

Partial Neutralization of the Acidogenic Western Diet with Potassium Citrate Increases Bone Mass in Postmenopausal Women with Osteopenia

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Chronic acid loads are an obligate consequence of the high animal/grain protein content of the Western diet. The effect of this diet-induced metabolic acidosis on bone mass is controversial. In a randomized, prospective, controlled, double-blind trial, 161 postmenopausal women (age 58.6 ± 4.8 yr) with low bone mass (T score -1 to -4) were randomly assigned to 30 mEq of oral potassium (K) citrate (Kcitrate) or 30 mEq of K chloride (KCl) daily. The primary end point was the intergroup difference in mean percentage change in bone mineral density (BMD) at lumbar spine (L2 through L4) after 12 mo. Compared with the women who received KCl, women who received Kcitrate exhibited an intergroup increase in BMD (\pm SE) of $1.87 \pm 0.50\%$ at L2 through L4 ($P < 0.001$), of $1.39 \pm 0.48\%$ ($P < 0.001$) at femoral neck, and of $1.98 \pm 0.51\%$ ($P < 0.001$) at total hip. Significant secondary end point intragroup changes also were found: Kcitrate increased L2 through L4 BMD significantly from baseline at months 3, 9, and 12 and reached a month 12 increase of $0.89 \pm 0.30\%$ ($P < 0.05$), whereas the KCl arm showed a decreased L2 through L4 BMD by $-0.98 \pm 0.38\%$ ($P < 0.05$), significant only at month 12. Intergroup differences for distal radius and total body were NS. The Kcitrate-treated group demonstrated a sustained and significant reduction in urinary calcium excretion and a significant increase in urinary citrate excretion, with increased citrate excretion indicative of sustained systemic alkalinization. Urinary bone resorption marker excretion rates were significantly reduced by Kcitrate, and for deoxypyridinoline, the intergroup difference was significant. Urinary net acid excretion correlated inversely and significantly with the change in BMD in a subset of patients. Large and significant reductions in BP were observed for both K supplements during the entire 12 mo. Bone mass can be increased significantly in postmenopausal women with osteopenia by increasing their daily alkali intake as citrate, and the effect is independent of reported skeletal effects of K.

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Osteoporosis has emerged as an important and rapidly accruing risk factor for morbidity, mortality, and health care resource utilization in Western countries. The reasons for the high prevalence of osteoporosis in Western societies are incompletely understood, but lifestyle factors (*e.g.*, diet) are thought to be important. An important characteristic of the modern Western diet, when compared with ancestral diet forms, is that it imposes an acid load on the body *via* acid-generating proteins, a characteristic that is tightly coupled with a low potassium (K) content (1,2).

Chronic acid loads have resulted in decreased bone calcium or wet weight in bone samples in some (3), albeit not all, animal studies (4,5) and in humans have resulted in negative calcium balance with hypercalciuria attributed to loss of bone mineral (6). *In vitro* studies using rat calvariae have shown that incubation in medium that mimics chronic metabolic acidosis results

in both noncellular and cell-mediated effects on bone: Acutely, there is physicochemical dissolution of bone (liberating calcium, phosphate, and carbonate), a potentially homeostatic process that might attenuate acidosis. Chronically, complex cellular effects are thought to predominate and include inhibition of bone formation and stimulation of bone resorption *via* acidosis-induced stimulation of osteoblastic prostaglandin E_2 synthesis and consequent stimulation of the RANK/RANKL signaling pathway (7,8). In aggregate, these effects might be extrapolated *in vivo* to decrease bone mass and bone quality.

It generally is not appreciated that the acid load that is induced by the Western diet typically is on the order of 25 to 125 mmol/d protons and is present for the entire lifespan (9,10). Quantitatively, this “normal,” diet-induced acid load is large and can approach 50% of that induced in short-term (1 to 3 wk) human mineral acid-loading studies (11,12). Importantly, short-term neutralization of these diet-induced acid loads during oral intake of alkali salts resulted in calcium retention and bone marker changes that were compatible with decreased bone resorption in both postmenopausal women and healthy young adults (13,14).

Epidemiologic studies have suggested a relationship among acidogenic diets and decreases in bone mineral density (BMD) as well as increased fracture incidence (15–17). Contrasting

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with these observations, however, diets that are high in or supplemented by animal proteins (thereby increasing the dietary acid load) also have been associated with anabolic effects on bone (increased bone mass, decreased fracture [18,19]) possibly by dietary protein-induced stimulation of IGF-1 and/or stimulated intestinal calcium absorption, although such data sets generally have included diets that are below recommended protein levels and thus may reflect, in part, correction of malnutrition (18–20). The effect of alterations in either acid or protein intake on bone mass remains controversial, and there are no reported controlled clinical trials on the independent effect of altered acid intake on bone mass in humans.

On the basis of the hypothesis that the acidogenic Western diet provides at least part of the pathophysiologic basis of osteoporosis, we evaluated the bone mass response to chronic alkali ingestion in humans without renal disease. We report that chronic alkali treatment resulted in a significant increase in lumbar spine and hip BMD.

Materials and Methods

Study Participants

We recruited nonvegetarian, postmenopausal women (T scores at lumbar spine, L2 through L4, of -1 to -4) who were <70 yr of age and at least 5 yr postmenopausal. We excluded women with any electrolyte or acid-base disorder, a serum creatinine >120 $\mu\text{mol/L}$, gastrointestinal disease, nephrolithiasis, or a history of nonvertebral osteoporotic fractures. Excluded concomitant medications included glucocorticoids, thiazide diuretics, K-sparing diuretics, cyclooxygenase-1 or -2 inhibitors, and any osteoporotic treatment within the last 3 yr. The participants were instructed to maintain their self-selected diet as a constant intake and to maintain their self-selected exercise regimen.

The University's institutional review board ("Ethikkommission Beider Basel") approved the study protocol. All participants provided written informed consent and were compensated for their participation.

