More acidic dietary acid-base load is associated with reduced calcaneal broadband ultrasound attenuation in women but not in men: results from the EPIC-Norfolk cohort study[1–3]

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ABSTRACT

Background: Dietary patterns that promote mild metabolic acidosis may have a negative effect on bone density.

Objective: We investigated the relation between a measure of dietary acid-base load, potential renal acid load (PRAL), and calcaneal broadband ultrasound attenuation (BUA) after adjustment for confounders and also compared the results with different estimates of acid-base load.

Design: A cross-sectional study was conducted in 14 563 men and women aged 42–82 y living in Norfolk, United Kingdom, in which measures of calcaneal BUA and dietary PRAL were estimated by using the European Prospective Investigation into Cancer and Nutrition Norfolk (EPIC-Norfolk) food-frequency questionnaire.

Results: A more acidic dietary intake (high PRAL) was significantly associated with lower calcaneal BUA in women but not in men; there was a difference of ≈2% in BUA between the highest and lowest quintiles of PRAL, independent of age, body mass index, smoking habit, physical activity, diagnosed osteoporosis, and history of fracture, and (in women) hormone replacement therapy. No relation was observed between history of fracture or incident fracture and PRAL. Those with the greatest PRAL had higher intakes of meat, fish, eggs, and cereal and cereal products and lower intakes of fruit and vegetables, tea, and coffee.

Conclusion: PRAL was inversely associated with bone ultrasound measures in women, but the magnitude of the association was relatively small compared with other known risk factors. Further longitudinal studies are required to establish whether, in the long term, these small effects are important in overall fracture risk in populations. *Am J Clin Nutr* 2007;85:1134–41.

KEY WORDS Acid-base balance, diet, broadband ultrasound attenuation, BUA, bone density, potential renal acid load, PRAL, European Prospective Investigation into Cancer and Nutrition Norfolk, EPIC-Norfolk

INTRODUCTION

Osteoporosis and osteoporotic fractures have substantial clinical and health effects. An estimated 75 million persons in Europe, Japan, and the United States are affected by osteoporosis (1, 2). Osteoporosis is characterized by low bone density and microarchitectural deterioration of bone tissue, and there is increasing interest in behavioral factors that may influence bone density.

The acid-base balance in the body is important to bone health and is modifiable by diet (3–5). The organic acids that are produced during metabolism and the hepatic oxidation of sulfur-containing amino acids (cysteine and methionine) lower blood pH through increased production of hydrogen ions (5, 6). Alkaline dietary salts contain the cations (potassium, calcium, and magnesium) and act as buffers for organic acids that have the potential to raise pH. If insufficient levels of buffering capacity are available from alkaline salts, mild metabolic acidosis develops, even in healthy persons (5, 7, 8). In healthy persons in whom acid production is increased experimentally, acid production increases more than does renal net acid excretion, so that acid balances become positive (8).

The suggested mechanism for the negative effect of increased metabolic acidosis on bone is bone resorption (8–12). Mature osteoclasts are activated, and osteoclastic calcium mobilization is stimulated so that bone matrix mineralization is inhibited (10, 13, 14). Because bone provides a large reservoir of buffering capacity from the content of carbonate and hydroxyapatite salts, mobilization of this reservoir ameliorates acidosis (13–15). The release of $CaCO_3$ is stimulated by acidosis, and increased excretion of calcium and markers of bone resorption has been found during metabolic acidosis (5, 8, 16, 17), which indicates that buffering by bone is a mechanism by which large changes in pH are avoided in the body (8, 13). Although indirect evidence is available for the effect of metabolic acidosis on bone from in vivo intervention studies and in vitro experiments, studies of bone density in general populations are limited—2 in women, 1 in children, and none in men (18–20). The dietary potential renal

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[2] Supported by program grants from the Medical Research Council UK and Cancer Research UK and by the European Union, Stroke Association, British Heart Foundation, the UK Department of Health, Food Standards Agency, and the Wellcome Trust.

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Received July 21, 2006.

Accepted for publication November 21, 2006.

acid load (PRAL) is one measure of acid-base load. It is calculated by taking into account the mineral and protein composition of foods, the average intestinal absorption rates of nutrients, sulfur metabolism, and urinary excretion of organic acids (21, 22).

The purpose of the present study was to investigate the cross-sectional relation between a dietary measure of acid-base load (PRAL), calcaneal broadband ultrasound attenuation (BUA; a measure of bone density), and incident fracture risk in a general population of men and women aged 42-82 y in the Norfolk area of the United Kingdom. Second, because adequate intakes of calcium and protein were shown to determine bone density, we investigated whether a relation existed between intakes of calcium and protein and the effect of dietary acid-base load (23–25). Third, because different methods of calculating dietary acid-base load are available, we wanted to compare the effect of different methods of estimation, specifically the net rate of noncarbonic endogenous acid production (NEAP) and the protein-to-potassium ratio (protein:potassium), on BUA (4, 7).

