# Dietary intakes and urinary excretion of calcium and acids: a cross-sectional study of women in China[1-3]

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**ABSTRACT** The relationship between dietary intakes and urinary calcium was examined in a cross-sectional survey of 764 middle-aged and elderly women with markedly different dietary patterns and lifestyles. Urinary calcium was correlated positively with urinary acids, including titratable acid ($r = 0.46$, $P < 0.0001$), ammonia ($r = 0.42$, $P < 0.0001$), and sulfate ($r = 0.52$, $P < 0.0001$). Urinary excretions of calcium and acids were correlated positively with intakes of animal and nondairy animal protein but were correlated negatively with plant-protein intake, possibly because of the alkaline nature of plant foods. Further analyses showed that urinary calcium and acids were associated positively with acid-forming foods and were associated negatively with plant foods. These results indicate that under free-living conditions urinary calcium excretion is likely determined by the acid-base status of the total diet, including among other factors the contribution of sulfur amino acids to urinary acid production. *Am J Clin Nutr* 1993;58:398–406.

**KEY WORDS** Urine, calcium, renal acid, protein, animal foods, osteoporosis

## Introduction

Both high-protein feeding (1–3) and metabolic acidosis induced by ammonium chloride (4, 5) have been shown in experimental human and animal studies to increase renal acid load, concomitant with an increase in urinary calcium excretion. Because intestinal calcium absorption is not increased simultaneously, urinary calcium loss often leads to an adverse calcium balance (6–10). Diets rich in acid-forming foods may impose an acid burden that draws upon the buffering capacity of body calcium, thus leading to bone resorption (11, 12).

It has been hypothesized that the lifelong ingestion of acid-ash foods, as are provided by some Western diets, might be one of the risk factors involved in the development of osteoporosis (13, 14). Studies in support of this hypothesis have linked a lower bone density with animal food consumption in Eskimos (15) and in omnivores (16–18). However, few epidemiologic studies have focused on and systematically reported this association. Abelow et al (19) recently summarized hip fracture data from 34 published cross-cultural studies conducted separately in 16 countries, indicating a positive association between incidence rates of hip fracture and animal-protein intakes. On the basis of the acid-forming features of animal protein, they put forward the metabolic acid–osteoporosis hypothesis.

In 1989, a cross-sectional study was conducted in 764 middle-aged and elderly women in five different counties in China to investigate possible associations between dietary intakes and osteoporosis as specifically evaluated by bone density measurements (20). A marked variation in dietary patterns and lifestyles was shown among women residing in five rural counties selected on the basis of the results of a nationwide nutrition survey of 65 counties in 1983 (21). The stability and simplicity of the dietary composition in these areas allowed us to examine the effects of lifelong dietary features, especially the consumption of acid-forming foods, on urinary calcium excretion under free-living conditions and to test the metabolic-acid hypothesis proposed by Abelow et al (19). In this paper we present the results of correlation analyses of dietary components and urinary excretion of acids and calcium. The relationship between bone density and dietary calcium from this survey was reported elsewhere (20).

## Methods

### *Subject selection*

Selection of survey counties and subjects was described in detail in a previous publication (20). Briefly, 764 women between the ages of 35 and 75 y were selected from two pastoral counties (Xianghuangqi and Tuoli, counties YA and WA, respectively) and three nonpastoral counties (Jiexiu, Cangxi, and Changle; counties CD, SB, and LC, respectively). Relevant characteristics of the survey subjects in each county are described in **Table 1.** The anthropometric variables were not significantly different among these five counties. However, women in county SB had a smaller body size and lower body weight than women in the other counties.

A general physical examination was conducted by local medical personnel in the public health clinic, and anthropometric measurements, including body weight, height, and bone density

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TABLE 1
Characteristics of middle-aged and elderly Chinese women residing in the five survey counties*

| Variables | Xianghuangqi ($n$ = 143) | Tuoli ($n$ = 175) | Jiexiu ($n$ = 165) | Cangxi ($n$ = 164) | Changle ($n$ = 180) |
|---|---|---|---|---|---|
| Age (y) | 52 ± 11 | 51 ± 11 | 54 ± 11 | 52 ± 11 | 53 ± 12 |
| Weight (kg) | 50 ± 8[b] | 53 ± 9[a] | 54 ± 9[a] | 45 ± 6[c] | 50 ± 9[b] |
| Height (cm) | 151 ± 6 | 152 ± 6 | 154 ± 6 | 150 ± 6 | 154 ± 7 |
| Age at menopause (y) | 47 ± 4[a] | 45 ± 5[b] | 48 ± 5[a] | 47 ± 8[a] | 47 ± 5[a] |

* $\bar{x}$ ± SD. Means not sharing the same letter superscript are significantly different, $P < 0.05$ (Duncan's multiple-range test).

were recorded simultaneously. All of the subjects were disease free and did not take any medication known to be relevant to calcium metabolism.

Selection of human subjects was approved by the Human Subjects Committee at Cornell University and the Chinese Academy of Preventive Medicine. The experimental protocol was fully explained to the subjects and signed approval forms were obtained before initiating the survey.