Treatments

The active study treatment was 10 mmol of trivalent K citrate (Kcitrate, wax matrix tablets, Urocit-K; Mission Pharmacal, San Antonio, TX) in three divided daytime doses, yielding 30 mmol of K and 30 mmol of potential base (bicarbonate) daily. Control participants received 30 mmol of K chloride (KCl) in wax matrix tablets of identical appearance and taste. Both groups received CaCO_3 (500 mg of Ca) and 400 IU of vitamin D_3 (Calperos D_3 ; Robapharm, Allschwil, Switzerland) daily.

Study Design

A total of 181 women were randomly assigned to receive either blinded Kcitrate or KCl for 12 mo. BMD was assessed twice at baseline (1 wk apart at all sites) and after 3, 6, 9, and 12 mo for spine and hip regions and after 6 and 12 mo for distal one third radius and total body BMD. Blood was collected twice at baseline (1 wk apart) and after 6 and 12 mo. Blinded study drug was labeled and dispensed by a study pharmacist; all other personnel remained blinded throughout. Two-hour morning fasting urine samples were collected twice at baseline and after 3, 6, 9, and 12 mo. Systolic and diastolic BP (Korotkoff disappearance) were measured by arm cuff inflation while in a comfortable sitting position at each visit.

Efficacy Outcome Variables

The primary end point was the intergroup difference in percentage change in areal BMD (g/cm^2) at the lumbar spine (L2 through L4) at month 12, assessed as the visit mean of same-day duplicate BMD values (participant repositioned after leaving table) by dual-energy x-ray absorptiometry (Lunar DPXL, Madison, WI). The short-term precision coefficient of variation for areal density in our unit is 1.3% (L2 through L4). Citrate, pyridinoline (PYR), and deoxypyridinoline (DPD) were measured in 2-h fasting morning urine, and all values were corrected for the urinary creatinine concentration. Because 24-h outpatient urinary Na, Cl, K, and urea N values are collected inaccurately (21), fasting morning solute/creatinine ratios were used. Blood was obtained in the morning fasting state and immediately refrigerator-centrifuged, and the serum was stored at -30°C .

Assays

Urinary citrate was measured by ion chromatography with a Metrohm IC 761 ion chromatograph using a Metrosep A Supp 4 column (Metrohm, AG, Herisau, Switzerland), eluted with $\text{NaCO}_3/\text{HCO}_3^-$, using chemical suppression and conductivity detection. Peak area was computed by automated integration (IC Net 2.3 Software, Metrohm). Intra- and interassay coefficient of variation was $<2\%$. Urinary total PYR and DPD were analyzed by HPLC (22).

Serum N-terminal and mid-region (N-MID) osteocalcin (23), intact parathyroid hormone (23), bone-specific alkaline phosphatase (BSAP) (24), and βC -telopeptide of type I collagen or c-terminal telopeptide (CTX) (24) were determined by immunochemiluminescence. Serum 25-OH vitamin D was determined with an IDS Ocleia ELISA kit (Boldon, UK). In a consecutive subset of 22 patients, 24-h urinary net acid excretion (NAE ; $\text{NH}_4^+ + \text{titratable acid} - \text{HCO}_3^-$) was measured at the month 12 visit (12).

Compliance and Safety Assessment

Study drug compliance was assessed by the pharmacist's tablet count. Patients were queried for interval adverse events at each quarterly visit.

Statistical Analyses

Randomization was unstratified and unblocked. The prespecified population for the primary end point (intergroup difference in mean percentage change in BMD at L2 through L4 at month 12) comprises all 161 participants who provided baseline and month 12 BMD data. In fact, the month 12 data included all participants who had not withdrawn before month 12. Using reported values for the SD of the primary end point of 3.8% (25) and a two-sided α of 0.05, the study had $>90\%$ power to detect a 2% intergroup difference (SD and effect size values similar to those obtained herein). Secondary end points included intragroup month 12 comparisons of percentage change in BMD at L2 through L4, total hip and femoral neck, and intergroup comparisons for total hip and femoral neck at month 12. BMD changes at other sites, bone metabolism markers, and excretion values were exploratory. No interim analysis was prespecified or performed. The primary end point, baseline intergroup comparisons, and exploratory secondary intergroup comparisons were tested by nonparametric Kruskal-Wallis. Secondary/exploratory within-group differences were tested using the nonparametric Wilcoxon rank sum test. Missing values were not carried forward or imputed because of the monotonic changes expected and its customary practice in the BMD field (in fact, all missing values were due to early dropout). Statistical analyses used SPSS, version 12 (SPSS, Chicago, IL).

Table 1. Baseline characteristics of the 161 women^a

| Characteristic | KCl (<i>n</i> = 79) | Kcitrates (<i>n</i> = 82) | <i>P</i> |
|--|----------------------|----------------------------|----------|
| Age (yr) | 58.7 ± 4.4 | 58.6 ± 4.9 | 0.695 |
| Years since menopause | 10.6 ± 4.1 | 10.5 ± 4.2 | 0.701 |
| Systolic BP (mmHg) | 131.1 ± 2.2 | 134.9 ± 1.9 | 0.114 |
| Diastolic BP (mmHg) | 80.7 ± 1.1 | 82.3 ± 1.0 | 0.152 |
| Body mass index (kg/m ²) | 25.0 ± 4.3 | 24.7 ± 4.2 | 0.593 |
| Cigarette smoking (%) | 18.8 | 25.0 | |
| Creatinine clearance ^b (ml/min) | 95.7 ± 7.1 | 94.3 ± 7.5 | 0.205 |
| 25-OH-vitamin D (nmol/L) | 76.0 ± 30.1 | 69.9 ± 32.5 | 0.249 |
| Citrate/creatinine (mg/mmol) | 67.2 ± 3.5 | 74.7 ± 3.9 | 0.328 |
| iPTH (pmol/L) | 4.52 ± 2.30 | 4.27 ± 1.51 | 0.392 |
| BSAP (μg/ml) | 11.1 ± 3.7 | 11.1 ± 3.6 | 0.955 |
| C-telopeptide (ng/ml) | 0.2753 ± 0.1427 | 0.2856 ± 0.2013 | 0.707 |
| Deoxypyridinoline/creatinine (nmol/mmol) | 12.6 ± 4.2 | 13.1 ± 6.8 | 0.554 |
| Pyridinoline/creatinine (nmol/mmol) | 69.9 ± 12.2 | 51.0 ± 20.4 | 0.266 |
| BMD lumbar spine (g/cm ²) | 1.027 ± 0.133 | 1.042 ± 0.117 | 0.435 |
| BMD total hip (g/cm ²) | 0.880 ± 0.108 | 0.896 ± 0.103 | 0.349 |
| BMD femoral neck (g/cm ²) | 0.804 ± 0.097 | 0.822 ± 0.089 | 0.217 |
| T scores lumbar spine | -2.10 ± 0.61 | -2.05 ± 0.58 | 0.451 |

^aData are means ± SD. BMD, bone mineral density; BSAP, bone-specific alkaline phosphatase; iPTH, intact parathyroid hormone; Kcitrates, potassium citrate; KCl, potassium chloride.

