Lower estimates of net endogenous noncarbonic acid production are positively associated with indexes of bone health in premenopausal and perimenopausal women[1–4]

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ABSTRACT

Background: The link between acid-base homeostasis and skeletal integrity has gained increasing prominence in the literature. Estimation of the net rate of endogenous noncarbonic acid production (NEAP) from dietary protein and potassium content enables exploration of the effects of dietary acidity or alkalinity on bone.

Objective: The study aimed to ascertain whether lower dietary acidity (lower dietary protein intake but higher potassium intake—ie, low estimate of NEAP) was associated with greater axial and peripheral bone mass and less bone turnover, independent of key confounding factors.

Design: Baseline (cross-sectional) results of a population-based study were examined further. The database includes spine and hip bone mineral density (BMD) in 1056 premenopausal or perimenopausal women aged 45–54 y and forearm bone mass and the urinary markers of bone resorption in 62 women. A validated food-frequency questionnaire was used to measure dietary intakes.

Results: Lower estimates of energy-adjusted NEAP were correlated with greater spine and hip BMD and greater forearm bone mass ($P < 0.02$ to $P < 0.05$). Hip and forearm bone mass decreased significantly across increasing quartiles of energy-adjusted NEAP ($P < 0.02$ to $P < 0.03$), and trends at the spine were similar ($P < 0.09$). Differences remained significant after adjustment for age, weight, height, and menstrual status. Lower estimates of energy-adjusted NEAP were also correlated with lower excretion of deoxypyridinoline and were significant predictors of spine and forearm bone mass.

Conclusions: These novel findings provide evidence of a positive link between a ratio of lower protein to higher potassium dietary intake (ie, less dietary acid) and skeletal integrity. *Am J Clin Nutr* 2004;79:131–8.

KEY WORDS Bone health, potassium, protein, dietary acid, dietary alkali, acid-base homeostasis

INTRODUCTION

There is an urgent requirement for the implementation of public health strategies to target prevention of poor skeletal health on a population-wide basis (1). As an exogenous factor, nutrition has a critical role to play in the optimization of bone health because nutrition is amenable to change and has relevant public health implications (2). The development of novel nutritional strategies remains a top priority.

The importance of acid-base homeostasis to skeletal integrity has been gaining increasing prominence in the literature (3). Theoretical considerations of the role that alkaline bone mineral may play in the defense against acidosis date back as far as the early 19th century (4). Natural, pathologic, and experimental states of acid loading, acidosis, or both have been associated with negative calcium balance (5) and greater bone loss (6). At the cellular level, a reduction in extracellular pH has been shown to have a direct enhancement on osteoclastic activity, with the result of increased development of resorption pits in bone (7). Observational, experimental, clinical, and intervention studies over the past decade suggested a positive link between the consumption of alkali-forming foods (ie, fruit and vegetables) and skeletal health (8).

Determination of the acid-base content of diets consumed by individuals and populations is a useful way to quantify the link between acid-base balance and skeletal health. On a daily basis, humans eat substances that both generate and consume protons, and, as a net result, consumption of a normal Western diet is

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associated with chronic, low-grade metabolic acidosis (9). The severity of the associated metabolic acidosis is determined, in part, by the net rate of endogenous noncarbonic acid production (NEAP), which varies with diet. Because 24-h urine collections are impractical in population-based studies, an alternative is to examine the net acid content of the diet. Frassetto et al (10) found that the protein-to-potassium ratio predicts net acid excretion, which, in turn, predicts calcium excretion. They propose a simple algorithm to determine the NEAP by measuring the acidifying effect of protein (via sulfate excretion) and the alkalizing effect of potassium (via provision of salts of weak organic acids).

The principal aim of this study was to examine, for the first time, the association between NEAP and indexes of bone health by using a population-based cohort of premenopausal and perimenopausal women. The specific objectives were to address whether low dietary acidity (lower dietary protein intake but higher potassium intake—ie, a low estimate of NEAP) was associated with greater axial and peripheral bone mass and less bone turnover, independent of key confounding factors.

SUBJECTS AND METHODS

Study design and subject selection

Subjects in this cross-sectional study were the baseline participants in the longitudinal Aberdeen Prospective Osteoporosis Screening Study (APOSS), in whom we previously showed positive associations between the nutrients contained in abundance in fruit and vegetables and the indexes of bone health (11, 12). In brief, subjects reported in this analysis ($n = 1056$) were largely (92%) premenopausal women aged 45–54 y who were randomly selected from a population health register to take part in an osteoporosis study as detailed previously (13). The women had not taken any medication or had no condition likely to affect their bone metabolism. The study was approved by the Grampian Research Ethics Committee.