SUBJECTS AND METHODS

Subjects

Approximately 25 000 men and women aged 40–79 y living in the general community participated in a baseline examination in Norfolk, United Kingdom, between 1993 and 1997 as part of the European Prospective Investigation into Cancer (EPIC), a 10-country collaboration on diet and cancer, and in the EPIC-Norfolk study of other health outcomes (26). Between 1997 and 2000, men and women then aged 42–82 y were invited to attend a second visit at which quantitative ultrasound measurements of the calcaneum were conducted by trained nurses according to standard protocols (27, 28).

All participants gave written informed consent and then were asked to complete a self-administered food-frequency questionnaire (FFQ) and a detailed health and lifestyle questionnaire (29, 30). The FFQ was designed to estimate habitual intake during the previous year, and nutrients were computed with the use of an in-house program, the CAFE (Compositional Analyses from Frequency Estimates) program (29). Ethical permission was granted by the Norfolk and Norwich Ethics Committee.

History of fracture was established from the answers to the question, "Has your doctor ever told you that you have any of the following? " The question was followed by a list of conditions that included osteoporosis, hip fracture, wrist fracture after age 20 y, and vertebral fracture (spinal fractures). Participants were followed for health events and the number of incident fractures by site (hip, wrist, spine, and other). The number of incident fractures was obtained between date of entry to the study (1997–2000) and mid-2005 for participants admitted to the hospital with the use of the unique National Health Service number by linking to the East Norfolk Health Authority database (ENCORE), which identifies all hospital contacts throughout England and Wales for Norfolk residents.

Quantitative ultrasound scanning was used to measure BUA and velocity of sound of the calcaneum with the use of the CUBA clinical machine (McCue Ultrasonics, Winchester, United Kingdom) at least twice on each foot, as described elsewhere (27, 28). Five machines were used, and each was calibrated daily with its physical phantom and monthly with a roving phantom and on one operator's calcaneum. We previously examined the data for evidence of systematic differences in temperature and machine by using multivariate regression models of BUA on age, weight, height, body mass index (BMI; in kg/m^2), smoking habit, use of hormone replacement therapy (HRT) for women, and previous fracture history. We found that no further adjustments for temperature, machine, or machine drift informed the fit of the data; therefore, the data are presented unadjusted for temperature (27). Height and weight were measured to the nearest 0.1 cm and 0.2 kg, respectively, while participants were wearing light clothing and no shoes (26, 27).

The PRAL index was calculated by using individual nutrients derived from the FFQ by using the following formula (22, 29):

$$\begin{aligned}\text{PRAL (in mEq/d)} = {} & [(\text{mg phosphorus/d} \times 0.0366) \\ & + (\text{g protein/d} \times 0.4888)] - [(\text{mg potassium/d} \\ & \times 0.0205) + (\text{mg calcium/d} \times 0.0125) \\ & + (\text{mg magnesium/d} \times 0.0263)] \quad (1)\end{aligned}$$

The PRAL index was divided into sex-specific quintiles for analysis. Chloride and sodium are sometimes included in the PRAL index but were excluded in these analyses because of their equimolar content in dietary salt and because urine chloride and sodium excretion concentrations are similar. The food types that are the main contributors to the nutrients included in the PRAL index were also derived from the FFQ.

Because previous studies also used other measures of acid-base load and because it was indicated that protein:potassium is the main determinant of PRAL, protein:potassium was calculated (protein g/d divided by potassium mEq/d), and renal net acid excretion, hereafter referred to as NEAP, was also calculated according to equations determined by Frassetto et al (7) (unadjusted for energy). The analyses were repeated with the use of these 2 measures, as in the following equation:

$$\begin{aligned}\text{NEAP (in mEq/d)} = {} & 54.5 \text{ (g protein/mEq potassium)} \\ & - 10.2 \quad (2)\end{aligned}$$

Physical activity level was estimated by using a validated score devised from a question about work-based and recreational physical activity. The scores represented physical activity levels described as inactive, moderately inactive, moderately active, and active (31).

We compared mean BUA in men and women in the different quintiles of PRAL before and after adjustment for age, BMI, smoking status, physical activity, and previously diagnosed osteoporosis and for HRT status in women; these factors were previously established as having a significant relation to calcaneal BUA within this population (27, 32). Men and women were studied separately because we previously found important sex differences in age-related bone loss in this population (21). Because the effect of acid-base load may be expected to differ depending on adequate intakes of calcium and because protein could also affect bone health independently of acid-base load, the analyses were repeated and additionally adjusted for calcium and protein. Analyses were also repeated and included adjustment for total energy intake. We also repeated the analyses with the use of velocity of sound. However, because the results did not differ

