Diet and bone mineral density study in postmenopausal women from the TwinsUK registry shows a negative association with a traditional English dietary pattern and a positive association with wine[1–4]

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ABSTRACT

Background: The effect of diet on bone mineral density (BMD) remains controversial, mainly because of difficulties in isolating dietary factors from the confounding influences of age, lifestyle, and genetic factors.

Objective: The aim of this study was to use a novel method to examine the relation between BMD and diet.

Design: A co-twin control study design with linear regression modeling was used to test for associations between BMD and habitual intakes of calcium, vitamin D, protein, and alcohol plus 5 previously identified dietary patterns in postmenopausal women from the TwinsUK registry. This approach exploited the unique matching of twins to provide an estimate of an association that was not confounded by age, genetic background, or shared lifestyle.

Results: In >2000 postmenopausal women (BMD data on 1019, 1218, and 1232 twin pairs at the hip neck, hip, and spine, respectively), we observed a positive association between alcohol intake (from wine but not from beer or spirits) and spine BMD ($P = 0.01$) and a negative association with a traditional 20th-century English diet at the hip neck ($P = 0.01$). Both associations remained borderline significant after adjustment for mean twin-pair intakes ($P = 0.04$ and $P = 0.055$, respectively). Other dietary patterns and intakes of calcium, vitamin D, and protein were unrelated to BMD.

Conclusion: Our results showed that diet has an independent but subtle effect on BMD; wine intake was positively associated with spine BMD, whereas a traditional (20th-century) English diet had a negative association with hip BMD.   *Am J Clin Nutr* doi: 10.3945/ajcn.111.019992.

INTRODUCTION

The results of many studies suggest that diet plays a critical role in modulating bone mineral density (BMD) in the achievement of peak bone mass (1) and in changing the rate of bone loss in postmenopausal women and elderly men (2). However, interactions between diet, other environmental variables, and genotype can result in the misclassification of dietary modulators of bone health. The primary objective of this study was to examine the diet of 1232 pairs of postmenopausal twins by using a validated food-frequency questionnaire (3) and to relate dietary pattern scores and intakes of a limited selection of dietary constituents reported to modulate BMD (calcium, vitamin D, protein, and alcohol) to measures of BMD in the hip and spine. The association has been studied in adult female twins by using

a co-twin control study design. This approach allows the strength of the association with diet to be estimated after any possible confounding effects of age, genotype, and shared environmental factors were taken into account.

SUBJECTS AND METHODS

Study subjects

Study subjects were twins enlisted in the TwinsUK registry, which is a national register of adult twins. Twins were recruited to the registry as volunteers through a series of media campaigns (4) and were not chosen for disease-specific investigations. In this study, we analyzed data from 1074 monozygotic and 1390 dizygotic pairs of twins who were postmenopausal women (UK residents) without metabolic disorders that were likely to alter the dietary intake or require its modification and who attended the Guy's and St Thomas' Hospital for clinical assessment between 1993 and 2004. The members of the TwinsUK registry have been shown not to differ from age-matched singleton women in the distribution of common traits and outcomes, including BMD (5), and to have dietary intakes comparable with other Western populations (3). The twins were brought up together and lived apart in adult life. All participants gave written, informed consent, and the Guy's and St Thomas' Hospital ethics committee approved the study.

Phenotypic variables

Participants completed a validated 131-item food-frequency questionnaire that was previously developed for the EPIC

(European Prospective Investigation into Cancer and Nutrition) study (6), from which nutrient intakes were determined by using an established nutrient database. For each food group, the frequency of intake (servings/wk) was adjusted for the total energy intake by using the residual method; energy-adjusted intakes were standardized to z scores, and these were used in the principal components analysis (PCA) (3). Five dietary patterns were calculated, which were referred to as fruit and vegetables, high alcohol, traditional English, dieting, and low meat. As artificial PCA-generated scores, these were independent variables standardized to have a mean of zero and an SD of one in the whole twin population. In addition, mean daily intakes of 4 specific nutrients that have previously been reported to modulate BMD, calcium, vitamin D, protein (7), and alcohol (8) were estimated for each participant. These 4 nutrients were chosen to focus the analysis on the reported association and to limit the number of independent tests. Dual-energy X-ray absorptiometry (QDR 2000W; Hologic) was used to measure the BMD of participants for the total hip, femoral neck, and lumbar spine. BMI was calculated by dividing weight in kilograms by the square of height in meters. Subjects also completed questionnaires that included questions on supplement use, menopausal history, smoking history, and physical activity (coded as inactive, moderately active, and active).