Measurements of bone health indexes

Bone mineral density (BMD) was assessed in 1056 women with the use of dual-energy X-ray absorptiometry (DXA) (Norland XR-26; Norland Corporation, Fort Atkinson, WI) at the lumbar spine (LS; lumbar vertebrae 2–4) and left femur [femoral neck (FN), femoral trochanter (FT), and femoral Ward's area (FW)]. The precision (expressed as CV) of this technique in our unit was 0.9% for the LS and 2.7% for the FN (14). Peripheral quantitative computed tomography (pQCT) was performed in a subset of 62 women at the ultradistal radius of the nondominant forearm by using a Stratec XCT-960 scanner (Stratec Medizintechnik, Berlin). The CV values were 1.24% for forearm total bone mass, 1.33% for forearm trabecular bone mass, and 1.88% for forearm cortical bone mass (15).

After subjects fasted overnight, urine and blood specimens were obtained from the subset of 62 women for measurement of markers of bone resorption (pyridinium crosslinks) and bone formation (osteocalcin), the full details of which were reported previously (12). In brief, 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 (16). The precision of this technique was 2.7% for pyridinoline and 1.7% for deoxypyridinoline. Serum osteocalcin was measured by an in-house enzyme-linked immunosorbent assay similar to one described previously (17) that used rabbit antiserum that was raised against purified bovine osteocalcin and that had full cross-reactivity with human osteocalcin and a CV of $<10\%$.

Current dietary intake and estimation of NEAP

Usual current dietary intake (over the previous 12 mo) was assessed by using a food-frequency questionnaire (FFQ) as previously detailed (11, 12). In brief, the FFQ was developed and validated against 7-d weighed records (18) and biochemical markers of antioxidant status (19), and its short-term and long-term reproducibility was tested (20).

NEAP was estimated in this population group by examining the ratio of protein to potassium intake normalized to a diet of 8.29 MJ (1982 kcal), which was the mean intake of these women. The simple algorithm proposed by Frassetto et al (10) was then applied:

$$\text{Renal net acid excretion} = 54.5 \times (\text{protein intake/potassium intake}) - 10.2 \quad (1)$$

The concept of NEAP is based on the considerations of the acidifying effect of protein, mainly through sulfate excretion, and the alkalizing effect of potassium, which results from the dietary intake of potassium as salts of weak organic acid.

Assessment of nondietary confounding factors

We measured the weight (by using balance scales; Seca, Hamburg, Germany) and height (by using a stadiometer; Holtain Ltd, Crymych, United Kingdom) of each subject. Information concerning age of menarche, parity, menstrual status, socioeconomic status, smoking habits, present and past physical activity, alcohol consumption, and caffeine consumption was assessed with the use of a questionnaire as reported previously (11, 12).

Statistical analysis

Statistical analysis was performed by using SPSS software (version 11; SPSS Inc, Chicago), and descriptive statistics (means, medians, SDs, and ranges) were determined for all variables. Data were checked for normality by using the Kolmogorov-Smirnov test. Because only age, weight, height, and menstrual status were identified as the confounding factors in our previous studies (11, 12), only those variables were controlled for in the analysis of the relation between NEAP and indexes of bone health. Intakes of protein and potassium were adjusted for total energy intake by normalizing all intakes to a diet of 8.29 MJ (or 1982 kcal). Pearson's correlations and partial correlations (with adjustment for age, weight, height, and menstrual status) were calculated between NEAP and each BMD site, the excretion of pyridinoline and deoxypyridinoline, and the osteocalcin concentration. Equal numbers, obtained from the corresponding values for each quartile cutoff, divided NEAP values into quartiles, and the mean values for BMD at each site and for bone resorption and formation were calculated. Differences between these bone health indexes were assessed by using the F test for linearity and the multiple-range test (one-way analysis of variance with Tukey's test), which is

TABLE 1
Characteristics of the study population[1]

