British Journal of Nutrition (2014), **112**, 1706–1714
doi:10.1017/S0007114514002773

Associations between the dietary intake of antioxidant nutrients and the risk of hip fracture in elderly Chinese: a case–control study

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(Submitted 26 December 2013 – Final revision received 4 August 2014 – Accepted 6 August 2014 – First published online 7 October 2014)

Abstract

The role of oxidative stress in skeletal health is unclear. The present study investigated whether a high dietary intake of antioxidant nutrients (vitamins C and E, β-carotene, animal-derived vitamin A, retinol equivalents, Zn and Se) is associated with a reduced risk of hip fracture in elderly Chinese. This 1:1 matched case–control study involved 726 elderly Chinese with hip fracture and 726 control subjects, recruited between June 2009 and May 2013. Face-to-face interviews were conducted to determine habitual dietary intakes of the above-mentioned seven nutrients based on a seventy-nine-item FFQ and information on various covariates, and an antioxidant score was calculated. After adjustment for potential covariates, dose-dependent inverse associations were observed between the dietary intake of vitamin C, vitamin E, β-carotene, and Se and antioxidant score and the risk of hip fracture (P for trend $\leq 0{\cdot}005$). The OR of hip fracture for the highest (*v.* lowest) quartile of intake were 0·39 (95 % CI 0·28, 0·56) for vitamin C, 0·23 (95 % CI 0·16, 0·33) for vitamin E, 0·51 (95 % CI 0·36, 0·73) for β-carotene, 0·43 (95 % CI 0·26, 0·70) for Se and 0·24 (95 % CI 0·17, 0·36) for the antioxidant score. A moderate-to-high dietary intake of retinol equivalents in quartiles 2–4 (*v.* 1) was found to be associated with a lower risk of hip fracture (OR range: 0·51–0·63, $P<0{\cdot}05$). No significant association was observed between dietary Zn or animal-derived vitamin A intake and hip fracture risk (P for trend $>0{\cdot}20$). In conclusion, a higher dietary intake of vitamins C and E, β-carotene, and Se and a moderate-to-high dietary intake of retinol equivalents are associated with a lower risk of hip fracture in elderly Chinese.

Key words: Antioxidant nutrients: Dietary intakes: Hip fractures: Case–control studies: Chinese

Osteoporotic fractures contribute significantly to the societal disease burden[1]. Hip fracture is considered to be the most severe type of osteoporotic fracture due to the high morbidity, mortality and economic cost[2–4]. Therefore, prevention strategies for hip fracture are particularly important.

Some studies have suggested that oxidative stress plays an important role in bone resorption. Oxidative stress has been shown in basic studies to increase osteoclastic resorption by inducing the activation of NF-κB[5,6], and 8-iso-$PGF_2\alpha$ (a biomarker of oxidative stress) concentrations have been reported to be negatively associated with bone mineral density (BMD) in observational studies[7].

Previous studies[8–10] have shown that higher consumption of fruit and vegetables is associated with higher BMD and bone mass and a reduced risk of fractures. Fruit and vegetables are major sources of antioxidants, such as vitamin C and β-carotene. Therefore, a high intake of fruit and vegetables may be a proxy for a high intake of antioxidants. Several epidemiological studies have investigated the relationships between the retinol equivalent of animal-derived vitamin A and plant-derived β-carotene, vitamin C, vitamin E, Zn, and Se and BMD or fracture, but the findings have been inconsistent[11–19]. Many studies have found a positive association between antioxidant consumption and bone health[11,14,15]. In contrast, some studies have shown retinol-equivalent and animal-derived vitamin A to be associated with a low BMD or risk of fracture[16,17]; in the Women's Health Initiative Study, no significant association was found between retinol equivalents, vitamin C, vitamin E or Se and BMD[12], and a longitudinal study also failed to establish a relationship between Zn and BMD[19]. Conflicting research findings suggest a potentially complex relationship between serum or plasma antioxidant concentrations and skeletal health[20–23]. The different study populations, study designs and sample sizes used may explain the discrepant observations in the studies reported thus far. The majority of previous studies have been conducted in

Abbreviations: BMD, bone mineral density; OC, oral contraceptives; USNBH, Utah Study of Nutrition and Bone Health.

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Western populations, and less is known about the association between the intake of antioxidants and skeletal health in Asian populations. The present study investigated the associations between the consumption of antioxidant nutrients, including vitamins C (mg/d) and E (mg/d), retinol equivalents (μg retinol equivalents/d), animal-derived vitamin A (μg/d), β-carotene (μg/d), Zn (mg/d) and Se (μg/d), and the risk of hip fracture in elderly Chinese.

Participants and methods

Study population

The present case–control study was conducted between June 2009 and May 2013 in Guangzhou, Guangdong Province, China. The total study group comprised 1452 recruits from four participating hospitals and communities. A detailed description of the study design has been published previously[10,24].