Statistical methods

The residual method (9) was used to adjust for total energy intake. These energy-adjusted dietary variables were standardized to have a mean of zero and an SD of one. Analyses were adjusted for age and the square of age, BMI, smoking, and physical activity. SEs derived from all models were adjusted for clustering by twin pair.

Linear regression analysis was first undertaken by treating the twins as individuals, which allowed comparison with results from singleton populations, as follows:

$$E(Y_{ij}) = \beta_0 + \beta_c X_{ij} \qquad (1)$$

where Y_{ij} and X_{ij} represent the BMD (Y) and dietary variable (X), respectively, of twin j from pair i. β_c represents the expected change in the transformed BMD per 1-SD increase in the energy-adjusted dietary intake in individuals.

Second, following the approach recommended by Begg and Parides (10), the association on BMD of each dietary factor was examined in a model that additionally included the twin-pair mean X_i for the dietary factor

$$E(Y_{ij}) = \beta_0 + \beta_i X_{ij} + \beta_t \bar{X}_i \qquad (2)$$

Here, β_t can be interpreted as the effect of a 1-SD increase in the pair-averaged dietary factor on BMD with the individual dietary factor held fixed. β_i can be interpreted as the effect of a 1-SD increase in the individual's dietary factor on BMD with the pair average held fixed.

A key point is that this model enabled us to examine confounding by family (pair)-level influences. The pair mean of the dietary factor might act as a surrogate for influential family-level attributes, both environmental and genetic. If β_i is significant, but

β_t is not significant, this suggests that we have identified a robust relation that is not due to the shared genotype or environmental confounding by unknown, common factors. In contrast, where β_c and β_t are significant, but β_i is not significant, this suggests that any apparent relation seen in singletons may be due to unidentified environmental or genetic confounding.

P values for all analyses were adjusted for multiple testing by using a permutation-based approach in which the BMD values were swapped between twin pairs, keeping all other variables unchanged, thus retaining the twin-pair structure of both the food and BMD data. Analyses were carried out for 10,000 replications to approximate the distribution of P values when there was no association between dietary variables and BMD. This distribution was used to adjust raw P values. Analyses were carried out with Stata 10 software (StatCorp).

RESULTS

Data were available for between 2038 and 2464 individual twins (of whom 1074 were monozygotic and 1390 were dizygotic) depending on the BMD site measured (**Table 1**). Twins were postmenopausal women with a mean ($\pm$SD) age of 56.3 $\pm$ 11.9 y. Mean ($\pm$SD) BMI was 26.0 $\pm$ 4.5. Mean ($\pm$SD) BMDs for the hip neck, total hip, and lumbar spine were 0.77 $\pm$ 0.12, 0.87 $\pm$ 0.15, and 0.95 $\pm$ 0.15 g/cm^2, respectively (Table 1). The estimated dietary intake was based on duplicate food questionnaires completed at different time points for just over one-half of participants (57%), which provided a better estimate of the habitual intake. The mean interval between first and last food questionnaires by subjects who completed more than one questionnaire was 9.6 y. The mean ($\pm$SD) daily dietary intakes of calcium, vitamin D, protein, and alcohol for all individuals are shown in Table 1; 84% of participants reported taking

TABLE 1
Characteristics of twins[1]

	Values
Age (y) (n = 2464)	56.3 $\pm$ 11.9
BMI (kg/m^2) (n = 2464)	26.0 $\pm$ 4.5
Hip neck BMD^2 (g/cm^2) (n = 2038)	0.77 $\pm$ 0.12
Total hip BMD (g/cm^2) (n = 2436)	0.87 $\pm$ 0.15
Lumbar spine BMD (g/cm^2) (n = 2464)	0.95 $\pm$ 0.15
Alcohol (g/d) (n = 2464)	9.2 $\pm$ 12.3
Calcium (mg/d) (n = 2464)	1104.5 $\pm$ 356.1
Protein (g/d) (n = 2464)	81.3 $\pm$ 20.4
Vitamin D (μg/d) (n = 2464)	2.6 $\pm$ 1.2
Energy-adjusted alcohol (g/d) (n = 2464)	9.2 $\pm$ 12.2
Energy-adjusted calcium (mg/d) (n = 2464)	1111.0 $\pm$ 250.9
Energy-adjusted protein (g/d) (n = 2464)	81.8 $\pm$ 11.4
Energy-adjusted vitamin D (μg/d) (n = 2464)	2.6 $\pm$ 1.0
Smoking status [n (%)]	
Never	1401 (56.9)
Former	814 (33.0)
Current	249 (10.1)
Physical activity [n (%)]	
Inactive	806 (34.9)
Moderately active	1203 (48.8)
Active	401 (16.3)

[1] All values are means $\pm$ SDs.
[2] BMD, bone mineral density.

calcium supplements, and 89% of participants reported taking vitamin D supplements.