Characteristics	Value
Age (y)	47.2 ± 1.89 (45–54)
BMI (kg/m^2)	24.9 ± 4.1 (15.8–44.0)
Energy (MJ)	8.29 ± 2.16 (3.20–16.8)
EN:EQ	1.435 ± 0.417 (1.01–3.00)
Protein (g)	82.5 ± 23.6[2] (20.0–231.0)
Potassium (mg)	3395 ± 812[3] (1475–6897)
Energy-adjusted NEAP[4]	0.161 ± 0.024 (0.067–0.263)

[1] $\bar{x}$ ± SD; range in parentheses. EN:EQ, energy equivalent (ratio of energy intake to basal metabolic rate); NEAP, net rate of endogenous noncarbonic acid production. n = 1056.
[2] Reference nutrient intake for protein = 45 g.
[3] Reference nutrient intake for potassium = 3500 mg.
[4] Estimate of NEAP using an algorithm in mEq · d^{-1} · 8.29 MJ^{-1}.

based on 95% CIs. Analysis of covariance was used to assess differences after adjustment for important confounding factors, as detailed above. Stepwise multiple regression analysis was also used to determine whether the estimate of NEAP was an independent predictor of indexes of bone health; age, weight, height, and menstrual status were also entered into the equation.

RESULTS

Descriptive data

The age; anthropometric data; dietary intakes of energy, protein, and potassium; and estimates of NEAP (as protein: potassium intake and as the renal net acid excretion algorithm) are shown in **Table 1** for all 1056 subjects. Crude and energy-adjusted intakes of protein and potassium were normally distributed. The energy equivalent [EN:EQ; ie, the ratio of energy intake to basal metabolic rate (BMR)] is also shown. The women were of average height and weight for the local population, and daily intakes of protein and potassium were well within the reference nutrient intakes for women in the United Kingdom between the ages of 19–50 y and >50 y (21). The EN:EQ was 1.435.

Quartile analysis between estimation of NEAP and lumbar spine, hip, and forearm bone mass

Estimates of energy-adjusted NEAP were grouped into quartiles, and the mean BMD at each site was calculated. Both FN and FW BMD decreased significantly across the increasing quartiles of energy-adjusted NEAP estimate ($P < 0.02$ and $P < 0.03$, respectively), and similar (nonsignificant) trends were found for LS BMD ($P < 0.096$). Differences were found between the highest and lowest quartiles of NEAP at the FN (**Figure 1**) and FW (**Figure 2**) BMD sites ($P < 0.04$), and there were similar, nonsignificant trends at the LS (**Figure 3**) and FT (**Figure 4**) BMD sites ($P < 0.1$). Differences in bone mass were of the following magnitudes: LS BMD, 0.038 g/cm^2; FN BMD, 0.021 g/cm^2; FW BMD, 0.038 g/cm^2; and FT BMD, 0.018 g/cm^2; these values represented a 2–4% reduction in LS and hip bone mass due to lower dietary acidity. The subjects were subsequently divided into 5, 8, and 10 equal groups. BMD values at all sites measured were significantly and consistently higher for the lowest estimate of NEAP, and BMD values were significantly and consistently lower for the highest estimate of NEAP, both of which suggest a linear trend.

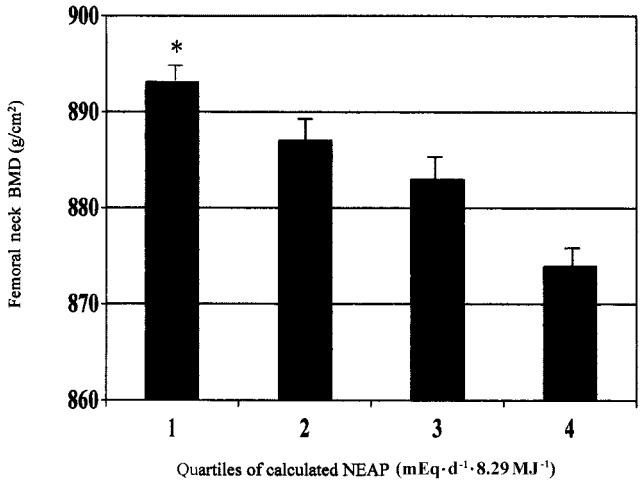

FIGURE 1. Mean (±SEM) femoral neck bone mineral density (BMD) by quartile (Q; n = 264) of calculated net rate of endogenous noncarbonic acid production (NEAP; mEq · d^{-1} · 8.29 MJ^{-1}). The test for linearity among all quartiles was significant, $P < 0.02$. Mean BMD values for Q1–Q4 were 852, 848, 841, and 824 g/cm^2, respectively. *Significantly different from Q4, $P < 0.04$ (ANOVA with Tukey's test, adjusted for age, weight, height, and menopausal status).

