Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: a randomized controlled trial[1–4]

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ABSTRACT

Background: Alkali provision may explain why fruit and vegetables benefit bone health.

Objective: We aimed to determine the effects of alkali-providing potassium citrate (double-blind) and fruit and vegetable intake (single-blind) on bone turnover over 2 y.

Design: We conducted a randomized placebo-controlled trial in 276 postmenopausal women (aged 55–65 y). Women were randomly assigned to 4 groups: high-dose potassium citrate (55.5 mEq/d), low-dose potassium citrate (18.5 mEq/d), placebo, and 300 g additional fruit and vegetables/d (equivalent of 18.5 mEq alkali). Serum and fasted urine for bone markers were collected at baseline and at 3, 6, 12, 18, and 24 mo. An additional urine sample was collected at 4–6 wk. Bone mineral density (BMD) was measured at baseline and 2 y.

Results: Repeated-measures ANOVA showed no difference between groups for urinary free deoxypyridinoline cross-links relative to creatinine (fDPD/Cr), serum *N*-terminal propeptide of type 1 collagen, or beta *C*-terminal telopeptide, although, at 4–6 wk, fDPD/Cr was lower in the high-dose potassium citrate group ($P = 0.04$). Mean ± SD spine BMD loss in the placebo group (1.8 ± 3.9%) did not differ significantly from that in the treatment groups (2.1 ± 3.2%; $P = 0.88$). Hip BMD loss in the placebo and low-dose potassium citrate groups was 1.3 ± 2.3% and 2.2 ± 2.3%, respectively ($P = 0.14$).

Conclusions: Two-year potassium citrate supplementation does not reduce bone turnover or increase BMD in healthy postmenopausal women, which suggests that alkali provision does not explain any long-term benefit of fruit and vegetable intake on bone. *Am J Clin Nutr* 2008;88:465–74.

INTRODUCTION

The role of the acid-base balance in human health is well documented. Of late, there has been renewed interest in how alterations in acid-base balance affect bone metabolism. Evidence from cellular studies (1, 2), observational population studies (3, 4), and short-term intervention trials (<3 mo) using alkaline salts of potassium (which mimic the acid-balancing properties of fruit and vegetables) (5–7) and diet (8, 9) suggest beneficial effects in reducing bone turnover. It has been suggested that long-term exposure to a diet that produces excess acid, which results in a gradual release of alkaline salts from the bone, may be a cause of osteoporosis (10). Fruit and vegetables provide alkaline metabolites, which could theoretically balance the excess acidity produced by eating a Westernized "acid-producing" diet.

Studies carried out in 996 late premenopausal Scottish women showed that nutrients associated with fruit and vegetable intake were associated with greater bone mineral density [BMD (11)], and less bone loss (12). Dietary acidity estimated from a food-frequency questionnaire was associated with reduced bone resorption in >3000 early postmenopausal women (4). The aim of the present double-blind placebo-controlled trial of potassium citrate, combined with a single-blind fruit and vegetable intervention, was to determine over 2 y whether the putative benefits of fruit and vegetables for bone health are due to the organic salts of potassium that these foods provide.

SUBJECTS AND METHODS

Subjects

Subjects were recruited from an ongoing longitudinal study of 49–54-y-old women selected from community health index records in 1990–1993 (4). Women who completed dietary questionnaires in 1998–2000 ($n = 3239$) were eligible to take part in the present study. Exclusion criteria were the lowest 10% of estimated dietary acidity (13), treatment for osteoporosis including previous bisphosphonate treatment, rare "ss" Col1A homozygosity (because of increased bone loss) (14), severe disease, malabsorption, difficulty in swallowing tablets or capsules,

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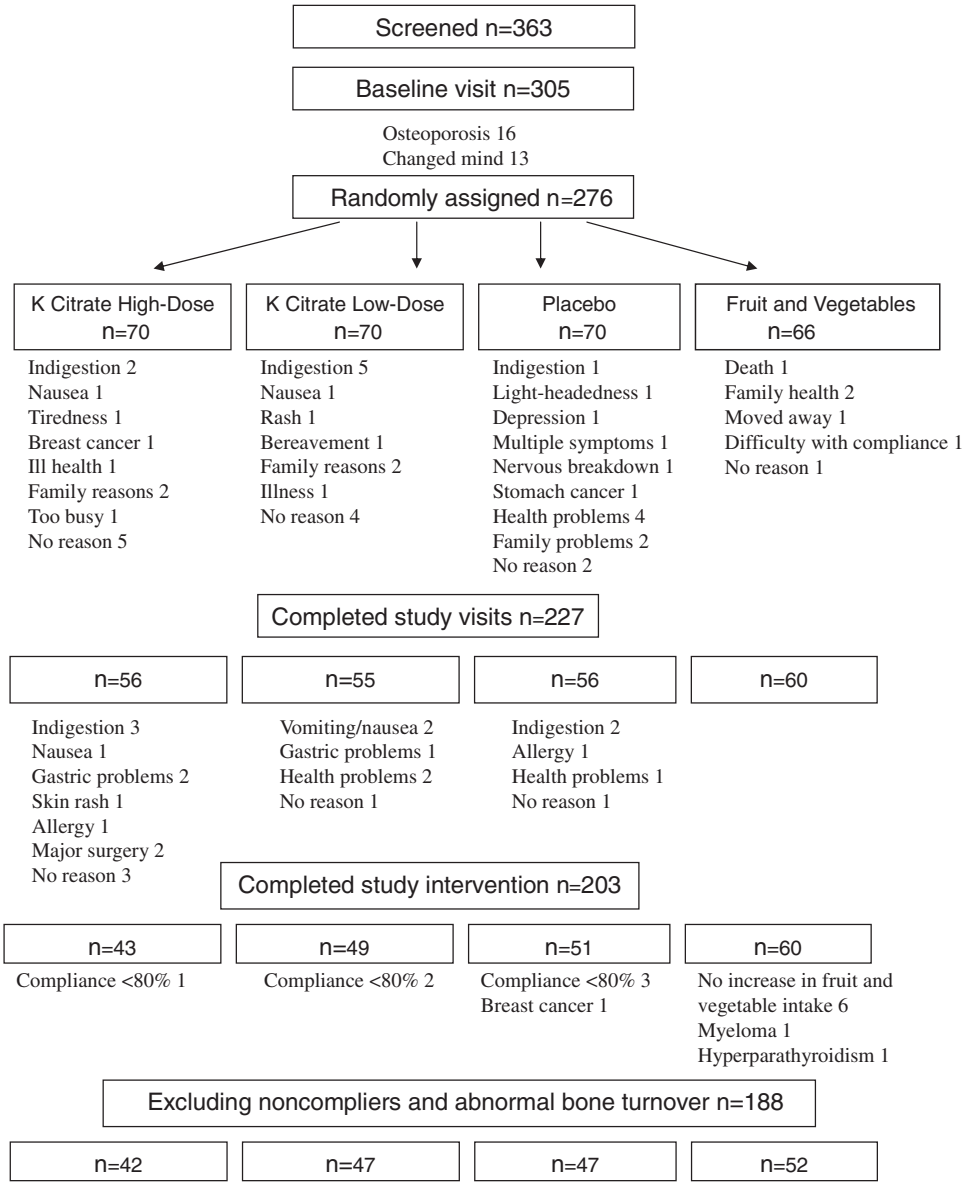