Associations of BMD at each site with the derived score for 5 patterns of dietary exposure from our PCA (fruit and vegetables, high alcohol, traditional 20th-century English, dieting, and low meat) and the 4 selected dietary variables are shown in **Table 2**. β Coefficients are shown for 2 models as follows: model 1 treated twins as individuals (β_c), and model 2 included individual observations (β_i shown) and the twin-pair mean of the dietary variable (β_t not shown). These models allowed for the examination of possible confounding by environmental or genetic factors shared by twins.

The analysis of dietary patterns showed that the traditional English pattern had a significant negative effect on BMD at all 3 sites after multivariate adjustment (Table 2), but the association only remained significant for the hip neck ($P = 0.01$) after adjustment for multiple comparisons. This association for the hip neck was present even after adjustment for twin-pair intake means ($P = 0.012$; model 2), although the adjusted P value was only of borderline significance ($P = 0.055$). None of the other dietary patterns had a significant association on BMD. For the 4 selected dietary components (calcium, vitamin D, protein, and alcohol), only alcohol intake had a significant association with BMD; higher alcohol intakes were associated with increased BMD in the spine after adjustment for multiple comparisons and when the twin-pair mean was included in the same model. When each type of alcohol was modeled separately for the hip neck, energy-adjusted servings of wine were associated with increased BMD ($\beta = 0.044$, $P = 0.01$), but beer and spirits showed no association. After differences between twins in model 2 were taken into account, no additional association was seen with the twin-pair mean itself for scores of either alcohol or the

TABLE 2
Regression analyses of pattern scores, selected nutrients, and BMD[1]

Dietary pattern or nutrient and sites	No. of pairs	Model 1[2]			Model 2[3]		
		β_c (95% CI)	P	Adjusted P[4]	β_i (95% CI)	P	Adjusted P[4]
Fruit and vegetable pattern score							
Spine	1232	0.020 (−0.018, 0.058)	0.309	0.850	0.022 (−0.029, 0.073)	0.393	0.920
Total hip	1218	0.030 (−0.002, 0.062)	0.064	0.287	0.027 (−0.015, 0.068)	0.205	0.695
Hip neck	1019	0.029 (−0.008, 0.066)	0.123	0.480	0.017 (−0.032, 0.065)	0.501	0.970
High-alcohol pattern score							
Spine	1232	0.023 (−0.014, 0.059)	0.225	0.730	0.028 (−0.019, 0.074)	0.241	0.750
Total hip	1218	0.004 (−0.032, 0.040)	0.839	1.000	0.028 (−0.020, 0.076)	0.250	0.776
Hip neck	1019	0.030 (−0.009, 0.070)	0.132	0.506	0.021 (−0.029, 0.072)	0.410	0.933
Traditional English pattern score							
Spine	1232	−0.035 (−0.069, 0.000)	0.048	0.224	−0.016 (−0.060, 0.028)	0.485	0.964
Total hip	1218	−0.039 (−0.070, −0.008)	0.014	0.064	−0.028 (−0.070, 0.013)	0.180	0.638
Hip neck	1019	−0.055 (−0.090, −0.020)	0.002	0.010	−0.055 (−0.098, −0.012)	0.012	0.055
Dieting pattern score							
Spine	1232	−0.020 (−0.055, 0.014)	0.244	0.765	−0.041 (−0.086, 0.004)	0.071	0.314
Total hip	1218	−0.009 (−0.042, 0.023)	0.575	0.987	−0.041 (−0.085, 0.003)	0.068	0.287
Hip neck	1019	−0.004 (−0.039, 0.032)	0.842	1.000	−0.015 (−0.061, 0.031)	0.518	0.976
Low-meat pattern score							
Spine	1232	−0.043 (−0.080, −0.007)	0.020	0.103	−0.046 (−0.092, −0.001)	0.047	0.217
Total hip	1218	−0.023 (−0.059, 0.013)	0.202	0.692	−0.030 (−0.070, 0.010)	0.146	0.548
Hip neck	1019	−0.022 (−0.058, 0.015)	0.246	0.750	−0.011 (−0.058, 0.035)	0.633	0.994
Energy-adjusted alcohol (g)							
Spine	1232	0.050 (0.017, 0.083)	0.003	0.014	0.057 (0.015, 0.099)	0.008	0.040
Total hip	1218	0.023 (−0.010, 0.056)	0.173	0.626	0.039 (−0.003, 0.081)	0.068	0.286
Hip neck	1019	0.045 (0.005, 0.085)	0.028	0.135	0.034 (−0.018, 0.086)	0.200	0.675
Energy-adjusted calcium (mg)							
Spine	1232	0.003 (−0.034, 0.039)	0.890	1.000	−0.002 (−0.049, 0.045)	0.925	1.000
Total hip	1218	0.016 (−0.015, 0.047)	0.319	0.867	−0.016 (−0.054, 0.022)	0.398	0.932
Hip neck	1019	0.011 (−0.023, 0.046)	0.526	0.975	0.001 (−0.041, 0.042)	0.978	1.000
Energy-adjusted protein (g)							
Spine	1232	0.012 (−0.023, 0.046)	0.502	0.970	0.029 (−0.014, 0.072)	0.189	0.651
Total hip	1218	−0.005 (−0.036, 0.025)	0.738	0.999	−0.013 (−0.047, 0.022)	0.465	0.964
Hip neck	1019	−0.027 (−0.060, 0.005)	0.102	0.415	−0.033 (−0.071, 0.005)	0.086	0.365
Energy-adjusted vitamin D (µg)							
Spine	1232	0.001 (−0.033, 0.035)	0.950	1.000	0.010 (−0.030, 0.049)	0.633	0.993
Total hip	1218	0.027 (−0.005, 0.059)	0.096	0.401	0.025 (−0.015, 0.064)	0.215	0.716
Hip neck	1019	−0.022 (−0.053, 0.008)	0.157	0.571	−0.026 (−0.061, 0.010)	0.155	0.572

