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Association of dietary and serum vitamin E with bone mineral density in middle-aged and elderly Chinese adults: a cross-sectional study

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Abstract

Previous studies have suggested that vitamin E (VE) may affect bone health, but the findings have been inconclusive. We examined the relationship between VE status (in both diet and serum) and bone mineral density (BMD) among Chinese adults. This community-based study included 3203 adults (2178 women and 1025 men) aged 40–75 years from Guangzhou, People's Republic of China. General and dietary intake information were collected using structured questionnaire interviews. The serum α-tocopherol (TF) level was quantified by reversed-phase HPLC. The BMD of the whole body, the lumbar spine and left hip sites (total, neck, trochanter, intertrochanter and Ward's triangle) were measured using dual-energy X-ray absorptiometry. In women, the dietary intake of VE was significantly and positively associated with BMD at the lumbar spine, total hip, intertrochanter and femur neck sites after adjusting for covariates (P_{trend}: 0·001–0·017). Women in quartile 3 of VE intake typically had the highest BMD; the covariate-adjusted mean BMD were 2·5, 3·06, 3·41 and 3·54 % higher, respectively, in quartile 3 (*v.* 1) at the four above-mentioned sites. Similar positive associations were observed between cholesterol-adjusted serum α-TF levels and BMD at each of the studied bone sites (P_{trend}: 0·001–0·022). The covariate-adjusted mean BMD were 1·24–4·83 % greater in quartile 4 (*v.* 1) in women. However, no significant associations were seen between the VE levels (dietary or serum) and the BMD at any site in men. In conclusion, greater consumption and higher serum levels of VE are associated with greater BMD in Chinese women but not in Chinese men.

Key words: Dietary vitamin E: Serum α-tocopherol: Bone mineral density: Chinese: Cross-sectional studies

Osteoporosis is a major worldwide public health problem. In 2014, about fifty-four million Americans had osteoporosis and low bone mass[1]. It was estimated that 40 % of 50-year-old Caucasian women in the USA would suffer an osteoporotic fracture in their remaining lifetime[2]. Although the Chinese population generally has lower risks of hip fractures compared with Western populations, a rapid increase in the incidence of hip fractures has been observed in the mainland Chinese population in the past 20 years[3]. Therefore, exploration of preventive measures for osteoporosis in older Chinese adults is essential and urgent.

Vitamin E (VE) includes α-, β-, γ- and δ-tocopherol (TF) and their respective tocotrienols. α-TF is the most abundant subtype and has the highest bioavailability[4]. Previous studies have suggested that oxidative stress and inflammation may serve as important mechanistic pathways in the pathogenesis of osteoporosis[5,6] by promoting osteoclastogenesis and inhibit bone formation[7,8]. VE, as an important antioxidant and immune regulatory nutrient, may be beneficial to the maintenance of bone health[9,10]. An increasing number of studies have investigated the potential beneficial effects of VE against osteoporosis. Previous studies found that VE could reverse the oxidant-induced alterations in chondrocytes[11] and promote bone formation in rats[12]. A few human studies found that higher dietary VE intake and/or greater proportion of the use of VE (α-TF) supplements were associated with a reduced risk for fractures in 61 433 women and 1138 men[13], as well as in 1215 men and women in the Utah Study[14]. Favourable associations between serum α-TF and bone mineral density (BMD) were also observed in a cross-sectional study[15]. However, a null association between dietary/serum α-TF and BMD was observed in the Women's Health Initiative study[16]. Moreover, Hamidi *et al.*[17] noted a detrimental association of higher levels of α-TF in diet, supplements and in serum with bone formation. Therefore, the VE–bone association remains speculative in humans.

In a recent review, high-dose α-TF by supplementation might have detrimental effects via affecting the normal function of vitamin K and other VE isomers, and the pro-oxidant effects[18]. Considering relatively higher proportion use of VE supplements (14·7–22·9 %) and multivitamin (31·3–39·8 %) in the Western

Abbreviations: BMD, bone mineral density; TF, tocopherol; VE, vitamin E.

