Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health?[1–3]

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ABSTRACT

Background: The role of nutritional influences on bone health remains largely undefined because most studies have focused attention on calcium intake.

Objective: We reported previously that intakes of nutrients found in abundance in fruit and vegetables are positively associated with bone health. We examined this finding further by considering axial and peripheral bone mass and markers of bone metabolism.

Design: This was a cross-sectional study of 62 healthy women aged 45–55 y. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry at the lumbar spine and femoral neck and by peripheral quantitative computed tomography at the ultradistal radial total, trabecular, and cortical sites. Bone resorption was calculated by measuring urinary excretion of pyridinoline and deoxypyridinoline and bone formation by measuring serum osteocalcin. Nutrient intakes were assessed by using a validated food-frequency questionnaire; other lifestyle factors were assessed by additional questions.

Results: After present energy intake was controlled for, higher intakes of magnesium, potassium, and alcohol were associated with higher total bone mass by Pearson correlation ($P < 0.05$ to $P < 0.005$). Femoral neck BMD was higher in women who had consumed high amounts of fruit in their childhood than in women who had consumed medium or low amounts ($P < 0.01$). In a regression analysis with age, weight, height, menstrual status, and dietary intake entered into the model, magnesium intake accounted for 12.3% of the variation in pyridinoline excretion and 12% of the variation in deoxypyridinoline excretion. Alcohol and potassium intakes accounted for 18.1% of the variation in total forearm bone mass.

Conclusion: The BMD results confirm our previous work (but at peripheral bone mass sites), and our findings associating bone resorption with dietary factors provide further evidence of a positive link between fruit and vegetable consumption and bone health. *Am J Clin Nutr* 2000;71:142–51.

KEY WORDS Bone mass, bone turnover, pyridinium cross-links, energy-adjusted nutrient intakes, potassium, magnesium, vitamin C, β-carotene, fruit, vegetables, acid-base balance, women

INTRODUCTION

Bone mineral density (BMD) and bone metabolism are affected by genetic, endocrine, mechanical, and nutritional factors, with extensive interactions between the different factors (1, 2). Our understanding of the influence of nutrition on bone health is limited because most studies to date concentrated primarily on the role of calcium in bone health and paid less attention to other micronutrients. A diet low in calcium is likely to be deficient in many other micronutrients as well, but few studies have addressed these potential relations. Furthermore, the effects of dietary intake on bone metabolism have received scant attention in the literature (3).

In a cross-sectional study, our group reported that intakes of zinc, magnesium, potassium, fiber, and vitamin C are associated with higher bone mass in premenopausal women (4). These relations were independent of the important confounding factors of weight, height, total energy intake, smoking, and physical activity. This led us to conclude that there may be a positive link between fruit and vegetable consumption and bone health because all of these nutrients are found in abundance in these 2 food groups and additional plausible mechanisms for the benefits of fruit and vegetables have been proposed (5). Other investigators have also suggested a role for these nutrients (6–8).

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The purpose of the present study was to investigate further the association between the micronutrients identified previously as being important to BMD (in particular, potassium, magnesium, fiber, vitamin C, β-carotene, phosphorus, and zinc) and indexes of bone health. We considered not only axial and peripheral bone mass but also markers of bone metabolism in a group of healthy women aged 45–54 y. Concomitantly, the effects of endocrine and hormonal influences on bone mass and bone metabolism were assessed and are reported elsewhere (9).

SUBJECTS AND METHODS

Study design and selection of subjects

Subjects in the present investigation were women aged 45–55 y who were randomly selected from a population health register to take part in an osteoporosis screening study (10) as detailed previously (4). The women had not taken any medications or suffered from any condition likely to affect their bone metabolism. Subjects who took part in this cross-sectional study were consecutive attendees of the screening study who agreed to return to undergo further investigations, including assessment of bone metabolism and forearm BMD, and to complete questionnaires on endocrine and hormonal status, dietary intake, menstrual and smoking history, and physical activity levels. These women had not taken part in any previous investigations of bone health. The effects of endocrine and hormonal influences on bone mass and bone metabolism are reported elsewhere (9). A total of 65 women were studied and were grouped as either premenopausal (regular menses in past 6 mo), perimenopausal (irregular menses in past 6 mo), or postmenopausal (absence of menses in past 6 mo). The classification of menstrual status was confirmed by measuring follicle stimulating hormone (9). The length of time between the initial BMD scan and measurement of bone metabolism was <1 mo. Informed consent was obtained from each subject and the study was approved by the Joint Ethics Committee of Grampian Health Board and the University of Aberdeen.

Anthropometric and bone mass measurements

Each woman's weight (while wearing light clothing and no shoes) was recorded by using a set of balance scales (Seca, Hamburg, Germany) calibrated to 0.05 kg. Height was measured with a stadiometer (Holtain Ltd, Crymych, United Kingdom). BMD was assessed by dual-energy X-ray absorptiometry [(DXA) Norland XR-26; Norland Corporation, Fort Atkinson, WI] at the lumbar spine (lumbar vertebrae 2–4) and left femur (femoral neck, femoral trochanter, and femoral Ward's triangle). The DXA instrument was calibrated fully and the standard measurement techniques adhered to strictly (11). The CVs of this technique in our hands were 0.9% for the lumbar spine and 2.7% for the femoral neck (12).

