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Association between serum vitamin B_6 concentration and risk of osteoporosis in the middle-aged and older people in China: a cross-sectional study

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ABSTRACT

Objective To determine the relationship between serum vitamin B_6 (Vit B_6) concentration and the status of bone mineral density and identify the relationship between serum Vit B6 and bone metabolism parameters in middle-aged and older people in China.

Design The present study was a cross-sectional study within the framework of an ongoing prospective population-based cohort study.

Setting and participants A total of 1829 residents (men ≥50 years and women ≥45 years) from two subdistricts were recruited from July 2015 to February 2016 in Shanghai, China.

Measures Recruited residents were grouped (control, osteopenia and osteoporosis) according to their lumbar spine bone mineral density, measured through dual-energy X-ray absorptiometry. Serum Vit B_6 concentrations, bone turnover marker concentrations and calcium and phosphorus metabolism parameters were assessed.

Results No significant linear trend between serum Vit B_6 concentrations and lumbar bone mass was observed in the men. In the women, the average osteoporosis risk was 61% higher at serum Vit B_6 concentrations of <19.2 µg/L than at those of >26.9 µg/L (OR 1.61, 95% CI 1.00 to 2.58). However, there was no significance after controlling of serum 25-hydroxy-vitamin D concentration and parathyroid hormone concentration, respectively. In the osteoporotic women, the serum Vit B_6 concentration was significantly negatively correlated to concentrations of bone turnover marker including N-terminal propeptide of type I collagen, β-C-terminal telopeptide of type I collagen and osteocalcin. It was also positively related to the serum 25-hydroxy-vitamin D concentration and inversely related to the serum parathyroid hormone concentration.

Conclusions A relatively low serum Vit B_6 concentration, even in the normal range, may be a risk factor for osteoporosis in postmenopausal women, which is dependent on serum 25-hydroxy-vitamin D concentration and parathyroid hormone concentration.

Trial registration number NCT02958020; Post-results.

Strengths and limitations of this study

- This was the first study on the relationship between serum vitamin B_6 (Vit B_6) concentration and the status of bone mineral density (BMD) and bone turnover in the Asian population.
- This was a cross-section study with limited strength.
- No participants in this study were diagnosed as having Vit B_6 deficiency; therefore, the risk of Vit B_6 deficiency for osteoporosis could not be analysed.
- For the sake of economic costs and limited time for on-site investigations, only lumbar spine BMD was measured in this study, which might lead to a certain degree of bias.
- Other factors, which were not recorded in this study, may also have influences on serum vitamin concentrations and bone metabolism, such as medications affecting gastrointestinal absorption function, dietary intakes and physical activities.

INTRODUCTION

Osteoporosis, a systemic skeletal disease, increases bone fragility and the risk of fracture by reducing bone mass and causing microarchitectural deterioration.[1] It is a growing health concern worldwide and the leading cause of fractures. Several risk factors for osteoporosis, such as age, obesity, smoking and drinking, have been identified.[2] Recently, studies have demonstrated that low dietary intake of vitamin B_6 (Vit B_6) or low blood Vit B_6 concentrations might be a novel and potentially modifiable risk factor for osteoporosis.[3 4]

The vitamin B family includes thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folic acid or folate (B_9) and cobalamin (B_{12}).[5] Low levels of vitamin B_{12} and folate have been proved to be associated with low bone mineral density (BMD) and a higher risk of fractures in the elderly.[6] As a member of vitamin B family, Vit B_6 is a water-soluble vitamin that is naturally present in many foods. Pyridoxine, pyridoxal, pyridoxamine and their

phosphorylated derivatives are collectively/generically known as Vit B_6. Vit B_6 acts as a coenzyme in the metabolism of amino acids, carbohydrates, neurotransmitters and lipids, and it performs various functions in the body.[7]

An in vitro study of B-vitamins on the activity of human osteoclasts revealed that low concentrations of Vit B_6 promoted osteoclast activity, thus indicating the role of low Vit B_6 concentrations in bone degradation.[8] Vit B_6 deficiency was reported to reduce new bone formation and cause an imbalance in the coupling between osteoblasts and osteoclasts in fracture healing in rats.[9] In addition, Vit B_6 is an essential coenzyme for lysyl oxidase in collagen crosslinking.[10] Animal studies have shown that Vit B_6 deficiency might impair crosslink formation, which subsequently adversely affects the mechanical properties of bones and contributes to bone fragility.[11] These preclinical studies have suggested crucial roles of Vit B_6 in maintaining bone health.

Several observational studies have suggested that an inadequate dietary intake of Vit B_6 or low plasma Vit B_6 concentrations contribute to high bone loss, low BMD and a relatively high risk of osteoporotic fracture in older people,[3 4 12] whereas other studies have concluded that the incidence of osteoporosis and circulating Vit B_6 concentration are not significantly associated.[13–15] Notably, almost all these studies were conducted in Western populations.[3 4 13–15] Because the dietary and lifestyle characteristics of Asian populations differ from those of Western populations,[14] no evidence is currently available on the association between serum Vit B_6 concentrations and osteoporosis in Asian populations, particularly in the Chinese population.