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population[19], Chinese population may provide a unique opportunity for the study of an association between VE (in diet and blood) and bone health, with minimum confounding from the supplementation of VE (2·9 %) or multivitamins (6·1 %)[20]. The present study examined the relation between dietary intakes and serum levels of VE and BMD among middle-aged and elderly Chinese adults.

Methods

Study participants

This study was conducted on the basis of a community-based cohort study aimed at assessing the determinants of cardiometabolic outcomes and osteoporosis that was established between 2008 and 2010. A total of 3169 participants between 40 and 75 years of age were recruited from urban Guangzhou, People's Republic of China, through community advertisements and subject referral, as reported in previous studies[21]. The participants were required to have been residents of Guangzhou for at least 5 years. We excluded subjects with previously confirmed conditions such as CVD, diabetes, renal failure, cancer, metabolic bone disease, chronic glucocorticoid use or a history of spine or hip fracture. Between April 2011 and January 2013, 2465 of the 3169 participants completed the second survey. A total of 871 participants were newly recruited using the same selection criteria and recruitment methods between February 2013 and August 2013. General information and information on habitual dietary intake and physical activity were collected at each survey, but the BMD was determined only at the second survey. We excluded participants with missing data for BMD (n 44) and for diet (n 71) and those who claimed implausibly high (≥16 736 kJ/d (≥4000 kcal/d)) or low (≤2092 kJ/d (≤500 kcal/d)) energy intake (n 18). Eventually, 3203 participants, including 2347 subjects who attended the second survey and 856 newly recruited subjects, were included for this study. The cross-sectional data of those who completed the follow-up survey and of the newly recruited subjects were used for this study. Written informed consent was obtained from each of the participants at their initial enrolment and during the follow-up survey. The study was approved by the Ethics Committee of the School of Public Health at Sun Yat-sen University.

Questionnaire interview

A structured questionnaire[22] was used to collect data on socio-demographic characteristics (e.g. education, occupation and marital status), lifestyle habits (e.g. smoking, alcohol consumption, tea consumption and physical activity) and habitual dietary consumption in the 12 months before the interview, multivitamin supplements (e.g. Centrum (Wyeth), Gold Theragran (Bristol-Myers Squibb), 21SUPER-VITA (Hangzhou Minsheng Pharmaceutical Group Co., Ltd); containing VE: 5–20 mg/piece), history of chronic diseases and medications and on history of menstruation (for women) at the baseline (2008–2010) and the follow-up (2011–2013) survey. We defined smokers as having smoked ≥1 cigarette/d, alcohol drinkers as having alcohol consumption ≥ once/week and tea drinkers as having had tea beverage ≥ twice/week for at least 6 consecutive months. Ca and multivitamin supplement user were defined as having taken the relevant pills daily for at least 1 month. Postmenopausal women were defined as at least 12 months since the last menstrual cycle. Physical activity was estimated as metabolic equivalent (MET-h/d) spent in standing, walking, engaging in mild, moderate or vigorous physical activity, carrying a load and walking upstairs (excluding sleeping and sitting) in a typical week over the previous year by using a nineteen-item questionnaire[23]. Correlation coefficient for the repeated measures of MET at baseline and follow-up was 0·666 in 2347 subjects followed up. Face-to-face interviews were conducted by trained interviewers with relevant knowledge before anthropometric and BMD measurements and laboratory assays were performed.

We used a seventy-nine-item quantitative FFQ to estimate the subjects' usual dietary consumption during the past year[24] at baseline (2008–2010) and follow-up (2011–2013). Most common foods were listed on the FFQ, and the subjects reported their intake of each food or item per day, week, month or year (or as never). For seasonal foods, the participants were asked to report the number of months of the year they had consumed each item. Photographs of commonly consumed foods (for 100 g or for each serving) and standard tableware were available to help quantify the foods consumed. The validity and reproducibility of the FFQ, with six 3-d energy-adjusted diet records and twenty-six nutrients, have been confirmed among the local population (the correlation coefficient between the FFQ and six 3-d dietary records was 0·25 for VE)[24]. The nutrients were calculated from the 2002 *China Food Composition Table*[25].