Peripheral quantitative computed tomography (pQCT) was performed at the ultradistal radius of the nondominant forearm by using a Stratec XCT-960 scanner (Stratec Medizintechnik, Berlin). The measurement scan was taken at a distance of 4% of the total ulnar length proximal to the ulna styloid process. Total, trabecular, and cortical forearm BMD were measured. Because these measurements are truly volumetric, results are expressed in g/cm^3. In vivo, short-term precision at our center expressed as the CV was 1.24% for total BMD, 1.33% for trabecular BMD, and 1.88% for cortical BMD in young, healthy females (13).

Markers of bone metabolism

Urine and blood specimens were obtained after subjects fasted overnight. Urine samples were transferred to sterile tubes and stored at −20°C until analyzed for pyridinoline and deoxypyridinoline. Blood was collected in evacuated tubes containing no additives, allowed to clot, and centrifuged at 3000 × *g* for 10 min at room temperature. The extracted serum was stored at −20°C until assayed for osteocalcin. Samples were measured within 12 wk to avoid significant degradation of osteocalcin.

Measurement of pyridinoline and deoxypyridinoline

Pyridinoline and deoxypyridinoline were analyzed by a fully automated method that uses solid-phase extraction and reversed-phase HPLC as described by Pratt et al (14). Urine samples were defrosted at room temperature, shaken, and then allowed to settle for 30 min. Portions of the urine samples were hydrolyzed with an equal volume of 12 mol HCl/L at 110°C overnight to convert all cross-links to the free form. The CV was 2.7% for pyridinoline excretion and 1.7% for deoxypyridinoline.

Measurement of osteocalcin

Serum osteocalcin was measured by enzyme-linked immunosorbent assay with rabbit antiserum raised against purified bovine osteocalcin that had full cross-reactivity with human osteocalcin (10). The CV was <10%. Care was taken during blood sampling to avoid hemolysis, which would affect the osteocalcin through proteases released from erythrocytes.

Usual dietary intake

Usual dietary intake (over the previous 12 mo) was assessed by using a food-frequency questionnaire (FFQ) that was developed and validated previously against 7-d weighed records and biochemical markers of antioxidant status (15, 16). The short-term (6 wk) and long-term (1 y) reproducibility of the FFQ was also tested in the study population previously (15–17). The FFQ is based on the Caerphilly FFQ (18), which was first adopted for use in the Scottish Heart Health Study (19) and later for the World Health Organization MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) survey. The present FFQ included far more detail on possible bone-related nutrients and other foods commonly consumed in northeast Scotland. To allow for greater detail on frequency of consumption, the variables "times per day" and "number of days per week" were included. The structure of the FFQ and the procedures for its correct completion were explained to each subject (by SAN) and a stamped, addressed envelope was provided for its return. The FFQs were coded and analyzed by using the Rowett Research Institute Nutritional Analysis Program (RONA), which is based on McCance and Widdowson's food-composition tables and supplements (20). Three women did not return their FFQs; therefore, results are presented for 62 women.

Past dietary habits

To assess past dietary intake, 2 age categories were chosen as being crucial stages in skeletal growth: childhood (≤12 y) and early adulthood (20–30 y). Although the ages of 12–20 y are also an important time for skeletal growth, when we pilot-tested the questions about past dietary intake we found that

TABLE 1
Subjects' anthropometric data and age of menarche[1]

Age (y)	47.3 ± 1.87 (45–54; 47)
Weight (kg)	65.3 ± 11.2 (47–98; 63.5)
Height (m)	1.616 ± 0.060 (1.503–1.733; 1.605)
BMI	25.0 ± 4.1 (18.1–36.6; 23.9)
Age of menarche (y)	13.2 ± 1.55 (10–18; 13)

[1] $\bar{x}$ ± SD; range and median in parentheses. n = 62 women.

women in the age range we were investigating (45–54 y) found 12–20 y a time of tremendous change and hence a much more difficult time to answer questions with certainty (16). Women were asked roughly how much milk they consumed daily and the frequency of consumption (number of times per day and number of days per week) of milk products, cheese, and fruit and vegetables (excluding potatoes). The aim of these questions was to enable us to classify women as low, medium, and high consumers of these foods. Consumption was categorized as low, medium, or high with reference to intakes of these foods in the United Kingdom (21). The consumption categories were as follows: for milk, low was <284 mL/d), medium was 285–568 mL/d, and high was >568 mL/d. For milk products, cheese, and fruit and vegetables, low was 1–4 times/d ≤2 d/wk, medium was 1–4 times/d 3–4 d/wk, and high was 1–4 times/d ≥5 d/wk.

Nondietary questions

Current activity levels were assessed through a series of questions concerning work and leisure activities and physical activity levels were calculated by using the James and Schofield equations (22). To assess past physical activity levels, the childhood and early adulthood age categories were used. Subjects were asked to record the amount of time they spent walking per day and the number of times a week they were physically active (short of breath and sweating) for ≥20 min. Information on current and past smoking habits was collected by asking subjects about the number of cigarettes they currently smoked or used to smoke per day and the number of years they had smoked. Subjects were also asked about their level of educational attainment as a marker of socioeconomic status and their age of menarche.

Statistical analysis

SPSS/PC+ (version 6.0; SPSS Inc, Chicago) was used for all statistical analyses. Descriptive statistics (means, medians, SDs, and ranges) were determined for all variables. The relation between nondietary factors, bone mass, and bone metabolism was examined by using Pearson correlation coefficients. The nondietary factors included age, weight, height, age of menarche, menstrual status, parity, smoking habits, present and past physical activity levels, socioeconomic status, caffeine intake, and alcohol consumption. The factors identified as being significant in this study and in our previous study (9) were age, weight, height, and menstrual status; thus, only these variables were controlled for in all subsequent nutritional analyses. To account for the problems of multiple testing, only those factors significant at the 1% level were considered to be statistically significant.