TABLE 1
Characteristics of the population stratified by quintiles (Q) of potential renal acid load (PRAL) in 6375 men and 8188 women aged 42–82 y

	PRAL						
	All	Q1	Q2	Q3	Q4	Q5	*P* for trend[2]
Men							
n	6375	1275	1275	1275	1275	1275	
Age (y)	62.9 ± 9.0[3]	62.0 ± 9.0	62.9 ± 8.9	62.9 ± 8.9	63.4 ± 9.2	63.4 ± 9.0	< 0.01
BMI (kg/m^2)	26.9 ± 3.3	26.9 ± 3.3	27.0 ± 3.3	26.8 ± 3.2	26.9 ± 3.4	26.8 ± 3.5	0.36
Current smokers [*n* (%)]	502 (7.9)	104 (8.2)	93 (7.3)	87 (6.8)	101 (7.9)	115 (9.0)	0.32
Physical activity	2.42 ± 1.11	2.44 ± 1.09	2.42 ± 1.10	2.42 ± 1.12	2.43 ± 1.11	2.41 ± 1.15	0.59
History of fractures [*n* (%)]	363 (7.7)	76 (6.0)	84 (6.6)	71 (5.6)	65 (5.1)	67 (5.3)	0.16
Incident fractures [*n* (%)]	95 (1.5)	24 (1.9)	13 (1.0)	16 (1.3)	21 (1.6)	21 (1.6)	0.94
Women							
n	8188	1640	1639	1639	1639	1639	
Age (y)	61.5 ± 9.0	61.1 ± 8.7	61.6 ± 9.0	61.8 ± 8.9	61.7 ± 9.1	61.4 ± 9.3	0.31
BMI (kg/m^2)	26.5 ± 4.4	26.5 ± 4.3	26.3 ± 4.3	26.6 ± 4.2	26.6 ± 4.4	26.6 ± 4.6	0.28
Current smokers [*n* (%)]	652 (7.9)	153 (9.3)	125 (7.6)	126 (7.7)	121 (7.4)	126 (7.7)	0.11
Physical activity	2.32 ± 1.04	2.39 ± 1.04	2.31 ± 1.03	2.33 ± 1.06	2.28 ± 1.01	2.32 ± 1.06	0.04
Current HRT [*n* (%)]	1734 (21.1)	372 (22.7)	362 (22.1)	325 (19.8)	344 (21.0)	331 (20.2)	0.05
History of fractures [*n* (%)]	607 (7.4)	109 (6.7)	126 (7.7)	142 (8.7)	107 (6.5)	123 (7.5)	0.79
Incident fractures [*n* (%)]	242 (3.0)	51 (3.1)	52 (3.2)	53 (3.2)	44 (2.7)	42 (2.6)	0.23

[1] PRALs were −101.28 to −14.07, −14.06 to −7.28, −7.27 to −1.60, −1.61–4.58, and 4.59–99.86 mEq/d in men and −119.71 to −16.27, −16.26 to −9.73, −9.74 to −4.11, −4.12–1.66, and 1.67–68.18 mEq/d in women in Q1, Q2, Q3, Q4, and Q5, respectively.

[2] Calculated with the use of ANOVA. *P* for trend was not calculated for quintiles of PRAL because this was the categorization variable. There was a significant interaction between sex and all variables except for smoking behavior ($P < 0.001$).

[3] $\bar{x}$ ± SD (all such values).

substantially from those for BUA, only data for BUA are presented here.

Because the effect of HRT on bone density in postmenopausal women is well established, the analyses were repeated after excluding women currently taking HRT and after adjustment for total energy intake. Because a history of fracture is also known to affect BUA, the analyses were repeated after the exclusion of persons with a history of fracture. Mean PRAL was calculated according to incident fracture status and was adjusted for age, BMI, total energy intake, previously diagnosed osteoporosis, physical activity, and smoking status and (in women) the use of HRT.

Intakes of nutrients and food types were stratified by quintiles of PRAL. In the United Kingdom, the main sources of potassium are potatoes and savory snacks, fruit and vegetables, and meat and meat products (33). The main sources of magnesium are cereals and cereal products, fruit and vegetables, and beverages (33). The main sources of protein and phosphorus are meat and meat products, milk and milk products, and cereals and cereal products (33).

Statistical analysis

Statistical analyses were performed with STATA statistical software (version 7.0; Stata Corp, College Station, TX). All analyses were stratified by sex. Mean BUA, adjusted for covariates, was calculated by analysis of covariance. Analysis of variance was used to test for differences among quintiles of PRAL for all variables except physical activity, smoking, and HRT status, for which chi-square tests were performed. Relative risks were calculated with the use of single-variable and multivariate logistic regressions.

In a further analysis to compare the relative magnitude of effect of PRAL with other known osteoporotic risk factors, the continuous variables were standardized and run in a multivariable regression. PRAL was analyzed by SD (*z* score), BMI by 3-unit groups, and age by 10-y groups. The inactive and moderately active categories of exercise were compared with the moderately active and active categories; current smoking was compared with no smoking; and the use of HRT medication was compared with no use of HRT medication.