^bCalculated using the formula of Cockcroft and Gault.

Results

Baseline Characteristics, Participant Disposition, Study Drug Compliance, and Adverse Events

Table 1 summarizes the participants' baseline characteristics. The women all were white and resided near Basel. Figure 1 depicts the screening and randomization process as well as the dropout rate and dropout reasons. A total of 161 participants completed the study. The dropout rate was higher in the KCl group, as a result chiefly of gastrointestinal events. Study drug tablet compliance in the Kcitrates group was 94% and in the KCl group was 93%. Accordingly, as illustrated by Table 2, fasting urinary K excretion increased similarly and significantly at all study time points in both groups. Fasting urinary citrate excretion was not altered significantly in the KCl group but showed a large, significant, and sustained increase in the Kcitrates group (Table 2). There was no significant difference in the fractional excretion of sodium both compared with baseline and within groups after 6 and 12 mo. Fasting urinary urea excretion was similar in both groups and did not change significantly during the study period, reflecting constant and comparable protein intake in both groups (Table 2).

Description of End Points

The primary end point (month 12 intergroup percentage change in L2 through L4 BMD) *P* value is the principal statistical finding of the trial. The significance values for the secondary end points of earlier L2 through L4 BMD differences and all total hip and femoral neck BMD intra- and intergroup differences are of lesser import for hypothesis testing but can be viewed as physiologically and clinically important, given their roles in determining the rapidity and consistency of efficacy.

Significance values for exploratory bone marker differences are of import only for mechanistic inference.

BMD and Bone Metabolism Markers

As illustrated in Figure 2, lumbar spine (L2 through L4) BMD, the primary end point, increased progressively and significantly in the Kcitrates group in contrast to a progressive decrease in the KCl group, resulting in an intergroup difference of 1.87 ± 0.50% (SEM, *P* < 0.001; *n* = 161) at month 12. For the secondary end points, significant intragroup changes also were found: Kcitrates increased L2 through L4 BMD significantly from baseline at months 3, 9, and 12 and reached a month 12 increase of 0.89 ± 0.30% (*P* < 0.05), whereas the KCl arm showed a decreased L2 through L4 BMD by -0.98 ± 0.38% (*P* < 0.05), significant only at month 12. Significantly higher intergroup BMD changes for the Kcitrates group at month 12 also were seen at total hip (1.98 ± 0.51%; *P* < 0.001) and femoral neck (1.39 ± 0.48%; *P* < 0.001). The greater intergroup BMD increments at these sites in the Kcitrates group became statistically significant between 6 and 9 mo and continued to increase through month 12. Intergroup BMD changes were not significantly different for either total body or distal one third of radius. For total body BMD, mean percentage change in BMD was -0.05 ± 0.2% by month 12 for KCl and -0.3 ± 0.2% for Kcitrates. For distal one third of the radius, the corresponding changes were -0.4 ± 0.4% and 0.1 ± 0.4%. Intragroup changes in total hip and femoral neck BMD showed significant decreases in the KCl group.

The remaining end points were exploratory. Serum BSAP, a marker of bone formation, increased similarly in both study

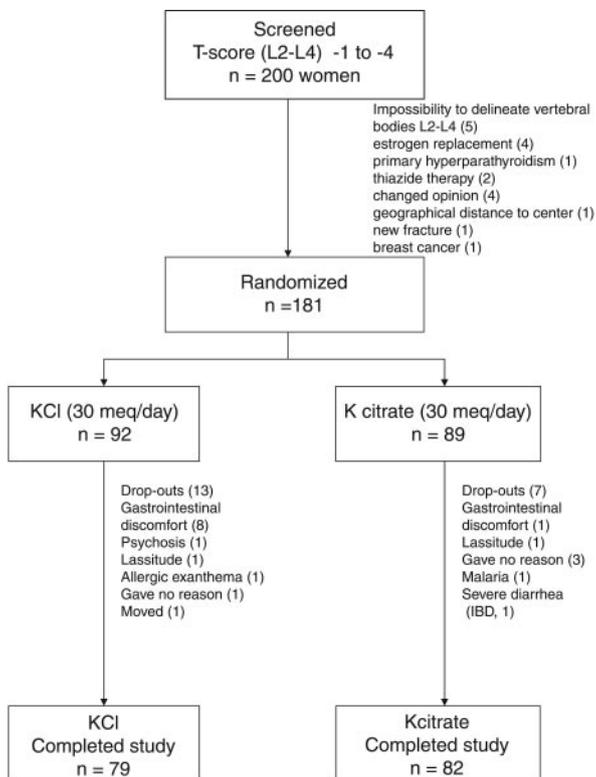


Figure 1. Screening/randomization process and dropout reasons.

groups, consistent with *in vitro* effects of K *per se* to stimulate bone collagen production/cell-mediated bone formation rate (26). The increase from baseline was statistically significant at both months 6 and 12 (Figure 3). Serum osteocalcin, a marker of bone formation/turnover, changed in the opposite direction from that of BSAP and exhibited a significant decrease in both groups. Urinary bone resorption markers (PYR and DPD) decreased significantly during the entire study in the Kcitrate group and increased in the KCl group (Figure 4). Although both mean PYR and DPD excretion changes were more negative at all time points for the Kcitrate group, this intergroup difference was significant only for DPD at month 3 ($P = 0.0024$). There were no significant changes in serum CTX, an additional marker of bone resorption (Figure 3); CTX was not measured at month 3.

