Meta-analysis of the quantity of calcium excretion associated with the net acid excretion of the modern diet under the acid-ash diet hypothesis\textsuperscript{1–4}

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ABSTRACT

Background: The acid-ash diet hypothesis of osteoporosis suggests that acid from the modern diet causes a demineralization of the skeleton, and mobilized bone calcium is excreted. A systematic approach has not been used to summarize the findings of the numerous studies about the hypothesis.

Objectives: The purpose of this meta-analysis was to estimate the quantity of net acid excretion and calciuria associated with the modern diet, to assess the association between acid excretion and calcium excretion, and to assess the influence of urine preservatives on calcium measurement.

Design: We systematically searched for trials of the acid-ash hypothesis and conducted a meta-analysis.

Results: Twenty-five of 105 studies met the inclusion criteria. The estimated quantity of net acid excretion from the weighted average of the control diets from 11 studies was 47 mEq/d. The increase in urinary calcium with a change in renal net acid excretion depended on whether the urine was acidic or alkaline ($P < 0.001$). A significant linear relation was observed between net acid excretion and calcium excretion for both acidic and alkaline urine ($P < 0.001$). The estimated change in urine calcium associated with a change of 47 mEq of net acid excretion in acidic urine was 1.6 mmol/d (66 mg/d) of calcium.

Conclusion: Evidence suggests a linear association between changes in calcium excretion in response to experimental changes in net acid excretion. However, this finding is not evidence that the source of the excreted calcium is bone or that this calciuria contributes to the development of osteoporosis. Am J Clin Nutr 2008; 88:1159–66.

INTRODUCTION

Cross-sectional studies suggest that osteoporosis develops from a gradual loss of bone mineral that is thought to begin as early as 25–30 y of age (1). A person with osteoporosis can readily experience a bone fracture, without trauma, and these fractures are associated with pain, disability, diminished quality of life, increased need for institutionalization, and increased rate of mortality (2–4). Ideally, as the pathogenesis of osteoporosis is understood, effective strategies to prevent the disease will be developed.

The acid-ash diet hypothesis of osteoporosis suggests that modern diets promote this disease through the metabolic production of acid that causes demineralization of the skeleton (5–8). Respected researchers, authors of medical textbooks and numerous review articles, as well as writers for lay audiences and complementary medicine have regarded the acid-ash hypothesis as the primary risk factor for bone health, and some advocate alternate diets and dietary supplements predicted under this hypothesis to reduce the risk of osteoporosis (9–15). According to this hypothesis, osteoporosis develops as the skeletal pool of calcium is gradually diminished over time as skeletal calcium is lost in the urine.

The chemical composition of urine is altered by diet; consequently, urine was used by many researchers to infer the extent of dietary acid anions consumed. In those studies net acid excretion (NAE) in urine is the calculated variable used to infer excess of dietary acid anions less dietary base cations (9, 16). Numerous studies have reported an association between NAE, a measure of acid excreted in urine, and the quantity of urinary calcium excreted. NAE was defined as NAE = titratable acid + $\mathrm{NH}_4^+ - \mathrm{HCO}_3^-$ and was manipulated by changing the diet or providing acidic or basic salts to subjects.

Calcium forms insoluble salts in urine with pH $> 6.5$ (17). The insoluble calcium is not measured when urine calcium is analyzed by laboratories (17, 18), which may create a measurement error for the association between the NAE and calcium excretion. It is possible that some or the entire amount of calcium seen with more acidic urine is due to better measurement of calcium in the acidic urine, and the presumed cause and effect relation could be due to confounding by measurement error.

To date, a systematic approach has not been used to summarize the findings from the numerous studies about the acid-ash diet hypothesis. The purpose of this study is to 1) estimate the quantity of net acid, 2) estimate calcium excretion in the urine associated with the modern diet, and 3) assess whether there is a linear

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association between NAE and calcium excretion among free-living adults. In addition, we assess whether the quantitative difference in calcium observed between acidic and alkaline urine might be due to lower solubility of calcium in alkaline urine.

**SUBJECTS AND METHODS**

**Literature search for the meta-analysis**

Literature relating to the acid-ash diet hypothesis was identified through computerized searches using, but not limited to, the following keywords or textwords: acid-base equilibrium, bone or bones, bone density, calcification, calcium, excretion, net acid excretion, acid excretion, biopsy, fracture(s), and bone mineral density. The databases searched included Medline back to 1966, Cochrane Database of Systematic Reviews, CINAHL back to 1982, EMBASE back to 1980, and the Cochrane Controlled Trials Register up to July 2007. Reference lists were reviewed for additional relevant studies.

