Original Research

Dietary Acid-Base Balance, Bone Resorption, and Calcium Excretion

Ramina Jajoo, MD, Lingyi Song, MS, Helen Rasmussen, RD, MS, Susan S. Harris, DSc, Bess Dawson-Hughes, MD

Division of Rheumatology, Tufts-New England Medical Center (R.J.), Bone Metabolism Laboratory (L.S., S.S.H., B.D.-H.), Nutrition Services Department, Metabolic Research Unit (H.R.), Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts

Key words: net acid excretion, calcium excretion, N-telopeptide, PTH

Objective: Metabolic studies reveal that acidogenic diets increase bone resorption acutely. This study was conducted to examine associations between diet-induced changes in net acid excretion (NAE) and changes in serum parathyroid hormone (PTH), bone resorption, and calcium excretion over a longer period of 60 days.

Methods: Forty healthy older men and women were given 0.75 g/kg of protein as meat, 600 mg of calcium, and 400 IU of vitamin D_3 daily and either cereal (acidogenic) or fruit and vegetable (alkalinogenic) foods as substitutes for some of the cereal in their usual diets. Blood and 24-hr urine measurements were made on days 14 (baseline), 44, and 74.

Results: In all subjects, change in renal NAE was correlated with changes in serum PTH ($r = 0.358$, $P = 0.023$), urinary N-telopeptide (NTX) ($r = 0.367$, $P = 0.020$), and urinary calcium excretion ($r_p = 0.381$, $P = 0.020$, after adjustment for diet group, change in PTH, and change in sodium excretion).

Conclusions: Diet changes that increase renal NAE are associated with increases in serum PTH, bone resorption, and calcium excretion over a 60-day period.

INTRODUCTION

American diets are considered to be a risk factor for osteoporosis and bone fractures in part due to their high potential acid content. The net endogenous acid produced from these diets, measured as urinary net acid excretion, is estimated to be 40–80 mEq/day [1,2]. Protein and cereal grains are metabolized to acidic residues whereas fruits and vegetables have an alkaline residue. Therefore the balance between intakes of these major dietary components will determine the net potential acid load of a diet [3]. An excess acid load is buffered by bone and in the process calcium is released.

Epidemiologic studies have observed that greater intakes of fruits and vegetables are associated with greater bone mineral density (BMD) [4,5] and lower urinary excretion of markers of bone resorption [5] but not with serum osteocalcin [5]. Short-term dietary interventions using metabolic diets have had mixed results. In one study, an acidogenic diet for 4 days lowered urinary and serum pH and increased urinary C-telopeptide and calcium excretion [6]. In a recent 8-week dietary study, however, a higher dietary protein intake increased urinary NAE but did not affect several markers of bone turnover, calcium retention or urinary calcium excretion [7]. Short-term administration of bicarbonate has resulted in decreases in urinary NAE, NTX [8,9], urinary hydroxyproline excretion [10], and urinary calcium excretion [9–11] and in one study, an increase in serum osteocalcin [10]. On the other hand acute ingestion of acid has been associated with increases in urinary NAE, hydroxyproline and calcium excretion [12].

It is important to determine whether the acid/base balance of the diet has a sustained effect on the skeleton. In this pilot study, we examine associations between diet-induced change in

Address correspondence to: Bess Dawson-Hughes, M.D., Bone Metabolism Laboratory at the Jean Mayer, USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington St. Boston, MA 02111. E-mail: Bess.Dawson-Hughes@Tufts.edu

Presented in part at the American Society of Bone and Mineral Research meeting in Seattle, WA, October, 2004.

This material is based on work supported by the National Institute of Health grant No. T32AR07050 and U.S. Department of Agriculture under agreement No. 59-1950-9001. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors, and do not necessarily reflect the view of the National Institute of Health or the U.S. Department of Agriculture.

renal NAE and changes in serum PTH, markers of bone turnover, and urinary calcium excretion in free-living subjects over a 60-day period.

MATERIALS AND METHODS

Subjects

Forty healthy men and women, age 50 and older, were enrolled and all subjects completed the study. Telephone prescreening with a short (11 question) food frequency questionnaire was used to identify subjects with usual dietary protein intakes of ≤0.75 g/kg/day and calcium intakes of ≤1000 mg/day and a brief medical history questionnaire was used to determine general eligibility. No subjects were taking estrogen, glucocorticoids, insulin or diuretics and none had a history of a disorder known to alter calcium or bone metabolism. Screening evaluation included DXA scans and blood and urine tests. Subjects were excluded if they had a femoral neck Z score <-2.0, 24-hour urine calcium >300 mg, or abnormal hepatic or renal function tests. The study protocol was approved by the Investigation Review Board at Tufts University and written informed consent was obtained from each subject.