Anthropometric and bone mineral density measurements

The weight and height of the participants were measured without shoes and while wearing light clothes. The BMI was then calculated. Dual-energy X-ray absorptiometry (Discovery W; Hologic Inc.) was used to measure BMD for the whole body, the lumbar spine (L1–L4) and left hip sites (total, neck, trochanter, intertrochanter and Ward's triangle) in April 2011 and August 2013[21]. The lumbar spine and left hip were scanned in a high-definition mode, whereas the whole body was scanned in the default mode. All scans were performed and analysed by the same well-trained professionals using Hologic Discovery software version 3.2. The *in vivo* CV for the BMD measurements in thirty participants after repositioning were 1·18 % (whole body), 0·87 % (lumbar spine), 1·02 % (total hip) and 1·92 % (femoral neck). The long-term CV of the measurements was 0·29 %, as calculated by daily testing of the phantom between April 2011 and August 2013.

Laboratory assay

Overnight fasting venous blood samples were collected at baseline (2008–2010). The separated serum samples were stored at −80°C until the analyses were performed. Serum α-TF was separated and quantified via reversed-phase HPLC according to the method of Burri *et al.*[26] with some modifications, between May 2013 and October 2014. Briefly, a 200-μl serum sample was

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deproteinised with 500 µl ethanol-butylated hydroxytoluene solution containing α-TF acetate as the internal standard, extracted twice using 2 ml hexane-butylated hydroxytoluene solution, dried under a stream of N and finally reconstituted in 200 µl mobile phase B (acetonitrile–methanol–tetrahydrofuran–ammonium acetate 55:35:5:5, v/v). Then, 50-µl samples were injected in a C18 analytical column at the room temperature. Peaks were detected at wavelengths of 292 nm for the α-TF through a Waters 2998 diode-array detector (Waters), with a day-to-day variation coefficient of approximately 6·3 % for α-TF. The serum total cholesterol levels were measured using the enzymatic colorimetric method in a Hitachi 7600–010 automated analyzer.

Statistical analysis

SPSS for Windows, version 17.0 (SPSS Inc.), was used for the analysis. A two-sided P value of <0·05 was considered to be statistically significant. Log transformation was conducted for the dietary data of VE, protein and Ca to achieve an approximately normal distribution. The dietary intake data were adjusted for total energy intake using the residual method[27]. The serum α-TF level was expressed as the ratio of serum α-TF:cholesterol[28,29]. The data were analysed separately by sex. Demographic and other characteristics of the study population were tabulated as means and standard deviations or as proportions. The differences between the groups were compared using t tests or ANOVA for continuous variables and χ^2 tests for categorical variables.

The subjects were categorised into quartiles by dietary intake of VE and by the ratio of serum α-TF:cholesterol. Multivariate ANOVA were used to compare the means of BMD between the quartiles by the VE indices. The Bonferroni's test was used for multiple comparisons. We adjusted only for age in model 1. Model 2 was further adjusted for BMI, education level (secondary or below, high school, college or above), household income (<4000, 4000–6000 and >6000 yuan/month/person), physical activity (MET-h/week), energy-adjusted dietary intake of protein, Ca, Ca supplement use (yes or no), multivitamin supplements use (yes or no), smoking status (yes or no), alcohol and tea consumption (yes or no), and oestrogen use (yes or no) and age at menopause (for women only).

Interaction analyses were conducted to examine whether the VE–BMD relationships were significantly modified by sex, multivitamin supplement use, education level and household income.