**Selection criteria for the literature**

Studies that examined the acid-ash diet hypothesis were included if they manipulated subjects’ acid-base intake through foods or supplemental salts such as potassium bicarbonate and reported the change of NAE and the outcome of calcium excretion. The databases searched included Medline back to 1966, Cochrane Database of Systematic Reviews, CINAHL back to 1982, EMBASE back to 1980, and the Cochrane Controlled Trials Register up to July 2007. Reference lists were reviewed for additional relevant studies.

**Table 1**

Studies included in the meta-analysis about change in net acid excretion and changes in calcium excretion or bone mineral density

| Study                  | Year | Intervention     | Subjects | Design | Blinded study | Accounted for losses | Calcium treatment | Calcium intake |
|------------------------|------|------------------|----------|--------|----------------|----------------------|-------------------|---------------|
| Weber et al (19)       | 1976 | NH₄Cl            | 6        | CO     | No             | No                   | 0                 | 1000          |
| Schuette et al (20)    | 1980 | Amount of protein| 11       | CO     | No             | No                   | 0                 | 800           |
| Hegsted et al (21)     | 1981 | Amount of protein| 6        | CO     | No             | No                   | 0                 | 500           |
| Lutz and Linkswiler (22) | 1981 | Amount of protein| 8        | CO     | No             | No                   | 0                 | 700           |
| Schuette et al (23)    | 1981 | Amount of protein| 8        | CO     | No             | No                   | 0                 | 500           |
| Lutz (24)              | 1984 | Amount of protein and NaHCO₃ | 6        | CO     | No             | No                   | 8                 | 500           |
| Lemann et al (25)      | 1986 | NH₄Cl            | 5        | CO     | No             | No                   | 0                 | 1300          |
| Breslau et al (26)     | 1988 | Type of protein  | 15/10    | LSD    | No             | No                   | 9                 | 400           |
| Lewis et al (27)       | 1989 | Calcium sources  | 8        | LSD    | No             | No                   | 0                 | 1600          |
| Trilok and Draper (28) | 1989 | Amount of protein| 8        | CO     | No             | No                   | 1                 | 800           |
| Remer and Manz (16)    | 1994 | Amount of protein and methionine | 6 | CO | No             | No                   | 3                 | ?             |
| Sebastian et al (9)    | 1994 | KHCO₃            | 18       | CO     | No             | No                   | 12                | 650           |
| Dahl et al (29)        | 1995 | Lentils          | 10       | RCO    | No             | No                   | ≥14               | Usual         |
| Frassetto et al (30)   | 2000 | KHCO₃            | 19       | CO     | No             | No                   | —                 | Usual         |
| Sellmeyer et al (31)   | 2002 | Potassium citrate | 60       | RCT    | Yes            | No                   | 18                | 500           |
| Maurer et al (32)      | 2003 | HCO₃⁻           | 9        | CO     | No             | No                   | 5                 | 1000          |
| Roughhead et al (33)   | 2003 | Amount of protein| 15       | RCO    | No             | Yes                  | 20                | 600           |
| Ince et al (34)        | 2004 | Amount of protein| 42       | RCO    | No             | Yes                  | 5                 | Same          |
| Marangella et al (35)  | 2004 | Potassium citrate | 52       | Trial  | No             | No                   | ?                 | ?             |
| Getman et al (36)      | 2005 | Cranberry juice  | 12       | RCO    | No             | No                   | 5                 | 400           |
| Kerstetter et al (37)  | 2005 | Amount of protein| 13       | RCO    | No             | No                   | 10                | 800           |
| Roughhead et al (38)   | 2005 | Meat or soy      | 13       | RCO    | No             | Yes                  | 21                | 700           |
| Spence et al (39)      | 2005 | Soy compared with milk protein | 15       | RCO    | Yes            | No                   | 14                | 1100          |
| Jajoo et al (40)       | 2006 | Grains or fruit and vegetables | 20       | RCT    | No             | No                   | 13                | ≥600          |
| Kerstetter et al (41)  | 2006 | Amount of protein| 20       | RCO    | No             | No                   | 14                | 800           |

1 None of the studies concealed the allocation to groups. CO, crossover study; RCO, randomized crossover study; LSD, Latin square design; RCT, randomized controlled trial; trial, nonrandomized trial.