Experimental Design And Diets

Subjects were asked to discontinue any usual vitamin D and calcium supplements from one week prior to enrollment to the end of the study. All subjects took one Posture-D tablet (600 mg of elemental calcium and 125 IU vitamin D_3) and a multivitamin (400 IU vitamin D_3) daily throughout the study (day 0–day 74) in order to reduce inter-subject variability in intakes and serum PTH levels. Calcium tri-phosphate was used because it is pH-neutral supplement and wouldn't affect NAE. On day 14, subjects were randomized to meat plus fruit and vegetable (F+V) food servings or meat plus cereal grain servings. Meat choices were beef (tenderloin, roast and hamburger), chicken and turkey breast, cod fish, pork tenderloin, and canned tuna; fresh fruit and vegetable choices were 100% fruit juices, bananas, blueberries, oranges, raisins, strawberries, broccoli, carrots, cauliflower, green beans, spinach, sweet potatoes, tomatoes, and zucchini; cereal/grain choices were couscous, pasta, rice, Pop Tarts™, and granola bars. All food supplements were prepared in the Metabolic Kitchen and portioned in daily amounts. They could be eaten at any time of the day. Blood and urine tests were made on days 14 (prior to the food supplements), 44, and 74 (end of the study).

All subjects were given servings of meat containing 0.75 g of protein/kg/d on days 14–74. Subjects assigned to F+V group were also provided with servings of fruits and vegetables that contained potential alkali in an amount calculated to neutralize the potential acid load (PRAL) of the meat [3]. Subjects assigned to the cereal group received foods that were low in potential alkali content, mainly grain cereals (for example, rice or pasta salad) with small amounts of fat in the form of olive oil for palatability. These foods were isocaloric with the fruit and vegetable substitutes. The cereal and F&V group food substitutes contained 825 ± 190 mg/day (21.1 ± 4.9 mmol/d) and 1583 ± 190 mg/day (40.6 ± 4.9 mmol/d) of potassium, respectively ($P < 0.001$). The calculated PRAL of the cereal group foods was acidic (mean = 28.6 ± 5.4 mEq/day) and that of the F&V foods was neutral (mean 0.1 ± 0.3 mEq/day; $P < 0.001$).

There were no significant group differences in age, sex, or body size. During the period that the food supplements were provided (day 14 to day 74), subjects were counseled by a dietitian to decrease the fat, added sugar, and grain content of their usual diets in order to maintain stable weight during the study. Analyses at the end of the study however revealed that there were deletions in other elements of the diet. The 24-hr urinary potassium content in the F/V group on day 74, 70.2 mmol, was below the expected level of 106 mmol (40 mmol from the added foods and 66 mmol in their basal diets), as shown in Table 1. Similarly, expected changes in NAE were not observed. NAE of the F/V group should have remained constant or declined somewhat (as acidogenic cereals in the self-selected diets were replaced with neutral food supplements), and yet it increased from 36.9 to 44.7 mmol/d (Table 1). These results indicate that the design was not fully implemented and that the diet neutralization portion of the experiment is invalid. Not surprisingly, there were no significant diet group differences in serum osteocalcin, calcium, and urinary

Table 1. Serum and Urine Measurements on Days 14 and 74 in the Two Study Groups

	Day 14	Day 74
Serum		
Intact PTH (pmol/L)		
Cereal group	4.9 ± 1.7	5.2 ± 1.8
F&V group	4.7 ± 1.2	4.5 ± 0.9
Total calcium (mmol/L)		
Cereal group	2.3 ± 0.1	2.4 ± 0.1
F&V group	2.3 ± 0.1	2.3 ± 0.1
24-Hour Urine		
Potassium (mmol/d)		
Cereal group	68.0 ± 17.6	59.2 ± 18.1
F&V group	66.1 ± 23.7	70.2 ± 24.7
Nitrogen (mmol/d)		
Cereal group	907 ± 163	1036 ± 236
F&V group	872 ± 298	994 ± 342
Calcium (mmol/d)		
Cereal group	4.06 ± 1.57	4.15 ± 1.87
F&V group	2.91 ± 1.30	3.40 ± 1.69
N-telopeptide (nmol)		
Cereal group	234 ± 188	247 ± 227
F&V group	149 ± 77	148 ± 78
Net Acid Excretion (mmol/d)		
Cereal group	39.1 ± 23.1	56.0 ± 23.0
F&V group	36.9 ± 25.1	44.7 ± 21.6

Values are mean ± SD.