Results

Characteristics of the study participants

The characteristics of the participants are shown in Table 1. A total of 2178 women and 1025 men were included in our study; among them, 1915 women and 886 men had serum α-TF data. The mean age was 59·8 (SD 5·5) years in women and 62·4 (SD 6·6) years in men. Men had a significantly higher BMD at all sites and a greater consumption of VE (19·5 *v.* 18·7 mg) but a lower ratio of serum α-TF:cholesterol (6·00 *v.* 6·25; $P<0·001$) compared with women (all $P<0·001$). In addition, the average dietary consumption of VE in both sexes was slightly higher than the adequate intake value (14 mg/d)[30]. The main sources (74 %) of dietary VE in the population of our study are from vegetable oils (27 %), legumes (12·7 %), rice (8·8 %), vegetables (7·8 %), nuts (6·8 %), fruits (5·4 %) and eggs (2·9 %) (data not shown).

Associations of vitamin E intake with bone mineral density

In women, multivariate ANOVA showed that dietary intake of VE was significantly and positively associated with the BMD for the lumbar spine, total hip, intertrochanter, and femur neck (all $P<0·05$), but not for the whole body, trochanter or Ward's triangle (P_{trend}: 0·083–0·127) after adjusting for the potential covariates in model 2 (Table 2). The subjects in quartile 3 tended to have the highest BMD among the quartiles, and their BMD were significantly higher than those in quartile 1 at each of the studied bone sites except for the whole body. The covariate-adjusted mean BMD were, 3·06, 3·41 and 3·54 % higher in quartile 3 (*v.* 1) of dietary VE at the total hip, intertrochanter and femur neck, respectively (all $P<0·05$) (Table 2). The associations tended to be more pronounced in the age-adjusted model (model 1) at the studied bone sites (P_{trend}: 0·001–0·019; online Supplementary Table S1). However, no significant relationship was observed between the dietary intake of VE and the BMD at any studied bone site in the two models in men (Table 2 and online Supplementary Table S1).

Associations of serum α-tocopherol concentration with bone mineral density

In women, positive dose–response associations were observed between the serum α-TF:cholesterol ratio and the BMD at each of the studied bone sites after adjusting for age (P_{trend}: 0·002–0·032; online Supplementary Table S2). Similar associations were obtained after adjusting for multiple potential covariates (P_{trend}: 0·001–0·022; Table 3). Similar BMD values were found in quartiles 3 and 4. The mean BMD were 1·24 % (whole body), 2·90 % (lumbar spine) and 2·04–4·83 % (hip sites) greater in quartile 4 than those in quartile 1. In men, however, no significant association was observed at any of the studied bone sites in models 1 and 2 ($P>0·05$) (Table 3; online Supplementary Table S2). Consistent results were found between the serum α-TF levels (uncorrected by cholesterol) and the BMD at each of the studied bone sites in both sexes (data not shown).

Interaction analysis

The associations between the VE levels (in diet and serum) and BMD at each of the studied bone sites did not differ significantly between the subgroups based on sex, multivitamin supplements use, education level or household income. P values for the interaction ranged between 0·221–0·575 for sex, 0·174–0·963 for multivitamin use, 0·057–0·897 for education level and 0·069–0·964 for household income (data not shown).

Discussion

In this population-based cross-sectional study, our results demonstrate that greater dietary intake of VE and serum

Table 1. Characteristics of study population by sex in Guangzhou, People's Republic of China*
(Mean values and standard deviations; numbers; medians and interquartile ranges (IQR); *n* 3203)