*Estimation of dietary intakes*

Dietary intakes of each woman were estimated by weighing foods consumed by the subject over a period of 3 d. Food weighing was conducted by members of each county survey team who had attended a 1-wk training course before the survey. Simple foods such as cheese, cake, bread, and pickled vegetables were directly weighed and recorded as the difference in food weight before and after each meal. To estimate the components of prepared mixed dishes the raw ingredients were weighed before cooking; after cooking, the whole ready-to-eat dish was measured by using a scoop or other small utensil as the measuring unit, which subsequently was used to measure the amount of the dish consumed by the subject. Intakes by the subject of the raw ingredients from the mixed dish were then calculated proportionally on the basis of the amount consumed by the subjects.

Nutrient intakes of subjects were calculated by using the recently revised *Chinese Food Composition Table* (22). Samples of food items not available in the food composition table were collected in the field and were frozen and shipped to Cornell University for nutrient analyses.

*Collection of urine and blood samples*

On the second day of the food survey each subject was asked to collect an overnight (1900–0700) urine sample in an acid-washed polyethylene container. After the total volume of urine samples was measured and recorded, two aliquot portions (50 mL each) of the urine samples were then made for laboratory analyses. One aliquot portion of the fresh sample was used for immediate determination of titratable acid at the local county health stations. The second aliquot portion was frozen at −20°C and shipped to Cornell University in Ithaca, NY for further analyses. Upon arrival at Cornell University urine samples were divided again for measurements of creatinine, ammonia, sulfate, phosphate, and minerals. The aliquot sample used for the measurements of minerals, including calcium, magnesium, potassium, and sodium, was acidified with 3% concentrated hydrochloric acid.

Fasting blood samples were drawn from the antecubital vein in the early morning and were separated according to standard procedures. The resulting plasma samples were frozen and sent to Cornell University for analyses of calcium, magnesium, and creatinine.

*Experimental methods*

Urinary titratable acid was determined on fresh urine samples by titrating the urine with 0.1% NaOH to bring the urine pH to 7.4 (23). Creatinine concentrations in the urine and plasma samples were measured by the method of Heinegard and Tiderstrom (24). Ammonia was determined by the method of Kun and Kearney (25), and urinary hydroxyproline was determined by the method of Goverde and Veenkamp (26).

Calcium, sodium, magnesium, and potassium were measured directly in the diluted samples with atomic-absorption spectrophotometry (model 2380; Perkin-Elmer, Norwalk, CT) according to the manual supplied by the manufacturer. Urinary sulfate was measured indirectly with the atomic spectrophotometric method by first precipitating urine samples with barium chloride, and urinary chloride by silver precipitation, as recommended by the manufacturer's manual. Phosphate was measured according to the method of Bartlett (27).

The hydroxyproline index was calculated as the ratio of urinary hydroxyproline (μmol) to creatinine (mmol). Creatinine clearance was estimated according to the procedure described by Zemel et al (28) and was corrected to a body surface area of 1.73 $m^2$ (29). Total renal acid was estimated as the sum of titratable acid and ammonia in the urine (3, 4).

*Statistical analyses*

The data were analyzed by using the *Statistical Analysis System* (SAS Institute Inc, Cary, NC) software package. Descriptive statistics including means and SDs were calculated for each variable of interest. The distribution of each variable was evaluated against the normal distribution, and natural logarithmic transformations were used to normalize highly skewed distributions, including those for nondairy animal protein and consumption of nondairy animal foods (NDAFs) for reliable statistical testing of the regression coefficients.

One-way analysis of variance followed by Duncan's multiple-range test were used to analyze differences in nutrient intake and urinary variables. Pearson product-moment correlation coefficients were used to assess associations between dietary components and urinary calcium excretion.

Multivariate regression analyses were then performed to assess the independent effects of dietary factors on urinary calcium excretion after age, body weight, and dietary calcium were adjusted for (30). This analysis was also conducted by stratifying the sample by county or location of the survey area. Because

there were no significant differences in these results, location was not included as a covariate in the models specified and only the results for subjects in all five counties are included here.

## Results

### *Dietary intakes*

Dietary patterns of women in each county are shown in **Figure 1.** Wheat flour was the staple food for women in counties YA, WA, and CD whereas rice constituted the major dietary component for women in counties SB and LC. Consumption of dairy foods and NDAFs were much higher for women in the two pastoral counties (YA and WA) than in the three nonpastoral counties (CD, SB, and LC). One of the nonpastoral counties (LC) was situated near the sea coast. Women in this county consumed a variety of fish and seafood, which were the major sources of NDAFs consumed. Intakes of vegetables and fruits were much higher in the three nonpastoral counties than in the two pastoral counties.

