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Effect of apolipoprotein E genotype on vitamin K status in healthy older adults from China and the UK

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The vitamin K concentration in the circulation and the availability of vitamin K to bone may be affected by factors influencing lipoprotein metabolism, such as apoE genotype. The relationships between markers of vitamin K status, bone mineral content and apoE genotype were studied in healthy older men and women aged 60–83 years, 177 from Shenyang, China, and 132 from Cambridge, UK. Fasting plasma was analysed for vitamin K_1, triacylglycerol, total osteocalcin, undercarboxylated osteocalcin (ucOC) and apoE genotype. Hip bone mineral content was measured using dual-energy X-ray absorptiometry. Subjects were grouped according to apoE genotype as E2/3, E3/3 and [E3/4 + E4/4]. The mean plasma vitamin K_1 concentration of the three genotype groups was significantly higher and the percentage ucOC was lower in the Chinese than in the British subjects ($P<0{\cdot}01$). A higher vitamin K_1 concentration was found in subjects with [E3/4 + E4/4] than those with either E2/3 or E3/3 in Cambridge (32·2 (SE 14·6) %, $P=0{\cdot}03$; 24·6 (SE 10·7) %, $P=0{\cdot}02$). Similar trends were observed although were not statistically significant in Shenyang (26·5 (18·9) %, $P=0{\cdot}16$; 23·1 (13·0) %, $P=0{\cdot}08$). Subjects with [E3/4 + E4/4] had a lower percentage ucOC (total osteocalcin adjusted) than did those with either E2/3 or E3/3 in Shenyang (65·1 (27·2) %, $P=0{\cdot}02$; 49·6 (19·9) %, $P=0{\cdot}01$ respectively) but not in Cambridge. This study demonstrates that a superior vitamin K status is associated with the apoE4 genotype in healthy older individuals from China and the UK.

Apo E: Vitamin K_1: Undercarboxylated osteocalcin: Bone: Older adults

Vitamin K is important for bone health through the vitamin K-dependent γ-carboxylation of the bone protein osteocalcin. The proportion of osteocalcin that is not fully γ-carboxylated is a predictor of bone mineral density and fracture incidence (Szulc *et al.* 1996; Booth *et al.* 2003), suggesting that poor vitamin K status may be a risk factor for osteoporosis (Binkley & Suttie, 1995; Vergnaud *et al.* 1997).

Vitamin K_1 (phylloquinone), the predominant dietary and circulating form of vitamin K, is mainly transported in the circulation by triacylglycerol-rich lipoproteins (TRL; Lamon-Fava *et al.* 1998). It is likely that the availability of vitamin K_1 to bone is affected by factors influencing lipoprotein metabolism. One of these factors is apoE, which acts as a ligand for the uptake of lipoproteins into target tissues such as liver and bone (Kohlmeier *et al.* 1996; Newman *et al.* 2002).

The apoE gene is polymorphic. Three common alleles code for three apoE isoforms: E2, E3 and E4, allowing for six possible combinations (E2/2, E2/3, E2/4, E3/3, E3/4 and E4/4). ApoE4 polymorphism has been linked to a lower bone mineral density and an increased risk of osteoporotic fracture (Shiraki *et al.* 1997; Kohlmeier *et al.* 1998; Cauley *et al.* 1999). There are, however, also studies showing a lack of association between apoE genotype and bone mineral density, bone loss or osteoporotic fracture (Booth *et al.* 2000; Heikkinen *et al.* 2000; von Muhlen *et al.* 2001). Research in haemodialysis patients suggested that individuals with the apoE4 genotype had an accelerated hepatic clearance of TRL-vitamin K and therefore less vitamin K available to bone (Saupe *et al.* 1993; Kohlmeier *et al.* 1996). This was, however, challenged by a later study in healthy men, which suggested that the clearance of TRL remnants was impaired in people with the apoE3/4 genotype (Bergeron & Havel, 1996).