Nutrient intakes were adjusted for total energy intake by using the residual method of Willett (23). When nutrient values were found to be skewed, log transformations (ln) were undertaken. Pearson correlations and partial correlations (with adjustment for age, weight, height, and menstrual status) were calculated for each nutrient for each BMD site, for excretion of pyridinoline and deoxypyridinoline, and for osteocalcin concentration.

To determine whether any of the nutrients were independent predictors of bone mass or markers of bone metabolism, age, weight, height, and menstrual status were entered into a forward stepwise multiple regression model together with intakes of important micronutrients (calcium, potassium, phosphorus, magnesium, fiber, β-carotene, zinc, and vitamin C). To accommodate our small sample size and the potential for multicollinearity among nutrition variables, a staged approach to the analysis was undertaken with only 2 energy-adjusted nutrient intakes at any one time. This model allowed the strongest independently predictive factors to be identified. Ninety-five percent CIs for the regression coefficients are presented.

Furthermore, in addition to considering the nutrients as continuous variables, nutrient intakes were grouped into quartiles and the mean BMD at each site and mean bone resorption and bone formation markers were calculated. Differences in these indexes of bone health were assessed by using a multiple range test [one-way analysis of variance (ANOVA) with Scheffe range], which is based on 95% CI limits. Analysis of covariance (ANCOVA) was used to assess differences after adjustment for the important confounding factors. The association between past dietary intake, bone mass, and bone metabolism was examined by both ANOVA and ANCOVA. To examine whether past food intakes were associated with current energy, calcium, or milk intakes, the chi-square test was used.

RESULTS

Descriptive data

The subjects' anthropometric data and age of menarche are shown in **Table 1**. Women were of average height and weight for the local population and the mean age of menarche was 13 y. The BMD and bone metabolism variables are shown in **Table 2**. These values were approximately normally distributed. The mean daily intake of nutrients (including vitamin and mineral supplements, untransformed data) are shown in **Table 3**. Mean daily intakes were well within the reference nutrient intake

TABLE 2
Lumbar spine, hip, and forearm bone mineral density (BMD) and bone metabolism variables[1]

Lumbar spine BMD (g/cm^2)	1.066 ± 0.128 (0.803–1.434; 1.053)
Femoral neck BMD (g/cm^2)	0.874 ± 0.099 (0.690–1.168; 0.874)
Femoral trochanter BMD (g/cm^2)	0.711 ± 0.092 (0.455–1.015; 0.700)
Femoral Ward's triangle BMD (g/cm^2)	0.690 ± 0.102 (0.492–0.972; 0.671)
Forearm total BMD (mg/cm^3)	391.2 ± 43.5 (308.2–469.1; 385.9)
Forearm trabecular BMD (mg/cm^3)	188.2 ± 31.6 (96.4–253.4; 185.2)
Forearm cortical BMD (mg/cm^3)	559.5 ± 55.6 (442.2–711.9; 554.4)
Urinary Pyd (nmol/mmol Cr)	47.4 ± 11.4 (27.8–78.7; 45.6)
Urinary Dpd (nmol/mmol Cr)	12.1 ± 4.2 (5.5–25.0; 11.3)
Serum OC (μg/L)	5.49 ± 1.65 (2.09–9.44; 5.53)

[1] $\bar{x}$ ± SD; range and median in parentheses. n = 62 women. Pyd, pyridinoline; Cr, creatinine; Dpd, deoxypyridinoline; OC, osteocalcin.

TABLE 3
Nutrient intakes[1]

	Value	RNI or EAR
Energy (MJ)	8.49 ± 2.36 (3.20–14.45; 8.17)	8.0
Protein (g)	84.3 ± 24.6 (36.2–170.8; 81.7)	46.5
Fat (g)	77.5 ± 28.6 (20.2–169.6; 73.4)	73.5
Calcium (mg)	1101 ± 377 (483–2136; 1003)	700
NSP (g)	15.9 ± 5.88 (5.9–33.3; 15.6)	18
Vitamin D (μg)	3.41 ± 2.51 (0.38–9.19; 2.84)	7.0
Vitamin C (mg)	103.4 ± 65.6 (23.8–453.2; 86.3)	40
Sodium (mg)	2784 ± 879 (1174–5321; 2661)	1600
Potassium (mg)	3404 ± 814 (1629–6076; 3317)	3500
Phosphorus (mg)	1536 ± 445 (711–2986; 1479)	550
Ca:P	0.72 ± 0.11 (0.43–0.95; 0.71)	—
β-Carotene (μg)	1979.2 ± 1100.3 (497.0–7131.0; 1767.0)	—
Iron (mg)	13.1 ± 4.4 (3.8–23.9; 12.8)	14.8
Magnesium (mg)	326 ± 90 (142–601; 317)	270
Zinc (mg)	10.4 ± 3.23 (3.9–22.9; 10.1)	7.0

[1] $\bar{x}$ ± SD; range and median in parentheses. n = 62 women. RNI, reference nutrient intake; EAR, estimated energy requirement (for energy only); NSP, nonstarch polysaccharide.

intervals for UK women between the ages of 19–50 and >50 y, as appropriate (24). Five women took vitamin and mineral supplements including calcium (n = 1), iron (n = 2), and evening primrose oil (n = 2). The mean energy equivalent (energy intake/basal metabolic rate) was 1.44.