Renal Calcium and Phosphate Excretion

Fasting urinary calcium excretion decreased significantly in the Kcitrate group *versus* KCl group by months 6 and 9, and a nominal reduction persisted throughout the study (Figure 5A). No significant intragroup changes in calcium excretion were observed. Fasting urinary phosphate excretion was similar in both groups but became significantly reduced in the Kcitrate group only at month 12. In circumstances of constant intake, a decrease in fasting calcium and phosphate excretion is thought to represent a net influx of these ions into bone. As shown in Figure 5B, the fractional excretion rates for both Ca and PO₄

became significantly lower in the Kcitrate group, indicating that Kcitrate treatment increased renal Ca and PO₄ avidity.

Relationship between Renal NAE and Changes in BMD

Renal NAE is a good estimate for endogenous acid production (reflecting acid load [9]) and, therefore, should correlate inversely with changes in bone mass. Twenty-four-hour NAE (measured in a subset of 22 women at month 12) indeed was correlated negatively and significantly with the 12-mo percentage change in BMD at L2 through L4 (Figure 6). The mean values in Figure 6 for NAE were 35 ± 8 (SEM) and 6 ± 9 in the KCl and Kcitrate groups, respectively, suggesting that, on average, NAE was nearly fully neutralized at the 30-mmol/d alkali dosage in this subset. An additional index of systemic alkalinization, urinary citrate excretion, remained essentially unchanged in the KCl group throughout the study, whereas it increased significantly in the Kcitrate group (Table 2).

Effect on BP

As early as month 3 in both treatment groups, significant and sustained decreases in both systolic and diastolic BP were observed (Figure 7). By month 12 for Kcitrate, systolic BP had fallen by 7.9 ± 1.8 mmHg ($P < 0.001$) and diastolic pressure had fallen by 6.4 ± 1.1 mmHg ($P < 0.001$), accompanied by similar systolic 7.8 ± 2.4 ($P < 0.001$) and diastolic 5.2 ± 1.0 mmHg ($P < 0.001$) decrements for KCl.

Discussion

Despite more than 70 yr of sustained interest in the possibility that chronic metabolic acidosis might decrease bone mass, central questions regarding the effect of acid-base alterations on human bone physiology and pathophysiology remain unanswered (4,27,28). First, does chronic metabolic acidosis of any magnitude decrease bone mass? Second, does the low-grade chronic metabolic acidosis that is induced by the acidogenic Western diet result in osteoporosis? Third, can neutralization of dietary acid result in increased bone mass in normal humans or those with osteopenia?

Indirect support for a possible role of chronic metabolic acidosis to reduce bone mass comes from small, uncontrolled studies in humans with nonazotemic renal disease (distal renal tubular acidosis [29,30]). Chronic dietary acid loads were shown to result in significant and reversible negative calcium balance (6), and experimental increases in animal protein intake or its chief acidogenic constituent, methionine, within the range that is characteristic of the Western diet were shown to cause both negative calcium balance and increased systemic acid load (31,32). Compatible with and strongly supportive of these observations, short-term neutralization of endogenous acid production by oral ingestion of bicarbonate for 7 to 18 d in both postmenopausal women and young healthy adults resulted in calcium retention and—on the basis of analysis of bone markers—in inhibition of bone resorption (13,14).

Whether the observed increase in BMD by prolonged alkali administration in this study is due primarily to an effect on bone formation remains to be clarified. Our results demonstrate a dissociation of two commonly used formation markers; BSAP

Table 2. Effect of KCl and Kcitrate treatment on fasting urinary sodium, chloride, K, citrate, and urea excretion

| Parameter | Urinary Na/Creatinine (mmol/mmol) | | Urinary K/creatinine (mmol/mmol) | | Urinary Cl/Creatinine (mmol/mmol) | | Urinary Citrate/Creatinine (mg/mmol) | | Urinary Urea/Creatinine (mmol/mmol) | |
|---|-----------------------------------|----------------|----------------------------------|--------------------------|-----------------------------------|----------------|--------------------------------------|---------------------------|-------------------------------------|----------------|
| | KCl Group | Kcitrate Group | KCl Group | Kcitrate Group | KCl Group | Kcitrate Group | KCl Group | Kcitrate Group | KCl Group | Kcitrate Group |
| Baseline | 13.52 ± 0.63 | 12.95 ± 0.61 | 8.10 ± 0.35 | 8.08 ± 0.42 | 15.10 ± 0.71 | 14.82 ± 0.60 | 67.2 ± 3.5 | 74.7 ± 3.9 | 30.4 ± 1.9 | 30.4 ± 1.7 |
| Change <i>versus</i> baseline at month 3 | 0.82 ± 0.93 | 0.43 ± 0.81 | 2.93 ± 0.54 ^b | 2.71 ± 0.51 ^b | 3.46 ± 1.0 | 0.37 ± 0.81 | 2.3 ± 2.3 | 18.2 ± 2.9 ^{b,c} | -0.10 ± 0.81 | -0.10 ± 0.70 |
| Change <i>versus</i> baseline at month 6 | -0.32 ± 0.82 | 0.41 ± 0.70 | 2.32 ± 0.50 ^b | 2.14 ± 0.54 ^b | 2.09 ± 0.97 | 0.20 ± 0.77 | 3.0 ± 2.9 | 15.9 ± 3.4 ^{b,c} | -0.79 ± 0.23 | -0.79 ± 0.41 |
| Change <i>versus</i> baseline at month 9 | 0.17 ± 0.72 | 0.28 ± 0.83 | 2.49 ± 0.65 ^b | 2.32 ± 0.54 ^b | 2.61 ± 0.94 | 0.13 ± 0.96 | 4.0 ± 2.6 | 11.3 ± 3.2 ^{b,c} | 0.78 ± 0.39 | 0.78 ± 0.30 |
| Change <i>versus</i> baseline at month 12 | -0.37 ± 0.84 | -0.06 ± 0.87 | 2.10 ± 0.55 ^b | 1.94 ± 0.50 ^b | 1.49 ± 1.02 | -0.04 ± 0.87 | 1.8 ± 3.6 | 2.5 ± 3.6 ^{b,c} | 0.06 ± 0.32 | -0.03 ± 0.41 |

^aTo convert solute/creatinine excretion ratios from mmol solute/mmol creatinine to mmol solute/g creatinine, multiply by 8.85.