Both forearm total and forearm cortical bone mass decreased significantly across increasing quartiles of energy-adjusted NEAP estimate ($P < 0.02$). A significant difference was found between the lowest quartile and the third quartile in both forearm total (**Figure 5**) and forearm cortical (**Figure 6**) BMD ($P < 0.05$); the difference between the lowest and highest quartiles was not significant ($P < 0.12$). Findings remained significant after adjustment for important confounding factors.

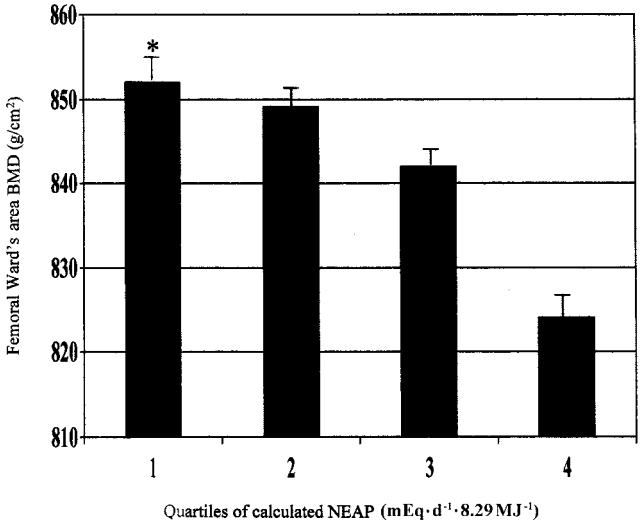

FIGURE 2. Mean (±SEM) femoral Ward's area bone mineral density (BMD) by quartile (Q; n = 264) of calculated net rate of endogenous noncarbonic acid production (NEAP; mEq · d^{-1} · 8.29 MJ^{-1}). The test for linearity among all quartiles was significant, $P < 0.03$. Mean BMD values for Q1–Q4 were 893, 888, 883, and 873 g/cm^2, respectively. *Significantly different from Q4, $P < 0.04$ (ANOVA with Tukey's test, adjusted for age, weight, height, and menopausal status).

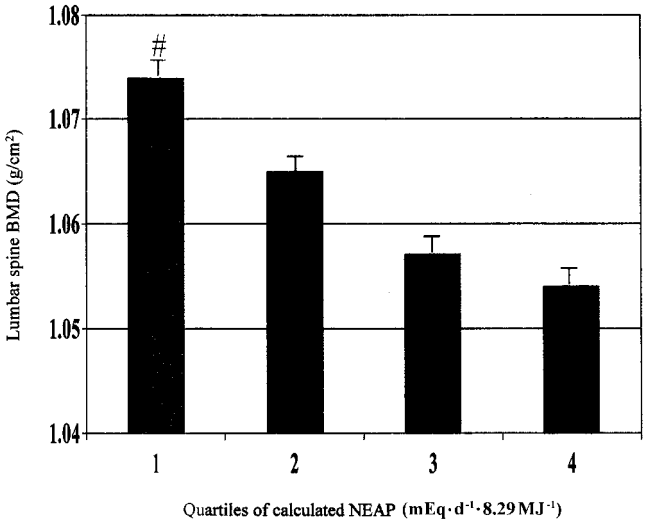

FIGURE 3. Mean (±SEM) lumbar spine bone mineral density (BMD) by quartile (Q; $n = 264$) of calculated net rate of endogenous noncarbonic acid production (NEAP; mEq $\cdot$ d^{-1} $\cdot$ 8.29 MJ^{-1}). The test for linearity among all quartiles showed a nonsignificant trend, $P < 0.096$. Mean BMD values for Q1–Q4 were 1074, 1065, 1058, and 1053 g/cm^2, respectively. #Trend for significant difference from Q4, $P < 0.1$ (ANOVA with Tukey's test, adjusted for age, weight, height, and menopausal status).

Groups were subsequently divided into a second set of 5, 8, and 10 equal groups. Only the lowest estimate of NEAP showed a consistently higher bone mass value when compared with the other quintile or decile NEAP groups. Thereafter the effect was much less marked, which indicated a threshold rather than a linear effect as we had noted for DXA. No differences were found at the peripheral forearm trabecular site.