Correlations between lumbar spine, hip, and forearm BMD and markers of bone metabolism

Correlations between the anthropometric data; age of menarche; lumbar spine, hip, and forearm BMD; and bone metabolism are shown in **Table 4**. BMD values at the lumbar spine and 3 hip sites were highly correlated with each other. Total forearm BMD was significantly correlated with both trabecular and cortical forearm BMD. There was a poor correlation between pQCT and DXA measurements, which was discussed elsewhere (25). Pyridinoline was significantly correlated with deoxypyridinoline. Weight was significantly correlated with most of the BMD sites. No significant relations were found between markers of bone metabolism and lumbar spine, hip, or forearm BMD.

Relation between nutrient intakes and bone metabolism

Correlation coefficients between energy-adjusted nutrient intakes and markers of bone resorption and formation are shown in **Table 5**. Potassium, magnesium, and phosphorus were significantly negatively correlated with pyridinoline excretion (**Figures 1** and **2**). Potassium, magnesium, β-carotene, and fiber were significantly negatively correlated with deoxypyridinoline excretion. A similar but nonsignificant (after adjustment for multiple testing) trend was seen for vitamin C intake and deoxypyridinoline excretion (P < 0.02). Total energy intake was positively correlated with serum osteocalcin concentrations. Correlation coefficients and P values were virtually unaffected after adjustment for the confounding factors.

Nutrient intakes were then grouped into quartiles and the mean values for bone resorption and formation markers calculated. Mean pyridinoline excretion was significantly lower with higher intakes of potassium (P < 0.01; **Figure 3**), magnesium (P < 0.04), and β-carotene (P < 0.05). Mean deoxypyridinoline excretion was significantly lower with higher intakes of potassium (P < 0.01; **Figure 4**), magnesium (P < 0.05; **Figure 5**), β-carotene (P < 0.05; **Figure 6**), and vitamin C (P < 0.02). Significant differences were found in pyridinoline and deoxypyridinoline excretion between women in the lowest and highest quartiles of potassium (P < 0.01) and magnesium (P < 0.05) intakes. A similar but nonsignificant result was found for β-carotene. Small, nonsignificant differences were also seen in pyridinoline and deoxypyridinoline excretion between the lowest and highest quartiles of fiber and vitamin C intakes. Adjust-

TABLE 4
Pearson correlation coefficients between anthropometric data, age of menarche, bone mineral density, and markers of bone metabolism[1]

	Anthropometric data				Lumbar spine and hip BMD				Forearm BMD			Markers of bone metabolism		
	Wt	Ht	BMI	Age of menarche	LS	FN	FT	FW	Total	Trab	Cort	OC	Pyd	Dpd
Age (y)	0.09	0.00	0.09	0.18	−0.17	−0.26	−0.15	−0.26	0.18	0.05	0.06	−0.06	−0.03	−0.01
Wt (kg)		0.27	0.91[2]	−0.23	0.30[3]	0.39[3]	0.45[3]	0.25	0.17	0.04	0.07	−0.16	0.04	−0.01
Ht (m)			−0.15	−0.02	0.19	0.27	0.30[3]	0.14	0.05	0.03	0.11	−0.03	−0.05	−1.2
BMI (kg/m^2)				−0.23	0.18	0.28[3]	0.33[3]	0.19	0.17	0.18	0.19	−0.15	0.05	0.04
Age of menarche (y)					−0.15	−0.23	−0.30[3]	−0.24	−0.16	−0.15	−0.02	−0.27	0.04	−0.10
LS (g/cm^2)						0.52[2]	0.60[2]	0.57[2]	0.20	0.37[3]	−0.05	−0.04	0.08	−0.09
FN (g/cm^2)							0.85[2]	0.88[2]	0.12	0.27	−0.05	−0.03	0.12	−0.03
FT (g/cm^2)								0.82[2]	0.22	0.37	−0.06	−0.08	0.09	−0.08
FW (g/cm^2)									0.23	0.36	−0.04	−0.10	0.14	−0.03
Forearm total (mg/cm^3)										0.50[2]	0.59[2]	−0.03	−0.02	0.04
Forearm trab (mg/cm^3)											−0.34[3]	0.07	0.15	−0.05
Forearm cort (mg/cm^3)												−0.04	0.05	−0.05
OC (μg/L)													−0.22	0.04
Pyd (nmol/mmol Cr)														0.67[2]
Dpd (nmol/mmol Cr)														

[1] Wt, weight; Ht, height; LS, lumbar spine; FN, femoral neck; FT, femoral trochanter; FW, femoral Ward's triangle; trab, trabecular; cort, cortical; OC, osteocalcin; Pyd, urinary pyridinoline excretion; Dpd, urinary deoxypyridinoline excretion; Cr, creatinine.
[2] P< 0.001.
[3] P < 0.01.