In brief, case participants were newly diagnosed (within the previous 2 weeks) with hip fracture on the basis of X-ray examination. Patients with any of the following conditions were excluded from the study: (1) a high-energy or pathological fracture; (2) a change in dietary habits within the previous 5 years; (3) a chronic disease such as diabetes, CVD, cancer, cognitive disorder, liver cirrhosis, thyroid disorder, renal failure or chronic diarrhoea; (4) current use of exogenous oestrogen, thiazine, corticosteroid or other medications; (5) poor vision that might affect routine activities. Control subjects were individually matched (1:1) by sex and age (±3 years) from the same hospitals or the local communities in the same cities. The controls were recruited through local advertisements and subjects' referrals and interviewed within 3 months of the enrolment of the corresponding cases. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the School of Public Health of Sun Yat-sen University (no. 3 in 2009). Written informed consent was obtained from all study participants.

Data collection

Trained interviewers with relevant medical knowledge conducted face-to-face interviews with the study participants and also took anthropometric measurements. Participants' sociodemographic characteristics, lifestyle habits, medical history, family history, years since menopause for females, and dietary and supplement intakes were determined by means of structured questionnaires and recorded. Each interviewer completed an equal proportion of interviews between the case group and the control group.

Calculation of dietary antioxidant intakes

Dietary intake information was collected by means of a modified semi-quantitative FFQ, which was used to determine the frequency ('never', 'per year', 'per month', 'per week' and 'per day') of consumption and amount of seventy-nine food items consumed over the previous year, with a colour picture booklet used as a guide for portion sizes. The average daily intake of a given food item was multiplied by its nutrient content based on the Chinese Food Composition Table 2002[25]. For each study participant, daily intake of energy and nutrients was then calculated by totalling the values across all food items. Retinol equivalents included both animal-derived vitamin A and fruit- and vegetable-derived β-carotene, which can be converted to retinol, and the intake was expressed as retinol equivalents (1 μg retinol equivalent = 1 μg of retinol = 12 μg of β-carotene). The validity and reproducibility of the FFQ have been reported elsewhere[26]. Correlation coefficients for energy-adjusted nutrients assessed from the questionnaires and six 3 d dietary records in 12 months were computed in the local population, and the correlation coefficients for vitamins A, C and E, retinol and carotene were found to be 0·32, 0·32, 0·25, 0·31 and 0·32, respectively[26].

Assessment of covariates

A face-to-face interview was conducted by a trained interviewer using a structured questionnaire to collect information on the following: age (year); sex (male/female); use of oral contraceptives (OC) and oestrogen (yes or no); education level (primary school or below, secondary school, and high school or above); occupation (full non-physical work, main non-physical work, and main physical labour, full physical labour, and others); household income (Yuan/month per person: ≤500, 501–2000, 2001–3000, and >3000); family history of fracture (yes/no); smoking (yes/no); passive smoking (yes/no); alcohol drinking (yes/no); Ca supplement use (yes/no); multivitamin supplement use (yes/no); daily energy intake and intake of selected dietary nutrients (energy-adjusted protein and Ca and P). Subjects who smoked at least one cigarette per d or drank alcohol once a week for at least 6 months were defined as smokers or drinkers. Subjects who had been exposed to other people's tobacco smoking for at least 5 min daily in the previous 5 years were defined as passive smokers. Body height (cm) and weight (kg) were measured in the controls dressed in light clothing and without shoes and self-reported by the case participants. BMI (kg/m^2) was then calculated. Daily physical activity (metabolic equivalent h/d) was estimated using a 24 h physical activity questionnaire containing nineteen items[27].

Statistical analyses

The characteristics of the case participants and control subjects were compared using the t test for continuous variables and Pearson's χ^2 test for categorical variables. The distribution of energy and nutrient intake data was normalised by log transformation. The dietary intakes of all nutrients were adjusted for total energy intake using a residual method[28]. An antioxidant score (ranging between 4 and 16) was calculated by summing the quartile points of each nutrient to evaluate the combined association of vitamins C and E, β-carotene, and Se. The participants were then categorised into quartiles (Q1–Q4) of intake according to the consumption of each energy-adjusted antioxidant or the score in control subjects, and the cut-offs

were applied for the classification of the case participants. The lowest quartile (Q1) was used as the reference.

As the matching of socio-economic factors (education level, household income and occupation) was not successful, both non-conditional and conditional regression methods were used and compared. Non-conditional logistic regression, as in the INTERHEART study[29], was used to estimate the association between the intake of selected antioxidants and the risk of hip fracture as it is a more conservative approach.