**Table 2** shows the daily nutrient intakes of women in the five survey counties. Intakes of protein, calcium, phosphorous, and vitamin A were significantly higher for women in county YA than in the other four counties ($P < 0.05$). Calcium intake of women in county WA was not significantly different from that in two of the nonpastoral counties (SB and CD). However, in this pastoral county 37.6% of the dietary calcium was from milk and dairy sources. Virtually no milk or dairy products were consumed in counties SB, CD, and LC. Women in county SB consumed more energy and carbohydrate (primarily derived from rice, wheat, and sweet potatoes) than women in other counties ($P < 0.05$), reflecting their greater levels of physical labor (working in the fields). Consistent with the results in Figure 1, nutrients derived primarily from plant foods, including ascorbic acid, $\beta$-carotene, and carbohydrate, were relatively higher in the three non-pastoral counties than in the pastoral counties (Table 2).

### *Urinary calcium and acids*

Average excretions of calcium, acids, and other minerals in the 12-h overnight urine samples are presented in **Table 3.** Women in counties YA, WA, and LC excreted relatively higher amounts of calcium and acids in their urine than did women in other two counties. Urinary calcium levels reported here are consistent with those reported in The Gambia and South Africa (2.4–5.7 mmol/24 h) (31) and those reported by Wen et al (32) in 655 middle-aged and elderly males and females residing in five areas in China (2.2–11.8 mmol/24 h) but are much lower than those in Western countries (31), even in county YA, which had high dietary calcium intake. The low urinary calcium excretion in Chinese women may be the result of long-term adaptation to low calcium intake, low bioavailability of dietary calcium, and low NDAF intakes as reported in this communication (*see* Discussion).

Correlation analyses (**Table 4**) showed highly significant associations between urinary calcium and acid excretions ($r = 0.42$–$0.58$, $P < 0.0001$). Calcium excretion also was positively correlated with the corrected creatinine clearance ($r = 0.40$, $P < 0.0001$), a urinary variable related to protein-induced hypercalciuria in human laboratory studies (33, 34).

### *Dietary intakes and urinary excretion of calcium and acid*

**Table 5** presents the univariate correlation coefficients between urinary variables and nutrient intakes. Urinary calcium was associated positively with total protein, animal protein, and nondairy animal protein ($r = 0.19$–$0.28$, $P < 0.001$) but was associated negatively with plant protein and indicators of plant food intakes such as $\beta$-carotene, carbohydrate, fiber, and ascorbic acid. Animal-protein intakes also were correlated positively with creatinine clearance and acid excretions, including titratable acid, ammonia, and sulfate.

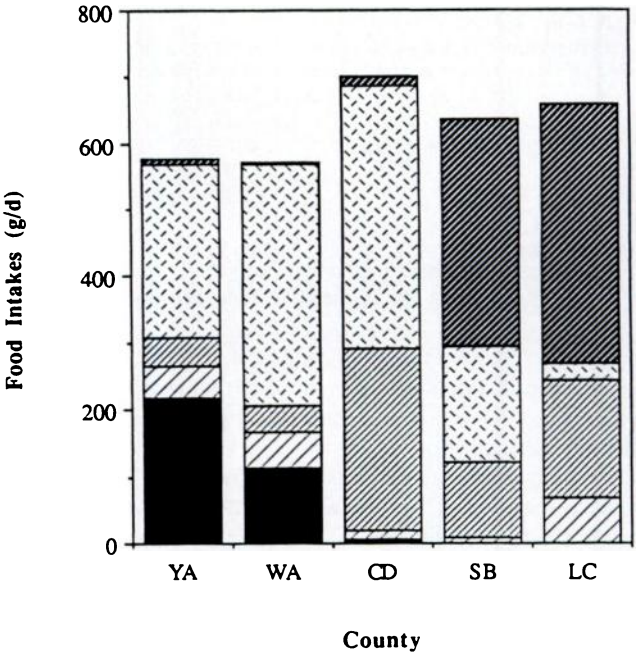


FIG 1. Dietary patterns of middle-aged and elderly women in the five survey counties: YA, Xianghuangqi; WA, Tuoli; CD, Jiexiu; SB, Cangxi; LC, Changle. Intakes of milk and dairy foods were expressed as grams dry weight per day. ■, Rice; ▨, wheat; □, vegetables; ▨, nondairy animal foods; ■, dairy foods.

**Table 6** shows the standardized coefficients of multivariate regression analyses. After age, weight, and dietary calcium were adjusted for, urinary calcium remained positively related to animal protein ($P < 0.001$) and nondairy animal protein ($P < 0.001$) and inversely related to plant-protein intake ($P < 0.001$), indicating that these associations were independent of any effects of dietary calcium and age. However, total dietary protein was not associated with urinary calcium excretion when dietary calcium was included in the model.

To address further the broader effect of diets on urinary calcium excretion, foods consumed by these women were grouped into 11 categories on the basis of food sources, and the foods were examined for their associations with urinary calcium and total renal acid. The standardized regression coefficients after age and dietary calcium were adjusted for are shown in **Table 7.** Generally, urinary acid and calcium excretions were correlated positively with foods that are prone to form acids in the body and were correlated negatively with foods that are alkaline ash.