Middle-aged and older people from Shanghai, China, with normal BMD, osteopenia and osteoporosis were recruited as participants for the present study. Serum Vit B_6 concentrations, bone turnover marker concentrations and parameters related to calcium and phosphorus metabolism were measured. The relationship between the serum Vit B_6 concentration and BMD and that between serum Vit B_6 concentration and bone turnover marker concentrations were analysed.

METHODS

Study design

The present study was a cross-sectional study within the framework of an ongoing prospective population-based cohort study (clinical trial registration number NCT02958020). Residents from two subdistricts in Shanghai, China (Lujiazui subdistrict and Longhua subdistrict) participated in this study from July 2015 to February 2016. All the participants provided signed written informed consent prior to participation.

Inclusion and exclusion criteria

Inclusion criteria: permanent residents of the aforementioned subdistricts (women aged ≥45 years and men aged ≥50 years), who agreed to provide signed written informed consent, were enrolled. *Exclusion criteria*: (1) residents with severe mental diseases or acute infectious diseases and who could not cooperate during the survey; (2) lactating or pregnant women; (3) premenopausal women because Z-scores, but not T-scores, are used for the diagnosis of osteoporosis among premenopausal women; (4) residents with severe physical diseases that affect bone metabolism or gastrointestinal absorption including chronic diarrhoea, severe liver dysfunction or renal failure, gastrectomy and enterectomy; (5) residents were taking vitamin D (Vit D), vitamin B or multivitamin supplements, or receiving medication that affected bone metabolism, including hormone drugs/hormonal replacement therapy and antiosteoporosis drugs.

Questionnaires and physical examinations

A total of 2285 residents were included in the survey. Data on the age, sex, height, body weight (in the standing position wearing indoor clothes without shoes), menopause, daily smoking frequency, daily alcohol consumption and ongoing medications of the participants were recorded. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2). After the execution of further screening, 456 residents were excluded because they were either taking Vit D, vitamin B or multivitamin supplements, or receiving medication that affected bone metabolism, including hormone drugs and antiosteoporosis drugs, at the time of the study. Finally, 1829 participants were enrolled for the final analysis.

Assessment of BMD and grouping

Lumbar spine BMD was evaluated as spine is the site of rapid bone loss.[16 17] When enrolled, the BMD of the lumbar spine (L1–L4) of the participants was measured using a dual-energy X-ray absorptiometry densitometer (Hologic Discovery CI, Bedford, Massachusetts, USA) and expressed as the T-score (number of SD above or below the mean BMD value at the patient's age or the mean BMD of healthy adults aged 30 years, respectively). The participants were divided into three groups, namely control, osteopenia and osteoporosis, according to the total BMD of the lumbar spine. Participants with T-scores ≥1.0 were included in the control group. Moreover, participants with T-scores between −1.0 and −2.5 were diagnosed as having osteopenia and included in the osteopenia group. Participants with T-scores ≤−2.5 were diagnosed as having osteoporosis and included in the osteoporosis group.

Serological detection

Venous blood was drawn from all the participants in the morning after 10 hours of fasting. The serum samples were collected within 2 hours after the blood collection. The blood was gently mixed and centrifuged at 3000 rpm for 15 min at room temperature to separate the serum. The serum was immediately stored at 4°C and tested within 24 hours. Serum concentration of pyridoxal 5′-phosphate, one of the most salient active forms of Vit B_6 in the body, was measured through high-efficiency

liquid chromatography using an ultraperformance liquid chromatograph (Agilent 1290 Infinity, Santa Clara, California, USA).[18] The concentrations of osteocalcin (OST, 12149133122), β-C-terminal telopeptide of type I collagen (β-CTX, 11972308122) and N-terminal propeptide of type I collagen (PINP, 03141071190) were measured through an electrochemiluminescence immunoassay by using an automatic biochemical analyser (cobas 8000 e602, Roche, Basel, Switzerland). The concentration of alkaline phosphatase (ALP) was measured through a continuous monitoring technique by using an automatic biochemical analyser (modular P800, Roche). A sensitive and specific high-performance liquid chromatography-tandem mass spectrometry method executed using a liquid chromatography-tandem mass spectrometer (API4000, AB SCIX, Framingham, Massachusetts, USA) was applied to measure the concentrations of total serum 25(OH)D, including both 25(OH)D_3 and 25(OH)D_2.[19] The total concentrations of serum calcium and phosphorus were measured using the o-cresolphtalein-complexone method and through phosphomolybdate ultraviolet colorimetry, respectively, by using an automatic biochemical analyser (modular P800, Roche). The concentration of parathyroid hormone (PTH) (11972103160) was detected through a chemiluminescence immunoassay by using an automatic biochemical analyser (cobas 8000 e602, Roche). The concentration of fibroblast growth factor 23 (FGF23) was measured through an ELISA performed using a human FGF23 ELISA kit (CSB-E10113h, Cusabio Biotech, Wuhan, China) and a micro plate reader (MK3, Thermo, Waltham, Massachusetts, USA).

Inquirer quality assurance

All research assistants, interviewers and physical examiners were trained centrally by the professional epidemiologists and experienced staffs of the central coordinating centre over a 2-week period.

Statistical analyssis

For baseline characteristics analysis, missing values were recorded as N/A and excluded from the final analysis using listwise deletion if the participants refused BMD assessment, physical examinations or part of the questionnaire. Sensitivity analysis was performed in case of missing values over 10% of the total values.