NAE, NTX, or calcium excretion or in changes in these measures. However there were substantial individual changes in NAE during the study (as shown in the figures). Herein we report associations of baseline NAE and 60-day change in NAE with baseline and change in serum PTH, biochemical markers of bone turnover, and calcium excretion in the group as a whole.

Diet Assessments

Nutrient calculations were performed using Nutrition Data System for Research (NDS-R) software version 4.04 32, developed by the Nutrition Coordinating Center, University of Minnesota, MN. A description of the nutrient database analytical values is reported elsewhere [13]. Potential renal acid load (PRAL) of the food substitutes was calculated by the method of Remer [3].

Dietary intake of protein, calcium, phosphorus, magnesium, vitamin D, and total energy over the preceding two months was assessed on the screening visit (representing baseline intake) and on day 74 with Fred Hutchinson Food Frequency Questionnaire [14]. They were self-administered on site and reviewed for completeness by a dietitian. Supplemental calcium was not recorded on this questionnaire.

Biochemical Measurements

Bloods were drawn after an 8-hour fast. Serum 25(OH)D was measured with radioimmunoassay kits from Diasorin (Stillwater, MN), serum PTH and osteocalcin with radioimmunometric assay kits, and serum IGF-1 with radioimmunoassay kits from Nichols Institute Diagnostics (San Juan Capistrano, CA). Urinary N-telopeptide levels were measured with competitive-inhibition enzyme-linked immunoabsorbent assay kits from Ostex International (Seattle, WA). Serum ionized and total calcium concentrations were measured with ion-selective electrodes measurement kit from Medica (Bedford, MA). Urinary sodium, potassium and calcium were measured by direct-current plasma emission spectroscopy with a Spectraspan 6 (Beckman Instruments, Palo Alto, CA). The intra- and inter-assay CVs of the above assays are 2.7 and 6.8% respectively. Urinary nitrogen was measured with a model FP-2000 nitrogen/protein determinator (LECO, St. Joseph, MI). This instrument employs a Dumas combustion method and detection using a thermal conductivity cell. It measures nitrogen with a precision of 15 ppm. Serum cortisol and 24-hour urinary free cortisol were assayed with solid-phase radioimmunoassay kits (Coat-A-Count Cortisol Kit, Diagnostic products Corp., Los Angeles, CA) with intra- and inter-assay CVs of 4.8 and 5.2% respectively. Osteoprotegerin and receptor activator of nuclear factor-kB ligand (RANKL) were measured using enzyme-linked immunosorbent assay from Osteogenic Core Technologies (Torrance, CA) with intra- and inter-assay CVs of 4.5 and 7.4% respectively. Samples from individual subjects were batched except for serum ionized calcium and serum and urinary creatinine analyses. NAE was measured in 24-hr urine collections by a modification of the Jorgensen titration method [15], as described by Chan [16]. Net acid excretion (NAE) = Titratable acid (TA) + Ammonium (NH_4^+) − Bicarbonate (HCO_3^-). Briefly, TA − HCO_3 was assessed after addition of HCl, boiling the sample, and then titrating the sample to neutral pH. To measure the NH_4^+, formol was added to the sample to release a H^+ from NH_4^+ and the sample was again titrated to neutral pH. All titrations were carried out with a TIM 900 Titration Manager by Radiometer Analytical (Hach Inc, Loveland, CO). The precision of NAE measurements in our laboratory was determined by analyzing aliquots of a single 24-hr urine collection on 15 different days. The aliquots were stored frozen at −20 degrees Celsius for 4 to 9 months and thawed only once. The CV of the NAE measurements was 10.9%. The CV of the TA measurements on the same 15 samples was 5.0%.

Statistical Analysis

Pearson correlations of baseline measurements and of their changes over 60 days in all subjects were performed and their corresponding P-values reported. The partial correlation coefficients were examined after adjusting for potential confounders in ANCOVA models. All results were the mean ± SD. All P-values were two-sided, and $P < 0.05$ was considered statistically significant. Analyses were conducted with SPSS version 11.5 (SPSS Inc, Chicago).