To quantify the effects of dietary acid-base load on urinary acid-base excretion, a further analysis of urine pH in a subsample of 363 men and women aged 43–79 y was conducted with the use of a pH meter (model 3310; Jenway, Dunmow, United Kingdom) in 24-h urine collections. The pH was regressed against quintiles of PRAL.

RESULTS

Bone ultrasound measurements and data from the FFQ were available for 6375 men and 8188 women. There were 95 incident fractures in men and 242 in women (**Table 1**).

Mean daily PRAL was significantly lower (ie, more alkaline) in women than in men ($P = 0.001$) (Table 1). For each quintile of PRAL, mean PRAL was more acidic in men than women, and in men the CV was higher (men: 253%; women: 160%), which indicated greater variability in values (Table 1). In men, increasing PRAL was significantly associated with age ($P < 0.01$), whereas, in women, it was significantly associated with physical activity ($P = 0.04$) and use of HRT medication ($P = 0.05$). In the substudy of PRAL and pH measured in 24-h urine, the β was −0.08 units of pH per quintile of PRAL, which was significant ($P < 0.001$).

When classified by quintiles of PRAL, the trends for the nutrients comprising PRAL and also NEAP and protein:potassium were significant ($P < 0.001$) (**Table 2**). Men and women in the top quintile

TABLE 2
Intake of nutrients contributing to the potential renal acid load (PRAL) index and net rate of noncarbonic endogenous acid production (NEAP) and protein-to-potassium ratio stratified by quintile (Q) of PRAL in 6375 men and 8188 women aged 42–82 y[1]

	All	Q1	Q2	Q3	Q4	Q5[2]
Men						
n	6375	1275	1275	1275	1275	1275
PRAL (mEq/d)	−4.77 ± 12.09	−21.65 ± 7.64	−10.38 ± 1.95	−4.36 ± 1.63	1.36 ± 1.79	11.17 ± 7.37
Protein (g/d)	85.3 ± 21.8	78.0 ± 19.7	78.1 ± 18.2	81.8 ± 18.9	87.7 ± 19.1	100.9 ± 23.9
Phosphorus (mg/d)	1524 ± 377	1471 ± 368	1432 ± 343	1469 ± 346	1538 ± 358	1709 ± 401
Calcium (mg/d)	1049 ± 298	1033 ± 310	1002 ± 284	1024 ± 280	1057 ± 288	1127 ± 312
Magnesium (mg/d)	346 ± 91	378 ± 91	338 ± 83	331 ± 88	332 ± 89	349 ± 95
Potassium (mg/d)	3904 ± 897	4430 ± 967	3880 ± 797	3736 ± 814	3700 ± 828	3775 ± 860
NEAP (mEq/d)	46.9 ± 9.1	35.3 ± 4.4	41.8 ± 2.7	46.5 ± 2.5	51.5 ± 2.8	59.4 ± 6.3
Protein:potassium	1.0 ± 0.2	0.8 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	1.3 ± 0.1
Women						
n	8188	1638	1638	1637	1638	1637
PRAL (mEq/d)	−7.61 ± 12.18	−24.89 ± 9.75	−12.77 ± 1.88	−6.85 ± 1.59	−1.33 ± 1.65	7.81 ± 5.89
Protein (g/d)	81.6 ± 20.7	75.2 ± 20.4	76.2 ± 18.3	79.3 ± 18.7	82.7 ± 18.6	94.8 ± 21.0
Phosphorus (mg/d)	1462 ± 360	1423 ± 373	1397 ± 329	1430 ± 346	1455 ± 342	1604 ± 371
Calcium (mg/d)	998 ± 288	1002 ± 305	969 ± 267	987 ± 281	987 ± 275	1046 ± 304
Magnesium (mg/d)	337 ± 87	370 ± 93	336 ± 79	327 ± 84	319 ± 81	333 ± 88
Potassium (mg/d)	3887 ± 917	4463 ± 1065	3910 ± 786	3757 ± 810	3623 ± 810	3679 ± 828
NEAP (mEq/d)	44.8 ± 8.9	33.4 ± 4.8	40.0 ± 2.9	44.4 ± 2.5	49.0 ± 2.4	57.0 ± 5.8
Protein:potassium	1.0 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	1.1 ± 0.0	1.2 ± 0.1

[1] PRALs were −101.28 to −14.07, −14.06 to −7.28, −7.27 to −1.60, −1.61–4.58, and 4.59–99.86 mEq/d in men and −119.71 to −16.27, −16.26 to −9.73, −9.74 to −4.11, −4.12–1.66, and 1.67–68.18 mEq/d in women in Q1, Q2, Q3, Q4, and Q5, respectively.
[2] P for trend < 0.001 between Q1 and Q5 of PRAL all nutrients and for NEAP and protein:potassium. There was a significant interaction between sex and all variables except potassium ($P < 0.001$).
[3] $\bar{x} \pm$ SD (all such values).

of PRAL (most acidic) consumed approximately twice as much meat, approximately one-quarter as much fish, and ≈40% more cereals and cereal products than did those in the bottom quintile (**Table 3**). Intakes of fruit and vegetables and of tea and coffee in the top quintile of PRAL were ≈60% and ≈80%, respectively, of those in the lowest quintile. Trends in intake of food types associated with quintile of PRAL were significant ($P < 0.001$).