All statistical analyses were performed using SPSS V.19.0 (SPSS, Chicago, Illinois, USA). For all analyses, we categorised the participants into the osteoporosis, osteopenia and normal groups according to their lumbar BMD values. The distribution normality was tested using the Kolmogorov-Smirnov test. The Kruskal-Wallis test was used to compare non-normally distributed data between any two groups (normal, osteopenia and osteoporosis), according to the gender. Analysis of variance was used to compare the normal distribution data among the groups by gender. The X^2 test was applied for the comparison of categorical variables. The associations between Vit B_6 values and ranks of lumbar BMD (rank 0, normal, T-scores ≥1.0; rank 1, osteopenia, −2.5<T scores <−1; rank 2, osteoporosis, T-scores ≤2.5) were analysed using linear regression.

The values of serum Vit B_6 concentrations were converted into ordinal quartiles, and a logistic regression model was used to examine the associations between values of the serum Vit B_6 concentration quartiles and ranks of osteoporosis (rank 0, normal; rank 1, osteoporosis). We estimated the ORs and 95% CIs using the highest quartile as the reference category in multivariate models. The covariates included BMI, age, serum 25(OH)D concentration and serum PTH concentration.

We also examined the associations between serum Vit B_6 concentrations and bone metabolism parameters, after additional adjustments for age and BMI, by using partial Pearson's correlation. All p values <0.05 were considered to be statistically significant.

Patient and public involvement

The questionnaire was tested with participants. The outcomes of BMD and serum parameters were disseminated to the participants with printed reports. There was no other patient involvement in preparation or review of this study.

RESULTS

The baseline characteristics of the participants in the control, osteopenia and osteoporosis groups are listed in table 1. Among the women, the mean age in the osteoporosis group was significantly increased than those in the control and osteopenia groups (p=0.000). Among the men, the mean age in the osteopenia group was lower than that in the control group (p=0.038). Among the men and women, the mean BMI values in the osteoporosis and osteopenia groups were significantly lower than that in control group (p=0.001 in women; p=0.000 in men). In addition, among the women, the BMI in the osteoporosis group was significantly lower than that in the osteopenia group (p=0.008). Meanwhile, the alcohol consumption and daily smoking frequencies did not differ significantly among the control, osteopenia and osteoporosis groups in the men and women (p>0.05).

The median of serum Vit B_6 concentration in the entire population was 22.00 µg/L (from 7.10 to 163.30 µg/L), with men showing 20.70 µg/L (from 7.10 to 163.30 µg/L) and women 22.80 µg/L (from 8.00 to 158.70 µg/L). Table 2 presents the concentrations of serum Vit B_6; concentrations of bone turnover markers, including serum PINP, β-CTX, OST and ALP and concentrations of serum calcium, phosphorus, 25(OH)D, PTH and FGF23 in the control, osteopenia and osteoporosis groups. The overall serum Vit B_6 concentrations in the control, osteopenia and osteoporosis groups were similar in both men and women, although there were limited statistical significances between the osteoporosis group and other two groups in the women (compared with control group, p=0.028; compared with osteopenia group, p=0.017).

Table 1 Comparison of baseline characteristics among control, osteopenia and osteoporosis groups

Characteristics	Women (n=1206)			Men (n=623)		
	Control (n=338)	Osteopenia (n=544)	Osteoporosis (n=324)	Control (n=368)	Osteopenia (n=180)	Osteoporosis (n=75)
Age at screening (years)	61.5 (6.26)	62.0 (5.74)	64.7 (6.11)*†	66.5 (5.84)	65.3 (6.19)*	66.8 (6.37)
BMI (kg/m^2)	25.4 (4.71) (n=327)	24.9 (9.79)* (n=526)	23.4 (5.42)*† (n=310)	24.9 (2.94) (n=362)	23.6 (2.68)* (n=178)	23.0 (3.63)* (n=75)
BMD (g/cm^2), median (IQR)	1.02 (0.97–1.08) (n=337)	0.86 (0.82–0.90)* (n=543)	0.71 (0.66–0.75)*† (n=324)	1.13 (1.04–1.20) (n=365)	0.90 (0.87–0.94)* (n=179)	0.75 (0.72–0.80)*† (n=75)
Alcohol intake (>1 drink per day) (%)	0.90 (n=319)	2.00 (n=507)	0.70 (n=302)	12.1 (n=347)	16.3 (n=172)	11.8 (n=68)
Current smokers (%)	2.20 (n=315)	1.20 (n=509)	0.70 (n=305)	19.6 (n=357)	24.2 (n=178)	25.0 (n=72)

Data are expressed as mean (SD) unless indicated otherwise. Number of values for every single analysis was listed if there were missing values.
*Compared with control group, p<0.05.
†Compared with osteopenia group, p<0.05.
BMD, bone mineral density; BMI, body mass index.