TABLE 5
Pearson correlation coefficents between energy-adjusted nutrient intake and markers of bone metabolism[1]

	Energy (MJ)	Ca (mg)	NSP (g)	β-Carotene (μg)	Mg (mg)	K (mg)	P (mg)	Vitamin C (mg)
Pyd (nmol/mmol Cr)	0.11	−0.31[2]	−0.22	−0.25	−0.37[3]	−0.31[2]	−0.36[3]	−0.21
Dpd (nmol/mmol Cr)	0.17	−0.09	−0.30[2]	−0.32[2]	−0.37[3]	−0.31[2]	−0.23	−0.26
OC (μg/L)	0.31[2]	0.011	0.06	0.09	0.14	0.14	0.17	0.14

[1] NSP, nonstarch polysaccharide; Pyd, urinary pyridinoline excretion; Dpd, urinary deoxypyridinoline excretion; Cr, creatinine; OC, serum osteocalcin. Adjustment for age, weight, height, and menopausal status did not alter the level or significance of the correlation coefficients.
[2] $P < 0.01$.
[3] $P < 0.005$.

ment for the given confounding factors with ANCOVA did not alter the results.

Relation between nutrient intakes and lumbar spine and hip BMD

No significant correlations were found between current nutrient intakes and lumbar spine or hip BMD. Additionally, no significant differences were found in BMD between women who consumed low amounts of milk, milk products, cheese, or vegetables in their childhood or early adulthood and those who consumed medium or high amounts. However, significant differences ($P < 0.01$) were seen in femoral neck BMD between women with high childhood intakes of fruit and those with medium or low intakes [femoral neck BMD (mean ± SD; 95% CI in parentheses): low, -0.852 ± 0.077 g/cm^2 (-0.821, -0.882); medium, -0.862 ± 0.096 g/cm^2 (-0.825, -0.904); high, -0.957 ± 0.125 g/cm^2 (-0.868, -1.046)]. These differences remained significant after adjustment for the confounding factors. Differences were also seen at the femoral trochanter and femoral Ward's triangle BMD sites but these were not significant. Similar trends were found between women who in early adulthood consumed low, medium, or high amounts of fruit, but these were not significant. Reported high intakes of milk and fruit during childhood and early adulthood were significantly and positively related to current energy, calcium, and milk intakes ($P < 0.0001$, chi-square test). However, adjustment for current calcium intake and past milk intake did not significantly alter the relation between past fruit intake and bone mass. Caffeine consumption was not related to BMD.

Relation between nutrient intakes and forearm BMD

Correlation coefficients between nutrient intakes and forearm BMD are shown in **Table 6**. Potassium and alcohol intakes were positively correlated with total forearm BMD. A similar but nonsignificant trend (after correction for multiple testing) was seen for magnesium, phosphorus, and calcium intakes ($P < 0.05$). Only alcohol intake was significantly positively correlated with forearm trabecular BMD. Although not significant, a similar association was seen between magnesium and potassium intakes and forearm cortical BMD ($P < 0.05$). Correlation coefficients and P values were virtually unaffected by adjustment for the confounding factors.

Nutrient intakes were then grouped into quartiles and the mean values for forearm BMD measurements were calculated. Mean total BMD was significantly greater with higher intakes of potassium, magnesium, fiber, and alcohol ($P < 0.05$ to $P < 0.005$). Mean cortical BMD increased significantly with increased intakes of magnesium, potassium, and alcohol ($P < 0.05$ to $P < 0.01$). Adjustments for the given factors by ANCOVA did not alter the

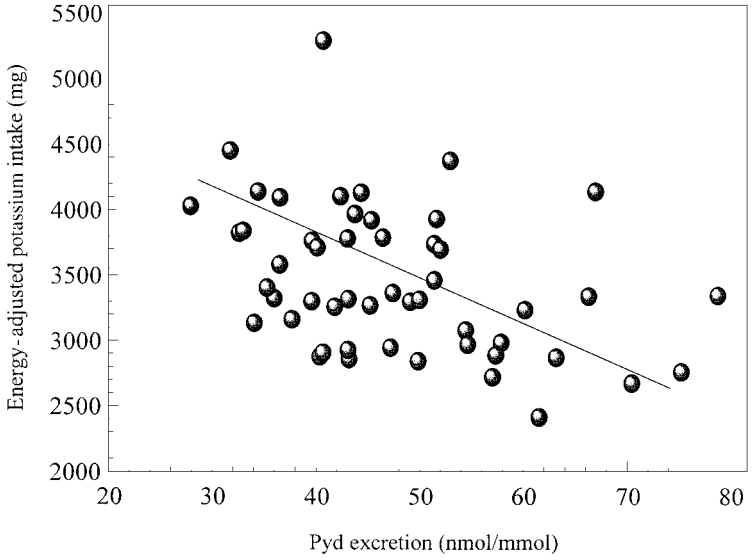

FIGURE 1. Correlation between energy-adjusted potassium intake and pyridinoline (Pyd) excretion. $r = -0.31$, $P < 0.02$. The correlation coefficients were unaltered after adjustment for age, weight, height, and menopausal status.