^b*P* < 0.025 *versus* corresponding baseline values.

^c*P* < 0.020, Kcitrate *versus* KCl group at months 3 through 12.

increased in both K-supplemented groups, whereas osteocalcin declined over 12 mo. This pattern is similar to that reported in a 3-mo bone marker study of daily Kcitrate in women with osteopenia (33). Although both markers are produced by osteoblasts, BSAP expression has been reported to appear early in the formation cycle, whereas osteocalcin is expressed by more active, mature osteoblasts (34). Whether K has effects on bone formation and whether they might be selective for an osteoblastic subset are not known. The observation of a possible increase in bone formation rate in the Kcitrate group, evidenced by the increasing BSAP levels, however, would be expected to amplify any net increase in bone mass that is attributable to suppression of resorption.

The observed increase in BMD may be related more to an antiresorption effect than to formation. The suppression of PYR and DPD excretion is consistent with previous uncontrolled observations during shorter periods of treatment (13,14). We did not assess markers of bone metabolism in the first 2 wk of this trial to confirm the larger effects that were seen previously in that time frame. Taken together, it is likely that cellular effects (formation and resorption of bone matrix) that are exhibited as bone marker changes are attenuated after the early weeks of alkali administration, but confirmation of this thesis will require more detailed studies.

It also is possible that increases in the degree and the uniformity of mineralization, further amplified by the physicochemical, non-cell-mediated mineralizing properties of alkali administration (7), may have increased BMD in the Kcitrate group beyond that accounted for by an antiresorptive effect on the amount of bone matrix. Such effects during antiresorptive treatment were described recently in alendronate-treated women with osteoporosis (35,36). The finding that an appreciable proportion of bisphosphonate effects on BMD can be attributed to noncellular mineralization effects, coupled with previous data showing independent effects of acidity on mineralization, provides strong support for the thesis that the effects of alkali in our study might be attributable fully to enhanced matrix mineralization and largely independent of cell-mediated events. In fact, a recent report using primary rat osteoblasts in culture found that induction of a physiologic degree of metabolic acidosis resulted in profound reductions in matrix mineralization in the complete absence of any detectable reduction in collagen

synthesis, directly supportive of a possible increase in BMD by a mineralization effect of alkali in our study (37). A significant but subtle mineralization defect that was assessed by bone biopsy histomorphometry was reported to be associated with metabolic acidosis in predialysis patients with chronic kidney disease (38). The more florid hypomineralizing condition, osteomalacia, also is associated with metabolic acidosis but has been reported only when other hypomineralizing factors also are present (*e.g.*, Fanconi syndrome, hypophosphatemia) (39).

Our observed net lumbar spine BMD increase (1.9%) is large and compares favorably with the month 12 increase of raloxifene (1.7%), although it is less than for the approved daily dose of ibandronate (3.9% [40,41]). Importantly, our observed changes using potentially bone-anabolic KCl as comparator may underestimate placebo-controlled efficacy (26,42). Our KCl arm's decrease in lumbar spine BMD, however, is consistent with 12-mo data for raloxifene placebo (-0.5% [40]) and other reports with even nominally greater (-1.2 to -2.8%) decreases in placebo BMD than found herein (43,44). Irrespective of the precise magnitude of the control arm result, the present BMD efficacy predicts fracture efficacy comparable to that of highly effective approved pharmaceutical agents.

Na excretion was unchanged and nearly identical in both study arms (Table 2). Therefore, our finding that KCl caused no hypercalciuria is consistent with previous reports showing that increased NaCl intake but not KCl results in significant hypercalciuria in humans (42). Citrate excretion increased significantly and remained increased in the Kcitrate group throughout the trial (Table 2). Because it has been established rigorously in humans that chronic administration of either Kcitrate or KHCO₃ causes similar decrements in NAE as well as similar increments in urinary citrate excretion, the finding in our study that citrate excretion increased is attributable to administration of citrate as an alkali precursor and not to a failure to metabolize administered citrate (45). Urinary citrate excretion is thought to increase and decrease in parallel with proximal tubule intracellular pH; therefore, the alkali-induced increases in urinary citrate excretion in our study provide an index of the effect of systemic alkalization to increase proximal tubule pH and thereby provide additional evidence for persistent systemic alkalization in this trial beyond that afforded by the decrease in urinary NAE in a subset (46). The finding that