Among the men, the serum Vit B_6 concentrations did not differ significantly among the three groups (p>0.05). Among the women, the concentrations of the bone turnover markers in the osteopenia and osteoporosis groups were evidently higher than those in the control group, including serum PINP (osteopenia vs control, p=0.000; osteoporosis vs control, p=0.000), β-CTX (osteopenia vs control, p=0.000; osteoporosis vs control, p=0.000), OST (osteopenia vs control, p=0.000; osteoporosis vs control, p=0.000) and ALP (osteopenia vs control, p=0.010; osteoporosis vs control, p=0.000); the concentrations of these bone turnover markers in the osteoporosis group were also higher than those in osteopenia group, including serum PINP (osteoporosis vs osteopenia, p=0.001), β-CTX (osteoporosis vs osteopenia, p=0.001), OST (osteoporosis vs osteopenia, p=0.013) and ALP (osteoporosis vs osteopenia, p=0.028). Furthermore, among the women, the serum 25(OH)D concentration in the osteoporosis group was significantly lower than the concentrations in the control and osteopenia groups (osteoporosis vs control, p=0.000; osteoporosis vs osteopenia, p=0.009). For men, the serum concentrations of PINP (p=0.002),

Table 2 Comparison of serum Vit B_6 and serological metabolism parameters among control, osteopenia and osteoporosis groups

Characteristics	Women (n=1206)			Men (n=623)		
	Control (n=338)	Osteopenia (n=544)	Osteoporosis (n=324)	Control (n=368)	Osteopenia (n=180)	Osteoporosis (n=75)
Vit B_6 (μg/L)	24.9 (8.90)	25.1 (11.4)	25.0 (14.0)*†	23.3 (12.6)	23.8 (15.1)	22.4 (13.3)
PINP (ng/mL)	42.8 (12.8)	48.6 (23.7)*	52.3 (17.0)*†	34.3 (13.9)	36.4 (12.8)	41.3 (18.5)*
β-CTX (ng/mL)	0.36 (0.12)	0.43 (0.17)*	0.46 (0.16)*†	0.30 (0.13)	0.34 (0.15)*	0.39 (0.18)*
OST (ng/mL)	16.9 (4.88)	19.2 (6.59)*	20.7 (7.36)*†	14.2 (5.46)	14.9 (5.08)	16.3 (6.13)*
ALP (U/L)	75.9 (18.3)	79.3 (19.2)*	83.2 (21.4)*†	73.9 (19.9)	72.1 (18.9)	78.9 (27.4)
P (mmol/L)	1.15 (0.13)	1.15 (0.13)	1.15 (0.13)	1.01 (0.19)	1.02 (0.15)	1.03 (0.19)
Ca (mmol/L)	2.31 (0.09)	2.31 (0.10)	2.30 (0.09)	2.28 (0.09)	2.28 (0.10)	2.27 (0.11)‡
25(OH)D (ng/mL)	18.7 (6.29)	18.2 (6.50)	17.0 (6.38)*†	19.4 (6.74)	18.8 (6.35)	18.1 (6.22)
PTH (pmol/L)	4.28 (1.54)	4.33 (1.60)	4.55 (1.88)	4.14 (1.46)	4.03 (1.53)	4.17 (1.67)
FGF23 (pg/mL)	3.58 (7.66)	3.42 (6.19)	3.06 (6.89)	3.79 (6.30)	3.86 (6.08)	4.23 (11.3)

Data are expressed as mean (SD), analysed by Kruskal-Wallis test.
*Compared with control group, p<0.05.
†Compared with osteopenia group, p<0.05.
‡Compared variance among the three group used analysis of variance test.
ALP, alkaline phosphatase; β-CTX, β-C-terminal telopeptide of type I collagen; Ca, calcium; FGF23, fibroblast growth factor 23; OST, osteocalcin; P, phosphorus; PINP, N-terminal propeptide of type I collagen; PTH, parathyroid hormone; Vit B_6, vitamin B_6.

Table 3 OR (95% CI) for the risk of osteoporosis by quartiles of serum Vit B_6 concentrations in women

	Model 1	Model 2	Model 3	Model 4
p^1 for linear trend	0.88	0.82	0.28	0.67
Quartiles of Vit B_6, µg/L				
p^2 for linear trend across quartiles				
First quartile (<19.2) (n=175)	1.77 (1.14 to 2.07)*	1.61 (1.00 to 2.58)*	1.35 (0.83 to 2.22)	1.54 (0.95 to 2.49)
Second quartile (19.2 to 22.8) (n=171)	1.55 (0.99 to 2.41)	1.46 (0.90 to 2.36)	1.27 (0.78 to 2.08)	1.42 (0.88 to 2.23)
Third quartile (22.8 to 26.9) (n=166)	1.33 (0.85 to 2.07)	1.38 (0.85 to 2.22)	1.23 (0.75 to 2.00)	1.35 (0.83 to 2.18)
Fourth quartile (>26.9) (n=150)	1	1	1	1

p^1, linear regression based on serum Vit B_6 values and ranks of lumbar bone mass (rank 0, normal; rank 1, osteopenia; rank 2, osteoporosis); p^2, OR (95% CI) test of linear trend based on logistic regression values of the Vit B_6 quartiles and ranks of osteoporosis (rank 0, normal; rank 1, osteoporosis).
Model 1, unadjusted; model 2, multivariate adjustment by BMI and age; model 3, multivariate adjustment by BMI, age and 25(OH)D; model 4, multivariate adjustment by BMI, age and PTH.
*P<0.05.
BMI, body mass index; Vit B_6, vitamin B_6.