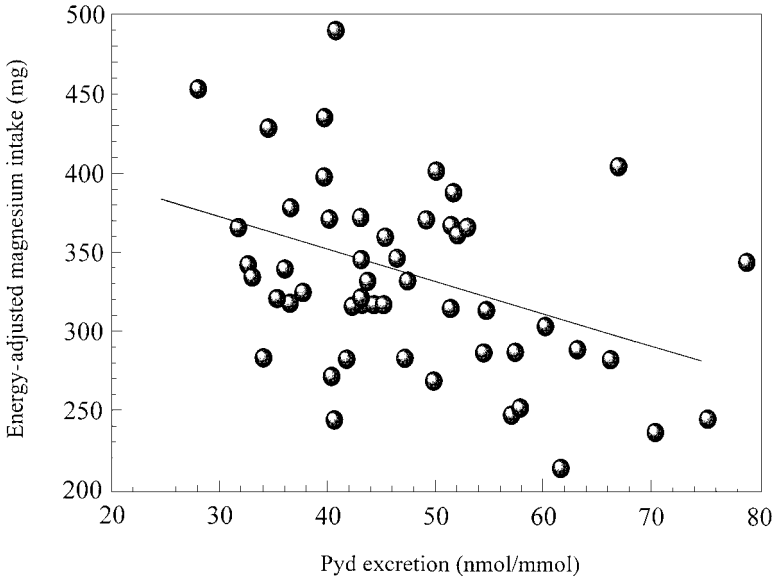

FIGURE 2. Correlation between energy-adjusted magnesium intake and pyridinoline (Pyd) excretion. $r = -0.37$, $P < 0.005$ The correlation coefficients were unaltered after adjustment for age, weight, height, and menopausal status.

results. No significant differences in forearm BMD were found according to past intakes of foods.

RELATION OF OTHER NONDIETARY VARIABLES TO BMD AND METABOLISM

No significant correlations were found between present or past physical activity levels and BMD or markers of bone metabolism. There were also no significant differences in these variables between smokers and nonsmokers. Differences were found in nutrient intake between smokers and nonsmokers: fat intake was higher in smokers than in nonsmokers ($P < 0.002$), whereas intakes of vitamin C, potassium, calcium, and NSP were lower (NS).

Regression analysis

Age, weight, height, menstrual status, and dietary intake were examined as independent variables for each of the bone mass sites measured and for each marker of bone metabolism by using forward stepwise multiple regression analysis to determine the best predictors. As expected from the bivariate correlations, only weight was a significant predictor at the femoral neck and femoral trochanter BMD sites. There were no significant predictors of lumbar spine BMD.

For total forearm BMD, alcohol and potassium intakes were highlighted as independent predictors, accounting for a combined 18.1% of the variation (alcohol, 10.7%; potassium, 7.4%). For forearm trabecular BMD, only alcohol intake was found to be important, accounting for 8.7% of the variation in bone mass. There was no direct effect of any of the nutrients on forearm cortical BMD. The regression equations and 95% CIs are shown in **Table 7**.

Energy-adjusted magnesium intake was found to be the strongest predictor of pyridinoline excretion, accounting for 12.3% of the variation in excretion. When potassium intake was entered into the equation without magnesium, it was also highlighted as an important independent predictor, accounting for 8% of the variation. For deoxypyridinoline excretion, magnesium was again found to be the strongest predictor, accounting for 12.1% of the variation in excretion. Other nutrients highlighted as important predictors when magnesium was not included in the equation were potassium (7.7%), β-carotene (8%), and fiber (7%). For osteocalcin, total energy intake was the independent predictor.

DISCUSSION

In the present study we examined the association between dietary intake and indexes of bone health, namely both axial (measured by DXA) and peripheral (measured by pQCT) BMD

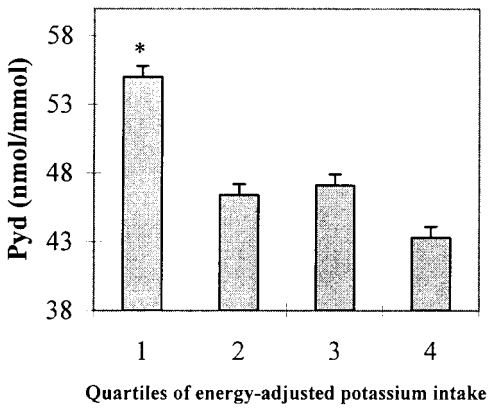

FIGURE 3. Mean (±SEM) decrease in pyridinoline (Pyd) excretion with quartile of energy-adjusted potassium intake. The test for linearity among all quartiles was significant, $P < 0.01$ (F test). *Significantly different from quartile 4, $P < 0.05$ (ANCOVA adjusted for age, weight, height, and menopausal status).

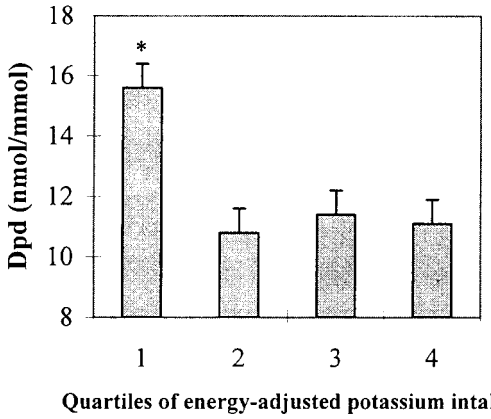

FIGURE 4. Mean (±SEM) decrease in deoxypyridinoline (Dpd) excretion with quartile of energy-adjusted potassium intake. The test for linearity among all quartiles was significant, $P < 0.01$ (F test). *Significantly different from quartile 4, $P < 0.01$ (ANCOVA adjusted for age, weight, height, and menopausal status).