There are very few studies on the effect of the apoE genotype on vitamin K status in healthy populations. We previously demonstrated that the incidence of hip fracture in China was low compared with that in Western countries (Yan *et al.* 1999). To explore whether differences in vitamin K nutrition might underlie differences in fracture incidence between Asian and European populations, we conducted two parallel studies investigating the influence of vitamin K status on bone health in older people in Shenyang, northern China, and Cambridge, UK. These studies showed that older people in Shenyang had significantly higher vitamin K_1 intakes, higher plasma vitamin K_1 concentrations and lower proportions of undercarboxylated osteocalcin (ucOC) compared with their British counterparts in Cambridge (Yan *et al.* 2004). The aim of the present study was to explore the influence of apoE genotype on markers of vitamin K status in these two groups of older people with a very different vitamin K status. In addition, the relationship between bone mineral status at the hip and apoE genotype was examined.

Abbreviations: BMC, bone mineral content; tOC, total osteocalcin; TRL, triacylglycerol-rich lipoproteins; ucOC, undercarboxylated osteocalcin.

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Subject and methods

Subjects

All subjects were from a study investigating ethnic differences in bone health in older Chinese and British adults conducted collaboratively by Shenyang Medical College, Shenyang, northern China, and Medical Research Council Human Nutrition Research, Cambridge, UK (Yan *et al.* 2004). Shenyang is one of the largest cities in north-eastern China, as previously detailed (Yan *et al.* 1999, 2004).

The Chinese subjects were eighty-five men (means and standard deviations: age 66·8 (SD 4·6) years, weight 68·9 (SD 9·3) kg, height 166·9 (SD 6·1) cm) and ninety-two women (means and standard deviations: age 64·4 (SD 4·2) years, weight 60·0 (SD 10·2) kg, height 155·1 (SD 5·0) cm) recruited by presentations at universities and factories in Shenyang. The British subjects were sixty-six men (means and standard deviations: age 69·0 (SD 6·1) years, weight 78·6 (SD 9·7) kg, height 173·3 (SD 6·3) cm) and sixty-six women (means and standard deviations: age 67·9 (SD 6·5) years, weight 69·5 (SD 12·2) kg, height 159·7 (SD 7·1) cm) recruited by posters in GP surgeries in Cambridge. All subjects were free of health problems or medications known to alter calcium or bone metabolism, such as thyroid disorders, diabetes, cancer and clotting disorders, and steroid use. Ethical approval for the Shenyang study was given by the Academic Committee of Shenyang Medical College and for the Cambridge study by the NHS Cambridge Local Research Ethics Committee. All participants provided informed written consent.

Laboratory analyses

Blood samples were collected between 07.00 h and 09.00 h after an overnight fast. Plasma was separated from blood cells by a refrigerated centrifuge (Mistral 6000, Sanyo Gallenkamp PLC, Leicester, UK) and both plasma and blood cells were stored at −80 °C until analysis. Plasma samples from Shenyang were transported on dry ice to Human Nutrition Research in Cambridge for the analysis of vitamin K_1, triacylglycerol, total osteocalcin (tOC) and ucOC.

ApoE genotyping was based on a previously described method (Wenham *et al.* 1991). DNA extracted from whole blood (Qiagen Ltd, Crawley, West Sussex, UK) was amplified using Taq polymerase (PCR Core System, Promega, UK) and thirty cycles. The 227 bp product encompassing the polymorphic nucleotides 3745 and 3883 was restricted for 4 h at 37°C with 20 units of the restriction endonuclease Cfo (Promega, Southampton, UK). Genotype was determined using a 4 % agarose gel (Invitrogen Ltd, Paisley, UK). The apoE genotyping of the Shenyang samples was conducted at Shenyang Medical College, and that of the Cambridge samples was conducted at Human Nutrition Research using the same procedures and materials. A cross-calibration of ten samples was performed, which confirmed that the genotyping by both laboratories was identical.

Plasma vitamin K_1 concentration was measured by HPLC with fluorometric detection, as previously described in detail (Wang *et al.* 2004). In brief, vitamin K_1 compounds were isolated from 0·25 ml plasma by liquid–liquid extraction, followed by solid-phase extraction. An internal standard of vitamin $K_{1(25)}$ (a synthetic homologue of vitamin K_1; Immundiagnostik AG, Bensheim, Germany) was used to compensate for losses of vitamin K_1 during the extraction process and subsequent chromatographic procedures. The detection limit for 0·25 ml plasma was 0·02 ng/ml. To ensure the quality assurance in routine analysis, three plasma pools containing three different levels of phylloquinone, 0·4, 1·4 and 3·4 nmol/l, were prepared as the quality control samples. The intra-assay CV were 5·2 %, 8·2 % and 3·0 %, and the inter-assay CV 16 %, 12 % and 8·1 %, respectively. Triacylglycerol concentration was measured by a colorimetric method (Thermo Clinical Labsystems Oy, Espoo, Finland). The intra- and interassay CV were 1·3 % and 5·0 %, respectively.