Antioxidant nutrient intakes and antioxidant scores were analysed as continuous variables as well as categorical quartile variables to calculate OR and 95% CI. The lowest quartile was considered as the reference quartile in the categorical variable analysis. Trend tests were carried out by modelling the mean values of the intake of each antioxidant nutrient or antioxidant score in the control groups as a continuous variable. Models were adjusted for age, sex + drugs (defined as men and women using OC or oestrogen and women not using OC and oestrogen; model 1). Subsequent models were further adjusted for BMI, education level, occupation, household income, family history of fracture, smoking and alcohol drinking, passive smoking, Ca and multivitamin supplement use, physical activity, daily intakes of energy and energy-adjusted protein and Ca and P (model 2). All covariates were selected using the forward stepwise method.

Interaction analyses were conducted to explore whether the above associations varied in different sexes (male or female) and with the source of the control subjects (community or hospital), and the stratified results by sex (male or female) and the source of the control subjects are reported. Years since menopause, former use of oestrogen and use of OC were further adjusted by female sex in the multivariate analysis. Interaction between sex and the source of the control subjects and the antioxidants studied was tested using the likelihood ratio test. A two-sided P value $<0{\cdot}05$ was considered significant. Considering type 1 error caused by multiple testing, P values were adjusted using Bonferroni correction. P adjusted$=0{\cdot}05$/number of tests. All analyses were conducted using SPSS version 17.0 (SPSS, Inc.).

Results

Study participants

A total of 1402 potential case patients and 1215 potential control subjects from participating hospitals and local communities were screened, and 501 (35·7%) of the former and 355 (29·2%) of the latter were found to not meet the study criteria. An additional 175 case patients and 134 control subjects who did meet the eligibility criteria were excluded for the following reasons: difficulty in communicating (forty-eight case patients and twenty-one control subjects); unreasonable energy intake (nineteen case patients and sixteen control subjects; reasonable range: 3347–16 736 kJ/d (800–4000 kcal/d) for males and 2092–14 644 kJ/d (500–3500 kcal/d) for females); refusal to participate (108 case patients and twenty-four control subjects); a history of fracture (seventy-three control subjects). Thus, 726 case patients and 726 control subjects (542 recruited from local communities and 184 recruited from hospitals) were included in the final analysis. Among the community-based controls, 6% were attendants or relatives of a patient from a non-hip fracture ward without any diseases related to the studied factors or bone health and 94% were recruited from the local communities of the same cities from where the cases came from.

The cases and controls had similar ages of 70 and 71 years in men and women, respectively. The mean values of dietary intakes of selected antioxidant nutrients were 474 and 476 μg retinol equivalents/d, 102 and 106 mg/d for vitamin C, 11 and 10 mg/d for vitamin E, 12·2 and 11·5 mg/d for Zn, and 48 and 48 μg/d for Se among men and women in the control group (Table 1). The dietary intake levels met 59 and 68% (retinol equivalents), 79 and 71% (vitamin E), and 96–106% (vitamin C, Zn and Se) of the values recommended by the Chinese Nutrition Society in 2000.

The characteristics of the participants stratified by case–control status are also given in Table 1. Overall, patients with hip fracture were more likely than control subjects to have a low BMI, to have low levels of education and household income, to engage in physical work, to be smokers, and to have consumed fewer multivitamin and Ca supplements. A trend of low physical activity and low OC and oestrogen use was observed in the female case patients.

Associations between the dietary intake of the studied antioxidant nutrients and the risk of hip fracture

In model 1, a significant inverse association was observed between the dietary intake of each studied antioxidant nutrient and antioxidant score and the risk of hip fracture (P for trend $<0{\cdot}001$–$0{\cdot}035$; Table 2). After adjustment for age, sex, BMI, socio-economic factors, family history of fracture, lifestyle habits, Ca and multivitamin supplement use, physical activity and some dietary factors, the dose-dependent inverse associations between the dietary intake of vitamin C, vitamin E, β-carotene, and Se and antioxidant score and the risk of hip fracture were found to remain significant (P for trend $\leq 0{\cdot}005$). The OR for the highest *v.* lowest quartiles of vitamin C, vitamin E, β-carotene, and Se intake and antioxidant score were 0·39 (95% CI 0·28, 0·56), 0·23 (95% CI 0·16, 0·33), 0·51 (95% CI 0·36, 0·73), 0·43 (95% CI 0·26, 0·70) and 0·24 (95% CI 0·17, 0·36), respectively. A moderate-to-high dietary intake of retinol equivalents (animal- and plant-derived retinol combined) in quartiles 2–4 (*v.* quartile 1) was found to be associated with a reduced hip fracture risk (OR range: 0·51–0·63, all $P<0{\cdot}05$). No significant association was found between the dietary intake of other antioxidant nutrients (animal-derived vitamin A and Zn) and the risk of hip fracture (P for trend 0·661 and 0·277, respectively). Similar results were obtained in the conditional logistic regression analyses (Table S1, available online) as well as when exposure variables were analysed as continuous variables (P range $<0{\cdot}001$–$0{\cdot}040$ for vitamin C, vitamin E, Se, and β-carotene and antioxidant score; Table S2, available online).