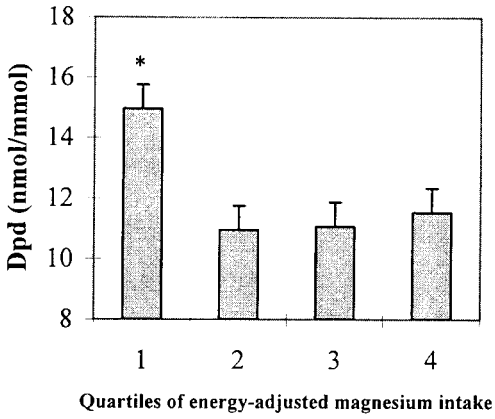

FIGURE 5. Mean (±SEM) decrease in deoxypyridinoline (Dpd) excretion with quartile of energy-adjusted magnesium intake. The test for linearity among all quartiles was significant, $P < 0.01$ (F test). *Significantly different from quartile 4, $P < 0.05$ (ANCOVA adjusted for age, weight, height, and menopausal status).

and bone metabolism (pyridinium cross-links as markers of bone resorption and osteocalcin for bone formation). This investigation is the first-reported cross-sectional study to examine the effect of nutritional factors on markers of bone metabolism by using a well-validated FFQ and pyridinium cross-links, which are now well recognized as specific markers of bone resorption (26–28). Previous studies of the effects of dietary factors on bone resorption used urinary hydroxyproline (29). Furthermore, in only 2 other studies investigating the relation between nutrition and bone health were adjustments made for total energy intake in the analyses (30, 31). The importance of such an adjustment when examining diet-disease relations was stressed by Willett (23). Furthermore, adjustment for total energy intake may be particularly important in relation to bone health, for which physical activity may be an important confounder.

Subjects for this study were selected from women who had participated in the Osteoporosis Screening Programme, as indicated in our earlier study (4). The response rate in the overall screening program was ≈75% depending on the invitation method used (10, 32). The rigorous application of the study exclusion criteria ensured that the subjects were unlikely to be compromised with regard to their bone health.

The study was designed such that bone mass, markers of bone metabolism, and dietary intake were measured within a short time period (<6 wk) so that they could be reliably related to one another. Note, however, that bone metabolism markers were measured in only one sample. Diurnal variations in both cross-link excretion and serum osteocalcin (33) have been shown and it would have been ideal to have collected repeat blood and urine specimens. Samples from all women were taken after an overnight fast, however, and thus drawn at standardized times. The FFQ was generally well answered and the energy equivalent (energy intake/calculated basal metabolic rate) was well within the range established for satisfactory completion (34).

The main limitations of this study are that the design was cross-sectional and the number of subjects studied was relatively small. Thus, only associations rather than causal relations between diet and bone health can be inferred. Interestingly, however, not only do our bone mass findings confirm those that we reported previously (4), but the bone metabolism results add novelty to our conclusions; that is, intakes of nutrients found in abundance in fruit and vegetables, namely, potassium, β-carotene, vitamin C, and magnesium, were positively associated with bone health (and a similar but not significant trend was found for fiber), and these relations remained significant after adjustment for age, weight, height, and menstrual status.

The relation shown in this study between dietary intake and bone metabolism indexes provides further intriguing evidence of the links between nutrients and bone metabolism (4). Low intakes of potassium, β-carotene, magnesium, and vitamin C were associated with increased bone resorption. Similar but not significant findings were seen for fiber intake. This relation between dietary intake and bone metabolism was strong as evidenced by the negative correlations and regression analysis,

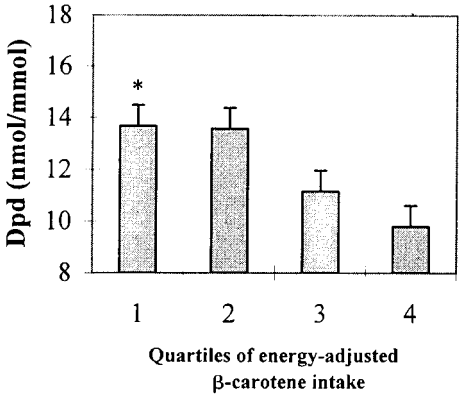

FIGURE 6. Mean (±SEM) decrease in deoxypyridinoline (Dpd) excretion with quartile of energy-adjusted β-carotene intake. The test for linearity among all quartiles was significant, $P < 0.01$ (F test). *Significance of difference from quartile 4: $P < 0.07$ (ANCOVA adjusted for age, weight, height, and menopausal status).

TABLE 6
Pearson correlation coefficients between energy-adjusted nutrient intake and forearm bone mineral density[1]

	Energy(MJ)	Ca (mg)	NSP (g)	β-Carotene (μg)	Mg (mg)	K (mg)	P (mg)	Vitamin C (mg)	Alcohol (g)
Total (mg/cm^3)	0.06	0.24	0.22	0.17	0.27	0.30[2]	0.25	0.11	0.35[3]
Trabecular (mg/cm^3)	0.11	0.23	0.12	0.11	0.05	0.17	0.08	0.09	0.32[2]
Cortical (mg/cm^3)	0.09	0.17	0.22	0.23	0.26	0.25	0.11	0.16	0.18

[1] NSP, nonstarch polysaccharide. Adjustment for age, weight, height, and menopausal status did not alter the level or significance of the correlation coefficients.
[2] $P < 0.01$.
[3] $P < 0.005$.

which showed magnesium intake to explain 12% of variation in pyridinoline and deoxypyridinoline excretion. No such relations were found between nutrient intakes and bone formation as measured by serum osteocalcin, implying a more direct relation between nutrient concentrations and bone resorption markers, concentrations of which rise first in the transition between the pre- and postmenopausal state. It is important to note, however, that much of the variation in bone resorption remained unaccounted for and the small number of subjects we studied is a limiting factor. Thus, our results are best used in the generation of hypotheses, which require further investigation before any substantial conclusions can be drawn.