NDAFs, including meat, eggs, and fish, are known to be major contributors to the formation of metabolic acid (35). Their associations with urinary calcium excretions were examined further in **Figure 2.** To determine any possible confounding effect of dietary calcium, female subjects were grouped into quartiles of calcium intake. Within each calcium quartile, subjects were divided again into three subgroups according to dietary intakes of

TABLE 2
Average daily nutrient intake of women in the five survey counties*

| | Xianghuangqi ($n$ = 142) | Tuoli ($n$ = 147) | Jiexiu ($n$ = 156) | Cangxi ($n$ = 151) | Changle ($n$ = 168) |
|---|---|---|---|---|---|
| Macronutrients | | | | | |
| Protein (g) | 75 ± 27[a] | 51 ± 17[c] | 57 ± 15[b] | 57 ± 18[b] | 49 ± 13[c] |
| Fat (g) | 38 ± 18[a] | 42 ± 27[a] | 24 ± 12[b] | 26 ± 16[b] | 27 ± 15[b] |
| Carbohydrate (g) | 216 ± 59[c] | 228 ± 64[c] | 336 ± 93[b] | 498 ± 164[a] | 328 ± 84[b] |
| Energy (kJ) | 6301 ± 1703[c] | 6238 ± 1724[c] | 7460 ± 1899[b] | 10268 ± 3226[a] | 7314 ± 1766[b] |
| Fiber (g) | 5.7 ± 2.0[c] | 3.3 ± 2.0[d] | 13.4 ± 4.3[b] | 16.2 ± 7.0[a] | 6.1 ± 2.5[c] |
| Vitamins | | | | | |
| β-Carotene (mg) | 0.67 ± 0.40[d] | 0.47 ± 0.58[d] | 2.43 ± 2.01[b] | 3.27 ± 2.21[a] | 1.36 ± 1.94[c] |
| Thiamin (mg) | 0.58 ± 0.20[d] | 0.94 ± 0.27[c] | 1.32 ± 0.38[a] | 1.19 ± 0.37[b] | 0.61 ± 0.18[d] |
| Niacin (mg) | 7.3 ± 2.7[c] | 10.0 ± 4.1[d] | 11.4 ± 3.5[c] | 14.9 ± 4.8[a] | 12.7 ± 5.1[b] |
| Ascorbic acid (mg) | 20 ± 27[c] | 29 ± 38[c] | 144 ± 93[a] | 136 ± 86[a] | 64 ± 49[b] |
| A (μg)† | 348 ± 581[a] | 197 ± 939[b] | 17 ± 46[c] | 10 ± 23[c] | 38 ± 41[c] |
| Minerals | | | | | |
| Calcium | | | | | |
| (mg) | 724 ± 333[a] | 369 ± 153[b] | 359 ± 159[b] | 328 ± 133[b] | 230 ± 104[c] |
| (mmol) | 36.1 ± 16.6 | 18.4 ± 7.6 | 17.9 ± 7.9 | 16.4 ± 6.6 | 11.5 ± 5.2 |
| Phosphorus | | | | | |
| (mg) | 1130 ± 360[a] | 909 ± 235[c] | 996 ± 261[b] | 1003 ± 306[b] | 689 ± 168[d] |
| (mmol) | 65.7 ± 20.9 | 52.8 ± 13.7 | 57.9 ± 15.2 | 58.3 ± 17.8 | 40.0 ± 9.8 |
| Potassium | | | | | |
| (mg) | 1368 ± 389[b] | 1102 ± 409[c] | 1556 ± 480[a] | 1625 ± 581[a] | 1122 ± 370[c] |
| (mmol) | 35.0 ± 9.9 | 28.2 ± 10.5 | 39.8 ± 12.3 | 41.6 ± 14.9 | 28.7 ± 9.5 |
| Magnesium | | | | | |
| (mg) | 304 ± 80[b] | 190 ± 59[d] | 306 ± 86[b] | 333 ± 103[a] | 232 ± 62[c] |
| (mmol) | 25.0 ± 6.6 | 15.6 ± 4.9 | 25.2 ± 7.1 | 27.4 ± 8.5 | 19.1 ± 5.1 |

* $\bar{x}$ ± SD. Means not sharing the same letter superscript are significantly different, $P < 0.05$ (Duncan's multiple-range test).
† Preformed retinol in food.

NDAFs: none (0 g/d), low (1–50 g/d), and high (> 50 g/d). Women in the high NDAF groups consistently showed a higher excretion of urinary calcium than women in the lower NDAF groups, indicating that NDAFs may augment calcium excretion independent of dietary calcium intake.

## Discussion

This cross-sectional survey of pre- and postmenopausal women was conducted in five rural counties of China, where dietary patterns varied dramatically in terms of type and quantity of foods consumed (Fig 1). This paper attempts to address two main research questions: are differences in dietary composition related to urinary calcium output and what are the major dietary determinants of urinary calcium excretion?