Plasma tOC and ucOC were analysed by one-step ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark), as previously described (Yan *et al.* 2004). The binding of osteocalcin to hydroxyapatite was determined by incubating 60 μl freshly thawed plasma with 30 μl hydroxyapatite (15 μg/l; Calbiochem, Merck Biosciences, Darmstadt, Germany) in duplicate. The intra- and interassay CV were 4·1 % and 10·5 % for tOC, and 15·9 % and 14·3 % for ucOC, respectively. The detection limit for the hydroxyapatite-treated sample was 0·18 μg/l. ucOC concentrations were undetectable in the samples from nineteen Chinese subjects; a nominal concentration of 0·09 μg/l was assigned for these samples. The ucOC measured was expressed as both concentration (μg/l) and as a percentage of tOC.

Bone mineral content (BMC) and bone area at the femoral neck and trochanter were measured using dual-energy X-ray absorptiometry (DPX-L (software 1·3z) in Shenyang, and DPX MD (software 4·7 d) in Cambridge; GE Lunar, Madison, US). Cross-calibration of the two dual-energy X-ray absorptiometry machines was performed by scanning the European Spine Phantom, as previously detailed (Yan *et al.* 2003).

Data-handling and analysis

We previously showed that plasma vitamin K_1 concentration was positively related to vitamin K_1 intake and plasma triacylglycerol concentration, whereas percentage ucOC was positively related to tOC and negatively related to plasma vitamin K_1 concentration (Yan *et al.* 2004). These factors were all considered when a possible effect of apoE genotype was examined.

Statistical analysis was performed by Linear Model software in Data Desk 6.1.1 (Data Descriptions, Ithaca, NY; 1995). The χ^2 test was used to examine the differences in genotype. Multiple linear regression and analysis of covariance were used to examine the relationships between plasma biochemical markers and apoE genotype adjusting for potential confounding factors such as age, sex, country and others. Interaction terms were used to examine differences (slopes) in the relationships between vitamin K_1 (or percentage ucOC) and apoE genotype in the Chinese and British groups. To correct for skewed distributions and to permit an exploration of proportional relationships, all continuous variables except age were converted to natural logarithms. In natural logarithms, group differences × 100 correspond closely to percentage differences calculated as (difference/mean) × 100 (Prentice *et al.* 1991).

In our data analysis, a correction was made for tOC when expressing the relative proportion of ucOC. Conventionally, this is achieved by expressing ucOC concentration as a percentage of total OC concentration ([ucOC/tOC] × 100). This, however, implies a relationship between ucOC and tOC that is one of direct proportion. In the present study, this was shown not to be the case. This was because the relationship between ucOC and tOC concentration, examined by the regression analysis of

logged variables, was not directly proportional. The coefficient for the relationship in the British population was 1·17 (SE 9·6), $P<0·0001$) but that in the Chinese population was 2·19 (SE 20·0, $P<0·0001$; Yan *et al.* 2004), demonstrating that ucOC and tOC were related in an approximately squared manner, which meant that a 1 % increase in tOC resulted in a more than 2 % increase in ucOC. In order to fully adjust for the effect of tOC on ucOC, tOC was included in the analysis of covariance models and removed from the model when not significant at $P<0·05$.

For exploring the relationship between bone mineral status and apoE genotype, BMC adjusted for bone area, body weight and height (size-adjusted BMC) was used (Prentice *et al.* 1994). All statistical models were set up in the same way, i.e. full models were generated and then variables $P>0·05$ were removed by backward elimination to provide a parsimonious model.

Results

The distribution of apoE genotypes was in Hardy–Weinberg equilibrium in both the Chinese and British samples. The frequency of the apoE3/3 genotype was higher in Shenyang than in Cambridge ($P=0·002$). As a result, the frequency of total apoE4 allele was lower and that of total apoE3 allele higher in Shenyang than in Cambridge ($P<0·01$;Table 1). No individual with an apoE2/2 genotype was found in either population, and the E2/4 genotype was not present in the Chinese group. To make the investigation consistent and comparative across the two populations, the effect of apoE genotype on vitamin K status and BMC was restricted to the three common genotypes E2/3, E3/3 and [E3/4 + E4/4] (Tables 2 and 3). The five British subjects with E2/4 were therefore not included in further analyses.