FIGURE 1. Details of study recruitment and reasons for withdrawing from study: 227 women completed the study and continued to attend study visits, including those who discontinued the intervention (205 attended every visit). Two hundred three women completed the study intervention (189 attended every visit). Twenty-three additional women attended the final scan. Complete measurements were unavailable for 8 women because of hip replacements (right hip, $n = 4$; left hip, $n = 3$; both hips, $n = 1$), which left 250 subjects for analysis of lumbar spine bone mineral density and 242 for analysis of mean total hip. The exclusion of 3 women for abnormal bone turnover, 6 women for tablet protocol noncompliance, and 6 women for not increasing fruit and vegetable intake left 188 women for per-protocol analysis, 182 of whom were available for mean total hip measurements (174 attended every visit).

<5 y past the menopause, hormone replacement therapy (HRT) in the previous 6 mo, and current use of oral corticosteroids or potassium-sparing diuretics. We included women taking other types of diuretics or hypertension tablets and women on thyroxine treatment, provided their thyroid function was stable (as assessed by free T4 and thyroid-stimulating hormone concentrations), and their dose had not changed in the year before study entry. A summary of recruitment details is given in **Figure 1**.

All subjects gave written informed consent. Ethical approval was obtained from the Grampian Research Ethics Committee (02/0053). The trial was registered at controlled-trials.com as ISRCTN86186352.

Randomization and intervention

We assigned subjects to 1 of 4 groups by using an automated telephone service (Health Services Research Unit, University of Aberdeen). The groups were high-dose potassium citrate (55.5 mEq/d), low-dose potassium citrate (18.5 mEq/d), placebo, and 300 g additional fruit and vegetables/d (diet group). Minimization criteria included current smoking, vitamin D receptor genotype and apolipoprotein E genotype, and category of estimated dietary acidity (>39mEq/d, <39 but >36 mEq/d, or <36 but >33 mEq/d). Capsules containing tripotassium citrate (1 g) and identical placebo capsules were purchased (DHP Clinical Trial

Supplies, Abergavenny, United Kingdom). Women were required to take 3 capsules at breakfast time (2 from bottle A and one from bottle B) and 3 capsules with the evening meal. For the placebo group, both bottles contained placebo; for the high-dose group, both contained potassium citrate capsules, which provided a daily 2.16-g (55.5-mEq) dose of potassium or alkali. For the low-dose group, bottle A contained placebo, and bottle B contained potassium citrate, so that 6 capsules provided 0.72 g (18.5 mEq) potassium or alkali. For a subject randomly assigned to the diet group, a nutritionist (ACH) calculated the portions of fruit and vegetables to be eaten (usual daily intake from the completed food diary plus 300 g). The research nurse (RS) used a range of color photographs to explain what constituted a portion (15). Women were given a small financial contribution ($2/d) toward purchasing additional fruit and vegetables. Blood samples were analyzed in the local hospital laboratory to check for biochemical abnormalities (including serum potassium).

Dietary intake and anthropometric assessments

The European Prospective Study into Cancer and Nutrition (EPIC) food diary was completed for 4 d before randomization and at 1 y (16–18), and the diaries were analyzed by using WINDIETS software (version 2002; Robert Gordon University, Aberdeen, United Kingdom). Every 3 mo, all study participants completed a 3-d dietary checklist, which consisted of a one-page list of fruit, vegetables, and other foods the participant checked of when she had eaten them. Portion sizes were assessed (by RS) at each 3-mo visit. Women were weighed every 6 mo on balance scales (Seca, Hamburg, Germany), and height was measured at baseline and 2 y by using a stadiometer (Holtain Ltd, Crymych, United Kingdom). Physical activity level (PAL) was estimated by using questions from the Scottish Heart Health Study (19). Social deprivation category was assessed from postal codes (20).

Markers of bone health

Nonfasted blood samples taken the same time of day at baseline and at 3, 6, 12, 18, and 24 mo were analyzed. Serum *N*-terminal propeptide of type 1 collagen (P1NP) was measured by using an enzyme chemiluminescence immunoassay [ECLIA; Roche Products Ltd, Penzberg, Germany (sensitivity: 2 μg/L; interassay and intraassay CVs: <4% across the range 5–100 μg/L)]. Serum beta *C*-terminal telopeptide (CTX) was measured by using an ECLIA [Roche (interassay and intra-assay CVs: <4% across the range 0.01–2.0 ng/mL)]. Free deoxypyridinoline cross-links (fDPD) were measured in 2-h early-morning fasted urine samples at the same time-points, and an additional collected sample was received by mail 4–6 wk after the baseline visit. Each subject's samples were stored together and analyzed as a single batch by using a commercial immunoassay (Quidel; Oxford Biosystems UK, Oxford, United Kingdom), and the results of the analysis were expressed relative to creatinine (fDPD/Cr). BMD was measured at the spine and both hips at baseline and at 2 y by the same radiographer using a dual-energy X-ray absorptiometry (DXA) scanner (Lunar Prodigy; GE Medical Systems Inc, Madison WI). Daily phantom measurements were performed. Short-term precision was estimated by using duplicate measurements from 7 postmenopausal women (independent of the present study). The CV was 0.5% for spine (L2–L4) and 0.6% for total hip.

Compliance measures

Compliance was estimated at each 3-mo visit by capsule count for the potassium citrate and placebo groups and by dietary reporting for the fruit and vegetable arm. Blood samples for plasma vitamin C (reflecting recent intake of fruit and vegetables), whole cell folate (reflecting longer-term intake), and plasma homocysteine (a risk factor for fracture that would decrease with increased dietary folate) were collected at baseline and at 12 and 24 mo. Vitamin C in acidified plasma was determined by HPLC with ultraviolet detection (21). Total plasma homocysteine was measured by reverse-phase HPLC using the DC30 Hcy Homocysteine Assay Kit in combination with a DC30 analyzer (Drew Scientific, Barrow-in-Furness, United Kingdom). Whole cell folate was measured by radioimmunoassay (Simultrac Radioassay Kit; MP Biomedical, London, United Kingdom), and expressed in grams of hemoglobin. Hemoglobin was measured in the same sample with the use of the Total Hemoglobin Kit (Sigma Diagnostics, Poole, United Kingdom) with appropriate internal standards.