Although there is little information on the influence of dietary intake on bone metabolism markers, several theories may help to explain our findings. For potassium intake, the skeleton may play an important role in acid-base homeostasis by the mobilization of skeletal salts to balance the endogenous acid generated from acid-producing foods. Wachman and Berstein (35) hypothesized that the skeleton is a reservoir of labile bases that can be mobilized for the defense of blood pH and plasma bicarbonate concentrations. Potassium bicarbonate has been shown to decrease urinary calcium excretion (36, 37), improve calcium balance (38), decrease bone resorption, and increase the rate of bone formation (39). Increased plasma acidity or decreased plasma bicarbonate concentrations may stimulate bone resorption directly by mineral dissolution and indirectly by reducing the pH and the bicarbonate concentrations of osteoclasts (40). This would result in adhesion of the osteoclast cells to their bone resorptive sites and thus secretion of hydrogen ions into bone-resorbing fluid compartments (41–43). Acidosis may also inhibit osteoblast function and thus limit bone formation (44).

The negative relation found between vitamin C intake and bone resorption may be explained by the fact that ascorbic acid is essential for the hydroxylation of proline and lysine residues; hydroxylysine is also involved in the cross-linking necessary for normal collagen fiber formation (45). Defective hydroxylation increases the intracellular degradation of collagen precursors; as a result, the formation of various cross-linking amino acids in collagen is perturbed. The significant negative correlation between vitamin C and deoxypyridinoline, but not pyridinoline, may be because pyridinoline is derived from hydroxylysine within the collagen helix whereas deoxypyridinoline involves reaction with a lysyl residue. Thus, a relative deficiency of vitamin C is likely to affect deoxypyridinoline more strongly than pyridinoline, as has been shown in guinea pigs (46). The women in our study were unlikely to be deficient in vitamin C, however. Vitamin C (and other trace elements) may also be important for antioxidant capacity if the connective tissue of bone is a target for free radical damage. There is little documentation of such an effect, but further studies are certainly justified.

A higher intake of magnesium was associated with lower bone resorption and was highlighted as the best predictor of both pyridinoline and deoxypyridinoline excretion. Magnesium is extremely important in skeletal metabolism and there is a growing appreciation that magnesium deficiency may be a cause of osteoporosis (47, 48). In humans, the iliac crest and upper femur of osteoporotic patients have been shown to have 10% less skeletal magnesium than that found at these sites in healthy control subjects (49, 50) and low magnesium intakes have been associated with low vertebral BMD in postmenopausal women (51). The effects of magnesium may be explained by the impairment of a skeletal ATPase responsible for transporting potassium ions into the skeletal interstitium in exchange for hydrogen ion extrusion, which could result in pH imbalance and enhanced bone resorption.

When we examined the association between dietary intake and bone mass, a high past intake of fruit was significantly associated with higher femoral neck BMD; a similar but not significant trend was seen at the other BMD sites. These results, although subject to possible recall bias, support our previous findings (4) and may reflect the positive influence of a high long-term consumption of alkaline-forming foods on bone health. No similar trends were found with past intake of milk or milk products.

TABLE 7
Regression coefficents for nutrient intake, forearm bone mineral density (BMD), and markers of bone metabolism[1]

Dependent variable	Independent factor	Regression coefficient (95% CI)	Constant	R^2	P
				%	
Forearm total BMD	Alcohol intake	1.99 (0.548, 3.433)	298.6	18.1	0.03
	Potassium	0.022 (0.003, 0.042)			
Forearm trabecular BMD	Alcohol intake	1.32 (0.215, 2.426)	178.01	8.7	0.02
Pyd	Magnesium	−0.075 (−0.125, −0.025)	72.1	12.3	0.004
Dpd	Magnesium	−0.028 (−0.046, −0.009)	21.0	12.1	0.004
Osteocalcin	Energy	0.0009 (0.00014, 0.0017)	3.68	8.0	0.02

[1] Pyd, urinary pyridinoline excretion; Dpd, urinary deoxypyridinoline excretion.

The significant relations seen between intakes of the key nutrients contained in fruit and vegetables and forearm BMD lend credence to this hypothesis of a positive influence of alkaline-forming foods on bone health. The Pearson correlation coefficient findings were further strengthened by the identification of potassium as an independent predictor of total forearm BMD, accounting for 7% of the variation. However, this finding was not consistent at all of the forearm BMD sites and no such relation was seen between any of the nutrients and lumbar spine or femoral neck BMD. This lack of association with the axial BMD sites may be explained by the small number of subjects studied and hence a limited statistical power to detect the differences seen in our larger population (4).

The positive association found between alcohol intake and bone mass is an intriguing finding and supports those of our previous study. Mechanisms that have been suggested for an alcohol effect on bone mass include the induction of the adrenal production of androstenedione and its adrenal conversion to estrone (52). Although there is some support in the literature of a positive effect of moderate alcohol intake on postmenopausal bone health (53), the mechanisms involved for pre- and perimenopausal women remain unclear.

In conclusion, we identified several key nutrients (namely, potassium, magnesium, fiber, β-carotene, and vitamin C) and a high past intake of fruit as being important to bone health as assessed by axial and peripheral BMD and bone metabolism markers. Although our data are limited by the cross-sectional study design, they suggest a link between fruit and vegetable consumption and bone health. Further investigations of the relations found between these micronutrients, BMD, and bone turnover are warranted.

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