There is consistent evidence showing that increased urinary calcium excretion with elevated intakes of certain proteins (36, 37) or by acidification of the diet with ammonium chloride (4, 5) is related to the decreased renal reabsorption of calcium to buffer the metabolic acid produced under these conditions. In the former case, renal acid excretion appears to be derived primarily from the excessive oxidation of the sulfur-containing amino acids methionine and cysteine that are generally rich in these proteins (34, 38), although conversion of neutral food substances to organic acids (38) and incomplete oxidation of organic acids (34) also contribute to this endogenous acid formation. Two equivalents of hydrogen ions are formed when one mole of sulfur-containing amino acids is oxidized to sulfate (38). Indeed, sulfur-containing amino acids, when added to a low-protein diet consumed by eight young males, was found to account for a significant part of, but not the entire, decrease in the renal reabsorption of calcium produced by the high-protein diet (28). In contrast, supplementing a basic diet with an equivalent amount of sulfur from taurine, which is not metabolized to sulfate, failed to induce hypercalciuria (39). A positive association between urinary calcium, dietary sulfur, and urinary excretion of total renal acid and sulfate was observed in subjects consuming a high-protein diet (3, 36). Thus, the excess endogenous acid created by a high-protein diet, rather than the protein itself, may account for the large proportion of increased calcium excretion. Consistent with this hypothesis is the finding that the hypercalciuretic effect is greatly attenuated by adding basic sodium bicarbonate to a high-protein diet (40). Furthermore, in metabolic acidosis either induced by the administration of ammonium chloride (4) or observed during chronic azotemic renal disease (41), the increase in urinary acid excretion also was reported to be accompanied with urinary calcium loss. Apparently, increased renal excretion of acid directly augments urinary calcium loss.

In agreement with this acid-load hypothesis, the results obtained from this cross-sectional survey (Table 4) also showed significantly positive associations between urinary calcium and urinary acids, including titratable acid ($r$ = 0.46, $P < 0.0001$, $R^2$ = 0.21), ammonia ($r$ = 0.42, $P < 0.0001$, $R^2$ = 0.18), and sulfate ($r$ = 0.52, $P < 0.0001$, $R^2$ = 0.27). These data suggest that increased urinary calcium excretion for free-living women may be determined at least partially by the production of en-

TABLE 3
Average excretion of calcium, other minerals, and acids measured in 12-h overnight urine samples*

| | Xianghuangqi ($n$ = 141) | Tuoli ($n$ = 135) | Jiexiu ($n$ = 156) | Cangxi ($n$ = 146) | Changle ($n$ = 158) |
|---|---|---|---|---|---|
| Acids | | | | | |
| pH | 5.9 ± 0.5[b] | 5.9 ± 0.4[b] | 6.2 ± 0.5[a] | 6.3 ± 0.5[a] | 6.2 ± 0.5[a] |
| Titratable acid (mEq) | 11.0 ± 5.3[a] | 9.9 ± 4.6[b] | 6.5 ± 3.0[c] | 4.0 ± 2.5[d] | 7.3 ± 3.3[c] |
| Ammonia (mmol) | 16 ± 10[a] | 14 ± 8[b] | 10 ± 6[cd] | 9 ± 5[d] | 11 ± 7[c] |
| Phosphate (mmol) | 52.2 ± 21.4[a] | 44.2 ± 19.6[b] | 31.4 ± 12.5[c] | 20.3 ± 9.7[d] | 33.4 ± 13.5[c] |
| Sulfate (mmol) | 17.2 ± 7.3[a] | 11.5 ± 5.8[b] | 11.3 ± 4.5[b] | 5.1 ± 2.4[d] | 8.8 ± 4.4[c] |
| Minerals (mmol) | | | | | |
| Calcium | 2.0 ± 2.4[a] | 1.3 ± 0.8[c] | 1.1 ± 0.8[c] | 0.8 ± 0.6[d] | 1.5 ± 0.9[b] |
| Potassium | 11 ± 6[a] | 11 ± 7[a] | 11 ± 7[a] | 9 ± 5[b] | 9 ± 5[b] |
| Magnesium | 2.1 ± 1.0[a] | 2.2 ± 1.1[a] | 1.6 ± 0.9[b] | 1.2 ± 0.7[c] | 1.4 ± 0.7[c] |
| Sodium | 82 ± 37[b] | 93 ± 50[a] | 58 ± 29[c] | 60 ± 38[c] | 52 ± 27[d] |
| Chlorine | 71 ± 39[b] | 101 ± 55[a] | 61 ± 37[c] | 64 ± 37[bc] | 59 ± 32[c] |
| Other indexes | | | | | |
| Creatinine clearance (mL/s) | 1.05 ± 0.36[a] | 1.04 ± 0.60[a] | 0.68 ± 0.33[b] | 0.72 ± 0.36[b] | 1.00 ± 0.70[a] |
| HPRO index† | 13.6 ± 9.2[a] | 13.6 ± 7.9[b] | 10.7 ± 7.5[c] | 8.5 ± 4.9[c] | 12.2 ± 6.6[b] |

* $\bar{x}$ ± SD. Means not sharing the same letter superscript are significantly different for the five counties, $P < 0.05$ (Duncan's multiple-range test).
† Hydroxyproline (μmol)/creatinine (mmol).

dogenous acids, as observed in human metabolic studies (42) and animal experiments (43).