24-h Urine substudies

A subset of women ($n = 70$ at study start, $n = 57$ at study end) collected 24-h urine samples at baseline and at 3, 6, 12, 18, and 24 mo for measurement of urinary pH and concentrations of potassium, calcium, sodium, phosphate, urea, and creatinine.

Statistical analysis

Using a growth model to fit our cross-sectional FFQ and bone marker data, selected for women >5 y past menopause, we estimated that fDPD/Cr would be 5.54 nmol/mmol in the placebo group (assuming a daily intake of 400 g fruit and vegetables) and 4.51 nmol/mmol in the diet group (equivalent to the low-dose potassium citrate group). With the use of an SD of 1.7, the study would require 45 participants in each group for 80% power at the 5% significance level. For the secondary outcome, 42 subjects in the placebo and active groups would detect a difference of 2.5 ± 4% BMD (80% power, $P = 0.05$). We estimated an annual BMD loss of 0.75% in the placebo group, allowing an annual increase in spine BMD of 0.5% to be detected in the treatment arms. It is questionable whether smaller differences would be biologically significant.

SPSS for WINDOWS software (version 15; SPSS Institute, Chicago, IL) was used to test differences in bone turnover markers between groups by repeated-measures analysis of variance (ANOVA), and treatment × time interactions were tested by using Pillai's trace. One-way ANOVA was used to analyze the BMD change between the treatment groups. Analysis was carried out on an intention-to-treat basis and on a per-protocol basis for women with >80% compliance.

RESULTS

There were no significant differences in baseline characteristics of the study participants according to treatment group (**Table 1**). There was a trend for higher circulating P1NP in the low-dose potassium citrate group ($P = 0.06$), and more women in the fruit and vegetable (diet) group were taking blood pressure–lowering medication ($P = 0.10$).

TABLE 1
Subject characteristics at baseline visit[1]

Characteristics	Potassium citrate: High-dose group (55.5 mEq/d)	Potassium citrate: Low-dose group (18.5 mEq/d)	Placebo group	Fruit and vegetable (diet) group	P^2
Subjects (*n*)	70	70	70	66	
Age (y)	59.6 ± 2.2[3]	59.7 ± 2.2	59.7 ± 2.1	59.2 ± 2.1	0.47
Weight (kg)	71.9 ± 14.5	71.7 ± 13.4	74.5 ± 14.5	76.8 ± 14.8	0.13
BMI (kg/m^2)	27.7 ± 5.3	27.4 ± 4.8	29.1 ± 5.6	29.5 ± 5.3	0.05
Height (cm)	161.0 ± 6.2	161.6 ± 6.0	160.0 ± 5.4	161.1 ± 5.7	0.44
Time past menopause (y)	10.4 ± 4.6	11.4 ± 5.5	10.9 ± 5.7	10.0 ± 3.8	0.39
Systolic blood pressure (mm Hg)	130 ± 22	129 ± 16	127 ± 20	130 ± 14	0.62
Diastolic blood pressure (mm Hg)	78 ± 14	80 ± 11	78 ± 9	79 ± 9	0.63
Serum creatinine (μmol/L)	84 ± 10	83 ± 7	84 ± 9	86 ± 8	0.21
Calculated creatinine clearance (mL/min)	73 ± 16	72 ± 14	75 ± 17	76 ± 14	0.61
Dietary intakes from food diary (*n*)	69	69	70	66	
Protein (g/d)	77.2 ± 16.9	82.4 ± 20.1	77.6 ± 17.7	75.3 ± 17.7	0.13
Potassium (mg/d)	3236 ± 972	3491 ± 957	3325 ± 782	3299 ± 901	0.50
Calcium (mg/d)	812 ± 305	868 ± 244	868 ± 202	828 ± 262	0.65
Including supplements (mg/d)[4]	870 ± 346	917 ± 282	885 ± 248	854 ± 270	0.63
Vitamin C (mg/d)	178 ± 153	176 ± 154	152 ± 111	129 ± 71	0.09
Including supplements (mg/d)[5]	192 ± 165	203 ± 192	161 ± 127	159 ± 138	0.26
Estimated NEAP					
Frassetto mEq/d	42.0 ± 13.7	41.3 ± 13.5	39.6 ± 10.2	38.5 ± 9.5	0.29
Remer mEq/d	41.3 ± 16.0	40.2 ± 19.4	41.6 ± 11.8	41.2 ± 13.1	0.95
Calcium:phosphorous ratio	0.63 ± 0.13	0.65 ± 0.11	0.66 ± 0.10	0.64 ± 0.11	0.56
Lumbar spine (*n*)	70	70	70	66	
BMD (g/cm^2)	1.164 ± 0.182	1.182 ± 0.149	1.192 ± 0.158	1.179 ± 0.148	0.49
T score[6]	−0.28 ± 1.51	-0.13 ± 1.25	-0.08 ± 1.33	-0.08 ± 1.23	
Total hip (*n*)	68	69	69	65	
BMD (g/cm^2)	0.996 ± 0.124	0.970 ± 0.099	0.996 ± 0.122	0.996 ± 0.122	0.50
T score[6]	−0.09 ± 0.99	-0.29 ± 0.80	-0.07 ± 0.97	-0.14 ± 0.93	
Serum (*n*)	64	65	69	65	
P1NP (μg/L)	42.4 ± 17.5	48.8 ± 19.1	41.5 ± 14.2	42.7 ± 20.0	0.06
CTX (ng/mL)	0.21 ± 0.11	0.22 ± 0.10	0.19 ± 0.09	0.21 ± 0.12	0.56
Urinary fDPD/Cr (nmol/mmol)	8.1 ± 2.7	8.0 ± 3.4	7.4 ± 2.2	7.3 ± 2.3	0.30
Smoking (%)	15.7	14.3	17.1	13.6	0.94
APOE haplotypes ($n = 216$)					
22 or 23	7	9	9	10	
33	34	31	32	27	
Contains 4 alleles	13	15	15	14	0.97
VDR alleles					
BB	8	10	8	9	
Bb	26	24	26	25	
bb	18	18	16	17	0.98
Social deprivation category (%)					
1 (most affluent)	27.1	20.3	18.8	12.1	0.62
2	44.3	43.6	45.0	60.6	
3	8.6	7.2	11.6	6.1	
4	11.4	15.9	15.9	12.1	
5 (least affluent)	8.6	13.0	8.7	9.1	
Creatinine clearance < 50 mL/min (*n*)	2	4	0	2	0.25
Blood pressure–lowering medication (*n*)	14	15	13	23	0.10
Withdrawals (*n*)					
In study until the end	43	48	49	60	0.01
In study for 18 mo (started later)	0	1	2	1	
Discontinued treatment but still in study	13	6	5	0	
Withdrawn	14	15	14	5	