	Female			Male			
	Mean	SD	*n*	Mean	SD	*n*	*P*
Age (years)	59·8	5·5	2178	62·4	6·6	1025	<0·001
Height (cm)	155·1	5·4	2178	166·1	5·8	1025	<0·001
Weight (kg)	56·3	8·3	2178	66·1	9·9	1025	<0·001
BMI (kg/cm^2)	23·4	3·2	2178	23·9	3·0	1025	<0·001
Physical activity (MET-h/d)†	34·1	5·4	2178	33·8	6·1	1025	0·191
Education level (%)							
Secondary or below	30·2		657	28·5		292	<0·001
High school	48·9		1064	37·4		383	
College or above	21·0		457	34·1		350	
Household income (yuan/month/person) (%)							
<4000	86·3		1880	83·0		851	0·024
4000–6000	8·2		179	11·1		114	
>6000	5·5		119	5·9		60	
Menopause age (years)	49·8	3·8	2101			–	–
Oestrogen user (%)‡	6·3		138			–	–
Daily dietary intake							
Total energy intake (kJ/d)	6330	1766		7401	1962		
Total energy intake (kcal/d)	1513	422	2178	1769	469	1025	<0·001
VE (mg/d)			2178			1025	
Median	17·8			18·3			
IQR	14·1, 23·0			14·7, 23·8			
Log-VE (mg/d)	18·7	4·7	2178	19·5	5·3	1025	<0·001
Ca (mg/d)			2178			1025	
Median	560			516			
IQR	435, 712			400, 670			
Log-Ca (mg/d)	574	170	2178	544	174	1025	<0·001
Protein (g/d)			2178			1025	
Median	63·7			70·1			
IQR	52·5, 77·6			58·3, 86·4			
Log-protein (g/d)	64·3	9·7	2178	71·7	11·4	1025	<0·001
Serum laboratory assay							
α-TF (μmol/l)	35·0	11·3	1973	30·5	10·5	909	<0·001
Total cholesterol (mmol/l)	5·64	1·09	2092	5·11	1·00	982	<0·001
α-TF:total cholesterol§	6·25	1·89	1915	6·00	1·87	886	0·007
BMD (g/cm^2)							
Whole body	1·061	0·105	2178	1·180	0·102	1025	<0·001
Lumbar spine	0·851	0·146	2178	0·962	0·16	1025	<0·001
Hip							
Total	0·798	0·113	2178	0·900	0·116	1025	<0·001
Trochanter	0·597	0·088	2178	0·665	0·096	1025	<0·001
Intertrochanter	0·957	0·141	2178	1·071	0·141	1025	<0·001
Neck	0·664	0·106	2178	0·744	0·114	1025	<0·001
Ward's triangle	0·497	0·134	2178	0·515	0·131	1025	<0·001
Tea drinker (%)‖	48·4		1054	74·6		765	<0·001
Alcohol drinker (%)¶	3·7		81	17·5		179	<0·001
Current smoker (%)**	0·5		11	38·0		389	<0·001
Ca supplement user (%)††	34·1		743	20·4		209	<0·001
Multivitamin supplement user (%)††	21·3		464	13·6		138	<0·001

MET, metabolic equivalent; VE, vitamin E; TF, tocopherol; BMD, bone mineral density.
* The differences between groups were compared using *t* tests for continuous variables and χ^2 tests for categorical variables.
† Physical activity included occupational, leisure-time and household-chores, presented as MET-h/d (excluding sleeping and sitting time).
‡ Oestrogen user were defined as taking at least a month during menopausal or postmenopausal.
§ *α*-TF:total cholesterol, the ratio of serum *α*-TF:serum total cholesterol.
‖ Tea drinkers were defined as having had tea (e.g. green tea, black tea, oolong) at least twice a week for at least 6 consecutive months.
¶ Alcohol drinkers were defined as having had wine (beer, white wine, red wine) at least once a week for at least 6 consecutive months.
** Smokers were defined as having smoked at least one cigarette daily for at least 6 consecutive months.
†† Ca and multivitamin supplement users were defined as taking at least a month.

concentration of *α*-TF are associated with greater BMD in Chinese women but not in men. Our findings suggest that maintaining better VE nutritional status (in diet and in serum), above and beyond the current RDA in People's Republic of China, may be helpful for the prevention of osteoporosis in women.

In agreement with our findings, several observational studies have shown that VE (in diet and in serum) may play a protective role in bone health. Mata-Granados *et al.*[15] showed that the mean serum VE level was significantly lower in women with osteoporosis than in women with normal BMD (3·0 *v.* 3·5 μmol/mmol)

Table 2. Multiple covariate-adjusted mean bone mineral density (BMD) by quartiles (Q) of dietary vitamin E (VE) intake in 2178 women and 1025 men† (Mean values with their standard errors)