In addition to the decreased renal reabsorption of calcium, an increased glomerular filtration rate (creatinine clearance) was reported to account for ≈10–15% of the elevation in urinary calcium loss induced by a high-protein diet (33). Similarly, results from this study also showed that creatinine clearance was positively correlated with urinary calcium ($r$ = 0.42, $P < 0.0001$, Table 4), suggesting that creatinine clearance may be another important determinant of urinary calcium excretion for these free-living women.

Both in human metabolic studies (8–10) and in animal experiments (44, 45), increasing dietary protein intake at consistent intakes of calcium and phosphorus has been reported to enhance urinary excretion of calcium. Calcium loss under these controlled conditions induced by high-protein diets ranged from 60 to 150 mg/d, depending on the amounts and types of protein ingested. Kerstetter and Allen (46) compiled data from 16 human studies and found that the association between dietary protein intake and urinary calcium loss was linear even though these studies were conducted separately in different laboratories. Similar findings also were observed in the present population studies. Dietary protein intakes were positively associated with urinary calcium and urinary acids, including titratable acid, ammonia, and sulfate (Tables 5 and 6).

TABLE 4
Matrix of univariate correlation coefficients between urinary excretion variables measured in 12-h overnight urine samples*

| | Calcium | Titratable acid | Ammonia | Sulfate | Creatinine clearance |
|---|---|---|---|---|---|
| Titratable acid | 0.46 | | | | |
| Ammonia | 0.42 | 0.63 | | | |
| Sulfate | 0.52 | 0.64 | 0.53 | | |
| Creatinine clearance | 0.42 | 0.50 | 0.58 | 0.46 | |
| Phosphate | 0.58 | 0.75 | 0.58 | 0.77 | 0.57 |

* $n$ = 736. $P < 0.0001$.

The calciuretic effect of dietary protein appears to depend on protein source. Animal protein and nondairy animal protein were related positively and significantly to urinary calcium excretion ($r$ = 0.28 and 0.19, $P < 0.001$, respectively) whereas plant protein was inversely related ($r = -0.13$, $P < 0.001$, Table 5), even after age and dietary calcium intake were adjusted for (Table 6). Plant protein per se is not likely to cause such large differences in calciuretic effects compared with other sources of protein, because some plant proteins such as wheat gluten (8, 36) and soy protein (7) also have been found to cause negative calcium balance in human subjects. Other simultaneously ingested components of the diet may therefore contribute to so-called dietary protein–induced calciuria, depending on their basic or acidic properties. Most plant foods are known to be alkaline ash (35) and to reduce acidity of the urine (47, 48), thereby attenuating urinary calcium excretion. The negative association observed between plant protein and urinary calcium excretion in the present study thus may reflect the base-forming capacity of plant foods.

A comparison of the dietary components and urine acidity for women residing in counties SB and LC further elucidates this hypothesis. The dietary components of these two counties were quite similar, particularly for staple foods (Fig 1). However, the diets of women in county SB were rich in sweet potatoes and pickled vegetables, with very low intakes of animal foods, whereas meat and a large variety of seafood constituted a significant proportion of the diets of women in county LC. Correspondingly, it was found that women in county LC had more acidic urine and excreted more calcium than women in county SB (Table 3). Thus, greater consumption of animal foods (including fish) by women in county LC may at least partially account for the higher urinary excretion of acids and calcium, although higher fiber intake in county SB should also be taken into account.

TABLE 5
Univariate correlation coefficients between urinary variables and dietary intakes ($n$ = 725)

| Dietary intakes | Urinary variables | | | | | |
|---|---|---|---|---|---|---|
| | Calcium | Titratable acid | Ammonia | Sulfate | Creatinine clearance | HPRO index* |
| Protein (g/d) | | | | | | |
| Total | 0.25† | 0.23† | 0.24† | 0.22† | 0.08‡ | 0.06 |
| Animal | 0.28† | 0.33† | 0.27† | 0.30† | 0.16† | 0.15† |
| Nondairy animal | 0.19† | 0.18† | 0.17† | 0.09‡ | 0.12† | 0.09‡ |
| Plant | −0.13† | −0.24† | −0.15† | −0.21† | −0.15† | −0.17† |
| Carbohydrate (g/d) | −0.16† | −0.32† | −0.17† | −0.36† | −0.11† | −0.19† |
| Fiber (g/d) | −0.19† | −0.33† | −0.19† | −0.26† | −0.19† | −0.13† |
| β-Carotene (mg/d) | −0.19† | −0.25† | −0.14† | −0.20† | −0.17† | −0.09 |
| Thiamin (mg/d) | −0.18† | −0.18† | −0.11‡ | −0.18† | −0.17† | −0.11‡ |
| Ascorbic acid (mg/d) | −0.19† | −0.29† | −0.20† | −0.25† | −0.18† | −0.31† |
| Vitamin A (mg/d)§ | 0.12† | 0.14† | 0.09‡ | 0.13† | 0.04 | 0.10‡ |
| Calcium (mg/d) | 0.30† | 0.33† | 0.29† | 0.35† | 0.11† | 0.12† |
| Phosphorus (mg/d) | 0.16† | 0.16† | 0.18† | 0.16† | 0.02 | 0.00 |
| Sodium (mg/d) | 0.05 | 0.04 | 0.06 | 0.03 | 0.01 | −0.04 |

* Hydroxyproline (μmol)/creatinine (mmol).
† $P < 0.01$.
‡ $P < 0.05$.
§ Preformed retinol in foods.