[1] *n* = number of subjects in each analysis. NEAP, net endogenous acid production (Frassetto equation (13): Estimated NEAP = [54.5 × protein (g/d)] − 0.57 potassium (mEq/d) −10.2; Remer equation (22): Estimated NEAP (mEq/d) = PRAL (mEq/d) + OAest (mEq/d), where PRAL = 0.49 × protein (g/d) + 0.037 × phosphorous (mg/d) − 0.021 × potassium (mg/d) − 0.026 × magnesium (mg/d) − 0.013 × calcium (mg/d) and OAest = 41/1.73 × [0.00714 × height$^{0.715}$ (cm) × weight$^{0.425}$ (kg)]; VDR, vitamin D receptor; APOE, apolipoprotein E; P1NP, serum *N*-terminal propeptide of type 1 collagen; CTX, *C*-terminal telopeptide of type 1 collagen; fDPD/Cr, free deoxypyridinoline cross-links expressed relative to creatinine.
[2] Determined with ANOVA for the continuous variables and the chi-square test for the categorical variables.
[3] $\bar{x}$ ± SD (all such values).
[4] Twenty-six women had been taking over-the-counter supplements containing calcium (<500 mg/d). There was no difference in the distribution across the study groups ($P = 0.29$).
[5] Thirty-four women had taken a vitamin supplement that contained vitamin C (<200 mg/d for $n = 32$) with no difference in the distribution across study groups ($P = 0.41$).
[6] *T* score was based on the National Health and Nutrition Examination Survey population.

TABLE 2
Fruit and vegetable intake estimated from food diaries completed at baseline and 1 y and plasma vitamin C, whole cell folate, and plasma homocysteine according to treatment group

	Treatment group				
Visit	High-dose	Low-dose	Placebo	Fruit and vegetables (diet)	P^1
Baseline					
Completed food diaries (*n*)	70	70	69	66	
Fruit intake (g/d)	282 ± 168 (242, 323)[2]	316 ± 174 (274, 363)	298 ± 184 (255, 342)	287 ± 185 (242, 333)	0.69
Vegetable intake (g/d)[3]	156 ± 95 (134, 179)	166 ± 89 (145, 188)	160 ± 79 (141, 179)	176 ± 96 (153, 200)	0.27
Provided blood samples (*n*)	65	67	67	65	
Plasma vitamin C (μmol/L)	80.7 ± 33.5	87.5 ± 26.6	77.0 ± 29.6	83.7 ± 24.4	0.19
Whole cell folate/Hb (ng/g)	2314 ± 884	2285 ± 775	2279 ± 810	2256 ± 775	1.00
Homocysteine (μmol/L)	10.6 ± 3.2	10.8 ± 3.0	10.5 ± 3.7	12.1 ± 5.5	0.13
Year 1					
Completed food diaries (*n*)	56	56	56	63	
Fruit intake (g/d)	273 ± 199 (220, 327)	284 ± 148 (244, 323)	275 ± 197 (222, 328)	582 ± 259 (516, 647)[4]	<0.001
Vegetable intake (g/d)	148 ± 99 (121, 174)	174 ± 94 (149, 199)	143 ± 84 (120, 165)	218 ± 103 (192, 244)[4]	<0.001
Provided blood samples (*n*)	56	56	58	64	
Plasma vitamin C (μmol/L)	76.7 ± 29.0	87.7 ± 25.0	81.0 ± 31.6	89.0 ± 24.8	0.06
Whole cell folate/Hb (ng/g)	2372 ± 820	2377 ± 733	2343 ± 730	2367 ± 618	0.97
Homocysteine (μmol/L)	10.6 ± 2.7	10.4 ± 3.4	10.1 ± 3.0	11.3 ± 4.6	0.33
Difference between year 1 and baseline					
Completed food diaries (*n*)	56	56	56	63	
Fruit intake (g/d)[5]	−11 ± 191 (−62, 40)	−34 ± 148 (−74, 5)	−17 ± 184 (−66, 32)	291 ± 270 (223, 359)	<0.001
Vegetable intake (g/d)[5]	−13 ± 100 (−39, 14)	9 ± 103 (−19, 36)	−18 ± 98 (−44, 8)	39 ± 94 (15, 63)	0.007
Provided blood samples (*n*)	56	56	58	64	
Plasma vitamin C (μmol/L)	−3.7 ± 27.8	−1.2 ± 20.2	2.7 ± 26.4	5.9 ± 25.3[6]	0.17
Whole cell folate/Hb (ng/g)	118 ± 626	43 ± 419	96 ± 650	92 ± 593	0.92
Homocysteine (μmol/L)	−0.03 ± 2.03	−0.16 ± 1.35	−0.26 ± 1.61	−0.39 ± 2.47	0.79
Year 2 (no food diary)					
Provided blood samples (*n*)	54	56	56	59	
Plasma vitamin C (μmol/L)	82.5 ± 30.2	86.4 ± 29.2	86.9 ± 30.8	88.1 ± 24.7	0.76
Whole cell folate/Hb (ng/g)	2468 ± 994	2471 ± 704	2418 ± 796	2519 ± 913	0.01
Homocysteine (μmol/L)	10.8 ± 3.1	9.6 ± 2.6	9.5 ± 3.0	10.8 ± 3.9	0.03

[1] One-way ANOVA.
[2] $\bar{x}$ ± SD; 95% CI in parentheses (all such values).
[3] Vegetables did not include potatoes.
[4] Year 1 intake was significantly different from baseline intake for fruit and vegetable group (paired *t* test): $P = 0.002$ for fruit and <0.001 for vegetables.
[5] Differences refer only to those subjects who completed food diaries at baseline and year 1.
[6] There was a trend for year 1 plasma vitamin C to be higher than baseline in the fruit and vegetable intake group, $P = 0.07$ (paired *t* test).