Mean (±SD) BUA was 90.0 ± 17.5 dB/MHz in men and 72.1 ± 16.5 dB/MHz in women. Mean BUA was analyzed in the following 10-y age groups: 40–49 y (men: 92.9 ± 17.3 dB/MHz;

TABLE 3
Intake of selected food types stratified by quintile (Q) of potential renal acid load (PRAL) in 6375 men and 8188 women aged 42–82[1]

	All	Q1	Q2	Q3	Q4	Q5
Men						
n	6375	1275	1275	1275	1275	1275
Meat and meat products (g/d)	98 ± 52[2]	73 ± 40	82 ± 38	92 ± 41	106 ± 42	138 ± 66[3]
Dairy foods (g/d)	425 ± 182	408 ± 191	414 ± 180	422 ± 173	432 ± 177	449 ± 186[3]
Eggs (g/d)	13 ± 13	10 ± 9	11 ± 10	12 ± 12	14 ± 13	17 ± 17[3]
Fish (g/d)	37 ± 26	34 ± 24	34 ± 22	35 ± 23	38 ± 24	44 ± 32[3]
Fruit and vegetables (g/d)	417 ± 219	572 ± 297	428 ± 192	387 ± 171	365 ± 164	332 ± 158[3]
Cereals and cereal products (g/d)	291 ± 136	250 ± 119	262 ± 112	283 ± 132	309 ± 132	352 ± 157[3]
Tea and coffee (g/d)	1068 ± 381	1222 ± 432	1111 ± 377	1050 ± 342	1005 ± 339	954 ± 349[3]
Women						
n	8188	1638	1638	1637	1638	1637
Meat and meat products (g/d)	91 ± 49.0	65 ± 41	77 ± 40	86 ± 41	98 ± 41	129 ± 54[3]
Dairy foods (g/d)	413 ± 176	405 ± 184	403 ± 166	415 ± 177	416 ± 171	427 ± 178[3]
Eggs (g/d)	11 ± 11	9 ± 9	10 ± 10	10 ± 9	11 ± 10	14 ± 15[3]
Fish (g/d)	38 ± 26	35 ± 26	36 ± 24	38 ± 25	39 ± 25	45 ± 29[3]
Fruit and vegetables (g/d)	514 ± 260	725 ± 351	537 ± 211	479 ± 202	429 ± 180	400 ± 174[3]
Cereals and cereal products (g/d)	256 ± 118	226 ± 107	235 ± 101	252 ± 113	264 ± 115	304 ± 134[3]
Tea and coffee (g/d)	1041 ± 385	1202 ± 428	1090 ± 372	1034 ± 355	972 ± 345	909 ± 353[3]

[1] PRALs were −101.28 to −14.07, −14.06 to 7.28, −7.27 to −1.60, −1.61–4.58, and 4.59–99.86 mEq/d in men and −119.71 to −16.27, −16.26 to −7.73, −9.74 to −4.11, −4.12–1.66, and 1.67–68.18 mEq/d in women for Q1, Q2, Q3, Q4, and Q5, respectively.
[2] $\bar{x} \pm$ SD (all such values).
[3] P for trend <0.001 (ANOVA).

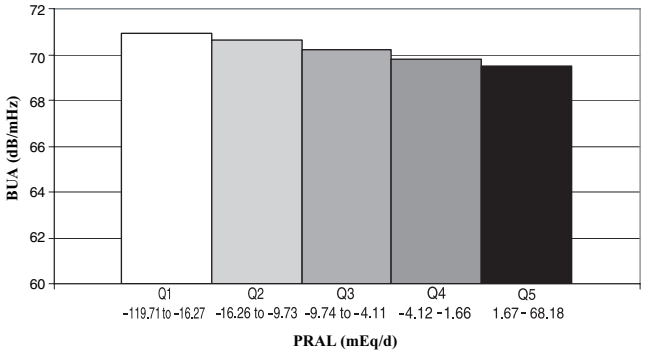

FIGURE 1. Relation of broadband ultrasound attenuation (BUA) by quintile (Q) of dietary potential renal acid load (PRAL) in all women after exclusion of women taking hormone replacement therapy. $\bar{x} \pm$ SD BUA was 70.9 ± 0.30 dB/MHz (Q1), 70.6 ± 0.21 dB/MHz (Q2), 70.2 ± 0.17 dB/MHz (Q3), 69.8 ± 0.21 dB/MHz (Q4), and 69.5 ± 0.30 dB/MHz (Q5); $P = 0.003$ with dietary PRAL for all women ($n = 6455$) after adjustment for age, BMI, physical activity, diagnosed osteoporosis, smoking status, and energy intake.