Most animal foods, including different kinds of meat, fish, and eggs, but not milk, have been shown to have predominantly acid-forming elements (35, 48) and are prone to produce acids in the body. Compared with plant foods, consumption of these animal foods may accelerate urinary calcium loss, not only because of the relatively high protein content of these foods but also because of their potential to induce the formation of urinary acid by other means. The results presented in Tables 5 and 6 indicate that urinary calcium was correlated negatively with nutrients derived from a diet rich in plant foods and correlated positively with nutrients derived primarily from animal foods. Indeed, direct correlation analyses between food intakes and urinary variables showed that urinary acids and calcium were correlated negatively with intakes of vegetables and fruits, whereas they were correlated positively with consumption of NDAFs (Table 7 and Fig 2). Rice, wheat, and corn have been reported to be acidic on the basis of the balance calculation of acid- and base-forming elements (35, 48). In this study, however, only rice was associated positively with urinary calcium (Table 7). Millet consumption also was found to be positively correlated with urinary calcium, probably because of the relatively high content of sulfur-containing amino acids in millet compared with vegetables and fruits (22).

Consistent with our results, Licata (49), in a controlled study, reported that urinary calcium excretion increased by 80% in

TABLE 6
Standardized regression coefficients: urinary calcium excretion and dietary protein intakes*

| Dietary protein intakes | Urinary calcium | Urinary calcium adjusted for | | | |
|---|---|---|---|---|---|
| | | Age | Dietary calcium | Weight | Age + dietary calcium |
| Total | 0.25† | 0.23† | 0.07 | 0.23† | 0.06 |
| Animal | 0.28† | 0.27† | 0.15‡ | 0.27† | 0.16‡ |
| Nondairy animal | 0.19† | 0.18† | 0.16† | 0.18† | 0.16† |
| Plant | −0.13† | −0.15† | −0.15† | −0.14† | −0.16† |

* $n$ = 725.
† $P < 0.001$.
‡ $P < 0.01$.

TABLE 7
Standardized regression coefficients: urinary calcium, total renal acid, and food categories after age and dietary calcium were adjusted for*

| Food category | $n$ | Total renal acid† (mEq) | Urinary calcium (mmol) |
|---|---|---|---|
| Cereals | | | |
| Rice | 362 | 0.01 | 0.17‡ |
| Wheat | 650 | 0.04 | −0.07 |
| Corn | 105 | −0.13 | −0.08 |
| Millet | 213 | 0.12 | 0.18§ |
| Vegetables | | | |
| Greens | 608 | −0.23‡ | −0.13§ |
| Pickles | 229 | −0.17‖ | −0.20§ |
| Roots | 415 | −0.22‡ | −0.19‡ |
| Soy | 113 | −0.16 | 0.16 |
| Dairy foods | 246 | −0.07 | 0.01 |
| Nondairy animal foods | 412 | 0.14§ | 0.17§ |
| Spices | 726 | −0.06 | 0.03 |

* $n$ = 725.
† Titratable acid plus ammonia.
‡ $P < 0.001$.
§ $P < 0.01$.
‖ $P < 0.05$.

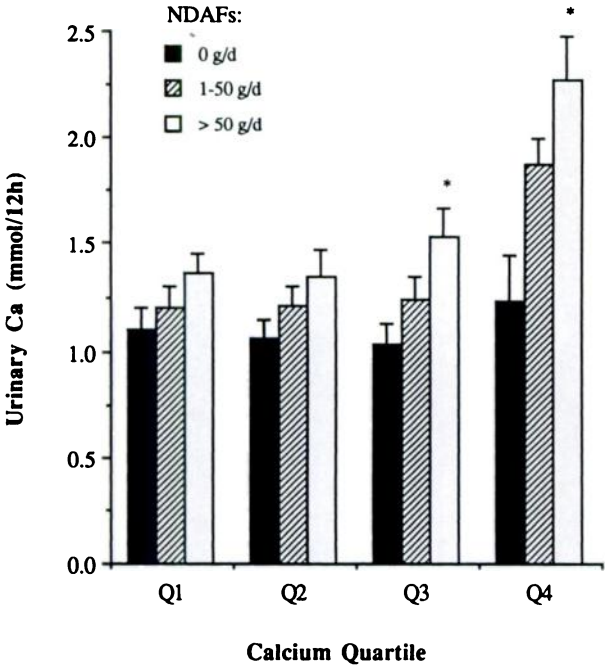


FIG 2. Urinary calcium excretion in middle-aged and elderly Chinese women grouped by calcium intake quartile and nondairy animal food (NDAF) intakes. Calcium quartile range ($\bar{x}$ ± SD): Q1, 104–221 mg/d (170 ± 31); Q2, 222–338 mg/d (281 ± 32); Q3, 339–520 mg/d (414 ± 52); and Q4, 521–1705 mg/d (781 ± 267). *Significantly different from NDAF = 0 g/d group in the same calcium quartile, $P < 0.05$.