The mean vitamin K_1 intake and plasma vitamin K_1 concentration of the three genotype groups were significantly higher in the Chinese than in the British subjects, and the plasma ucOC concentration and percentage ucOC were significantly lower (Table 2). A higher vitamin K intake was found in subjects with [E3/4 + E4/4] than in those with E3/3 in Shenyang (32·5 (SE 12·4) %, $P=0·01$]; whereas a higher vitamin K intake was found in subjects with E3/3 than in those with E2/3 in Cambridge (25·5 (SE 12·2) %, $P=0·04$). Neither plasma triacylglycerol nor

Table 1. Frequency of apoE genotypes and alleles in Shenyang and Cambridge subjects†

	Shenyang (*n* 177)		Cambridge (*n* 132)	
	n	%	*n*	%
Genotypes				
ApoE2/2	0	–	0	–
ApoE2/3	25 (M10, F15)	14·1	24 (M15, F9)	18·2
ApoE2/4	0	–	5 (M2, F3)**	3·8
ApoE3/3	123 (M62, F61)	69·5	69 (M31, F38)**	52·3
ApoE3/4	28 (M13, F15)	15·8	32 (M17, F15)	24·2
ApoE4/4	1 (F1)	0·6	2 (M1, F1)	1·5
ApoE alleles				
ApoE2	25	7·1	29	11·4
ApoE3	299	84·5	194**	73·1
ApoE4	30	8·4	41**	15·5

M, men; F, women.
† All distributions were in Hardy–Weinberg equilibrium. Comparisons between Shenyang and Cambridge made by χ^2 analyses.
** $P<0·01$.

Table 2. Plasma vitamin K_1, triacylglycerol and osteocalcin concentrations in Shenyang and Cambridge subjects according to apoE genotype (Variables transformed to natural logarithms and comparisons made by ANOVA with Scheffé *post hoc* analysis)

		Chinese						British					
		E2/3 (*n* 25)	SE	E3/3 (*n* 123)	SE	[E3/4 + E4/4] (*n* 29)	SE	E2/3 (*n* 24)	SE	E3/3 (*n* 69)	SE	[E3/4 + E4/4] (*n* 34)	SE
Vitamin K_1 intake (μg/d)	Geometric mean	252		233		320[a]		86**		112[b, **]		95**	
	Ln mean	5·53	0·13	5·45	0·05	5·77	0·10	4·46	0·09	4·72	0·07	4·55	0·07
Plasma vitamin K_1 (nmol/l)	Geometric mean	2·14		2·03		2·92		0·58**		0·67**		0·81[a, c, **]	
	Ln mean	0·76	0·13	0·71	0·06	1·07	0·15	−0·54	0·12	−0·40	0·06	−0·21	0·09
Triacylglycerol (mmol/l)	Geometric mean	1·34		1·23		1·48		1·23		1·06*		1·13*	
	in mean	0·29	0·11	0·21	0·05	0·39	0·11	0·21	0·10	0·06	0·04	0·12	0·08
Total osteocalcin (μg/l)	Geometric mean	15·8		15·0		16·4		17·6		20·1**		21·3**	
	Ln mean	2·76	0·09	2·71	0·04	2·80	0·05	2·87	0·06	3·00	0·05	3·06	0·08
Undercarboxylated osteocalcin (μg/l)	Geometric mean	2·56		1·93		1·48[a,c]		6·36**		5·87**		7·32**	
	Ln mean	0·94	0·21	0·66	0·12	0·39	0·29	1·85	0·11	1·77	0·09	1·99	0·13
% Undercarboxylated osteocalcin	Geometric mean	16·1		12·8		9·0[a,c]		33·8**		28·8**		29·1**	
	Ln mean	2·78	0·14	2·55	0·10	2·20	0·26	3·52	0·07	3·36	0·06	3·37	0·07

[a] E3/4 and E4/4 *v.* E3/3 within the same population, $P<0·05$.
[b] E3/3 *v.* E2/3 within the same population, $P<0·05$.
[c] E3/4 and E4/4 *v.* E2/3 within the same population, $P<0·05$.
* $P<0·05$, ** $P<0·01$ significantly different from those in Chinese subjects with the same genotype.