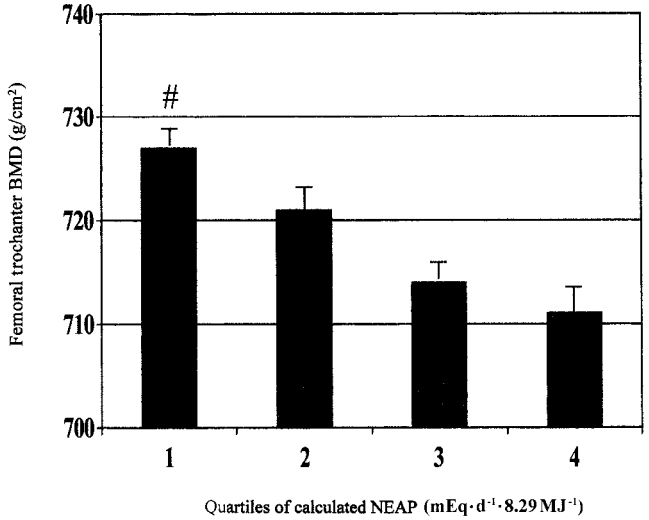

FIGURE 4. Mean (±SEM) femoral trochanter bone mineral density (BMD) by quartile (Q; $n = 264$) of calculated net rate of endogenous noncarbonic acid production (NEAP; mEq $\cdot$ d^{-1} $\cdot$ 8.29 MJ^{-1}). The test for linearity among all quartiles was not significant, $P < 0.15$. Mean BMD values for Q1–Q4 were 728, 721, 714, and 711 g/cm^2, respectively. #Trend for significant difference from Q4, $P < 0.1$ (ANOVA with Tukey's test, adjusted for age, weight, height, and menopausal status).

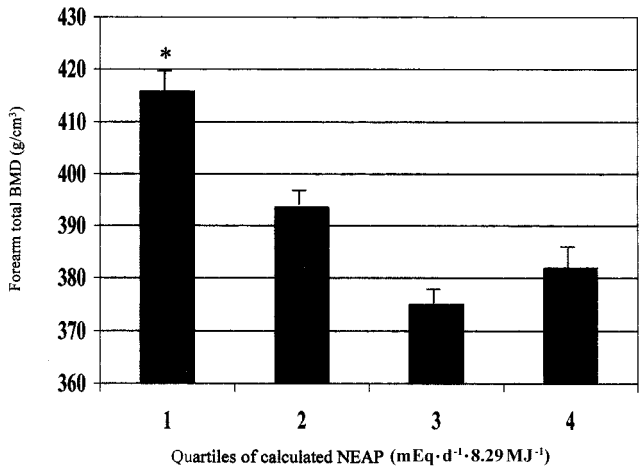

FIGURE 5. Mean (±SEM) forearm total bone mineral density (BMD) by quartile (Q; $n = 15$) of calculated net rate of endogenous noncarbonic acid production (NEAP; mEq $\cdot$ d^{-1} $\cdot$ 8.29 MJ^{-1}). The test for linearity among all quartiles was significant, $P < 0.02$. Mean BMD values for Q1–Q4 were 416, 392, 375, and 380 g/cm^3, respectively. *Significantly different from Q3, $P < 0.05$ (ANOVA with Tukey's test, adjusted for age, weight, height, and menopausal status).

Associations between estimation of NEAP and markers of bone metabolism

Lower estimates of energy-adjusted NEAP were associated with lower excretion of deoxypyridinoline ($r = 0.27$; $P < 0.05$), and a similar nonsignificant trend was seen with pyridinoline excretion in the subset of 62 women ($r = 0.24$; $P < 0.09$). Adjustment for the confounding factors weakened the association between NEAP and deoxypyridinoline ($P < 0.1$). The highest quartile of energy-adjusted NEAP estimate was found to have the highest pyridinoline and deoxypyridinoline excretion, and a significant difference was found between the second and highest quartile for these bone resorption markers