	Q of dietary VE intake									
	Q1		Q2		Q3		Q4			ANCOVA‡
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	% Diff	P_{trend}
Women										
n	544		545		545		544			
BMD (g/cm^2)										
Whole body	1·051	0·004	1·055	0·004	1·065	0·004	1·058	0·004	1·33	0·113
Lumbar spine	0·834	0·006	0·842	0·006	0·854	0·006	0·850	0·006	2·52	0·017
Hip										
Total	0·784	0·004	0·790	0·004	0·808***§	0·004	0·796	0·004	3·06	0·006
Trochanter	0·590	0·003	0·589	0·003	0·603*§	0·003	0·594	0·003	2·20	0·083
Intertrochanter	0·938	0·005	0·948	0·005	0·970***§	0·005	0·957	0·005	3·41	0·001
Neck	0·650	0·004	0·656	0·004	0·673***§	0·004	0·661	0·004	3·54	0·006
Ward's triangle	0·482	0·005	0·488	0·005	0·506**	0·005	0·488	0·005	4·98	0·127
Men										
n	256		256		257		256			
BMD (g/cm^2)										
Whole body	1·183	0·007	1·178	0·006	1·188	0·006	1·173	0·007	0·42	0·501
Lumbar spine	0·969	0·010	0·961	0·010	0·967	0·010	0·953	0·010	−0·21	0·373
Hip										
Total	0·902	0·007	0·896	0·007	0·901	0·007	0·899	0·007	−0·11	0·932
Trochanter	0·670	0·006	0·664	0·006	0·666	0·006	0·662	0·006	−0·60	0·414
Intertrochanter	1·070	0·009	1·066	0·008	1·074	0·008	1·071	0·008	0·37	0·792
Neck	0·744	0·007	0·746	0·006	0·744	0·007	0·742	0·007	0·00	0·774
Ward's triangle	0·515	0·008	0·507	0·008	0·518	0·008	0·521	0·008	0·58	0·430

% Diff., percentage difference = (Q3 − Q1)/Q1 × 100 %.
Mean value was significantly different from that for Q1: * $P<0·05$, ** $P<0·01$, *** $P<0·001$.
† The range of dietary VE intake in each quartile was: 7·7–15·6, 15·6–18·0, 18·0–21·3 and 21·4–66·7 mg/d in women; and 8·0–15·9, 15·9–18·7, 18·7–21·9 and 21·9–53·9 mg/d in men.
‡ In ANCOVA, we adjusted for age, BMI, education level, household income, physical activity, energy-adjusted intake of protein, Ca, Ca supplements, multivitamin supplement use, smoking, alcohol and tea drinking, oestrogen use and menopause age (for women only).
§ Mean value was significantly different from that for Q2 ($P<0·05$).

Table 3. Covariate-adjusted mean bone mineral density (BMD) by quartiles (Q) of serum *α*-tocopherol (TF):cholesterol ratio in 1915 women and 886 men† (Mean values with their standard errors)

	Q of the ratio of serum *α*-TF:cholesterol									
	Q1		Q2		Q3		Q4			ANCOVA‡
BMD (g/cm^2)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	% Diff	P_{trend}
Women										
Whole body	1·048	0·004	1·056	0·004	1·063	0·004	1·061	0·004	1·24	0·022
Lumbar spine	0·828	0·006	0·841	0·006	0·853*	0·006	0·854**	0·006	2·90	0·001
Hip										
Total	0·785	0·005	0·789	0·005	0·798	0·004	0·801	0·005	2·17	0·003
Trochanter	0·588	0·004	0·590	0·004	0·598	0·004	0·600	0·004	2·04	0·008
Intertrochanter	0·941	0·006	0·947	0·006	0·957	0·006	0·961	0·006	2·13	0·008
Neck	0·647	0·004	0·656	0·004	0·667*	0·004	0·665*	0·004	2·78	0·001
Ward's triangle	0·476	0·005	0·489	0·005	0·495	0·005	0·499*	0·005	4·83	0·002
Men										
Whole body	1·180	0·007	1·186	0·007	1·172	0·007	1·191	0·007	0·84	0·585
Lumbar spine	0·968	0·010	0·970	0·010	0·951	0·010	0·972	0·010	0·41	0·870
Hip										
Total	0·898	0·007	0·905	0·007	0·897	0·007	0·903	0·007	0·56	0·862
Trochanter	0·663	0·006	0·672	0·006	0·661	0·006	0·667	0·006	0·75	0·988
Intertrochanter	1·070	0·009	1·075	0·009	1·070	0·009	1·075	0·009	0·47	0·815
Neck	0·741	0·007	0·750	0·007	0·741	0·007	0·751	0·007	1·35	0·543
Ward's triangle	0·516	0·009	0·520	0·009	0·513	0·009	0·525	0·009	1·74	0·586