Table 3. Differences in plasma vitamin K_1 and percentage undercarboxylated osteocalcin (%ucOC) in Chinese and British subjects with different apoE genotype (Variables transformed to natural logarithms and percentage differences obtained by analysis of covariance with Scheffé *post hoc* analysis)

	E2/3 *v.* E3/3				[E3/4 + E4/4] *v.* E2/3				[E3/4 + E4/4] *v.* E3/3			
	Chinese (*n* 25 *v.* 123)		British (*n* 24 *v.* 69)		Chinese (*n* 29 *v.* 25)		British (*n* 34 *v.* 24)		Chinese (*n* 29 *v.* 123)		British (*n* 34 *v.* 69)	
	% difference	SE	% difference	SE	% difference	SE	% difference	SE	% difference	SE	% difference	SE
Plasma vitamin K_1 (nmol/l)	−0·4	13·7	−6·1	13·0	+26·5	18·9	+32·2	14·6[a]	+23·1	13·0	+24·6	10·7[a]
%ucOC	+14·0	18·9	+15·3	11·0	−65·1	27·2[b]	−15·0	10·0	−49·6	19·9[b]	+1·5	9·8

[a] Significantly different after adjusting for vitamin K_1 intake, triacylglycerol concentration and gender, $P<0·05$.
[b] Significantly different after adjusting for total osteocalcin and gender, $P<0·05$.

tOC concentration was significantly related to apoE genotype in any population. A higher plasma vitamin K_1 concentration was found in subjects with [E3/4 + E4/4] than in those with either E2/3 or E3/3 in Cambridge after adjusting for vitamin K_1 intake, plasma triacylglycerol concentration and gender (Table 3). Similar trends were observed although were not statistically significant in Shenyang (Table 3). The magnitude of the effect was similar in Cambridge and Shenyang (Table 3), and no interaction was found in the two-country combined data, suggesting that there was no evidence of a country difference in the relationship between apoE genotype and plasma vitamin K_1 concentration ($P=0·75$). Subjects with [E3/4 + E4/4] had a lower percentage ucOC than those with either E2/3 or E3/3 in Shenyang ($P=0·02$ and $P=0·01$, respectively, Table 3), but these associations were not significant in Cambridge ($P=0·14$ and $P=0·94$, respectively). There was no significant interaction in this relationship between counties despite a large apparent difference in response ($P=0·36$). Size-adjusted BMC at the hip was not significantly related to apoE genotype at either the femoral neck or trochanter in either population (data not shown).

Discussion

The present study of healthy older men and women has shown that vitamin K status, as measured using vitamin K_1 concentration and percentage ucOC in the plasma, is better in China than in Britain, and in individuals with one or two copies of the apoE4 allele, suggesting that these individuals may be at lower risk of osteoporotic fracture (Szulc *et al.* 1996; Vergnaud *et al.* 1997).

The possibility that apoE4 is a genetic risk factor for osteoporosis and fracture has been investigated in previous studies, but results are inconsistent. In contrast to our findings, some have demonstrated that older people with one or two apoE4 alleles have a lower lumbar spine bone mineral density (Shiraki *et al.* 1997; Cauley *et al.* 1999), and an increase in fracture risk (Kohlmeier *et al.* 1998; Cauley *et al.* 1999), compared with people with no apoE4 allele, but other results (Booth *et al.* 2000; Heikkinen *et al.* 2000; von Muhlen *et al.* 2001) do not support these findings.

Furthermore, our observation that a higher plasma vitamin K_1 concentration or a lower percentage ucOC was associated with apoE4 is different from that reported in haemodialysis patients. These studies showed that plasma vitamin K_1 concentration was highest among those individuals with one or two copies of the apoE2 allele, intermediate among those homozygous for apoE3, and lowest among those with one or two copies of the apoE4 allele (Saupe *et al.* 1993; Kohlmeier *et al.* 1995). It was suggested that this distribution is in accordance with the relationship between apoE genotype and the rate of hepatic clearance of chylomicron remnants from the circulation, the E4 allele being associated with the most rapid catabolism (Kohlmeier *et al.* 1996). However, a later study in healthy young men (Bergeron & Havel, 1996) showed that the clearance of TRL remnants was slower in subjects with the apoE3/4 compared with the apoE3/3 genotype. If this is true, the higher plasma vitamin K_1 concentration and lower percentage ucOC in the subjects with E4 allele found in the present study could be due to a slower clearance of TRL remnants from the circulation, and subsequently more vitamin K_1 rich-lipoprotein being available for uptake by bone.