In the stratified analysis, no significant interactions were found between the dietary intake of the studied antioxidant

Table 1. Demographics, lifestyle characteristics and selected hip fracture risk factors of the study population
(Mean values and standard deviations; number of participants and percentages)

	Men (*n* 177 pairs)					Women (*n* 549 pairs)				
	Controls		Cases			Controls		Cases		
	Mean	SD	Mean	SD	*P*	Mean	SD	Mean	SD	*P*
Continuous variables*										
Age (years)	69·8	7·1	70·2	7·1	0·560	71·2	6·8	71·3	6·9	0·822
BMI (kg/m^2)	23·3	2·4	21·1	2·2	<0·001	23·1	3·2	21·6	3·9	<0·001
Years since menopause (years)	–	–	–	–	–	21·1	9·4	21·8	9·7	0·242
Physical activity† (MET-h/d)	72	43·4	64·3	45·5	0·103	73·2	54·6	62·4	42·7	<0·001
Dietary intake										
Energy (MJ/d)	6·41	1·62	6·37	1·53	0·836	6·03	1·51	5·74	1·60	0·003
Protein (g/d)	62·7	19·1	58·7	20·5	0·060	59·4	17·9	52·6	18·5	<0·001
Ca (mg/d)	468	208	357	140	<0·001	473	199	372	193	<0·001
Retinol equivalents (μg retinol equivalents/d)	474	227	401	219	<0·001	476	211	395	200	0·003
Vitamin A (animal-derived) (μg/d)	161	113	162	141	0·976	166	127	146	114	0·007
β-Carotene (μg/d)	3749	2243	2909	1535	<0·001	3728	1986	2993	1797	<0·001
Vitamin C (mg/d)	102	60	77	40	<0·001	106	57	82	47	<0·001
Vitamin E (mg/d)	11·10	4·87	8·12	4·78	<0·001	10·07	4·46	7·71	4·11	<0·001
Zn (mg/d)	12·17	3·44	11·98	3·64	0·613	11·46	3·08	10·65	3·38	<0·001
Se (μg/d)	48·3	21·2	43·5	18·3	0·024	47·7	19·4	40·8	17·5	<0·001
	n	%	*n*	%		*n*	%	*n*	%	
Categorical variables‡										
Education level					<0·001					<0·001
Primary school or below	54	30·7	93	52·2		247	45·1	359	66·4	
Secondary school	40	22·7	28	15·7		96	17·5	57	10·5	
High school or above	82	46·6	57	32		205	37·4	125	23·1	
Household income (Yuan/month per person)					0·001					<0·001
≤500	4	2·2	15	8·5		15	2·7	69	12·1	
501–2000	41	23·2	67	37·9		165	30·1	225	41·4	
2000–3000	83	46·9	62	35		241	44	178	32·7	
>3000	49	27·7	33	18·6		127	23·2	75	13·8	
Occupation§					0·028					<0·001
Full non-physical work	42	23·7	40	22·5		98	17·9	82	15·1	
Main non-physical work	62	35	37	20·8		137	25	89	16·4	
Main physical labour	26	14·7	35	19·7		63	11·5	74	13·6	
Full physical labour	41	23·2	58	32·6		200	36·5	268	49·3	
Others	6	3·4	8	4·5		50	9·1	31	5·7	
Family history of fractures	14	7·9	27	15·2	0·032	69	12·6	87	15·9	0·114
Smoker‖	68	38·4	88	49·4	0·036	11	2	28	5·1	0·005
Passive smoking¶	20	11·3	50	28·1	<0·001	100	18·2	129	23·6	0·029
Alcohol drinker**	20	11·3	34	19·1	0·041	24	4·4	15	2·7	0·145
Ca supplement user	50	28·2	25	14	0·001	228	41·6	177	32·4	0·002
Multivitamin user	43	24·3	13	7·3	<0·001	137	25	50	9·1	<0·001
Oral contraceptive user	–	–	–	–	–	92	17	28	5·3	<0·001
Former oestrogen user	–	–	–	–	–	49	9	8	1·6	<0·001

MET, metabolic equivalent.
* Evaluated by *t* tests.
† Physical activities included daily occupational activities, leisure-time activities and household chores, evaluated by MET-h/d.
‡ Evaluated by χ^2 tests.
§ Occupation was categorised into five levels on the basis of labour model.
‖ Smoker was defined as having smoked at least one cigarette daily for at least 6 consecutive months.
¶ Passive smoking was defined as having been exposed to other people's tobacco smoking for at least 5 min daily in the previous 5 years.
** Alcohol drinker was defined as having had alcoholic beverages (wine, beer or Chinese spirits) at least once a week for at least 6 consecutive months.

nutrients and sex or source of the control subjects (Table 3; *P* interactions >0·004, 0·05/fourteen tests).