% Diff., percentage difference = (Q4 − Q1)/Q1 × 100 %.
Mean value was significantly different from that for Q1: * $P<0·05$, ** $P<0·01$.
† The range of serum *α*-TF:total cholesterol ratio in each quartile was: 0·24–5·14, 5·14–6·02, 6·03–7·12 and 7·12–24·45 in women; and 0·44–4·81, 4·81–5·63, 5·63–6·53 and 6·54–15·97 in men.
‡ In ANCOVA, we adjusted for age, BMI, education level, household income, physical activity, energy-adjusted intake of protein, Ca, Ca supplements, multivitamin supplement use, smoking, alcohol and tea drinking, oestrogen use and menopause age (for women only).

in a cross-sectional study on 232 early postmenopausal Spanish women. In a recent case–control study of 726 pairs of hip fracture cases and control patients in People's Republic of China, the highest (*v.* lowest) tertile of dietary VE intake was associated with a lower risk for hip fracture (OR 0·51; 95 % CI 0·36, 0·73)[22]. Similar results were observed in the Swedish Mammography Cohort (61 433 women) and the Uppsala Longitudinal Study of Adult Men (1138 men), in which hip fracture risks increased 1·86 (95 % CI 1·67, 2·06) times in women with the lowest (*v.* highest) quintile of dietary VE intake and 3·33 (95 % CI 1·43, 7·76) times in men with lower VE dietary intake (Q1–4 *v.* Q5); each 1SD decrease in the serum α-TF level was associated with a 1·58-fold (95 % CI 1·13, 2·22) increase in the risk for hip fracture in men[13].

However, such a favourable association of dietary VE intake with BMD at any of the sites was not found in the Women's Health Initiative Observational Study on 11 068 women between 50 and 79 years of age[16]. The Aberdeen Prospective Osteoporosis Screening Study showed that increased dietary VE intake was correlated with a greater loss of BMD at the femur neck and lumbar spine locations (r −0·110 and −0·100; all $P<0·01$) in 891 women between 45 and 55 years of age who were followed up for 5–7 years[31]. Because the subjects in the Women's Health Initiative Observational Study (mean 7·8 mg/d) and the Aberdeen Prospective Osteoporosis Screening Study (baseline mean 8·3 mg/d; follow-up mean 13·3 mg/d) consumed less VE from diet than did those in our population (about 19 mg/d), the lower dietary intake of VE may be responsible for the negative results in these studies. Macdonald *et al.*[31] reported that greater femoral neck BMD loss was associated with increased VE intake (including supplements) in 891 women. Another human interventional study on 25 patients with osteoporosis aged between 45 and 70 years found no significant change in the levels of a biomarker for serum bone formation (specific bone alkaline phosphatase) after 45 and 90 d of VE supplementation (400 mg/d)[32]. Overall, our findings are generally consistent with those of previous studies and support the hypothesis that better VE nutritional status may be beneficial to bone health.

The reasons for the between-study heterogeneity are uncertain. The differences in the proportion of high-dose VE supplementation (primarily α-TF) among previous studies might partly explain the heterogeneity. It is hypothesised that α-TF at high dose may have adverse effects on bone as it may interfere with vitamin K metabolism, block the entry of other VE isomers beneficial to bone into the circulation and become pro-oxidant itself[18]. To some extent, the beneficial effects of dietary VE may thus be offset by the supplementation of high-dose α-TF[33]. Furthermore, the small study size or the short observational period may have limited its power to detect significant differences in changes in BMD or fracture incidence in some previous studies. Other factors such as the study design, the covariates applied for adjustment and ethnic differences may also account, at least in part, for the different findings across these studies.