In addition, direct evidence that apoE plays an important role in the uptake of lipoprotein-borne vitamin K_1 into osteoblasts has

been reported (Newman *et al.* 2002). This study demonstrated that the osteoblast uptake of vitamin K was mediated by apoE in TRL-rich lipoproteins and heparan sulphate proteoglycans on the osteoblast surface. ApoE4 seems to stimulate cellular binding (Cullen *et al.* 1998) and uptake to a greater degree than other isoforms (Newman *et al.* 2002). The relationship between apoE genotype and percentage ucOC in the Chinese subjects would support this mechanism. The lack of a significant effect in British subjects may reflect their lower vitamin K status overall and consequently the smaller range of percentage ucOC observed.

The effect of apoE genotype on markers of vitamin K status in healthy populations has been little studied. The association between apoE4 and percentage ucOC adjusted for tOC found in the present study was different from that observed in a small pilot study in which a lower tOC-adjusted ucOC level was related to apoE2 in British and Chinese women (Beavan *et al.* 2005). The carboxylation of osteocalcin in the pilot study was investigated by RIA (Incstar Corporation, Stillwater, MN, USA). The mean tOC concentrations obtained were relatively low, 1–5μg/l, and close to the level of detection for the assay (Beavan *et al.* 2005). Owing to the already low tOC, ucOC concentrations after hydroxyapatite binding were very low, being not detectable in 62% of samples. In the present study, we used a more sensitive method: ELISA (Nordic Bioscience Diagnostics). Although the absolute values cannot be compared between different assays, the mean concentrations of tOC measured by the ELISA method (15–21μg/l) were much higher compared with the detection limit. ucOC were still undetectable in some Chinese samples, but the proportion was much lower (10·7%). We believe that the different techniques used in the two studies contributed significantly to the discrepancy between them, although other factors might also be involved.

We observed that a higher vitamin K intake was found in subjects with [E3/4 + E4/4] than in those with E3/3 in Shenyang, and in subjects with E3/3 than in those with E2/3 in Cambridge. When the effect of apoE genotype on plasma vitamin K concentration was examined, vitamin K intake was included in analysis of covariance models as one of the potentially confounding independent variables. Therefore, the effect of vitamin K intake had been eliminated. If there was anything in the British group, it should be the other way round because subjects with E3/3 had a relatively higher vitamin K intake but a lower plasma vitamin K concentration than those with [E3/4 + E4/4] (Tables 2 and 3).

We could not find any significant association between plasma triacylglycerol concentration and apoE genotype in this study, unlike some reports (Dallongeville *et al.* 1992). Vitamin K_1 is mainly carried by chylomicron remnants after a meal (Kohlmeier *et al.* 1996). The blood samples in our study were collected in the early morning after an overnight fast. This could explain why an association between plasma vitamin K_1 and triacylglycerol concentrations was not found (Kohlmeier *et al.* 1996). Second, fasting plasma triacylglycerol levels varied widely between individuals (Table 2). This variability could also mask a clear effect of apoE genotype on triacylglycerol levels.

The apoE4 allele has been associated with low bone mineral density (Shiraki *et al.* 1997) and bone fracture (Kohlmeier *et al.* 1998), which has been attributed to a modulation of vitamin K transport, although others have not been able to find these associations (Booth *et al.* 2000; Heikkinen *et al.* 2000; Stulc *et al.* 2000). In this study, we did not find any association between size-adjusted BMC at the hip and apoE genotype in any population. We appreciate, however, that the sample size of our study was limited for investigating a possible genotype effect on bone mineral status.

In summary, our study demonstrates that a superior vitamin K status, as demonstrated by either higher plasma vitamin K_1 concentration or lower percentage ucOC, is associated with the apoE4 genotype in healthy older individuals from China and the UK. The fact that these relationships or trends of the association are seen within two populations with very different vitamin K status suggests that it is mediated through effects of apoE on vitamin K transport to and uptake into bone.

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