women: 81.7 ± 15.8 dB/MHz), 50–59 y (men: 91.0 ± 17.3 dB/MHz; women: 78.3 ± 15.2 db/MHz), 60–69 y (men: 89.9 ± 17.1 dB/MHz; women: 70.0 ± 14.9 dB/MHz), and >70 y (men: 88.6 ± 18.2 dB/MHz; women: 62.2 ± 15.0 dB/MHz). Because little difference was observed after adjustment for energy intake, results are presented unadjusted for energy intake, except for those presented in **Figure 1**. No relation was observed between PRAL and BUA either before or after adjustment for covariates in men (**Table 4**). However, in women, a significant trend was observed between BUA and PRAL (ie, a more acidic dietary intake was associated with a lower BUA), which remained significant after adjustment for age and other covariates (Table 4). A 2.1% difference in BUA was observed between quintiles 1 and 5 of PRAL in women in the fully adjusted model. When women taking HRT were excluded from the analyses, the results did not differ significantly (Figure 1).

In men, no relation was observed between PRAL and history of fracture or incident fracture risk, but, in women, a nonsignificantly lower risk was observed between increasing quintiles of PRAL and incident fracture, after exclusion of those women with a history of fracture (Table 4). Mean PRAL according to incidence of fracture, after adjustment for covariates, was −8.75 mEq/d in female subjects with fractures and −7.59 mEq/d in female control subjects (P for difference = 0.20) and −4.83 mEq/d in male subjects with fractures and −4.77 mEq/d in male control subjects ($P = 0.96$).

When the analyses were repeated with NEAP and protein: potassium, the results did not differ significantly (data not shown). The correlation between the different measures of acid-base load was 0.93 ($P < 0.001$) for the relation between PRAL and protein-potassium and for that between PRAL and NEAP.

In the comparative regression analysis that used standardized variables all variables except smoking status were significant in

TABLE 4
Calcaneum broadband ultrasound attenuation (BUA) stratified by quintile (Q) of potential renal acid load (PRAL) in 6375 men and 8188 women aged 42–82 y, adjusted for age, BMI, physical activity, and smoking status and for hormone replacement therapy (HRT) status in women[1]

	PRAL					
	Q1	Q2	Q3	Q4	Q5	P for trend[2]
Men						
BUA (dB/MHz)[3]	90.0 ± 17.6	89.7 ± 17.2	90.4 ± 17.4	90.1 ± 17.7	89.9 ± 17.9	0.89
Model 1[4,5]	89.9 ± 0.38	90.0 ± 0.27	90.1 ± 0.22	90.1 ± 0.27	90.2 ± 0.38	0.58
Model 2[4,6]	89.4 ± 0.40	89.7 ± 0.27	90.0 ± 0.22	90.4 ± 0.27	90.7 ± 0.40	0.068
History of fractures	1.0	1.11 (0.81, 1.53)[7]	0.93 (0.67, 1.30)	0.85 (0.60, 1.19)	0.88 (0.63, 1.23)	0.14
Model 1[5]	1.0	1.10 (0.80, 1.51)	0.92 (0.66, 1.29)	0.83 (0.59, 1.17)	0.86 (0.62, 1.21)	0.14
Incident fractures	1.0	0.54 (0.27, 1.06)	0.66 (0.35, 1.25)	0.87 (0.48, 1.58)	0.87 (0.48, 1.58)	0.88
Model 1[5]	1.0	0.52 (0.26, 1.03)	0.65 (0.34, 1.23)	0.84 (0.46, 1.51)	0.84 (0.46, 1.51)	0.99
Model 3[8]	1.0	0.46 (0.22, 0.95)	0.63 (0.33, 1.21)	0.75 (0.40, 1.40)	0.85 (0.46, 1.57)	0.97
Women						
BUA (dB/MHz)[3]	73.2 ± 16.5	72.4 ± 16.2	72.1 ± 16.4	71.5 ± 16.7	71.5 ± 16.6	< 0.001
Model 1[4,5]	72.9 ± 0.27	72.5 ± 0.19	72.1 ± 0.16	71.7 ± 0.19	71.3 ± 0.27	< 0.001
Model 2[6]	72.8 ± 0.28	72.5 ± 0.20	72.1 ± 0.16	71.8 ± 0.20	71.4 ± 0.28	0.004
History of fractures	1.0	1.17 (0.90, 1.53)	1.33 (1.03, 1.73)	0.98 (0.74, 1.29)	1.14 (0.87, 1.49)	0.99
Model 1[5]	1.0	1.13 (0.86, 1.48)	1.28 (0.98, 1.67)	0.92 (0.59, 1.17)	1.09 (0.62, 1.21)	0.90
Incident fractures	1.0	1.02 (0.69, 1.51)	1.04 (0.70, 1.54)	0.86 (0.57, 1.29)	0.82 (0.54, 1.24)	0.23
Model 1[5]	1.0	0.97 (0.65, 1.43)	0.98 (0.66, 1.45)	0.81 (0.53, 1.22)	0.77 (0.51, 1.17)	0.14
Model 3[8]	1.0	0.86 (0.56, 1.34)	0.88 (0.57, 1.37)	0.90 (0.58, 1.38)	0.59 (0.36, 0.97)	0.08