Compliance

A capsule count was conducted at every 3-mo visit. Overall compliance was 77% for the intention-to-treat analysis, with 9 women taking <10% of the capsules. For those who completed the study, compliance was 93%, with 6 women taking <80% of the capsules. The routine biochemistry tests at baseline and at 3, 6, 12, 18, and 24 mo, which were intended to detect abnormalities, showed a trend for higher serum potassium in the high-dose potassium citrate group than in the other groups, although the concentration was still in the reference range.

The 1-y food diaries showed that, in the diet group only, mean fruit and vegetable intake had increased (**Table 2**), and there was a wide variation in daily intake (126–1470 g for fruit and 31–630 g for vegetables). Seven women ("noncompliers") had decreased their fruit and vegetable intake. Dietary checklists also showed an increase in fruit and vegetable intake in the diet group from 3 mo onwards but no change in the capsule groups (data not shown). There was no difference between groups in baseline measurements of plasma vitamin C, whole cell folate, or homocysteine. At 1 y, mean plasma vitamin C had increased in the diet group ($n = 64$) from 83.1 ± 24.0 to 89.0 ± 24.8 μmol/L ($P = 0.07$, paired *t* test); with exclusion of the noncompliers (which left $n = 57$), the increase was 82.9 ± 24.5 to 89.7 ± 25.8 μmol/L ($P = 0.05$). The increase from 84.5 ± 23.8 to 88.1 ± 24.7 μmol/L at 2 y was not significant [$P = 0.26$ ($n = 59$); with exclusion of noncompliers: $P = 0.22$ ($n = 52$)]. Plasma vitamin C had not changed in the capsule groups ($P = 0.72$). Mean whole cell folate had increased from baseline, but there was no statistically significant difference between the study groups (Table 2). Mean plasma homocysteine had decreased in all groups except the high-dose potassium citrate group (Table 2).

Bone turnover

There was no difference in bone turnover markers between groups at the baseline visit ($n = 263$), but P1NP was slightly

TABLE 3
Baseline mean and mean change from baseline for serum *C*-terminal telopeptide (CTX), serum *N*-terminal propeptide of type 1 collagen (P1NP), and urinary free deoxypyridoline cross-links relative to creatinine (fDPD/Cr)

	Treatment group				
Visit	High-dose Potassium citrate	Low-dose Potassium citrate	Placebo	Fruit and vegetables (diet)	P^1
Serum provided at each visit ($n = 202$)[2]					
P1NP (n)	50	51	47	54	
Baseline (μg/L)	42.2 ± 19.1[3]	51.6 ± 19.8	41.3 ± 13.9	42.2 ± 20.4	0.02
Change in P1NP from baseline (μg/L)					
3 mo	−5.4 ± 9.1	−3.8 ± 12.8	−3.7 ± 6.3	−4.3 ± 11.7	0.85
6 mo	−3.8 ± 10.9	−2.8 ± 13.1	−0.1 ± 9.4	−2.9 ± 14.6	0.50
12 mo	−3.0 ± 12.8	−4.6 ± 16.5	−1.4 ± 8.8	−1.9 ± 12.4	0.60
18 mo	−3.0 ± 12.4	−4.5 ± 15.2	0.8 ± 8.8	−1.1 ± 13.0	0.18
24 mo	−2.0 ± 15.4	−6.6 ± 17.4	−2.3 ± 8.3	−2.1 ± 17.2	0.35
CTX (n)	50	51	47	54	
Baseline (ng/mL)	0.205 ± 0.113	0.230 ± 0.110	0.196 ± 0.095	0.204 ± 0.129	0.46
Change in CTX from baseline (ng/mL)					
3 mo	−0.013 ± 0.065	0.005 ± 0.093	0.015 ± 0.072	0.002 ± 0.102	0.43
6 mo	0.009 ± 0.091	0.005 ± 0.092	0.006 ± 0.085	−0.005 ± 0.103	0.86
12 mo	0.000 ± 0.095	0.004 ± 0.088	0.019 ± 0.097	0.019 ± 0.104	0.65
18 mo	−0.011 ± 0.102	-0.024 ± 0.109	0.009 ± 0.085	0.007 ± 0.115	0.32
24 mo	−0.007 ± 0.105	−0.032 ± 0.098	0.008 ± 0.091	0.003 ± 0.109	0.21
Urine provided at each visit ($n = 182$)[2]					
fDPD/Cr (n)	46	44	42	50	
Baseline (nmol/mmol)	8.1 ± 3.4	7.5 ± 2.4	7.6 ± 2.0	7.2 ± 2.3	0.46
Change in fDPD/Cr from baseline (nmol/mmol)					
3 mo	−0.9 ± 3.2	−0.2 ± 2.0	0.2 ± 2.2	0.1 ± 2.1	0.15
6 mo	−0.5 ± 3.1	−0.2 ± 1.8	−0.2 ± 1.5	0.0 ± 2.7	0.75
12 mo	−0.7 ± 3.5	−0.5 ± 2.5	0.1 ± 2.6	−0.4 ± 1.8	0.57
18 mo	−0.7 ± 3.6	−0.4 ± 1.8	−0.6 ± 1.7	−0.1 ± 2.0	0.65
24 mo	−0.9 ± 3.2	−0.7 ± 1.7	−0.7 ± 1.9	−0.3 ± 2.0	0.61
Urine provided at early visits: mailed sample and 3-mo visit ($n = 150$)					
fDPD/Cr (n)	41	36	36	37	
Baseline (nmol/mmol)	8.2 ± 3.6	7.3 ± 1.9	7.4 ± 2.0	7.3 ± 1.8	0.33
Change in fDPD/Cr from baseline (nmol/mmol)					
1–1.5 mo[4]	−1.4 ± 4.1	0.2 ± 1.5	−0.4 ± 2.1	0.1 ± 1.5	0.03
3 mo	−0.9 ± 3.4	0.1 ± 1.9	−0.1 ± 2.1	−0.1 ± 1.6	0.21

[1] One-way ANOVA.

[2] The results shown are for one-way ANOVA of only the women who attended every visit (3, 6, 12, 18, and 24 mo) and excluding 3 women with abnormal bone turnover (1 each for hyperparathryodism; breast cancer and treatment changes; and a fracture and myeloma). Two women were in the fruit and vegetable (diet) group, and one was in the placebo group. One-way ANOVA using the total number of women (minus the 3 exclusions) at each visit gave similar results. Repeated-measures ANOVA of the bone markers at each visit showed no significant visit × treatment interaction for P1NP ($P = 0.79$), CTX ($P = 0.48$), or urinary fDPD/Cr ($P = 0.76$).

[3] $\bar{x}$ ± SD (all such values).