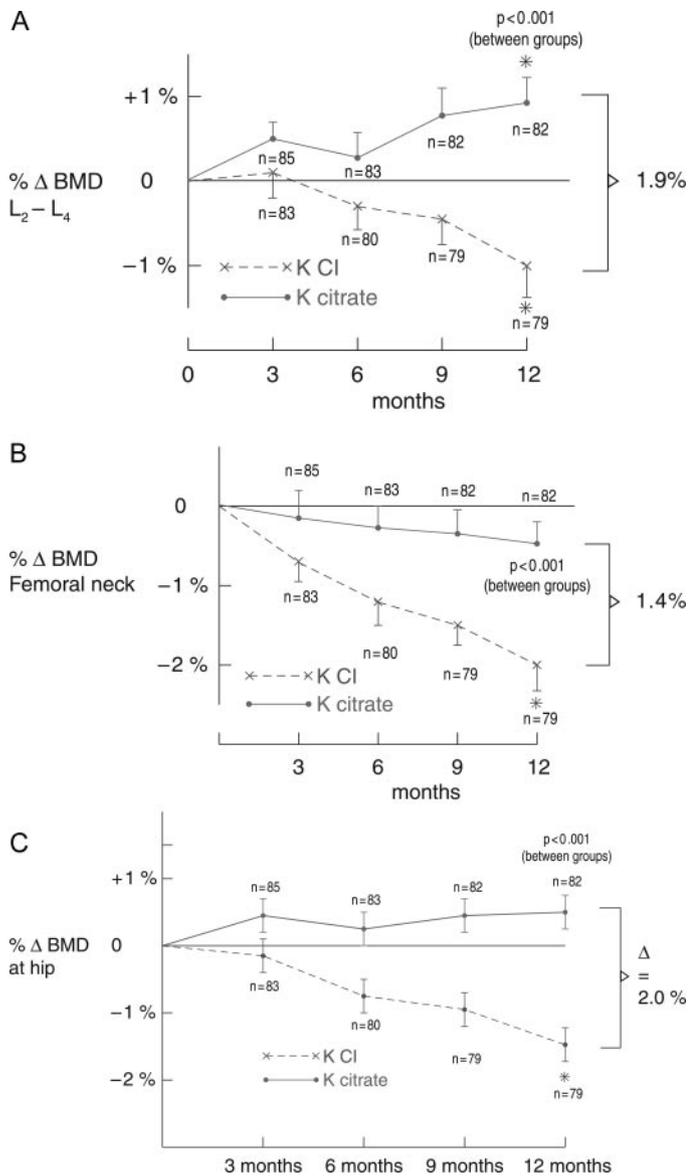


Figure 2. (A) Effect of potassium (K) citrate (Kcitrate) treatment on the percentage change in bone mineral density (BMD) measured at lumbar spine (L2 through L4). The *P* values indicate statistical significance for intergroup comparisons. Intragroup analysis shows that BMD changes were significant ($P < 0.05$) at months 3, 9, and 12 in the Kcitrate and at months 12 in the K chloride (KCl) group when compared with baseline values (*). Error bars show \pm SEM. (B) Effect of Kcitrate treatment on the percentage change in BMD measured at femoral neck. The *P* values indicate statistical significance for intergroup comparison. Intragroup analysis shows that BMD changes were significant ($P < 0.05$) at months 6, 9, and 12 mo in the KCl group when compared with baseline values (*). Error bars show \pm SEM. (C) Effect of Kcitrate treatment on the percentage change in BMD measured at total hip compared with baseline and control group receiving KCl. The *P* values indicate statistical significance for intergroup comparison. Intragroup analysis shows that BMD changes were significant ($P < 0.05$) at months 6, 9, and 12 mo in the KCl group when compared with baseline values. Error bars show \pm SEM.

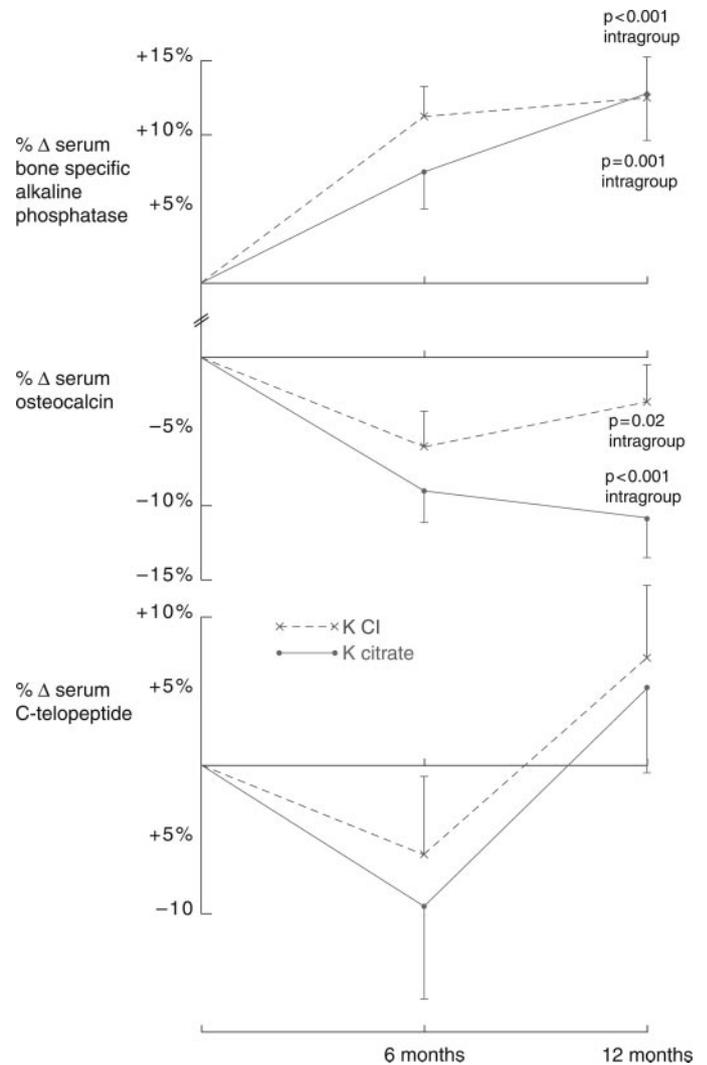


Figure 3. Effect of Kcitrate treatment on the percentage change in activity of bone-specific alkaline phosphatase (BSAP) and serum concentrations of osteocalcin and c-telopeptide compared with baseline and with control group receiving KCl. Error bars show \pm SEM.

initially (month 3) greater elevations in urinary citrate became gradually attenuated by month 12 possibly might be attributable to a delay in the rate of bone alkali salt uptake (including CO_3^- and dibasic phosphate salts of Ca), consistent with the later intergroup separation of BMD at lumbar spine.