β-CTX (p=0.000) and OST (p=0.003) in the osteoporosis group were also higher than those in the control group. In addition, most calcium and phosphorus metabolism markers, including calcium, phosphorus, serum 25(OH)D, PTH and FGF23, in the osteopenia and osteoporosis groups did not differ significantly from those in the control group (p>0.05).

The association between the risk of osteoporosis and low serum Vit B_6 concentration in both men and women was analysed (tables 3 and 4). Among the women, no significant linear trend was observed between the serum Vit B_6 concentration and ranks of lumbar bone mass, before and after adjustment for age and BMI (table 3, p>0.05). An analysis of the linear trend between the ordinal values of serum Vit B_6 concentration quartiles and osteoporosis showed that a serum Vit B_6 concentration in the first quartile (<19.2 µg/L) was a risk factor for osteoporosis (table 3; without adjustment, OR=1.77, 95% CI 1.14 to 2.07, p=0.011, p<0.05; after adjustment for age and BMI, OR=1.61, 95% CI 1.00 to 2.58, p=0.049, p<0.05); however, no significant association between serum Vit B_6 concentration and risk of osteoporosis was shown after adjustment with 25(OH)D and PTH, respectively (table 3; after adjustment for age, BMI and 25(OH)D, OR=1.35, 95% CI 0.83 to 2.22, p=0.231, p>0.05; after adjustment for age, BMI and PTH, OR=1.54, 95% CI 0.95 to 2.49, p=0.078,

Table 4 OR (95% CI) for the risk of osteoporosis by quartiles of serum Vit B_6 concentrations in men

	Model 1	Model 2	Model 3	Model 4
p^1 for linear trend	0.59	0.82	0.91	0.85
Quartiles of Vit B_6, µg/L				
p^2 for linear trend across quartiles				
First quartile (<17.5) (n=104)	1.96 (0.92 to 4.17)	1.72 (0.79 to 3.74)	1.59 (0.72 to 3.52)	1.69 (0.77 to 3.70)
Second quartile (17.5 to 20.7) (n=112)	1.63 (0.75 to 3.53)	1.54 (0.69 to 3.41)	1.45 (0.65 to 3.25)	1.52 (0.69 to 3.39)
Third quartile (20.7 to 24.6) (n=114)	1.67 (0.77 to 3.61)	1.69 (0.73 to 3.63)	1.57 (0.70 to 3.52)	1.63 (0.73 to 3.63)
Fourth quartile (≥24.6) (n=113)	1	1	1	1

p^1, linear regression based on Vit B_6 values and ranks of bone mass (rank 0, normal; rank 1, osteopenia; rank 2, osteoporosis); p^2, OR (95% CI) test of linear trend based on logistic regression values of the Vit B_6 quartiles and ranks of osteoporosis (rank 0, normal; rank 1, osteoporosis).
Model 1, unadjusted; model 2, multivariate adjustment by BMI and age; model 3, multivariate adjustment by BMI, age and 25(OH)D; model 4, multivariate adjustment by BMI, age and PTH.
BMI, body mass index; Vit B_6, vitamin B_6.

Table 5 Relationship between serum Vit B_6 concentrations and bone metabolism parameter concentrations in control, osteopenia and osteoporosis groups

	Correlation coefficient (women)			Correlation coefficient (men)		
	Control group	Osteopenia group	Osteoporosis group	Control group	Osteopenia group	Osteoporosis group
PINP	−0.008	0.018	−0.147*	−0.040	−0.081	−0.061
β-CTX	0.002	0.001	−0.122*	−0.037	−0.082	−0.122
OST	−0.022	0.010	−0.153*	−0.069	−0.156*	−0.132
ALP	−0.092	−0.117*	−0.085	−0.130*	−0.079	−0.078
P	0.179*	0.126*	0.039	0.092	0.062	0.131
Ca	0.139*	0.017	0.080	0.116*	0.154*	0.197
25(OH)D	0.354*	0.233*	0.319*	0.205*	0.292*	0.305*
PTH	−0.206*	−0.072	−0.148*	−0.155*	−0.120	−0.245*
FGF23	−0.062	−0.059	−0.019	0.024	0.076	−0.038

P values of the relationship between serum Vit B_6 concentrations and bone metabolism parameter concentrations.
*P<0.05.
ALP, alkaline phosphatase; β-CTX, β-C-terminal telopeptide of type I collagen; Ca, calcium; FGF23, fibroblast growth factor23; OST, osteocalcin; P, phosphorus; PINP, N-terminal propeptide of type I collagen; PTH, parathyroid hormone; Vit B_6, vitamin B_6.

p>0.05). These findings suggested that women with relative low serum Vit B_6 concentrations might be susceptible to osteoporosis, and the association depended on serum concentrations of 25(OH)D and PTH. Among the men, no significant linear trend was observed between serum Vit B_6 concentrations and the ranks of lumbar bone mass. In addition, no significant linear trend was observed between the ordinal values of serum Vit B_6 concentration quartiles and osteoporosis (table 4, p>0.05).