[1] Models 1 and 2 were adjusted for age, age squared, BMI, smoking, and physical activity. BMD, bone mineral density.
[2] Linear regression modeling of BMD on individual dietary variables only (β_c).
[3] Linear regression modeling of BMD on individual dietary variables (β_i) and their twin-pair means (β_t; not shown) in the same model.
[4] Values were adjusted for multiple comparisons by using permutation methods.

traditional English pattern (results not shown), which suggests that the relations observed were real and not due to confounding by shared factors.

DISCUSSION

Public health strategies to reduce the risk of osteoporosis focus on modifiable environmental factors that promote peak bone mass in young adulthood and minimize the rate of bone loss in later life. A number of dietary constituents have been proposed to have an impact on bone health, including calcium, silicon, vitamin D, vitamin K, fruit and vegetables, phytoestrogens, and meat (7).

In this study, the mean daily intake of calcium was 1104 mg Ca, which was higher than the mean of 823 mg Ca for women aged 50–64 y (UK National Diet and Nutrition Survey), and the mean vitamin D intake was 2.6 μg vitamin D/d, which was lower than the UK National Diet and Nutrition Survey mean of 3.5 μg vitamin D/d (11). Over 80% of participants reported taking vitamin D and calcium supplements, but we did not have detailed quantitative intake data. The dietary intake of vitamin D by participants in our study was very low, and the small SD indicated that the vast majority of women consumed diets that contained insufficient amounts of vitamin D. In contrast, the mean dietary calcium intake was high, albeit with a relatively wide range of intakes. MacInnis et al (14) reported a positive association between calcium intake (mean: 971 mg Ca/d) and forearm BMD (β = 2.74 ± 1.02, P < 0.01) in 63 postmenopausal twin pairs with a mean age of 58 y. However, one limitation of their study was the relatively modest number of twin pairs compared with the number of twin pairs in our study, which was much larger and involved >1000 twin pairs. It is possible that supplement use masked any associations between dietary intake and BMD. When we stratified data according to supplement use, results remained the same for participants who took supplements but were different in the group that did not take supplements, but the sample size was very small and, thus, difficult to interpret. Dose-response relations between calcium and vitamin D intakes and BMD in postmenopausal women have not been clearly established; findings from different studies were inconsistent, and the minimum effective amounts were difficult to ascertain because of confounding variables (12). However, a recent opinion from the European Food Safety Authority (13) proposed conditions for a health claim that related to BMD in women aged ≥50 y of a daily intake of ≥1200 mg Ca with or without 20 μg (800 IU) vitamin D. When confounding by genotype was eliminated, the absence of an association between calcium intake and BMD indicated that habitual diets together with calcium and vitamin D supplements, which were taken by most of the women, provided sufficient calcium for bone mineralization.