subjects when the diet was supplemented with lean beef and chicken to raise protein intake from 0.5–0.8 to 1.5–2.0 g/kg body wt. Licata also found that urinary calcium excretion was correlated with urinary sulfate ($r$ = 0.60) and ammonia ($r$ = 0.72). Schuette and Linkswiler (50) observed a negative calcium balance in eight young men when they ingested a high-meat diet provided as ground beef. Similar results also were observed by other investigators (51, 53) although calcium balance was not considerably affected in one of the reports (52). By raising daily protein intake from 67 to 107 g with egg white in seven young men, Zhao and Chen (39) recently observed a significant increase in urinary sulfate and a decrease in calcium retention. However, Spencer et al (54) noticed only a negligible calcium loss in the urine at the very beginning of a study in hospitalized patients when meat intakes were changed from normal to high amounts.

The reasons for this discrepancy in calciuretic effects are not clear. The lower calciuretic effect in the study by Spencer et al (54) was attributed to the presence of higher phosphorous in meat (54, 55) and the smaller magnitude of changes in protein intake between control and treatment groups (55). Different acid-base status provided by diets among these various studies also might be an alternative possibility. Variations in the proportion of vegetables and fruits in these diets, for example, may change the acid-base status of the urine and hence result in differences in calcium excretion. Although dairy foods are of animal origin, they are actually alkaline ash (35). A positive calcium balance was also reported in subjects whose diets were high in meat or simulated meat and were supplemented with dairy products (50). But no significant associations were observed for dairy foods in the present study after age and dietary calcium intake were adjusted for (Table 7). Therefore, under free-living conditions, urinary calcium output is a complicated process that is not only related to dietary protein intake but also is dependent on the acid-base balance of whole meals ingested by subjects. And under some circumstances, the acid-base balance of the diet may be more important than protein intake in determining the excretion of urinary calcium. From this point of view, a comprehensive study involving measurements of acid-base status of the total meal and the urine may be needed to clarify this issue of the calciuretic effect of meat consumption.

An important question that remains unresolved is whether the positive association observed between urinary calcium and food intakes is accounted for by dietary calcium. Increasing calcium intakes clearly have been shown to result in greater urinary calcium (56, 57). In the present study urinary calcium was also associated with calcium intake (Table 5, $r$ = 0.30, $P < 0.001$). However, as seen in Figure 2, when survey subjects were stratified into quartiles by dietary calcium, increased urinary calcium was observed at all intakes of NDAF. Actually, there were no marked associations between dietary calcium and urinary calcium excretion below the third dietary calcium quartile (< 520 mg/d). This may reflect the fact that at calcium intakes below the minimum calcium requirement body calcium is better conserved whereas excess calcium intake is more readily spilled into the urine. However, even with these low calcium intakes, increased urinary calcium excretion was still observed for women in the high NDAF group. These results indicate that the increased calciuretic effect resulting from high consumption of NDAF was independent of dietary calcium intakes. Indeed, when calcium intake was adjusted for in the multivariate regression analyses, consumption of NDAFs and nondairy animal protein still correlated positively with calcium excretion (Table 6).

Unlike the results reported in experimental animals (45), hypercalciuria induced by a protein-rich diet in human subjects is not compensated for by increased calcium absorption in the intestine. As a result, subjects often have a negative calcium balance, which should result in increased bone resorption. In this cross-sectional study, it is not possible to estimate calcium absorption from the intestine and thereby to examine the dietary effects on calcium balance in women. Nonetheless, comparisons at the county level suggest that a greater consumption of acid-forming foods may raise the calcium requirement for women. This is especially true for women in county LC. Calcium intake in this county was only 230 mg/d, less than one-third of the current Chinese recommended dietary allowance of 800 mg/d (58). Urinary calcium excretion in contrast, was quite high in this county, nearly twice that of nonpastoral county SB. Dietary conditions in this county may be deleterious to the bone health of the local women, given the relatively low calcium intake, greater consumption of acid-forming foods, and high excretion of urinary calcium.

In summary, results of this cross-sectional survey of 764 middle-aged and elderly women suggest that the urine content of acids and calcium is influenced considerably by the dietary intakes of the women in each county. Under free-living conditions, consumption of acid-forming foods may augment calcium excretion in the urine, probably because of acid loading in the urine indicated by positive associations between urinary calcium, acid excretion, consumption of acid-ash foods, and dietary intakes of animal protein and nondairy animal protein. Statistically

significant associations were still observed even when age and dietary calcium were adjusted for in the multivariate analyses.

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