The relationships between serum Vit B_6 concentration and the markers of bone turnover and those between serum Vit B_6 concentration and calcium and phosphorus metabolism in the different groups of women and men are listed in table 5. Notably, the serum Vit B_6 concentration exhibited significant negative relationships with most of the bone turnover markers in the women in the osteoporosis group (PINP, p=0.008; OST, p=0.006 and β-CTX, p=0.028). Additionally, the serum Vit B_6 concentration was significantly positively correlated with serum 25(OH)D concentrations in both women and men (women, control group, p=0.000 and osteoporosis group p=0.000; men, control group and osteoporosis group, p=0.000 and osteoporosis group p=0.008). The serum Vit B_6 concentration in most groups was also significantly inversely related to the PTH concentration (women, control group, p=0.000 and osteoporosis group p=0.007; men, control group and osteoporosis group, p=0.003 and osteoporosis group p=0.034).

DISCUSSION

Relationships among serum vit B_6 concentrations, reduced BMD and fracture

Vit B_6 is widely distributed in plant-based foods such as beans, cereals and brown rice,[20] which are the most common components of the daily diet of the Chinese population, particularly in middle-aged and older people. An adequate supply of dietary Vit B_6 sustains normal serum Vit B_6 concentrations. Serum Vit B_6 deficiency is defined as a serum Vit B_6 concentration of <20 nmol/L (4.94 µg/L).[21 22] Poor dietary intake or low circulating Vit B_6 concentrations have been considered as a potential risk factor for osteoporosis; however, the relation of Vit B_6 and BMD is debatable.

A longitudinal follow-up study of the Framingham Osteoporosis Study, in which >22% of the participants were diagnosed as having serum Vit B_6 deficiency, showed that bone loss was inversely associated with the serum Vit B_6 concentration in older people, and people with serum Vit B_6 deficiency exhibited lower femoral neck BMD values than did those with normal serum Vit B_6 concentrations.[4] Therefore, Vit B_6 deficiency might be a risk factor for decreased bone mass.

In our cross-sectional study on community-dwelling middle-aged and older people, we observed no significant differences in the serum Vit B_6 concentrations among the control, osteopenia and osteoporosis groups; furthermore, we observed no linear trend between the serum Vit B_6 concentrations and the ranks of lumbar bone mass. This finding is consistent with those of a previous study in postmenopausal British women.[15] In our study, the serum Vit B_6 concentrations of all the female and male participants were >7 µg/L, and the mean serum Vit B_6 concentrations of all the groups were >20 µg/L. The mean concentration of serum Vit B_6 in postmenopausal British women was approximately 45 nmol/L (11.12 µg/L), which is also considerably higher than the limit of serum Vit B_6 deficiency.

In this study, we found that the serum Vit B_6 concentration in the lowest quartile, which was determined to be considerably higher than the limit of serum Vit B_6

deficiency, might be a risk factor for osteoporosis in postmenopausal women. However, the association of serum Vit B_6 was limited and dependent on serum 25(OH)D and PTH concentrations.

The Rotterdam study also showed that the risk of fracture decreased with an increase in dietary Vit B_6 intake independent of BMD.[3] The Framingham Osteoporosis Study demonstrated that low plasma Vit B_6 concentrations were associated with a high risk of fracture in older people.[4] Additionally, another study confirmed a statistically significant inverse relationship between dietary pyridoxine intake and hip fracture risk among women.[12] Both bone density and quality are crucial factors for determining bone strength, and collagen crosslinks play a crucial role in maintaining bone quality.[23] Vit B_6 is essential for lysyl oxidase in collagen crosslinks,[10] and Vit B_6 deficiency can reduce the concentrations of crosslinking intermediates and impair collagen crosslink formation in the bones,[24–26] thus leading to low bone quality. This could be a reason (apart from reduced BMD) for the observed association between high bone fracture risk and low dietary Vit B6 intake or low circulating Vit B6 concentrations.[3 4 12]

This cross-sectional study did not include participants with Vit B_6 deficiency; therefore, the risk of Vit B_6 deficiency for osteoporosis could not be analysed. In addition, only lumbar spine BMD was measured due to economic and time limits; other variables which were not involved in this study such as dietary intakes and physical activities could also influences the change the association of serum Vit B_6 concentration and status of bone mass. All these limitations might lead to a certain degree of bias of this study.

Relationship between serum Vit B_6 concentration and bone turnover marker concentrations in postmenopausal osteoporosis

Postmenopausal osteoporosis occurs mainly due to the drastic reduction in endogenous oestrogen levels in women, which leads to an increase in the concentrations of circulating bone turnover markers. We found that the serum Vit B_6 concentration was significantly inversely associated with the concentration of bone turnover markers in the women in the osteoporosis group. Low concentration of Vit B_6 has demonstrated a stimulatory effect on osteoclast activity, although no significant effect on osteoclast number was revealed.[8] In addition, homocysteine (HCY) has been confirmed a significant correlation with bone resorption markers. High HCY level induced by low Vit B_6 concentration, could stimulate bone resorption, leading to a shift of bone metabolism towards bone resorption.[27] This is consistent with our finding that the serum Vit B_6 concentration was inversely associated with the concentration of bone resorption marker. Only a few studies have reported the relationship between serum Vit B_6 and bone formation markers with equivocal results. Holstein *et al* found that the concentrations of serum OST were significantly lower in individuals with low serum Vit B_6 concentrations than in those with high serum Vit B_6 concentrations.[25] Also plasma OST and bone-specific ALP activity were significantly decreased in Vit B_6-deficient animals.[28] In contrast, in vitro study with human primary osteoblasts found that decreasing B-vitamin concentrations was not accompanied by changes of ALP, OST, PINP as well as mineralised matrix formation.[29] It was reported that a decrease in bone resorption results in a rapid reduction in bone formation because of reduced release of coupling factors.[30] Therefore, there was possibly an indirect effect of serum Vit B_6 on bone formation, which is secondary to its regulation on bone resorption. However, additional studies are still required to clarify the mechanisms by which Vit B_6 affects bone turnover, especially bone formation, in menopausal osteoporotic women.