We chose KCl as comparator rather than true placebo to provide a control for experimental provision of alkali without confounding changes in K load (13,42). The choice of KCl was fostered by multiple considerations. First, unlike NaCl, there are no reports in any species of either calciuric or bone mass losses that are attributed to KCl. Second, multiple reports in humans have demonstrated consistently that chronic K administration results in hypocalciuria (13,42,47–49), and this finding has been interpreted to reflect positive calcium balance and skeletal anabolism. Moreover, KCl as well as KHCO_3 loading has been reported to result in hypocalciuria in healthy adults (42). Third, consistent with alkali-independent hypocalciuric

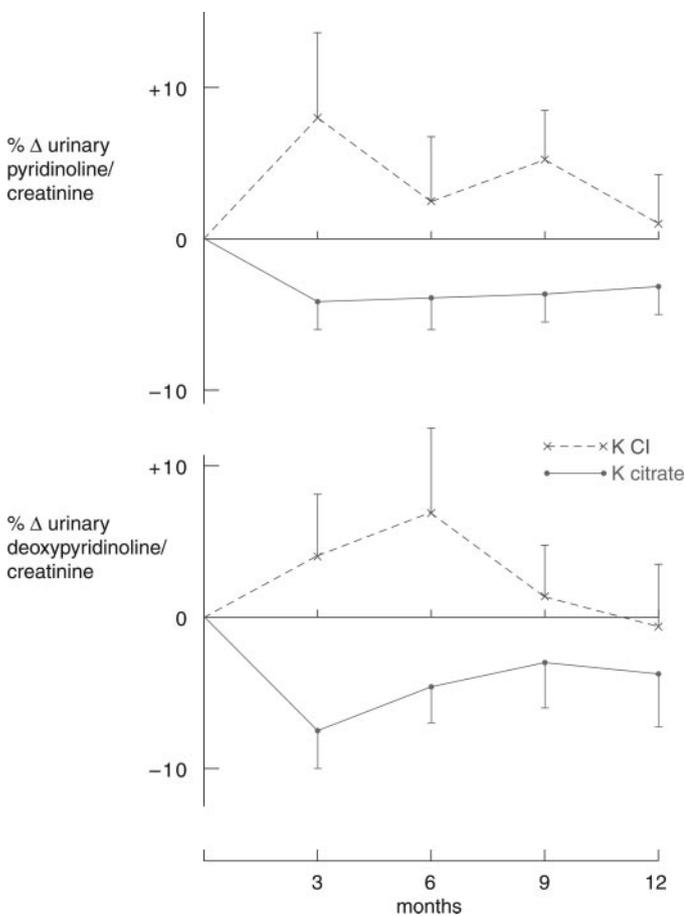


Figure 4. Effect of Kcitrate treatment on the percentage change in urinary excretion of pyridinoline and deoxypyridinoline compared with baseline and to control group receiving KCl. Error bars show ± SEM.

effects of K, higher femoral neck BMD was reported to be associated with a higher K intake in a population-based screening of Scottish premenopausal women (age >44), and the K intake effect on BMD explained a four-fold greater proportion of BMD variation than did calculated dietary acid production (10). In the longitudinal Framingham Osteoporosis Study in the elderly, high K intake also was significantly associated with higher baseline BMD in both genders, and for men, BMD loss over 4 yr was significantly less at higher K intakes despite a similar BMD-protective effect of high animal protein intake (50). Because the K-associated improved interval change in BMD could not be attributed to K associated with alkali, this observation also is supportive of anion-independent BMD-protective effects of K on bone mass. These *in vivo* results have been supported by the finding that *in vitro* addition of KCl to K-depleted medium in mouse calvariae inhibited bone Ca efflux, decreased markers of bone resorption, and increased bone collagen synthesis that were independent of extracellular pH or HCO₃⁻ concentration and therefore judged to be anion independent and K specific (26). Accordingly, comparing Kcitrate to KCl provides a potentially active comparator and a greater theoretical hurdle for bone mass accretion than an alternative trial using true (inactive) placebo.

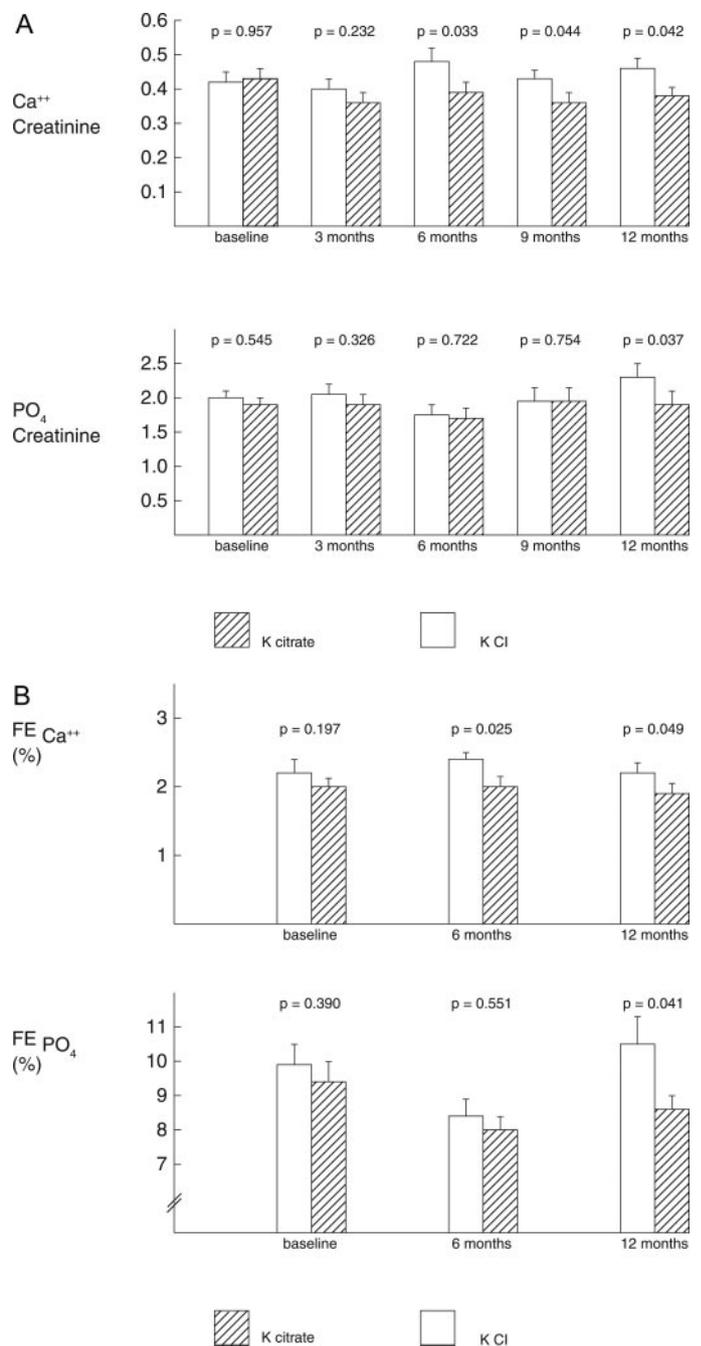


Figure 5. Effect of Kcitrate treatment on calcium and phosphate excretion (as Ca/creat and PO₄/creat in mmol/mmol) in fasting urine (A) and on the urinary fractional excretion rates of calcium and phosphate (B) compared with the control group receiving KCl. Fractional excretion was computed as (U/P_{Ca} ÷ U/P_{creat}) × 100. U/O_{con}, urine to plasma calcium concentration; U/P_{creat}, urine to plasma creatinine concentration. Error bars show ± SEM.