[1] PRALs were −101.28 to −14.07, −14.06 to −7.28, −7.27 to −1.60, −1.61–4.58, and 4.59–99.86 mEq/d in men and −119.71 to 16.27, −16.26 to −9.73, −9.74 to −4.11, −4.12–1.66, and 1.67–68.18 mEq/d in women in Q1, Q2, Q3, Q4, and Q5, respectively.
[2] P for trend calculated with use of ANOVA. Sex × BUA, sex × history of fractures, and sex × incident fractures interactions were significant ($P < 0.001$).
[3] All values in row are $\bar{x} \pm$ SD (except P value).
[4] All values in row are $\bar{x} \pm$ SE (except P value).
[5] Adjusted for age, BMI, physical activity, previously diagnosed osteoporosis, and smoking status and for HRT status in women.
[6] Adjusted for age, BMI, physical activity, previously diagnosed osteoporosis, calcium, protein intake, and smoking status and for HRT status in women.
[7] Odds ratio; 95% CIs in parentheses (all such values).
[8] Excluded persons with history of fractures and adjusted for age, BMI, physical activity, and smoking status and for HRT status in women ($n = 6018$ men; $n = 7588$ women).

TABLE 5
Mean calcaneum broadband ultrasound attenuation regression with age, BMI, smoking status, physical activity level, and potential renal acid load (PRAL) and (for women) use of hormone replacement therapy (HRT) in 6375 men and 8188 women aged 42–82 y

	Men			Women		
	β	95% CI	*P*	β	95% CI	*P*
Age (per 10-y group)	−1.46	−1.89, −0.92	<0.001	−7.60[1]	−7.96, −7.24	<0.001
BMI (per 3-unit group)	1.67	−1.28, 2.06	<0.001	3.11[1]	2.90, 3.33	<0.001
Smoking (yes or no)	−2.39	−3.98, −0.79	0.003	−1.02	−2.16, 0.11	0.078
Physical activity[2]	0.65	−0.22, 1.52	0.14	0.80	0.17, 1.44	0.013
PRAL (per SD)[3]	−0.06	−0.49, 0.37	0.78	−0.49	−0.80, −0.18	0.002
HRT (yes or no)				5.56	4.79, 6.32	<0.001

[1] Women were significantly different from men ($P < 0.001$).
[2] Inactive and moderately active compared with moderately active and active physical activity.
[3] SD = 12.2 mEq/d (*z* score).

women (**Table 5**). Age and the use of HRT were the variables most strongly associated with BUA in women, followed by BMI, physical activity, smoking status, and PRAL, whereas, in men, the effects were all much smaller but differed significantly from those in women for age and BMI.

DISCUSSION

In the present study, we found a significant negative association between a more acidic diet (more positive PRAL) and calcaneal BUA in women but not in men. We found no association with history of fracture or incident fracture in either men or women. To our knowledge, the present study is the first large population-based study of dietary acid-base load in men and premenopausal and postmenopausal women.

PRAL is a measure of the relative consumption of foodstuffs supplying the nutrients included in the acid load. The foods contributing the most to the acid load are meat, fish, milk and milk products, and eggs; cereal grains and foods are also main contributors (34). In the present study, there were significant ($P < 0.001$) trends of all food groups with PRAL, with higher intakes of meat, fish, eggs, and cereal and cereal products and lower intakes of fruit and vegetables, tea, and coffee between the highest quintile of PRAL and the lowest.

Mean PRAL was more alkaline in our study (3.68 ± 10.51 mEq/d; range: −46.2–84.9 mEq/d) than in another UK study (−7.60 mEq/d; range: −119.71–68.18 mEq/d; (35). The PRAL index does not include the contribution from organic acids, and, in populations with relatively alkaline intake, organic acids could be more important (13). However, because organic acids are calculated from body size and because BMI is the main determinant of BUA, we wanted to determine the effect of dietary acid-base load independent of body weight. Although the diet in the present study was more alkaline than that in another UK study, our average estimate of NEAP was 46 mEq/d, which is similar to that in the United States (48 mEq/d) (34).