Relationship between serum Vit B_6 concentration and 25(OH)D concentration and between serum Vit B_6 concentration and PTH concentration

This study also showed that the serum Vit B_6 concentration was significantly positively associated with the 25(OH)D concentration in the men and women, and it was inversely associated with the PTH concentration in all the women and men with normal bone mass or osteoporosis.

Serum 25(OH)D concentrations were significantly lower in animals with Vit D and Vit B_6 deficiencies than in animals with Vit D deficiency alone. Recovery of serum 25(OH)D concentration after Vit D administration was delayed under conditions of Vit B_6 deficiency.[31] Moreover, it was speculated that either the activity of renal 25-hydroxy-vitamin D-1-alpha-hydroxylase decreases or $1,25(OH)_2D$ turnover increases in a state of Vit B_6 deficiency.[28] Since serum Vit B_6 might regulate Vit D metabolism, it was inferred that Vit B_6 might indirectly regulate bone metabolism at least partially through its regulation on Vit D, which has dual effects on both bone formation and bone resorption.[32]

Thus far, studies have not revealed the regulatory effects of single Vit B_6 on PTH expression. Vit D exerts negative feedback effects on PTH secretion to prevent the overcorrection of low calcium concentrations. On binding, the Vit D/Vit D receptor complex in parathyroid gland cells translocates into the nucleus and inhibits PTH transcription. Vit D can also inhibit PTH cell proliferation.[33–35] From our results, we infer that Vit B6 might play a positive role in Vit D metabolism, consequently prohibiting PTH secretion. However, the effects and regulatory mechanisms of Vit B_6 on PTH are largely unknown.

Although the serum Vit B_6 concentration was significantly associated with 25(OH)D and PTH, and both PTH and Vit D play critical roles in the maintenance of calcium and phosphate homeostasis and maintenance of bone health,[36] this study revealed no significant relationship between the serum Vit B_6 concentration and the serum concentrations of calcium and phosphate. These findings are consistent with the results of a previous study, which showed that in rats with Vit B_6 deficiency, Vit D metabolism was impaired without changes in plasma calcium homeostasis or BMD.[28]

CONCLUSION

In conclusion, a relatively low serum Vit B_6 concentration might be a risk factor for osteoporosis in postmenopausal women, including those with normal serum Vit B_6 concentrations. However, the contribution of relatively low serum Vit B_6 concentration to osteoporosis risk was limited, and depended on serum concentrations of Vit D and PTH. Serum Vit B_6 might play a potential role in postmenopausal osteoporosis.

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Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval The study was conducted in accordance with considerations for the protection of participants' rights and was approved by the Ethics Board of Shanghai University of Traditional Chinese Medicine (no: 2014LCSY12).