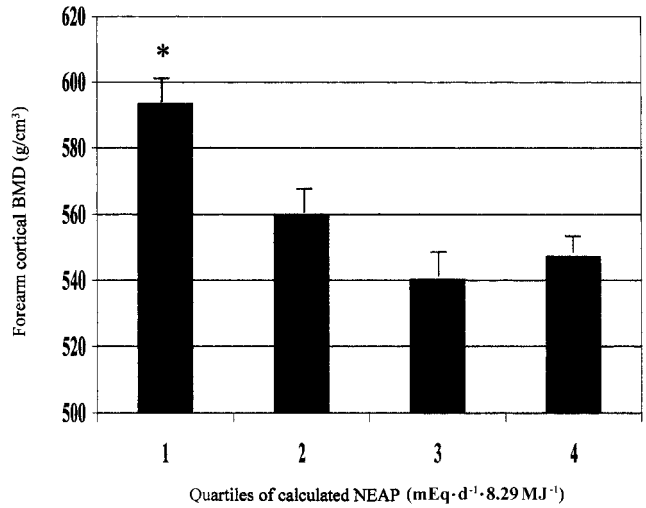

FIGURE 6. Mean (±SEM) forearm cortical bone mineral density (BMD) by quartile (Q; $n = 15$) of calculated net rate of endogenous noncarbonic acid production (NEAP; mEq $\cdot$ d^{-1} $\cdot$ 8.29 MJ^{-1}). The test for linearity among all quartiles was significant, $P < 0.02$. Mean BMD values for Q1–Q4 were 588, 560, 540, and 542 g/cm^3, respectively. *Significantly different from Q3, $P < 0.05$ (ANOVA with Tukey's test, adjusted for age, weight, height, and menopausal status).

TABLE 2
Difference in pyridinium crosslink excretion between quartiles (Q) of the energy-adjusted net rate of endogenous noncarbonic acid production (NEAP) estimate[1]

	NEAP estimate			
	Q1 (n = 15)	Q2 (n = 15)	Q3 (n = 16)	Q4 (n = 16)
		$nmol \cdot mmol^{-1} \cdot L^{-1}$		
Pyridinoline excretion	49.6 ± 12.4 (32.9–78.7)	42.8 ± 10.8[2] (32.5–75.2)	43.8 ± 9.8 (27.8–63.1)	54.3 ± 10.2 (37.6–70.4)
Deoxypyridinoline excretion	12.1 ± 3.3 (6.4–17.3)	10.6 ± 5.0[2] (6.6–25.0)	11.9 ± 3.7 (5.5–19.2)	14.4 ± 4.4 (8.3–23.5)

[1] $\bar{x}$ ± SD; range in parentheses.
[2] Significantly different from Q4, $P < 0.05$ (ANOVA with Scheffe's test).

($P < 0.05$; **Table 2**). In contrast with the findings for pQCT, the most marked effect was seen in the group with highest estimate of NEAP, which was associated with the greatest bone resorption. No associations were found between NEAP and serum osteocalcin concentrations.

Correlations between estimates of NEAP and lumbar spine, hip, and forearm bone mass

Correlations between energy-adjusted NEAP and LS, hip, and forearm bone mass are shown in **Table 3**. Lower estimates of NEAP were significantly associated with higher LS BMD ($P < 0.02$), FN BMD ($P < 0.05$), and FW BMD ($P < 0.05$). Lower estimates of NEAP were also found to be significantly associated with higher forearm cortical ($P < 0.03$) and forearm total ($P < 0.02$) bone mass in the subset of 62 women. Differences remained significant after adjustment for age, weight, height, and menstrual status. No associations were found at the forearm total BMD site.

Estimation of NEAP as an independent predictor of bone health

At the LS, NEAP was an independent predictor of bone mass after weight and height, with the equation as follows:

$$\text{LSBMD} = 0.269 + 0.00438\ \text{weight (kg)} + 0.00035\ \text{height (m)} - 0.385\ \text{NEAP (protein-to-potassium ratio/8.29 MJ)} \quad (2)$$

TABLE 3
Pearson's correlation coefficients between the energy-adjusted net rate of endogenous noncarbonic acid production (NEAP) estimate and spine, hip, and forearm bone mass[1]

	Energy-adjusted NEAP estimate
Bone mineral density (g/cm^2)[2]	
Lumbar spine	−0.085[3]
Femoral neck	−0.07[4]
Femoral trochanter	−0.05
Femoral Ward's area	−0.07[4]
Bone mass (g/cm^2)[5]	
Cortical forearm	−0.27[6]
Total forearm	−0.29[3]

[1] Adjusted for age, weight, height, and menstrual status.
[2] n = 1056.
[3] $P < 0.02$.
[4] $P < 0.05$.
[5] n = 62.
[6] $P < 0.03$.