RESULTS

The clinical characteristics of the 40 subjects are shown in Table 2. Eighteen men and 22 women participated; their mean age was 63.7 ± 8.4 years.

The self-reported nutrient intakes at the beginning and end of the study (including food substitutes) are summarized in Table 3. The subjects reported significant increases in total protein intake during the study, however, intake of potassium did not change significantly. Weight decreased by an average of 0.61 ± 1.58 kg during the study. Compliance, as recorded by

Table 2. Baseline Characteristics of the 40 Study Subjects

Characteristic	
Age (yrs)	63.7 ± 8.4
% Men	45
% Caucasian	95
Weight (kg)	76.1 ± 11.3
BMI (kg/cm^2)	26.5 ± 3.8
% Smokers	2.5
Alcohol (drinks/wk)	3.4 ± 5.0
Miles walked/wk	7.0 ± 8.0
Spine BMD (gm/cm^2)	1.1 ± 0.2
Femoral neck BMD (gm/cm^2)	0.92 ± 0.15

Values are mean ± SD.

Table 3. Daily Nutrient Intake at Screening and Day 74 in the 40 Subjects

Nutrient	Screening	Day 74
Energy (kcal)	1580 ± 700	1659 ± 549
Fat		
%Calories	35 ± 7	33 ± 7
gm	61 ± 32	62 ± 29
Protein		
%Calories	18 ± 4	23 ± 5
gm	67 ± 28	90 ± 21
Carbohydrate		
%Calories	47 ± 8	44 ± 8
gm	188 ± 93	181 ± 65
Calcium (mg)[a]	750 ± 427	642 ± 287
Magnesium (mg)	270 ± 99	283 ± 79
Phosphorus (mg)	1146 ± 485	1233 ± 347
Potassium (mg)	2574 ± 1018	2851 ± 753
Sodium (mg)	2518 ± 1077	2687 ± 808
Protein/potassium (g/mEq)	1.0 ± 0.2	1.3 ± 0.3

Values are mean ± SD.

[a] Based on the Hutchinson Food Frequency Questionnaire, and does not include calcium supplements.

the subjects in their diaries, exceeded 97% for the food substitutes and 98% for calcium and multivitamin pills.

Serum and 24-hour urine measurements on days 14, 44, and 74 are summarized in Table 4. As expected, nitrogen excretion increased significantly with the food substitutes (by 125.6 ± 246.9 mmol/d, $P = 0.003$), as did the urinary nitrogen to potassium ratio ($p < 0.001$). NAE also increased significantly by 11.9 ± 18.7 mmol/d, $P < 0.001$. Urine potassium excretion did not change significantly.

Associations of NAE with urine nitrogen, potassium and N/K ratio were examined in all subjects. At baseline (day 14, after 2 weeks on calcium and vitamin D supplements and prior to food substitutions), NAE was significantly correlated with urinary nitrogen excretion ($r = 0.636$, $P < 0.001$, $n = 40$) and with the urinary nitrogen to potassium ratio ($r = 0.528$, $P < 0.001$, Fig. 1-A) but not with urinary potassium excretion ($r = 0.068$, $P = 0.678$). Change in NAE (day 74–day 14) was significantly correlated with change in urinary nitrogen excretion ($r = 0.389$, $P = 0.013$) and with change in the urinary nitrogen to potassium ratio ($r = 0.453$, $P = 0.003$, Fig. 1-B) but not with change in urinary potassium excretion ($r = -0.104$, $P = 0.525$).

We next examined associations between NAE and serum PTH, markers of bone turnover, and urinary calcium excretion in all subjects. At baseline, NAE was significantly correlated with urinary N-telopeptide ($r = 0.428$, $P = 0.006$) but not with serum osteocalcin, PTH, or urinary calcium excretion ($r = 0.281$, $P = 0.079$, trend); adjustment for urinary sodium excretion did not alter these associations. Change in NAE (day 74–day 14) was significantly correlated with change in serum PTH ($r = 0.358$, $P = 0.023$; Fig. 2). An increase in NAE was also associated with an increase in urinary NTX ($r = 0.367$, $P = 0.020$; Fig. 3) and adjusting for change in serum PTH modestly altered this association ($r_p = 0.304$, $P = 0.060$). Change in PTH and change in urinary N-telopeptide were not significantly correlated either before ($r = 0.260$, $P = 0.106$) or after controlling for change in NAE ($r_p = 0.148$, $P = 0.369$). Change in NAE was not associated with change in serum osteocalcin ($r = -0.128$, $P = 0.431$). Adjusting for food

