south, intakes of animal-based foods were higher in urban than in rural areas, which is somewhat inconsistent with the urban-rural variations of plasma vitamin B-12 concentrations (no difference between urban and rural areas in the south, but lower in the urban area than in the rural area in the north). The reasons for the seasonal and urban-rural differences in plasma vitamin B-12 concentration are unclear. There may be contamination of vegetables with soil and related microorganisms, with vitamin B-12 contributing some to the intakes in summer or in rural areas. Severe atrophic gastritis (31) and malabsorption of vitamin B-12, which could be caused by bacterial overgrowth (32) and *Helicobacter pylori* infection (33,34), are also important risk factors of vitamin B-12 deficiency. However, these were not investigated in this study.

In the north, we observed a significant positive correlation between plasma folate and vitamin B-12 concentrations, similar to that in Guatemalan women (16). However, the correlation was not observed in the southern women, who had the highest status of both folate and vitamin B-12. Deficiency of folate and/or vitamin B-12 were both higher in the north, indicating that low folate and/or vitamin B-12 status tend to occur together, which may reflect the overall nutritional status of the population, having

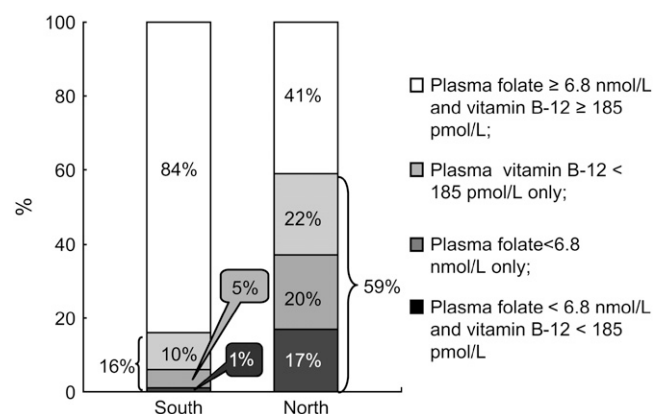


FIGURE 2 Percent deficiency of folate, vitamin B-12, or both in middle-aged Chinese men and women aged 35 to 64 y in the south (*n* = 1191) and in the north (*n* = 1216) of China. Folate deficiency was defined as plasma folate concentration <6.8 nmol/L; Vitamin B-12 deficiency was defined as plasma vitamin B-12 <185 pmol/L.

low intakes of both vegetables and animal-based foods, which is different from that in vegetarians (15) or special groups who adhere to a certain diets for cultural and religious reasons.

Our previous study indicated that low folate status might be a general health problem in the Chinese population (23,25). The present results indicate that vitamin B-12 deficiency is also common in Chinese adults. Our data showed that ~8% of women of reproductive age (35–44 y old) living in the south, where the incidence of NTD is low (~1/1000 births), and 37% in the north, where the incidence of NTD is high (5–6/1000 births) (25), had vitamin B-12 concentrations <185 pmol/L. Low vitamin B-12 status is a risk factor, independent of folate status, for NTD and vascular diseases, possibly by producing hyperhomocysteinemia (3–6). Taken together, the present findings and our previous studies suggest that an intervention study including both folic acid and vitamin B-12, rather than folic acid alone, is likely to be much more effective at reducing risk of NTD and hyperhomocysteinemia in the Chinese population, and it is more important in north of China. In addition, 21% of the northerners had plasma vitamin B-12 concentrations <148 pmol/L, which is a cut-off previously associated with megaloblastic anemia, suggesting, the need to evaluate the prevalence of megaloblastic anemia in the north. Also, the high prevalence of vitamin B-12 deficiency in the north raises the concern about “masking” the diagnosis of a vitamin B-12 deficiency or megaloblastic anemia if folic acid supplements are given or if folic acid fortification is implemented, in these areas.

One limitation of our study was the cross-sectional design, with samples selected from the north around Beijing and the south around Shanghai. These participants represent those living in the economically developed areas in the south and north of China. We speculate that worse conditions for folate and vitamin B-12 deficiency prevail in the economically underdeveloped areas in the country. Further investigation in these areas is needed. These data provide strong evidence for high prevalence of vitamin B-12 insufficiency in China, indicating the need for both of folate and vitamin B-12 intervention strategies.

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