The regression analysis explained only 13.5% of the variation in LS BMD; weight and height explained 13%, and estimates of NEAP explained a further 0.5% ($P < 0.001$). Partial correlation coefficients were significant for weight, height, and NEAP ($P < 0.001$, $P < 0.001$, and $P < 0.025$, respectively). By using the calculated regression equation, holding weight and height constant (using the mean values for the group), and looking at the difference in LS BMD between the minimum and maximum intakes of NEAP estimate, we found an 8% reduction in LS BMD. Absolute values were 0.923 g/cm^2 for the highest intake of NEAP and 0.999 g/cm^2 for the lowest intake of NEAP, a difference of 0.076 g/cm^2. At the forearm, NEAP was highlighted as an independent predictor of both cortical and total bone mass, accounting for 0.7% and 0.5% of the variation ($P < 0.001$). NEAP was not a predictor of bone mass at any of the hip sites measured and was not significant in the model for excretion of either pyridinoline or deoxypyridinoline ($P < 0.09$).

DISCUSSION

This study is the first reported investigation between estimates of NEAP and indexes of bone health—namely, axial and peripheral bone mass—and markers of bone metabolism in a population-based cohort of premenopausal and perimenopausal women. Our results indicate that diets with a lower protein content but higher potassium content (ie, lower acidity or higher alkalinity) are associated with greater bone mass and a tendency to less bone resorption. Results appeared to indicate a linear effect of NEAP for axial BMD (as assessed by DXA) but were more reflective of a threshold effect for peripheral bone mass (as assessed by pQCT). For bone resorption, the most marked effect (ie, the highest level of excretion) was seen in the group with the highest estimate of NEAP.

Quantifying the acid-base content of diets generally consumed by populations is critical for determining the diets' effects on bone status. It is considered that normal adult humans eating typical Western diets have chronic, low-grade metabolic acidosis (22). This acidosis is believed to occur because the amount of noncarbonic acids released into the systemic circulation after the metabolism of such a diet (eg, sulfuric acid from the metabolism of proteins) is greater than the amount of base released simultaneously (eg, bicarbonate from the metabolism of organic acid salts of potassium in fruit and vegetables) (23). Because the NEAP is difficult to measure directly, we used protein:potassium intake and then applied the simple algorithm that was shown by Frassetto et al (10) to

determine the net renal acid excretion (a predictor of calcium excretion).

Our data support the growing interest in the importance of acid-base homeostasis to skeletal integrity, theoretical considerations of which date back to the 1880s (24), and yet it is only more recently that the subject was more thoroughly reviewed (3, 4, 25). The pioneering work of Lemann et al (26) and Barzel (27) >3 decades ago extensively showed the effects of acid from the diet on bone mineral in both man and animals. Wachman and Bernstein (28) put forward a hypothesis that "the increased incidence of osteoporosis may represent, in part at least, the results of a lifelong utilization of the buffering capacity of the basic salts of bone for the constant assault against pH homeostasis." They suggested that long-term consumption of "a diet favoring alkaline-ash (ie, fruits and vegetables) may be important for osteoporosis prevention." Novel work by Arnett and Dempster (29) indicates a direct enhancement of osteoclastic activity after a reduction in extracellular pH, independent of PTH, and there is evidence that a small drop in pH, which brings pH close to the physiologic range, causes a tremendous burst in bone resorption (30, 31). It has been shown that the administration of either potassium bicarbonate administration (32) or potassium citrate (33) results in significant improvements in both calcium and bone metabolism.

A large number of population-based observational studies published in the past decade suggested an association between fruit and vegetable consumption and indexes of bone health (11, 12, 34–42), and the findings of the Dietary Approaches to Stop Hypertension and Dietary Approaches to Stop Hypertension–Sodium intervention trials support such a link (43–46). More recently, Buclin et al (47) examined the effect of dietary modification on calcium and bone metabolism. The acid-forming diet increased urinary calcium excretion by 74% and bone resorption, as measured by C-terminal peptide excretion, by 19% more than did the alkali-forming diet, both at baseline and after an oral calcium load.

It is important to note that the positive associations found between fruit and vegetable consumption and bone health may be due to some other, as yet unidentified dietary component rather than to alkali-excess effect (48). Convincing work by Muhlbauer et al (49) suggested that vegetables, herbs, and salads commonly consumed in the human diet affect bone resorption in the rat by a mechanism that is mediated not by the base excess of the vegetables, herbs, and salads but possibly through pharmacologically active compounds that are currently being explored (50).