Table 4. Serum and Urine Measurements on Days 14, 44 and 74 in the 40 Subjects

	Day 14	Day 44	Day 74	Change (Day 74–Day 14)	Significance of change (P value)
Serum					
Intact PTH (pmol/L)	4.8 ± 1.5	4.8 ± 1.3	4.9 ± 1.5	0.05 ± 0.99	0.751
Osteocalcin (nmol/L)	3.6 ± 1.1	3.6 ± 1.0	3.4 ± 1.0	−0.20 ± 0.49	0.016
Ionized calcium (mmol/L)	1.1 ± 0.04	1.1 ± 0.04	1.1 ± 0.03	−0.01 ± 0.03	0.044
Total calcium (mmol/L)	2.3 ± 0.08	2.3 ± 0.1	2.4 ± 0.08	0.04 ± 0.08	0.002
IGF-1 (nmol/L)	20.3 ± 6.8	21.4 ± 6.9	21.2 ± 6.4	0.9 ± 3.9	0.153
Osteoprotegerin (pg/ml)	55.5 ± 21.5	54.2 ± 20.5	54.2 ± 21.0	−1.25 ± 12.8	0.542
RANKL (pg/ml)	6.5 ± 13.3	6.6 ± 13.1	6.3 ± 11.9	−0.25 ± 2.8	0.576
Cortisol (nmol/l)	403.0 ± 104.9	405.7 ± 118.7	408.5 ± 110.4	5.5 ± 102.1	0.700
24-Hour Urine					
Potassium (mmol/d)	67.0 ± 20.8	66.1 ± 21.8	65.0 ± 22.2	−1.9 ± 20.1	0.545
Nitrogen (mmol/d)	889 ± 241	1033 ± 270	1014 ± 293	126 ± 247	0.003
Nitrogen/Potassium	14.1 ± 4.4	17.2 ± 7.5	16.6 ± 5.6	2.4 ± 4.5	0.001
Sodium (mmol/d)	128 ± 47	123 ± 54	114 ± 50	−14.0 ± 57.2	0.130
Calcium (mmol/d)	3.4 ± 1.5	3.6 ± 1.85	3.7 ± 1.8	0.3 ± 1.2	0.114
N-telopeptide (nmol/d)	190 ± 146	171 ± 111	195 ± 171	5.5 ± 70.4	0.625
Free Cortisol (nmol/d)	119 ± 44	112 ± 44	113 ± 55	−6.3 ± 34.7	0.249
Titratable acid (mmol/d)	18.3 ± 15.4	22.4 ± 14.9	25.0 ± 14.3	6.8 ± 12.4	0.001
Net acid excretion (mmol/d)	39.3 ± 25.2	47.0 ± 28.0	51.2 ± 23.8	11.9 ± 18.7	0.000
Creatinine (mmol/d)	10.7 ± 3.2		11.1 ± 3.3	0.47 ± 2.0	0.146

Values are mean ± SD.

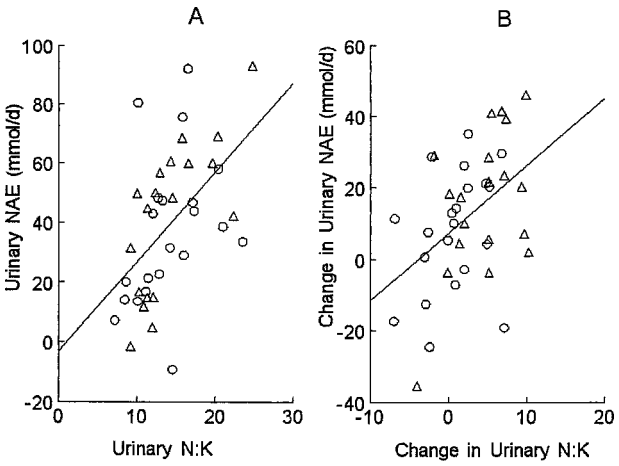

Fig. 1. A. Correlation between 24-hour urinary nitrogen to potassium ratio and 24-hour urinary net acid excretion (mmol/d) on day 14 (baseline) in 40 subjects ($r = 0.528$, $P < 0.001$). B. Correlation between change in the 24-hour urinary nitrogen to potassium ratio and change in 24-hour urinary net acid excretion (mmol/d) from day 14 to 74 in 40 subjects ($r = 0.453$, $P = 0.003$). Triangles represent subjects given meat and cereal food substitutes and circles represent subjects given meat and fruits and vegetables.