The size of association between BUA and dietary acid-base load in women between quintiles 1 and 5 of PRAL—the most alkaline and most acidic, respectively—was ≈2% after adjustment for known factors that affect BUA (ie, BMI, age, smoking status, exercise behaviors, previously diagnosed osteoporosis, and use of HRT medication in women) and after exclusion of subjects taking HRT medication and subjects with a history of fracture. When protein and calcium intakes were accounted for, there was little difference in the results. However, compared with other known risk factors, the effect of PRAL is small. In addition, no association was found with either a history of fracture or incident fracture.

The magnitude of the associations found are similar to those in another UK study, in which a 2–4% reduction in lumbar spine and hip bone density between quartiles 1 and 4 of dietary NEAP was found (1056 premenopausal and perimenopausal women aged 45–54 y) (19). In further analyses of premenopausal, perimenopausal, and postmenopausal women, no significant difference was observed in the bone mineral density of the lumbar spine or femoral neck with NEAP; however, in menstruating women, significant associations were observed with energy-adjusted NEAP at the femoral neck ($P = 0.04$) and with NEAP ($P = 0.026$) and energy-adjusted NEAP ($P = 0.014$) at the lumbar spine (18). In a study with children aged 6–18 y, those with a higher PRAL had significantly less cortical area ($P < 0.05$) and bone mineral content ($P < 0.01$) after adjustment for protein intake (20). PRAL accounted for 2% of the variation in bone indexes.

The correlations between the different dietary indexes of acidity—PRAL, NEAP, and protein:potassium—were high and were similar to those in another study (35). The results of analyses with the use of these indexes also did not differ significantly, which indicated that they capture the same elements of dietary acid-base load.

We found no association between dietary intake of PRAL and BUA in men in this population. It is well documented that bone density and BUA decrease with age in women. Although our previous study confirmed these findings in women, we found only small age-related differences in BUA in men (27, 36–43). Why women should be more sensitive than men to the effects of dietary acid load remains an open question. Bone loss in women is related to reduced production of circulating estrogen during and after menopause. It is possible that the effects of mild metabolic acidosis induced by diet could interact with the effects of estrogen withdrawal in women. Although the relation between PRAL and BUA in women was small, because there is an age-related decline in renal function that leads to development of low-grade diet-dependent metabolic acidosis, the effect of excess dietary acid could have a greater effect as people age (44).

The present study has several limitations. The cross-sectional associations limit the causal inferences that can be made, although there are biologically plausible mechanisms for the relation between dietary acid load and bone metabolism. It is unlikely that persons with different bone measures across the normal range would change their dietary intake. Of course, we cannot exclude confounding, although the associations in women were independent of known factors that influence bone health, such as smoking and BMI. The study could have lacked power to detect associations or could have underestimated any associations because of measurement errors in assessment of diet and bone health or because of the range of dietary intake or bone measures. Measurements with BUA were made of the calcaneum and not of the spine or hip. Nevertheless, previous associations with known osteoporotic risk factors and increased risk of incident fracture with BUA were shown in this population (27, 28). As with other studies reporting associations, we used an FFQ to measure diet; in a substudy, we found significant relations with PRAL and urinary pH, which indicated that the dietary measurement reflects the acid-base balance in the body.

Evidence that mild metabolic acidosis is detrimental to bone health has been available for some time (3, 4, 8, 45, 46), although the present study is the largest population study to show an association between a more acidic dietary load and bone health in women. Our findings concur with previous small-scale human intervention and animal studies that found measurable effects of dietary interventions designed to modify the acid-base load on measures of blood and urine pH and bone turnover (5, 8, 16, 17). Supplementation studies with alkaline salts have found effects on markers of bone turnover and urinary pH and net acid excretion (8, 16, 47). In addition, animal and in vitro studies have elucidated the mechanisms for the effect of metabolic acidosis on bone metabolism (10–12, 45, 48, 49) and have also established that metabolic—not respiratory—acidosis is responsible for the effect of modified (blood) pH on bone cells and that this effect is mediated by prostaglandin E_2 (12, 49).

In conclusion, we found that a more acidic PRAL was associated with a significantly lower calcaneal BUA in women but not in men. We also found no evidence that risk of history of fracture or incident fracture was affected in either men or women. Although the association in women was independent of other known factors that influence bone, the magnitude of these associations was relatively small compared with other known risk factors. However, longitudinal studies are required to establish whether, in the long term, these small effects are important to overall fracture risk in populations.

AAW provided the dietary data, performed the statistical analyses, and wrote the manuscript. KTK and SAB are principal investigators of the EPIC-Norfolk Study. All of the authors were involved in interpreting the data and contributed to writing the manuscript. None of the authors had any personal or financial conflict of interest.

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