The APOSS Nutrition baseline data set is a well-characterized, population-based cohort of premenopausal and perimenopausal women (11, 12). These studies were among the first to examine the association between nutritional factors and bone health, with measurements of BMD at clinically relevant sites, assessment of markers of bone metabolism, and the use of a well-validated dietary assessment tool. Adjustments were also made for key confounding factors, including total energy intake. Our strict exclusion criteria ensured that the data for analysis in this study were from subjects who were unlikely to have been compromised with regard to their bone health. In general the FFQ was well answered and the EN:EQ was within the range established for satisfactory completion (51). Adjustment for total energy intake is important when examining diet-disease relations in general (21), but it is especially important in relation to bone health, for which physical activity is an important confounder (52).

Our bone marker data are somewhat difficult to interpret because differences were seen only between the second and highest quartile and not between the lowest and the highest quartiles. This may be explained, in part, by the cross-sectional nature of the study design and our small sample size. Furthermore, it is well known that between-subject variability in the excretion of these bone metabolism markers is a common problem in the interpretation of bone metabolism data (53).

In this re-analysis, we found that a diet characteristic of a lower protein intake but a higher potassium intake is associated positively with indexes of bone health. These data do not suggest that dietary protein is detrimental to bone health, because even women in the lowest quartile of protein had protein intakes well above the EAR (mean intake: 82.5 g/d). Rather, these data indicate that dietary potassium (as the denominator in the NEAP algorithm) is the critical component—that is, diets that are characterized by less dietary acid (ie, are closer to neutral) are associated with better indexes of bone health. Although the variation in factors, including weight and height, account for most of the variation in BMD among subjects, estimates of NEAP still accounted for a significant fraction of the variation. A shift from the top quartile to the lowest quartile of dietary acidity as a group or from the top intake to the lowest intake of dietary acidity as an individual resulted in a better bone mass. We believe our findings concerning NEAP are critical because, in adults after the age of <30 y, both weight and height remain relatively stable; most of the influence of increasing height and weight on the setting of bone mass has been exerted by then. Thus in women who are postmenopausal, elderly, or both, with one-time measure of BMD, weight and height influences on BMD manifest strongly among subjects. However, it can be postulated that NEAP continues to wear away bone gradually and indefinitely after the age of 30 y, and this effect accelerates as the glomerular filtration rate drops with age (A Sebastian, personal communication, May 2003). In other words, weight and height differences among subjects would not likely transfer their influence on BMD in each individual over time, but NEAP, as an ongoing dynamic influence on bone, presumably does continue its influence on bone mass in each individual subject over time.

Of growing interest in the literature is the recognition that calcium plays a critical role in the relation between the balance of a beneficial versus detrimental effect of protein on the skeleton (54–57). It is possible that calcium supplements may be favorable to bone, not just through the additional mineral that they supply but also through their provision of additional alkali salts (58). Because the relation between dietary protein and skeletal health remains a controversial one (59), it might be prudent to suggest reanalysis of existing nutrition and bone health data sets to focus specifically on the effect of protein: potassium and protein:calcium on indexes of bone health.

There are limitations to our study data and thus to the reanalysis of the relation between NEAP and bone presented here. The study design for this analysis was cross-sectional; hence we can state only associations rather than relations, and consequently we cannot draw any firm conclusions about the influence of nutrition on bone health. The number of subjects studied for peripheral bone mass and markers of bone metab-

olism is small, and, although every effort was made to validate the dietary assessment tool, it is well established that FFQs have errors associated with them. Unfortunately, it was not possible within the study design to measure in a subgroup the true NEAP estimate from 24-h urine collections.

In conclusion, the findings of the present study suggest that lower estimates of NEAP (ie, diets of less acidity; lower protein:higher potassium content) are significantly associated with greater axial and peripheral bone mass and less bone resorption, an effect that it is independent of important confounding factors. These novel findings provide further evidence of a positive link between less dietary acid and skeletal integrity, and thus they lend additional support to the hypothesis that the skeleton plays an important role in maintaining acid-base homeostasis (60).

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SAN, JCM, MJG, SPR, and DMR were responsible for the design of the experiment; SAN, JCM, and MJG were responsible for data collection; SAN, HM, MKC, JCM, and MJG were responsible for data analysis; and all authors were responsible for writing the manuscript. None of the authors had any conflict of interest.

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