[4] Sample sent by mail 4–6 wk after baseline visit. Not all women sent in a urine sample by mail ($n = 150$ of the 182 subjects above). One-way ANOVA showed a significant difference in fDPD/Cr change from baseline at 4–6 wk, $P = 0.03$; the high-dose potassium citrate group differed significantly from the low-dose potassium citrate group, $P = 0.047$ (Bonferroni post hoc test). Repeated-measures ANOVA of the bone markers at each visit showed no significant visit × treatment interaction when all 3 visits (baseline, 4–6-wk, and 3-mo) were included ($P = 0.20$) but a significant interaction when only baseline and 4–6-wk visits were tested ($P = 0.039$).

higher in the low-dose potassium citrate group than in the high-dose potassium citrate group ($P = 0.06$, 0.02, and 0.01 at baseline, 3 mo, and 6 mo, respectively). Three women were excluded from subsequent analysis because of abnormal bone turnover due to hyperparathyroidism, fracture with myeloma (both in the diet group), and breast cancer treatment (placebo group). Repeated-measures ANOVA on an intention-to-treat basis comparing baseline and last visit only ($n = 260$) showed no significant visit × treatment interaction for serum P1NP ($P = 0.55$) or CTX ($P = 0.69$). Similarly, for fDPD/Cr ($n = 258$), there was no difference between groups ($P = 0.32$ for visit × treatment interaction). Repeated-measures ANOVA for the bone marker data that were available for every visit ($n = 202$) also showed no visit × treatment interaction for serum P1NP, CTX, or urinary fDPD/Cr ($n = 182$) ($P = 0.48$, 0.78, and 0.62 for log-transformed P1NP, CTX, and fDPD/Cr, respectively). The differences in bone markers from baseline according to treatment group are shown in **Table 3**. The outcome was the same when the analysis was repeated with the exclusion of noncompliers or women who were on blood pressure–lowering medication. One-way ANOVA of the additional urine sample (sent by mail 4–6 wk after the baseline visit) showed a difference between groups in fDPD/Cr from baseline ($P = 0.03$),

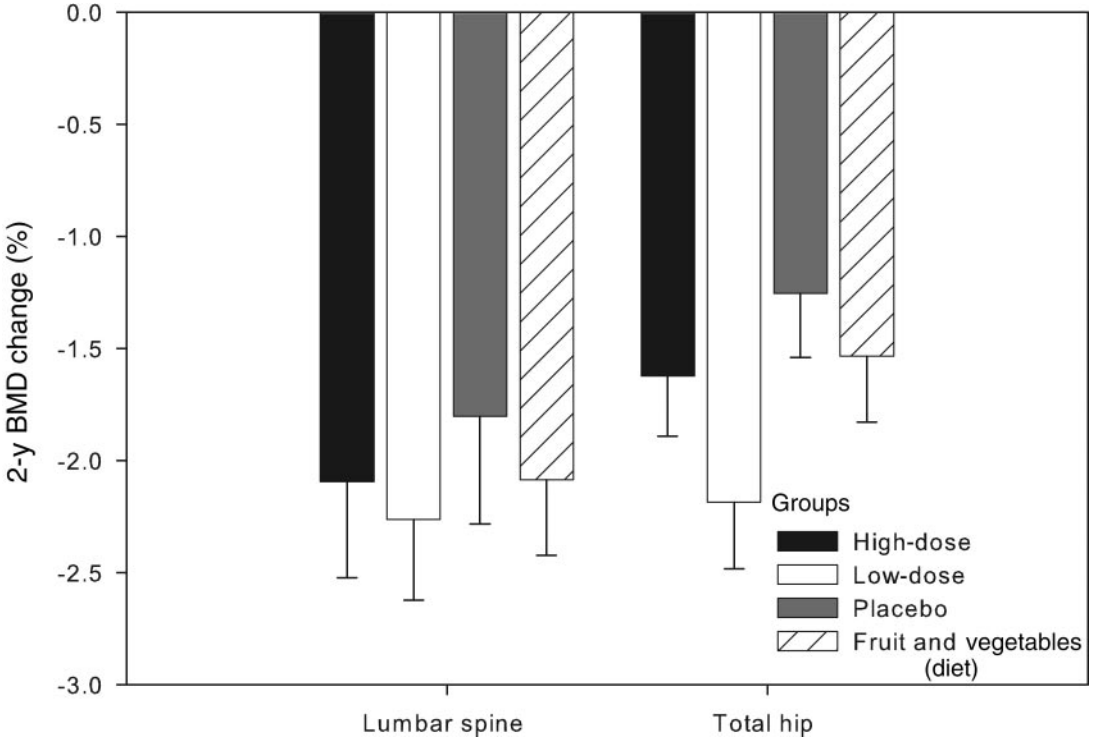

FIGURE 2. Mean (±SEM) percentage change over the duration of the study (2 y) in bone mineral density (BMD) at the lumbar spine (LS) (n = 60, 61, 66, and 63 in the high-dose potassium citrate, low-dose potassium citrate, placebo, and fruit and vegetable groups, respectively) and mean total hip (n = 58, 59, 65, and 60 in the high-dose potassium citrate, low-dose potassium citrate, placebo, and fruit and vegetable groups, respectively). $P = 0.88$ for LS, $P = 0.14$ for mean total hip (one-way ANOVA). The results were similar after adjustment for confounders (ie, age, weight, height, and social deprivation category). $P = 0.90$ for LS, $P = 0.21$ for mean total hip (ANCOVA).

with the high-dose potassium citrate group showing a greater reduction in mean fDPD/Cr than did the low-dose potassium citrate group ($P < 0.05$; ANOVA and Bonferroni). There was no difference between treatment groups at 3 mo ($P = 0.21$, ANOVA) and no significant visit × treatment interaction when baseline, 4–6 wk, and 3 mo measures were analyzed by repeated-measures ANOVA ($P = 0.20$). However, there was a significant visit × treatment interaction if only baseline and 4–6 wk samples were included in the repeated-measures ANOVA ($P = 0.04$).

Bone mineral density

Mean ± SD BMD loss at the spine in the placebo group was 1.8 ± 3.9% over 2 y. Apparently greater BMD loss (2.1 ± 3.2%) in the treatment groups was not significant ($P = 0.88$, ANOVA). For mean total hip, BMD loss was less in the placebo group (1.3 ± 2.3%) than in the low-dose potassium citrate group (2.2 ± 2.3%), but the difference was not significant ($P = 0.14$, ANOVA) (**Figure 2**). The per-protocol analysis showed similar results [spine: loss of 1.5 ± 3.4% in the placebo group and 2.5 ± 3.0% in the treatment groups ($P = 0.25$); total hip: loss of 1.1 ± 2.4% in the placebo group and 2.2 ± 2.4% in the low-dose potassium citrate group ($P = 0.11$, ANOVA)]. Adjustment for weight, height, age, and social deprivation category did not change the outcome (spine: $P = 0.90$; hip: $P = 0.21$, ANCOVA, data not shown). The same findings were observed after the performances of sensitivity analyses excluding women on blood pressure–lowering medication. Weight change was less than ±5% over the 2-y study, and there was no statistically significant difference between the treatment groups.