Several potential mechanisms may explain the favourable association. It has long been demonstrated that oxidative stress can stimulate osteoclastic differentiation and function by increasing the receptor activator of NF-κB (RANK) ligand (RANKL)[34]. After binding to RANK, RANKL stimulates the formation, activity and survival of osteoclasts, resulting in increased bone resorption[35,36]. Oxidative stress may also inhibit bone marrow stromal cell differentiation into osteoblasts via extracellular signal-regulated kinases and their dependent NF-κB-signalling pathway[37]. Oxidative stress may also negatively effect osteoblastogenesis by the Wnt-β-catenin pathway[38]. VE is well known for its antioxidative activities, including free radical scavenging, reduction of damage from reactive oxidant species and inhibition of lipid peroxidation[4,11]. In addition, VE may improve bone health through other approaches such as the improvement of Ca transport and utilisation[39] and the suppression of the bone-resorbing cytokines IL-1 and -6[40,41].

Our findings showed that the favourable VE–BMD association tended to be more pronounced in women than in men, although no significant VE–sex interaction ($P_{interaction}$ range: 0·221–0·575) was observed. Oxidative stress may play an important role in the development of osteoporosis as discussed above. Postmenopausal women often suffer from increased oxidative stress due to oestrogen deficiency. Previous studies suggested that women might be more sensitive to oxidative stress[42,43] and had higher levels of oxidative stress[44] compared with men. In addition, the lower level of serum α-TF and small study size in men than women might also limit us to detect the significantly favourable association in men. Therefore, there is more potential to observe the favourable VE–BMD association in women than in men in the present study.

This study has several strengths. First, the large sample size permitted precise results in women. Next, we assessed both dietary VE intake and the circulating level of α-TF. The consistent results between the dietary intake and the serum level enhance the validity of the favourable association between VE and BMD in our population. Third, we used the ratio of α-TF: cholesterol as the exposure variable of circulating VE to adjust for the influence of serum cholesterol[28]. Fourth, we carefully adjusted for a variety of covariates to avoid related potential confounding.

Several limitations of the present study should be considered. The main limitation is the cross-sectional study design. Although a causal relationship could not be determined, reverse causality could be avoided because we excluded participants with conditions that might have changed their dietary habits. Next, we did not consider VE supplements in the assessment of VE intake because we had not collected the details of the VE supplements. Instead, we adjusted for the use of multivitamin supplements that may contain VE. It was reported that only 2·9 % of old adults used VE supplements in Foshan, a middle city connected to Guangzhou in South China[20]. Therefore, the potential influence of VE supplements would be limited in this study. Third, the correlation coefficient between the FFQ and six 3-d dietary records for dietary VE values (0·25) was relatively lower in the present study. The associations of dietary intake of VE with that of BMD might thus be underestimated because of the large random error in the dietary assessments. Fourth, we recruited the participants through volunteer recruitment, which may have affected the representativeness of the results. Lastly, measurements of serum α-TF were taken only once, which provided a snapshot of nutrient status. However, studies have

reported no seasonal variations and good intra-individual stability of serum α-TF levels for relatively long periods[45,46].

In conclusion, our study adds to the evidence that supports a positive association of VE (in diet and serum) with higher BMD and a lower risk for osteoporosis in a large sample of middle-aged and elderly Chinese women but not in Chinese men. Considering the pitfalls of cross-sectional study design, further prospective studies are needed to verify the findings in our population.

Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114515004134

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Y.-m. C. conceived and designed the study and critically revised the manuscript; W.-q. S. analysed the data and wrote the paper; J. L. and Y. C. were responsible for laboratory assay; W.-q. S., Y.-y. Z. and K. G. collected the data. All the authors participated in paper writing, and read and approved the final version of the manuscript.

There are no conflicts of interest to declare.

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