Discussion

In the present study, a reduced risk of hip fracture was found to be associated with a high dietary intake of vitamins C and E, β-carotene, and Se and with a moderate dietary intake of retinol equivalents. Similar inverse associations were found when antioxidant scores were analysed.

Many epidemiological studies have found that vitamins C and E have beneficial effects on skeletal health[11,13,14]. A nested case–control study of 1120 elderly Swedish women aged 40–76 years included in the Swedish Mammography Cohort showed that a low intake of vitamins C and E increased the risk of hip fracture in current smokers after adjustment for

Table 2. Risk of hip fracture for quartiles (Q) of antioxidant intake in the study population
(Number of cases and controls; odds ratios and 95 % confidence intervals)

	Quartiles of dietary energy-adjusted intake				
Variables	Q1 (lowest)	Q2	Q3	Q4	*P* for trend*
Retinol equivalents					
Intake† (μg retinol equivalents/d)					
Male	216	356	459	678	
Female	226	354	465	673	
Cases (*n*)	324	134	147	120	
Controls (*n*)	181	182	181	181	
OR I‡	1·00	0·41	0·46	0·37	<0·001
95 % CI		0·31, 0·55	0·34, 0·61	0·28, 0·50	
OR II§	1·00	0·51	0·61	0·63	0·050
95 % CI		0·36, 0·72	0·43, 0·88	0·42, 0·98	
β-Carotene					
Intake (μg/d)					
Male	1882	2931	3900	5954	
Female	1622	2648	3987	6281	
Cases (*n*)	287	170	145	123	
Controls (*n*)	181	182	181	181	
OR I	1·00	0·59	0·50	0·43	<0·001
95 % CI		0·44, 0·78	0·38, 0·67	0·32, 0·57	
OR II	1·00	0·68	0·57	0·51	<0·001
95 % CI		0·49, 0·94	0·41, 0·79	0·36, 0·73	
Vitamin A (animal-derived)					
Intake (μg/d)					
Male	64	108	159	308	
Female	64	111	159	303	
Cases (*n*)	212	204	150	159	
Controls (*n*)	181	182	181	181	
OR I	1·00	0·95	0·71	0·75	0·035
95 % CI		0·72, 1·26	0·53, 0·96	0·56, 1·00	
OR II	1·00	1·24	1·08	1·14	0·661
95 % CI		0·89, 1·73	0·75, 1·56	0·80, 1·63	
Vitamin C					
Intake (mg/d)					
Male	55	84	110	167	
Female	49	77	110	171	
Cases (*n*)	311	165	138	111	
Controls (*n*)	181	182	181	181	
OR I	1·00	0·51	0·45	0·35	<0·001
95 % CI		0·39, 0·68	0·34, 0·59	0·26, 0·47	
OR II	1·00	0·52	0·55	0·39	<0·001
95 % CI		0·38, 0·72	0·39, 0·77	0·28, 0·56	
Vitamin E					
Intake (mg/d)					
Male	5·99	8·23	10·36	14·48	
Female	6·37	9·17	11·58	16·74	
Cases (*n*)	388	144	118	75	
Controls (*n*)	181	182	181	181	
OR I	1·00	0·38	0·31	0·19	<0·001
95 % CI		0·29, 0·50	0·23, 0·41	0·14, 0·27	
OR II	1·00	0·46	0·38	0·23	<0·001
95 % CI		0·34, 0·63	0·28, 0·53	0·16, 0·33	
Zn					
Intake (mg/d)					
Male	9·18	10·43	11·52	13·86	
Female	9·74	11·19	12·12	14·55	
Cases (*n*)	257	160	166	142	
Controls (*n*)	181	182	181	181	
OR I	1·00	0·62	0·65	0·54	<0·001
95 % CI		0·47, 0·83	0·49, 0·86	0·41, 0·73	
OR II	1·00	0·83	0·99	0·76	0·277
95 % CI		0·60, 1·15	0·70, 1·39	0·53, 1·10	
Se					
Intake (μg/d)					
Male	31·6	40·8	47·9	60·5	
Female	31·3	39·5	47·7	63·9	
Cases (*n*)	267	196	175	87	
Controls (*n*)	181	182	181	181	