The BP reductions that were obtained in this trial provide the first reported long-term K-supplemented BP data in normotensive individuals. Although our BP results are not placebo controlled, the results are comparable in magnitude to BP reductions that were seen in a shorter (8 wk) placebo-controlled

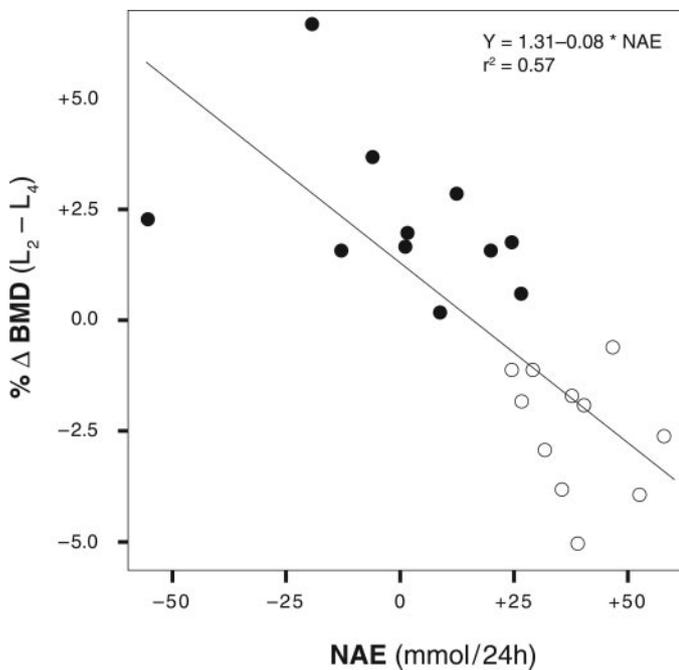


Figure 6. Relationship between urinary net acid excretion (instructed to collect full 24-h collection as outpatient) at 12 mo and percentage change in BMD measured at lumbar spine (L2 through L4) at 12 mo in a subset of women ($n = 22$). ○, data from women in the KCl group; ●, data from women in the Kcitrates group.

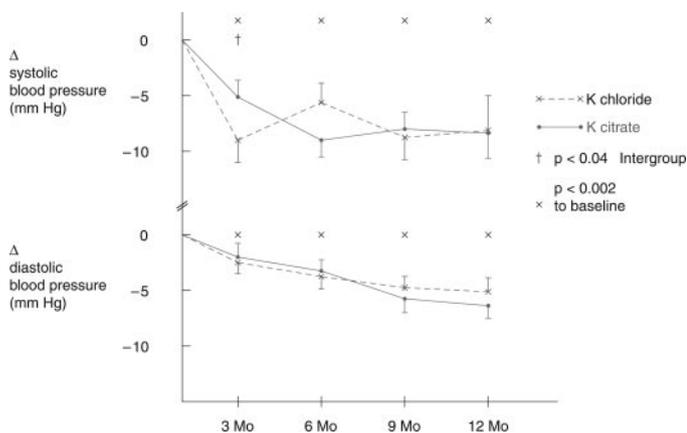


Figure 7. Effect of Kcitrates and KCl treatment (30 mmol/d in each treatment group) on the changes in systolic and diastolic BP. Error bars show \pm SEM.

study in hypertensive individuals using Kcitrates (51). It is interesting that although that placebo-controlled crossover study has been cited as positive for Kcitrates and negative for KCl (52), it was underpowered for BP effects, and the observed effect of KCl on diastolic BP was 67% of that observed with equivalent Kcitrates. Our observed diastolic decrease at month 12 for both supplements (-5 to -6 mmHg) is comparable to 8-wk changes that were observed in the Dietary Approaches to Stop Hypertension (DASH) trial on the effects of K-enriched foods in hypertensive individuals (-3 to -6 mmHg), suggesting that

Kcitrates treatment of women with osteopenia might provide long-term beneficial BP effects in tandem with a bone mass benefit (53).

The weaknesses of our study include its sample size for adverse events/safety (well powered for BMD efficacy); limitation to a specific class of osteopenia; lack of racial, gender, and ethnic diversity; and its conduct at a single center as well as inability to control rigorously for changes in physical activity/exercise. However, our study provides important insights into the pathophysiology of osteoporosis. As a proof-of-principle study, it demonstrates that neutralization of diet-induced endogenous acid production increases BMD, thereby proving the concept that such dietary acid loads are detrimental to bone mass and thus constitute a causative risk factor for bone loss in postmenopausal women with osteopenia. Whether fully neutralized high-protein intakes are superior to fully neutralized low-protein intakes remains unexplored. Further research also is required to examine the dosage-response and role of acid neutralization on bone mass in other patient populations (*e.g.*, men, the elderly, children before accelerated adolescent bone mass accretion) and in affecting fracture rates.

Conclusion

This study establishes that bone mass can be increased significantly in postmenopausal women with osteopenia by increasing their daily alkali intake as Kcitrates and that this effect is independent of reported *in vitro* skeletal effects of co-administered K. The magnitude of the effect is large, and the safety profile was found to be excellent, albeit based on a limited sample size. The results strongly support the thesis that neutralization of the modern Western diet will promote skeletal health. The associated BP effects of the K supplement provide additional incentive to move forward with controlled outcome trials using long-term Kcitrates treatment.

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