There was a significant correlation between the amount of fruit eaten at baseline and BMD at the hip ($r = 0.13$, $P = 0.04$) in the entire cohort. There was no relation between fruit or vegetable consumption at 1 y and the final BMD measurements in the entire cohort or the separate treatment groups.

Subgroup studies (24-h urine)

There was a significant difference in mean daily potassium excretion and urinary pH between the treatment groups at 3, 6, 12, 18, and 24 mo ($P = 0.001$, repeated-measures ANOVA) (**Table 4**). There was no significant difference in urinary sodium, phosphate, or urea by repeated- measures ANOVA (data not shown). For calcium, there was a significant visit × treatment interaction ($P = 0.03$): the high-dose potassium citrate group had significantly lower calcium excretion at 3 and 6 mo ($P = 0.02$ and 0.01, respectively) (Table 4).

DISCUSSION

To our knowledge, the present study is longest alkali-supplementation study for bone health and the first trial of a bone-loss intervention that balanced treatment groups for certain key genotypes. Despite minor but uncomfortable side effects (ie, indigestion and a bloated feeling), most women tolerated taking potassium citrate for 2 y, as was evident by the capsule count and, in the high-dose potassium citrate group, an increase in circulating potassium. The present study showed no persistent changes in bone markers over 2 y with a daily dose of 18.5 or 55.6 mEq potassium citrate. We observed a transient reduction in fDPD/Cr

TABLE 4
Urinary pH and change from baseline for 24-h urinary excretion of potassium and calcium according to treatment group

	Treatment group				
Visit	High-dose potassium citrate	Low-dose potassium citrate	Placebo	Fruit and vegetables (diet)	P^1
Attended each visit (n)[2]	12	13	12	18	
pH					
Baseline	6.52 ± 1.02[3]	6.53 ± 0.55	6.47 ± 0.56	6.32 ± 0.66	0.83
3 mo	7.51 ± 0.36	7.36 ± 0.56	6.45 ± 0.56	6.50 ± 0.66	<0.001[4]
6 mo	7.53 ± 0.54	7.05 ± 0.48	6.31 ± 0.45	6.34 ± 0.61	<0.001[4]
12 mo	7.57 ± 0.43	7.13 ± 0.76	6.38 ± 0.44	6.53 ± 0.71	<0.001[4]
18 mo	7.39 ± 0.83	7.06 ± 0.46	6.35 ± 0.64	6.51 ± 0.57	<0.001[4]
24 mo	7.40 ± 0.59	7.10 ± 0.54	6.48 ± 0.64	6.53 ± 0.55	<0.001[4]
Baseline daily potassium excretion (mmol)	63.1 ± 25.3	66.6 ± 18.6	55.0 ± 23.2	64.4 ± 25.8	0.64
Difference in daily potassium excretion from baseline (mmol)					
3 mo	38.1 ± 14.4	10.6 ± 26.3	4.1 ± 20.4	15.1 ± 20.4	0.002[4]
6 mo	48.7 ± 25.6	4.2 ± 19.2	2.4 ± 23.9	7.2 ± 25.2	<0.001[4]
12 mo	43.0 ± 18.2	11.6 ± 20.3	−2.5 ± 39.6	2.7 ± 28.6	0.001[4]
18 mo	53.7 ± 30.5	16.4 ± 17.6	−0.2 ± 28.9	3.6 ± 26.8	<0.001[4]
24 mo	42.7 ± 29.9	8.3 ± 28.5	−4.4 ± 27.4	5.8 ± 27.9	0.001[4]
Baseline daily calcium excretion (mmol)	3.15 ± 1.59	3.57 ± 1.75	2.94 ± 1.61	3.07 ± 1.90	0.81
Difference in daily calcium excretion from baseline (mmol)					
3 mo	−0.96 ± 1.10	−1.23 ± 1.73	0.83 ± 2.15	0.03 ± 1.26	0.007
6 mo	−1.17 ± 1.01	−0.42 ± 1.17	0.79 ± 1.91	0.18 ± 1.52	0.011[5]
12 mo	−1.04 ± 1.29	−0.14 ± 1.20	−0.24 ± 1.63	−0.60 ± 1.44	0.39
18 mo	−0.45 ± 1.05	−0.24 ± 1.31	−0.26 ± 1.28	−0.38 ± 1.21	0.97
24 mo	−1.09 ± 1.28	−0.78 ± 2.02	0.01 ± 1.50	−0.65 ± 1.28	0.36

[1] One-way ANOVA.

[2] The results shown are only for the women who attended every visit. Analysis using the total number of women at each visit gave similar results. Repeated-measures ANOVA showed visit × treatment interaction for urinary pH ($P = 0.001$), potassium excretion ($P = 0.001$), and calcium excretion ($P = 0.03$).

[3] $\bar{x} \pm$ SD (all such values).

[4] Post hoc analyses. For pH, the high-dose and low-dose potassium citrate groups were significantly different from the placebo and fruit and vegetable groups; for potassium excretion, the high-dose potassium citrate group was significantly different from other treatment groups.

[5] Post hoc analysis. For calcium excretion, the high-dose potassium citrate group was significantly different from the placebo ($P = 0.034$) and fruit and vegetable ($P = 0.038$) groups at 6 mo.

only in the high-dose potassium citrate group at 4–6 wk, and that value returned to baseline at 3 mo.