Table 2. *Continued*

Variables	Quartiles of dietary energy-adjusted intake				
	Q1 (lowest)	Q2	Q3	Q4	*P* for trend*
OR I	1·00	0·72	0·65	0·33	<0·001
95 % CI		0·55, 0·95	0·49, 0·86	0·24, 0·45	
OR II	1·00	0·87	0·95	0·43	0·005
95 % CI		0·62, 1·22	0·63, 1·41	0·26, 0·70	
Antioxidant score					
Score	5	8	11	14	
OR I	1·00	0·48	0·32	0·22	<0·001
95 % CI		0·36, 0·64	0·23, 0·43	0·16, 0·31	
OR II	1·00	0·47	0·36	0·24	<0·001
95 % CI		0·34, 0·65	0·26, 0·51	0·17, 0·36	

* Significant levels: $P<0·006$, adjusted using Bonferroni correction.
† Mean intake of vitamin A in male/female controls, which was adjusted for daily energy intake using the residual method, and the mean of daily energy intake was 5669 kJ (1355 kcal) for males and 5347 kJ (1278 kcal) for females.
‡ OR I: from unconditional logistic model adjusted for age and sex + drugs (defined as men and women using oral contraceptives (OC) or oestrogen and women not using OC and oestrogen).
§ OR II: from unconditional logistic model adjusted for age; sex + drugs; BMI; educational level; occupation; household income; family history of fracture; smoking; passive smoking; alcohol drinking; Ca supplement use; multivitamin supplement use; physical activity; daily energy intake; and dietary intake of selected nutrients (protein and Ca and P; energy-adjusted), and all covariates were selected using the forward stepwise method.

age, weight and other osteoporosis risk factors[13]. Similar results were obtained in the Utah Study of Nutrition and Bone Health (USNBH), which examined the risk of hip fracture in 2564 Americans[11], in the Framingham Osteoporosis Study of 4-year bone loss[14], in an interventional study of BMD[30] and in a cross-sectional study[22]. Consistent with the results of these studies, we found a significant inverse association between the increased intake of vitamins C and E and the risk of hip fracture. However, the protective effect exerted by vitamins C and E against hip fracture and BMD was not observed in the Women's Health Initiative Study[12], in the fracture intervention trial study[31], or in a case–control study of 329 American women after adjustment for important covariates[32]. The reasons for the between-study differences remain unclear. Differences in the study designs and in the methods used for dietary intake assessment, as well as the varied populations, might in part explain the discrepancies. Vitamins C and E are important dietary antioxidants. They might improve bone health by scavenging free radicals[33], which have been found to be involved in rodent bone metabolism and to enhance bone resorption[34]. In addition, there is much evidence suggesting that vitamins C and E play a role in the formation of collagen matrices[35,36]. Therefore, they are needed for normal bone development.

Our finding of an inverse association between the dietary intake of β-carotene and the risk of hip fracture is congruent with previously reported findings[11,37]. In the USNBH, Zhang *et al.*[11] observed an inverse association between the intake of β-carotene and the risk of osteoporotic hip fracture in 2564 Americans aged ≥ 50 years. Similar favourable effects of dietary β-carotene[37], serum β-carotene concentrations[38] and plasma β-carotene concentrations[21] on BMD or BMD changes have been found. Furthermore, a cross-sectional study showed dietary β-carotene intake to be inversely correlated with the excretion of deoxypyridinoline (a marker of bone resorption)[39]. However, neither dietary intake nor the serum concentration of β-carotene was found to be associated with hip fracture risk or bone loss in the Nurses' Health Study involving 72 337 postmenopausal women[17], in the Aberdeen Prospective Osteoporosis Screening Study[19], in the Uppsala Longitudinal Study of Adult Men[20], in the Framingham Osteoporosis Study[40] or in the Swedish Mammography Cohort[13]. Vitamin C and many other phytochemicals coexist with β-carotene in foods. Although the positive association between β-carotene and reduced hip fracture risk might be attributed in part to its antioxidant effects[33], many studies have shown phytochemicals such as lycopene to have positive effects on hip fracture risk[40] and BMD[21]. Further studies are needed to clarify the independent association of β-carotene with hip fracture risk by well adjusting for the coexisting phytochemicals.

Studies on the effect of retinol-equivalent intake on skeletal health have yielded inconsistent results. A population-based cohort study of 1526 American women aged ≥ 55 years showed an inverse U-shaped association between animal-derived vitamin A and BMD, particularly at the femoral neck[18]. Opotowsky & Bilezikian[41] also described a U-shaped association between serum vitamin A concentrations and hip fracture risk. Many studies have shown that retinol-equivalent and animal-derived vitamin A in high doses or high serum vitamin A concentrations accelerate bone loss and increase fracture risk[16,17,20]. Consistent with these findings, we found that a moderate intake of retinol equivalents had the strongest positive effect on hip fracture risk. However, we did not find a deleterious effect for the highest quartile, possibly because of a relatively low intake of retinol equivalents in the present study population in comparison with that in other populations[17,19], and the highest intake was observed for the plant-derived retinol equivalents. Nevertheless, several studies have failed to establish a relationship between vitamin A and skeletal health[21,42]. Several biological mechanisms might explain the potential U-shaped association. Vitamin A deficiency has been shown to increase both osteoclastic and osteoblastic activities, resulting in abnormal bone growth in animals[43]. On the other hand, there is much evidence in rodents showing that excessive vitamin A or synthetic retinoid