High-protein diets have been reported to be associated with calciuria (15) but also higher BMD (eg, in elderly women) (16). We showed no association between protein intakes (mean: 81.3 g/d) and BMD. Harrington et al (17) suggested that protein-induced urinary calcium loss is compensated for by increased bone resorption, but the homeostatic response may be influenced by VDR genotype. In our study, the confounding effects of genotype were removed, and therefore, we concluded that dietary protein was not a significant modulator of BMD in normal postmenopausal women.

The intake of alcohol (in g/d and/or frequency) was associated with higher spine BMD and hip-neck bone density before adjustment for multiple comparisons. These results confirmed findings of a previous study in a smaller cohort of twins (8) and supported the conclusions of a recent meta-analysis in which a moderate consumption of alcohol (>0.5 to <2 drinks/d) was associated with higher femoral bone density and lower risk of hip fracture (18). The mean daily intake of alcohol was 9.2 g/d (±12.3g), which equates to ~1–2 standard drinks (8 g alcohol/d in the United Kingdom; 14 g alcohol/d in the United States); our results indicate that the consumption of 1 unit (standard drink) alcohol/d increase bone density in the spine by 0.03 g/cm^2. The consumption of wine, but not of beer or spirits, was associated with higher BMD.

We observed that a so-called traditional 20th-century English diet, which consists of high intakes of fried fish, fried potatoes, legumes (eg, baked beans), red and processed meats, savory pies, and cruciferous vegetables (eg, cabbage and cauliflower), was associated with a lower BMD. This diet was defined in a previous publication from PCA (3) as 1 of 5 main dietary patterns, with the others being "fruit and vegetables," "high alcohol," "dieting," and "low meat." The traditional English diet, which is a diet more typical of that consumed in the 20th century by industrialized countries, was assumed to be less healthy because of the amount and type of fats and the micronutrient content (3). Tucker et al (19) examined the effects of dietary patterns on BMD in older adults who were participants in the Framingham Osteoporosis Study. The authors observed 6 distinct dietary patterns and divided subjects into groups, each of which contained 69–313 individuals. There was a significant positive association between BMD and fruit and vegetable intake in men and a negative association between BMD and high candy consumption in men and women. From the PCA carried out on our own data, we were unable to identify a diet equivalent to the high-candy diet and, thus, could not directly compare our results with those obtained from the Framingham cohort, but we were unable to show that a diet high in fruit and vegetables was associated with higher BMD. This results may possibly have been due to a difference in the absolute quantity of fruit and vegetables consumed; the mean percentage of energy intake from fruit and vegetables in the Framingham cohort was high (29.7%, with 19.9% being contributed by noncitrus fruit and fruit juice), whereas in our study, the mean percentages of energy intake from fruit and vegetables were 17.1% and 7.8%, respectively. In contrast, our results supported the observation of Tucker et al (19) of a positive effect of alcohol (wine) on BMD (radius) in women.

We were unable to provide evidence of causality or to exclude unmeasured confounding because of the observational study design. However, our use of the co-twin control design has allowed us to detect subtle environmental effects on BMD, which were not confounded by factors in the shared environment of the twins or by genotype. We also controlled for potential covariates in our multivariate model, but it is plausible that residual confounding existed. For 57% of our cohort, we had repeat measures of diet, which better represented usual consumption patterns and potentially strengthened the predictive value of our data set. We used the results from a previous work in which data from the food-frequency questionnaires of female twins were categorized

into 5 main dietary patterns by using PCA (3). The patterns were given a descriptive name that reflected the most common types of foods consumed. Current research on diet and health tends to focus more on dietary patterns rather than on individual constituents and nutrients because of the complex relation between diet and chronic diet-related diseases as reflected by food-based dietary guidelines. Our overall findings that moderate intakes of alcohol from wine were associated with a higher BMD, and the consumption of a traditional 20th-century English diet, as defined from our previous analysis of habitual dietary patterns, was associated with a lower BMD, independent of genotype, provide information that can be used for nutrition policies aimed at the reduction of risk of osteoporosis. However, intervention studies are recommended to confirm our findings, and additional research is required to understand the mechanisms for the effects on BMD.

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