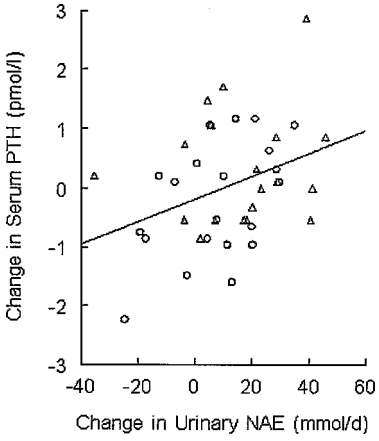

Fig. 2. Correlation between change in urinary net acid excretion (mmol/d) and change in serum PTH (pmol/l) from day 14 to 74 in 40 subjects ($r = 0.358$, $P = 0.023$). Triangles represent subjects given meat and cereal food substitutes and circles represent subjects given meat and fruits and vegetables.

substitute group did not alter any of these associations. Change in NAE was significantly correlated with change in urinary calcium excretion after adjustment for food substitute group, change in serum PTH, and change in urinary sodium excretion ($r_p = 0.381$, $P = 0.020$).

The urinary nitrogen to potassium ratio was associated with urinary calcium excretion at baseline ($r = 0.338$, $P = 0.013$) but changes in these 2 measures were not significantly correlated. Initial PTH and change in PTH were inversely correlated with basal and change in ionized and total calcium but none of these correlations reached significance except one (correlation

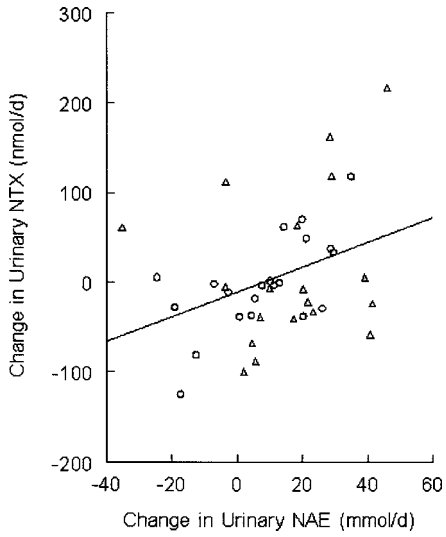

Fig. 3. Correlation between change in 24-hour urinary net acid excretion (mmol/d) and change in 24-hour urinary N-telopeptide (mmol/d) from day 14 to 74 in 40 subjects ($r = 0.367$, $P = 0.020$). Triangles represent subjects given meat and cereal food substitutes and circles represent subjects given meat and fruits and vegetables.

between baseline PTH and total calcium, $r = -347$, $P = 0.028$). Correlation coefficients for the others ranged from -0.109 to -0.292.

Urinary nitrogen excretion and serum IGF-1 were not significantly correlated on Day 14 ($r = 0.267$, $P = 0.095$) but the increase in urinary nitrogen excretion during the study was associated with an increase in serum IGF-1 ($r = 0.400$, $P = 0.011$). Neither baseline nor change in NAE was associated with baseline or change in serum IGF-1, osteoprotegerin, RANKL, or serum or urinary free cortisol. None of the results was significantly affected by gender.

DISCUSSION

This study reveals that diet-induced changes in renal NAE are associated with changes in indicators of bone health including biochemical markers of bone turnover and calcium excretion and possibly also PTH. The positive but modest association between change in NAE and change in PTH noted in this study has not been observed in previous human studies, including the shorter-term bicarbonate intervention studies [8–11] and an 8-week crossover dietary intervention study [7]. There is also evidence that acidosis directly enhances bone resorption in organ culture [17]. We are not aware that acid affects parathyroid glands in organ culture. Thus our findings may have been due to chance. However, there is evidence that metabolic acidosis acutely stimulates PTH secretion in dogs when the concentrations of calcium and magnesium are clamped, and that during hypocalcemia, metabolic acidosis increases the secretion rate and the half-life of PTH [18]. In a

subsequent study, Lopez et al [19] found that acute metabolic alkalosis increased calcium flux to bone and decreased PTH secretion acutely in dogs. In an *in vitro* study, Bushinsky et al. [17] showed that PTH releases calcium from bone at any pH. The observed association between change in NAE and change in PTH suggests that PTH may in part mediate the bone resorption that accompanies acidogenic diets. This finding will require confirmation in other clinical studies.