Table 3. Risk of hip fracture for quartiles (Q) of vitamin and mineral intakes stratified by sex and source of controls in the study population†‡ (Odds ratios and 95 % confidence intervals)

	Quartiles of dietary energy-adjusted intake								
	Q1	Q2		Q3		Q4 (highest)		*P* for trend	*P* for interaction*
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI		
Retinol equivalent									
Sex									0·787
Male	1·00	0·91	0·39, 2·14	2·01	0·87, 4·68	1·11	0·42, 2·89	0·574	
Female	1·00	0·51	0·34, 0·75	0·55	0·36, 0·83	0·66	0·42, 1·04	0·194	
Source of controls									0·690
Hospital	1·00	0·34	0·18, 0·65	0·3	0·15, 0·6	0·35	0·18, 0·72	0·001	
Community	1·00	0·60	0·40, 0·90	0·71	0·47, 1·09	0·76	0·48, 1·23	0·296	
β-Carotene									
Sex									0·941
Male	1·00	1·67	0·74, 3·76	1·85	0·80, 4·27	0·41	0·16, 1·06	0·039	
Female	1·00	0·49	0·33, 0·72	0·36	0·24, 0·53	0·43	0·29, 0·64	<0·001	
Source of controls									0·946
Hospital	1·00	0·66	0·35, 1·24	0·57	0·29, 1·11	0·43	0·21, 0·9	0·016	
Community	1·00	0·58	0·39, 0·86	0·47	0·31, 0·71	0·41	0·27, 0·62	<0·001	
Animal-derived vitamin A									
Sex									0·372
Male	1·00	1·89	0·86, 4·15	3·22	1·32, 7·87	2·23	0·93, 5·30	0·148	
Female	1·00	1·06	0·72, 1·55	0·93	0·62, 1·40	1·06	0·71, 1·58	0·807	
Source of controls									0·200
Hospital	1·00	0·88	0·44, 1·75	0·64	0·34, 1·23	0·46	0·25, 0·85	0·008	
Community	1·00	1·3	0·88, 1·92	1·21	0·78, 1·88	1·48	0·95, 2·31	0·124	
Vitamin C									
Sex									0·605
Male	1·00	0·69	0·31, 1·53	1·01	0·44, 2·32	0·25	0·10, 0·61	0·004	
Female	1·00	0·46	0·32, 0·67	0·42	0·28, 0·62	0·38	0·25, 0·56	<0·001	
Source of controls									0·354
Hospital	1·00	0·55	0·29, 1·03	0·72	0·36, 1·42	0·53	0·24, 1·19	0·076	
Community	1·00	0·49	0·33, 0·73	0·47	0·31, 0·7	0·32	0·21, 0·49	<0·001	
Vitamin E									
Sex									0·213
Male	1·00	0·53	0·26, 1·07	0·13	0·06, 0·31	0·19	0·08, 0·45	<0·001	
Female	1·00	0·50	0·34, 0·73	0·55	0·37, 0·81	0·30	0·19, 0·46	<0·001	
Source of controls									0·029
Hospital	1·00	0·4	0·23, 0·70	0·42	0·19, 0·92	0·52	0·24, 1·11	0·006	
Community	1·00	0·41	0·28, 0·62	0·29	0·2, 0·43	0·15	0·1, 0·23	<0·001	
Zn									
Sex									0·263
Male	1·00	2·09	0·96, 4·54	1·40	0·62, 3·19	1·80	0·74, 4·35	0·303	
Female	1·00	0·73	0·50, 1·07	0·98	0·66, 1·44	0·80	0·53, 1·23	0·443	
Source of controls									0·991
Hospital	1·00	0·53	0·3, 0·94	1·08	0·57, 2·07	0·78	0·34, 1·79	0·265	
Community	1·00	0·88	0·58, 1·32	0·91	0·60, 1·38	0·75	0·48, 1·16	0·240	
Se									
Sex									0·737
Male	1·00	0·57	0·27, 1·21	0·44	0·20, 0·98	0·19	0·07, 0·54	0·002	
Female	1·00	1·01	0·69, 1·47	1·19	0·80, 1·78	0·64	0·41, 0·99	0·001	
Source of controls									0·255
Hospital	1·00	0·73	0·41, 1·32	0·89	0·46, 1·73	0·42	0·2, 0·88	0·052	
Community	1·00	0·87	0·57, 1·31	0·88	0·54, 1·41	0·42	0·23, 0·76	0·013	