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REFERENCES

1. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, March 7-29, 2000: highlights of the conference. *South Med J* 2001;94:569–73.
2. Holm JP, Hyldstrup L, Jensen JB. Time trends in osteoporosis risk factor profiles: a comparative analysis of risk factors, comorbidities, and medications over twelve years. *Endocrine* 2016;54:241–55.
3. Yazdanpanah N, Zillikens MC, Rivadeneira F, *et al*. Effect of dietary B vitamins on BMD and risk of fracture in elderly men and women: the Rotterdam study. *Bone* 2007;41:987–94.
4. McLean RR, Jacques PF, Selhub J, *et al*. Plasma B vitamins, homocysteine, and their relation with bone loss and hip fracture in elderly men and women. *J Clin Endocrinol Metab* 2008;93:2206–12.
5. Fratoni V, Brandi ML. B vitamins, homocysteine and bone health. *Nutrients* 2015;7:2176–92.
6. van Wijngaarden JP, Doets EL, Szczecińska A, *et al*. Vitamin B12, folate, homocysteine, and bone health in adults and elderly people: a systematic review with meta-analyses. *J Nutr Metab* 2013;2013:1–19.
7. Hellmann H, Mooney S. Vitamin B6: a molecule for human health? *Molecules* 2010;15:442–59.
8. Herrmann M, Schmidt J, Umanskaya N, *et al*. Stimulation of osteoclast activity by low B-vitamin concentrations. *Bone* 2007;41:584–91.
9. Dodds RA, Catterall A, Bitensky L, *et al*. Abnormalities in fracture healing induced by vitamin B6-deficiency in rats. *Bone* 1986;7:489–95.
10. Bird TA, Levene CI. Lysyl oxidase: evidence that pyridoxal phosphate is a cofactor. *Biochem Biophys Res Commun* 1982;108:1172–80.
11. Massé PG, Rimnac CM, Yamauchi M, *et al*. Pyridoxine deficiency affects biomechanical properties of chick tibial bone. *Bone* 1996;18:567–74.
12. Dai Z, Wang R, Ang LW, *et al*. Dietary B vitamin intake and risk of hip fracture: the Singapore Chinese Health Study. *Osteoporos Int* 2013;24:2049–59.
13. Macdonald HM, McGuigan FE, Fraser WD, *et al*. Methylenetetrahydrofolate reductase polymorphism interacts with riboflavin intake to influence bone mineral density. *Bone* 2004;35:957–64.
14. Abrahamsen B, Madsen JS, Tofteng CL, *et al*. Are effects of MTHFR (C677T) genotype on BMD confined to women with low folate and riboflavin intake? Analysis of food records from the Danish osteoporosis prevention study. *Bone* 2005;36:577–83.
15. Baines M, Kredan MB, Usher J, *et al*. The association of homocysteine and its determinants MTHFR genotype, folate, vitamin B12 and vitamin B6 with bone mineral density in postmenopausal British women. *Bone* 2007;40:730–6.
16. Gallagher JC. Bone mineral density measurements: how often should bone mineral density be measured in postmenopausal women? Results from the Women's Health Initiative study. *Menopause* 2015;22:581–3.
17. Albright F, Smith PH, Richardson AM. Postmenopausal osteoporosis: its clinical features. *JAMA* 1941;116:2465–74.
18. Barthus RC, Mazo LH, Poppi RJ. Simultaneous determination of vitamins C, B6 and PP in pharmaceutics using differential pulse voltammetry with a glassy carbon electrode and multivariate calibration tools. *J Pharm Biomed Anal* 2005;38:94–9.
19. Heudi O, Trisconi MJ, Blake CJ. Simultaneous quantification of vitamins A, D3 and E in fortified infant formulae by liquid chromatography-mass spectrometry. *J Chromatogr A* 2004;1022:115–23.
20. Roth-Maier DA, Kettler SI, Kirchgessner M. Availability of vitamin B6 from different food sources. *Int J Food Sci Nutr* 2002;53:171–9.
21. Leklem JE. Vitamin B-6: a status report. *J Nutr* 1990;120 Suppl 11(4):1503–7.
22. Driskell JA. Vitamin B-6 requirements of humans. *Nutr Res* 1994;14:293–324.
23. Saito M, Fujii K, Marumo K. Degree of mineralization-related collagen crosslinking in the femoral neck cancellous bone in cases of hip fracture and controls. *Calcif Tissue Int* 2006;79:160–8.
24. Fujii K, Kajiwara T, Kurosu H. Effect of vitamin B6 deficiency on the crosslink formation of collagen. *FEBS Lett* 1979;97:193–5.
25. Holstein JH, Herrmann M, Splett C, *et al*. Low serum folate and vitamin B-6 are associated with an altered cancellous bone structure in humans. *Am J Clin Nutr* 2009;90:1440–5.
26. Paschalis EP, Tatakis DN, Robins S, *et al*. Lathyrism-induced alterations in collagen cross-links influence the mechanical properties of bone material without affecting the mineral. *Bone* 2011;49:1232–41.
27. Herrmann M, Peter Schmidt J, Umanskaya N, *et al*. The role of hyperhomocysteinemia as well as folate, vitamin B(6) and B(12) deficiencies in osteoporosis: a systematic review. *Clin Chem Lab Med* 2007;45:1621–32.
28. Massé PG, Delvin EE, Hauschka PV, *et al*. Perturbations in factors that modulate osteoblast functions in vitamin B6 deficiency. *Can J Physiol Pharmacol* 2000;78:904–11.
29. Herrmann M, Umanskaya N, Wildemann B, *et al*. Accumulation of homocysteine by decreasing concentrations of folate, vitamin B12 and B6 does not influence the activity of human osteoblasts in vitro. *Clin Chim Acta* 2007;384:129–34.
30. Pederson L, Ruan M, Westendorf JJ, *et al*. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. *Proc Natl Acad Sci U S A* 2008;105:20764–9.
31. Klimova OA, Sokol'nikov AA, Kodentsova VM, *et al*. Vitamin D and calcium metabolism in relation to different levels of vitamins B6 and D. *Vopr Pitan* 1991;4:56–9.
32. Carmeliet G, Dermauw V, Bouillon R. Vitamin D signaling in calcium and bone homeostasis: a delicate balance. *Best Pract Res Clin Endocrinol Metab* 2015;29:621–31.
33. Darwish HM, DeLuca HF. Identification of a transcription factor that binds to the promoter region of the human parathyroid hormone gene. *Arch Biochem Biophys* 1999;365:123–30.
34. Jääskeläinen T, Huhtakangas J, Mäenpää PH. Negative regulation of human parathyroid hormone gene promoter by vitamin D3 through nuclear factor Y. *Biochem Biophys Res Commun* 2005;328:831–7.
35. Chow EC, Quach HP, Vieth R, *et al*. Temporal changes in tissue 1α,25-dihydroxyvitamin D3, vitamin D receptor target genes, and calcium and PTH levels after 1,25(OH)2D3 treatment in mice. *Am J Physiol Endocrinol Metab* 2013;304:E977–E989.
36. Khundmiri SJ, Murray RD, Lederer E. PTH and Vitamin D. *Compr Physiol* 2016;6:561–601.