In this study, a diet-induced increase in NAE was positively associated with an increase in urinary NTX, suggesting that a dietary acid load stimulates bone resorption over a 60-day period. We know of no indication that an increase in NAE alters the renal handling of NTX. The observed link between change in dietary acid load and change in a marker of bone resorption is in agreement with several [6,9,10] but not all [7] studies. In a 7-day study, Maurer et al noted that when substituting $NaHCO_3$ and $KHCO_3$ for NaCl and KCl respectively, urinary NAE and excretion of deoxypyridinoline, pyridinoline and NTX were significantly reduced [9]. Similarly, Sebastian et al [10] found that administration of $KHCO_3$ decreased NAE and urinary hydroxyproline over 18 days in postmenopausal women. In a 4-day dietary intervention study in 8 young men, consumption of an acidogenic diet caused a 19% increase in urinary C-telopeptide [6]. In contrast, in the only available longer-term diet intervention study, Roughead et al [7] reported no change in urinary NTX after increasing the NAE during an 8-week study in postmenopausal women. Thus our findings are consistent with short-term alkali [9,10] and diet [6] intervention studies, and extend current evidence by suggesting that higher NAE has a sustained association with increased bone resorption over a 60-day period. The lack of association of NAE with serum osteocalcin, a biochemical marker of bone formation, in our study is in contrast to a previous short-term alkali-intervention study [10] but in agreement with a longer-term dietary intervention study [7]. The association of increase in NAE with increase in urinary NTX but not osteocalcin suggests that a more acidic environment may uncouple the bone remodeling process. This has been observed previously in rats [20,21].

In this study, a diet-induced increase in NAE was associated with an increase in urinary calcium excretion and this may have occurred by several mechanisms. The increase in dietary protein may have increased intestinal calcium absorption [22]. The increased NAE may have blocked the renal tubular response to PTH [23]. In addition, calciuria is an expected consequence of enhanced bone resorption. In this study, all subjects started taking the calcium supplements on day one and so were adapted to the higher calcium intakes by the time they began the food substitutions on day 14. The observed increase in urine calcium excretion is in agreement with short-term bicarbonate intervention studies but not with longer-term dietary studies. Lutz [24] found that adding $NaHCO_3$ to a moderate protein diet for 9 days decreased urinary calcium excretion. Sellmeyer et al. [8] noted that the addition of potassium citrate for 4 weeks in 60 postmenopausal women on high salt diets lowered NAE and urinary calcium excretion compared with the addition the placebo. However, in an 8-week crossover study, Roughead et al. [7] found no change in urinary calcium excretion after increasing renal NAE from 30 to 60 mEq/d by increasing dietary protein intake from 0.94 to 1.62 g/kg/d.

The association between NAE and the urinary nitrogen to potassium ratio is not surprising since nitrogen and potassium have opposing effects on NAE. This same phenomenon was noted in several short-term diet studies with regard to dietary intake of nitrogen (protein) and potassium (F&V). In one study plasma $[H^+]$ was determined by steady-state rate of endogenous noncarbonic acid production, which negatively correlated with dietary potassium content [25]. Frassetto et al. [26] noted that the dietary protein to potassium ratio accounted for 71% of the variation in renal NAE. Use of the ratio underscores the notion that it is the balance of nutrients in the diet that determines its ultimate pH.

The absence of expected changes in urinary potassium and NAE indicates subject noncompliance that appears to have occurred in the process of deleting foods from their self-selected diets (to some extent, fruits and vegetables rather than grains, added sugar, and fat alone appear to have been deleted). In this study, food substitution was not a successful means of precisely altering the acid-base balance of the diet over a sustained period. This is unfortunate because the alternative, long-term metabolic diet studies, requires access to metabolic research units and is extremely expensive.

In conclusion, a diet-induced increase in NAE is associated with an increase in bone resorption and urinary calcium excretion over a 60-day period in healthy elderly men and women with relatively high protein intakes. This study suggests that changes observed in 7 to 18 day metabolic studies may persist for up to 60 days. This study also raises the possibility that the diet-induced increase in bone resorption may involve PTH-dependent and independent mechanisms.

ACKNOWLEDGMENT

We are grateful to the staff of Metabolic Research Unit at Jean Mayer USDA Human Nutrition Research Center on aging at Tufts University for assistance in carrying out this study.