*Significant level: $P > 0·004$ (= 0·05/fourteen tests), adjusted using Bonferroni correction.
†Study size: male, 177 pairs; female, 549 pairs; hospital controls, 184 pairs; community controls, 542 pairs.
‡OR from multivariate unconditional logistic regression models. The following covariates were adjusted for: age; BMI; educational level; occupation; household income; family history of fracture; smoking; passive smoking; alcohol drinking; Ca supplement use; multivitamin supplement use; physical activity; daily energy intake; dietary intake of selected nutrients (protein and Ca and P; energy-adjusted). For women, years since menopause, oral contraceptive use, and former use of oestrogen were further adjusted for using the stepwise forward method.

is associated with increased osteoclastic bone resorption[44,45]. These findings suggest that retinol equivalents are required for skeletal growth, but hypervitaminosis A may have a deleterious effect on skeletal health.

Se supplementation can reinforce endogenous antioxidative systems[46]; thus, it may improve bone health by defending against oxidative stress. We found Se to be inversely associated with the risk of hip fracture, and this association has also been observed in other studies[11]. However, no significant association was found in the Swedish Mammography Cohort[13]. The null association might be attributed in part to the pronounced errors in the assessment of Se intake because

of the variation in the Se content of foods between different countries and different regions[47].

To assess the combined association of vitamins C and E, β-carotene, and Se, we further examined the association of the antioxidant score and the risk of hip fracture by summing the quartile points of each nutrient. A more significant inverse association was observed when compared with that observed for individual nutrients, except vitamin E, suggesting that the combination of vitamin C, β-carotene and Se might be more efficient than individual nutrients. We also assessed whether the inverse association was modified by sex and the source of the control subjects. No significant interaction was found after adjusting for the number of multiple tests, indicating similar associations across the subgroups by these variables. However, we had insufficient power to detect the interactions between some studied nutrients and sex or the source of the control subjects with regard to the risk of hip fracture. For interactions between antioxidant nutrients and sex, we had a power <50 % for retinol equivalents, β-carotene and vitamin C, but >90 % for the remaining antioxidant nutrients to detect an OR of 0·7, with an α error of 0·004, assuming an ordinal trend across quartiles of intake. For interactions between antioxidant nutrients and the source of the control subjects, we had a power <30 % for retinol equivalents and β-carotene and >90 % for the remaining exposures[48].

The present study has several limitations. In a case–control study, the time period between the exposure and the outcome is unclear. Nevertheless, this factor might have been mitigated in the present study because only new cases were selected; potential case patients and control subjects with a chronic disease that could have altered dietary habits or nutritional factors were excluded, and adults maintain relatively stable long-term dietary habits[49]. Moreover, recall bias might affect the results. We attempted to minimise recall bias through face-to-face interviews and by visual aids for the assessment of portion sizes. We also controlled interviewer bias by having each researcher interview a similar proportion of cases and controls. Another limitation was the assumptions made in the calculation of antioxidant values for food items. Antioxidant concentrations varied across foods that were combined in one item. When this occurred, we assigned the mean value of the contributing foods. In addition, limited information was collected about the dietary intake of oil, which is a rich source of vitamin E. Measurement errors could have resulted in the misclassification of the participants. However, such errors might have reduced rather than strengthened the observed association. In addition, the calculation of antioxidant score from the intake of antioxidant nutrients could lead to misclassification, and we assumed all the included antioxidants to have contributed equally to the association. Finally, as the study was hospital-based, selection bias could not be excluded completely, despite the fact that the case patients were recruited from various types of hospitals and controls were mainly recruited from the local communities.

In conclusion, the results of the present study suggest that a high dietary intake of vitamin C, vitamin E, β-carotene, and Se and a moderate-to-high dietary intake of retinol equivalents may protect against hip fracture in elderly Chinese.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114514002773

Acknowledgements

The authors are grateful to Dr Wei-fu Ouyang and Sulan Tu for helping with data collection and the doctors and nurses in the above-mentioned hospitals for facilitating both the recruitment of participants and the interviews.

The present study was supported by the National Natural Science Foundation of China (Y.-m. C., grant no. 81072299, 81273049 and 30872100). The sponsor had no role in the design and analysis of the study or in the writing of this article.

The authors' contributions are as follows: Y.-m. C. conceived and designed the study and critically revised the manuscript; L.-l. S. analysed the data and wrote the article; B.-l. L., H.-l. X., F. F., W.-z. Y., B.-h. W. and W.-q. X. carried out the study and data cleansing and wrote the article.

None of the authors has any conflicts of interest to declare.

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