The bone marker data from other studies of postmenopausal women have not always been consistent. In one study, an 18-d supplementation with potassium bicarbonate at 60–120 mEq/d reduced urinary hydroxyproline and increased circulating osteocalcin (5). In another study, reductions in CTX and *N*-terminal telopeptide (NTX) were observed with 40 mEq potassium citrate and calcium citrate (800 mg) over 2 wk, but no change was seen with potassium citrate alone, although urinary calcium decreased (23). The NTX increase induced by a high salt diet (225 mmol/d) was prevented with potassium citrate supplementation (90 mEqd) over 4 wk (6), but that study did not show the effect of potassium citrate supplementation at lower salt intakes. A reduction in bone markers was observed for 22 postmenopausal women in a 3-mo study using 4–8 g (37–74 mEq) potassium citrate/d. However, the initial fDPD concentration was higher in the treated group (9.1 nmol/mmol) than in a group of 24 age-matched women (6.3 nmol/mmol), which suggested that the groups were not well-matched (7). A 1-y study compared potassium citrate with potassium chloride (30 mEq/d) in osteoporotic and osteopenic postmenopausal women who had lower BMD (T score −1 to −4) and mean body mass index [(in kg/m^2) 25 ± 4.3] and higher bone turnover (24) than did our population. The bone turnover marker data in that 1-y study were inconsistent over time. but potassium citrate showed an increase in lumbar spine BMD of 0.89 ± 0.30%. The bone loss of 0.98 ± 0.38% in their potassium chloride group was similar to the 1% annual loss in our population. Jehle et al also gave supplements of calcium and vitamin D, but baseline dietary intakes were not assessed.

It is possible that potassium citrate together with extra calcium intake may benefit bone health in a population with low calcium intakes through an alkali × calcium interaction. Potassium citrate may have had no long-term influence on bone turnover in the present study because habitual calcium intake was sufficient. In a recent study of male rats, a decrease in calcium excretion was seen with no effect on long-term bone markers, BMD, or bone strength (25). The authors suggested that a lifelong excess of

dietary protein causes low-grade metabolic acidosis, but that the skeleton can be protected by an adequate calcium supply. Similarly, the decrease in 24-h urinary calcium excretion seen in a subset of the high-dose potassium citrate group in the present study had no long-term effect on bone turnover markers. Although noncompliance is possible, the 24-h urinary potassium data together with serum potassium and capsule count in the full group would not support that possibility. Other investigators have proposed that, in healthy persons, less calcium is absorbed in the intestine to compensate for the excretion of less calcium, and the body re-establishes its steady state (26,27). This may not be the case at low calcium intakes.

Our design was to test whether potassium citrate, at doses relevant to diet, prevented bone loss in normal postmenopausal women, rather than as a treatment for osteoporosis. The influence of nutrition on bone may be evident in already frail persons because they are more sensitive to nutritional interventions, in addition to having a poor diet throughout life. In a healthy population, other factors such as body weight, muscle mass, and good general health may have a stronger influence on bone than does diet.

The diet group was included in the study to compare the effects of alkali provision through diet (a diet containing additional vitamins, minerals, and phytochemicals) with those of alkali provision from potassium citrate (with no additional nutrients). The diet group was designed to be equivalent to the low-dose potassium citrate group, but the types of fruit and vegetables were not controlled. Food diaries and checklists suggested good compliance for most women, but the blood measurements did not corroborate this, despite most volunteers being highly committed to the study. Seven women decreased their intake of fruit and vegetables, but even women who reported good compliance, who assumed that they were meeting the required number of portions throughout the study, may not have met their targets every day. The findings were no different in women taking blood pressure–lowering medication. Alternative explanations are *1*) that, if plasma vitamin C was already high, extra fruit and vegetables may not have increased the concentration much further (28); *2*) there were between-subject differences in vitamin C metabolism; and *3*) the additional fruit consumed was relatively low in vitamin C. Serum folate may not accurately reflect compliance, because not all fruit and vegetables contain folate, and folate is found in other foods, including an increasing number of fortified foods. Similarly, B vitamins other than folate can reduce homocysteine (29).

Health benefits of fruit and vegetables may be confined to people who eat little or no fruit and vegetables, with no added benefit for those already eating a few portions a day. Alternatively, an additional 300 g fruit and vegetables/d may be insufficient to influence bone turnover. The 30-d ancillary Dietary Approaches to Stop Hypertension trial for bone markers did not provide dietary details (9), but the main trial provided diets for 8 wk with 6 additional servings/d compared with the run-in diet (30). This protocol was not considered feasible in a long-term trial in which the study participants purchased their own food. We observed a relation between fruit intake and BMD at the baseline visit in the present study, which is consistent with epidemiologic data from our own center and other centers (11, 12, 31). This finding may reflect a life-long effect of diet on bone health. The mechanism of acid-base balance was dismissed in a study reporting an association between fruit and vegetable intake and bone mineral status in adolescents and older women, because it found no association with estimates of renal acid excretion (32). Muhlbauer et al (33) showed that the bone-sparing effect of vegetables in rats did not occur through an acid-balancing mechanism. The types of extra fruit and vegetables eaten may not have been rich sources of bone-active constituents. If the bone-active components are confined to vegetables, the small increase in intake seen in the present study is unlikely to have influenced bone health.

Other limitations to the present study are that we did not control the normal diet (for pragmatic reasons), and we did not supplement with calcium and vitamin D. The same research nurse saw each subject to ensure consistency of measurements and dietary advice, but the timing prevented taking fasted blood samples. Recent eating is known to influence CTX concentrations, whereas there is a modest effect on P1NP (34). However, each participant was seen at the same time of day throughout the study, and we had fasted urine samples for the alternative bone resorption marker fDPD/Cr. The 24-h urine sample was collected only in a subgroup to avoid overburdening participants. The strengths of the present study are that it is the longest study to date investigating the influence of dietary alkali on bone health, that it used bone turnover assessments at regular intervals, and that it used the gold standard of BMD measurements. We included 2 doses of dietary alkali in the double-blind randomized controlled trial and a dietary arm so that the findings could be translated to public health advice.

In summary, neither potassium citrate at 18.5 or 55.6 mEq/d nor 300 g self-selected fruit and vegetables/d influenced bone turnover or prevented BMD loss over 2 y in healthy postmenopausal women. Further work is required to investigate whether particular fruit and vegetables are important and how much of each is optimal for bone health.

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The authors' responsibilities were as follows—HMM (principal investigator, who had full access to all of the data in the study and who takes responsibility for the integrity of the data and the accuracy of the data analysis): the study design, study management, and the writing of the manuscript. AJB: responsibility for the volunteers' welfare and assistance with the study design; DMR (director of the Aberdeen Prospective Osteoporosis Screening Study from where the volunteers were recruited): contributed to the study design; RS (the research nurse in charge of the study volunteers): active involvement in qualitative research; ACH: processed, analyzed, and assisted with the interpretation of the food diaries and entered data including dietary checklists; SLN: contributed to the design of the dietary checklists; LA: statistical analyses; GD: analysis and interpretation of the plasma vitamin C results; SD: analysis of samples for whole cell folate and plasma homocysteine and interpretation of the results; WDF: analysis of the 24-h urine subset and of all samples for bone formation and resorption markers; and all authors: critical appraisal of the manuscript. SLN was the principal grant holder on a

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