REFERENCES

1. Manz F, Kalhoff H, Remer T: Renal acid excretion in early infancy. Pediatr Nephrol 11:231–243, 1997.
2. Remer T, Dimitriou T, Manz F: Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. Am J Clin Nutr 77:1255–1260, 2003.
3. Remer T, Manz F: Potential renal acid load of foods and its influence on urine pH. J Am Diet Assoc 95:791–797, 1995.
4. Tucker KL, Hannan MT, Chen H, Cupples LA, Wilson PW, Kiel

DP: Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr 69:727–736, 1999.
5. New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, Grubb DA, Lee SJ, Reid DM: Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr 71:142–151, 2000.
6. Buclin T, Cosma M, Appenzeller M, Jacquet AF, Decosterd LA, Biollaz J, Burckhardt P: Diet acids and alkalis influence calcium retention in bone. Osteoporos Int 12:493–499, 2001.
7. Roughead ZK, Johnson LK, Lykken GI, Hunt JR: Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. J Nutr 133:1020–1026, 2003.
8. Sellmeyer DE, Schloetter M, Sebastian A: Potassium citrate prevents increased urine calcium excretion and bone resorption induced by a high sodium chloride diet. J Clin Endocrinol Metab 87:2008–2012, 2002.
9. Maurer M, Riesen W, Muser J, Hulter HN, Krapf R: Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. Am J Physiol-Renal 284:F32–F40, 2003.
10. Sebastian A, Morris Jr RC: Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. N Engl J Med 331;279, 1994.
11. Lemann Jr J, Gray RW, Pleuss JA: Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men. Kidney Int 35:688–695, 1989.
12. Lemann Jr J, Gray RW, Maierhofer WJ, Cheung HS: The importance of renal net acid excretion as a determinant of fasting urinary calcium excretion. Kidney Int 29:743–746, 1986.
13. Schakel SF, Sievert YA, Buzzard IM: Sources of data for developing and maintaining a nutrient database. J Am Diet Assoc 88:1268–1271, 1988.
14. Block G, Woods M, Potosky A, Clifford C: Validation of a self-administered diet history questionnaire using multiple diet records. J Clin Epidemiol 43:1327–1335, 1990.
15. Jorgensen K: Titrimetric determination of the net excretion of acid/base in urine. Scand J Clin Lab Invest 9:28–291, 1957.
16. Chan JC: The rapid determination of urinary titratable acid and ammonium and evaluation of freezing as a method of preservation. Clin Biochem 5:94–98, 1972.
17. Bushinsky DA, Frick KK: The effects of acid on bone. Curr Op Nephrol Hypertens 9:369–379, 2000.
18. Lopez I, Aguilera-Tejero E, Felsenfeld AJ, Estepa JC, Rodriguez M: Direct effect of acute metabolic and respiratory acidosis on parathyroid hormone secretion in the dog. J Bone Miner Res 17:1691–1700, 2002.
19. Lopez I, Rodriguez M, Felsenfeld AJ, Estepa JC, Aguilera-Tejero E: Direct suppressive effect of acute metabolic and respiratory alkalosis on parathyroid hormone secretion in the dog. J Bone Miner Res 18:1478–1485, 2003.
20. Arnett TR, Spowage M: Modulation of the resorptive activity of rat osteoclasts by small changes in extracellular pH near the physiological range. Bone 18:277–279, 1996.
21. Sprague SM, Krieger NS, Bushinsky DA: Greater inhibition of in vitro bone mineralization with metabolic than respiratory acidosis. Kidney Int 46:1199–1206, 1994.
22. Kerstetter JE, O'Brien KO, Caseria DM, Wall DE, Insogna KL: The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. J Clin Endocrinol Metab 90:26–31, 2005.
23. Beck N, Kim HP, Kim KS: Effect of metabolic acidosis on renal action of parathyroid hormone. Am J Physiol 228:1483–1488, 1975.
24. Lutz J: Calcium balance and acid-base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. Am J Clin Nutr 39:281–288, 1984.
25. Kurtz I, Maher T, Hulter HN, Schambelan M, Sebastian A: Effect of diet on plasma acid-base composition in normal humans. Kidney Int 24:670–680, 1983.
26. Frassetto LA, Todd KM, Morris Jr RC, Sebastian A: Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am J Clin Nutr 68:576–583, 1998.

Received February 8, 2005; revision accepted November 17, 2005.