2 Subjects received calcium before outcome measurement.
Acid-ash interventions included alteration of food or nutrient intakes or administration of acidic or alkaline salts, such as potassium bicarbonate or ammonium chloride. The manipulations to alter diet acid load included changes in food intake (16, 20–24, 26–29, 33, 34, 36–41); sulfur-containing amino acids (16, 23); supplements of potassium bicarbonate (9, 30), ammonium chloride (19, 25), or potassium citrate (31, 35); substitution of sodium or potassium chloride with the bicarbonate salts (22, 32); or a combination of food and salts (24, 27). None of the non-English language studies met the criteria for acceptance (43, 44).

Many studies, including some that were well quoted, were not included in the meta-analysis for the following reasons: no presentation of numerical results (44–47), no quantification of NAE (48–56), no measurement of urinary calcium (57, 58), or more than one intervention performed at the same time (59). Numerous studies were observational and were not included because there was no manipulation of intakes (15, 58, 60–83). Other studies did not qualify for the meta-analysis because the urine collection was for periods shorter than 24 h (43, 84–91), all of the subjects had a chronic condition (92–99), subjects were in a state of weight loss (100, 101), the studies only included children (15, 61, 63, 65–67, 69, 81, 87, 102–104), the studies only included animals (105–107), or they were in vitro animal bone studies (108–121). The search also located numerous narrative review articles on the acid-ash hypothesis (5, 7, 8, 13, 122–129).

Methodologic quality of the studies of the acid-ash diet hypothesis

The studies were assessed for the following 8 indicators of methodologic quality (130, 131): randomization to groups or order of treatments, concealment of randomization, blinding of intervention, complete follow-up, blinding of outcome measurement, intent-to-treat analysis, control of calcium intakes, and duration of control of the subject’s calcium intakes before urine measurements. Subjects were allocated to treatment groups or to the order of treatment by randomization in 12 of the 25 studies included in this review (Table 1). None of the studies described any concealment of allocation to groups. Only 3 studies mentioned any attempt to mask or blind subjects to their group allocation (31, 39, 132). None of the studies reported using an intention-to-treat analysis. In addition, only 8 of the studies reported whether all of the subjects completed the interventions (29, 31, 33–35, 38, 39, 41). Sixteen of the studies controlled the subject’s calcium intakes (Table 1), 11 of these for the recommended 7 d before measurement of the outcomes (133). In summary, the methodologic quality of the studies was limited; therefore, it is possible that the findings from these studies may provide biased estimates of the effect of the acid load on calcium excretion (131).

Methods of the meta-analysis

Some studies reported more than one intervention, and each comparison to the control was included in the meta-analysis; in all, 34 comparisons and 509 observations were included (Table 2). For those studies that measured the outcomes for one intervention at multiple points in time (33, 38), the outcomes were averaged together to provide one set of acid excretion and calcium values for each intervention. We made an estimate of the NAE of the modern diet by taking a weighted average of the control diets of the studies.

A regression analysis, weighted by study sample size, was used to assess whether there was evidence of a relation across the studies and to estimate the change of calcium excretion for every unit change of NAE (134) with the use of STATA 10 (Stata Corp, College Station, TX). Whether the urine was treated with acid to improve calcium solubility was considered a potential effect modifier for this regression analysis. Studies were categorized as acidic if 1) the urine was treated with acid before analysis or 2) if the mean urine pH was < 6.5 (17) in both treatment arms. Researchers were contacted to clarify whether the urine samples were treated with acid before analysis if this detail was not clear in the report (16, 22, 34). To estimate the relation between NAE and calcium excretion among free-living adults and to avoid overinfluence to the regression by extreme cases, the changes of NAE were restricted to those changes that could be achieved through diet of free-living adults who are not taking an acid supplement (NH4Cl); therefore, the 2 extreme cases of NAE > 200 were not included (19, 25). Repeating the regression without the restriction did not change the findings.

RESULTS

The estimated average quantity of NAE from the average of the control diets was 47 mEq/d (range = 31 (34) to 71 (9) mEq/d), based on the weighted average of 24-h urine measures (n = 208) of the control arm (which may represent the modern diet) from 11 studies that reported this information (9, 16, 19, 26, 29, 30, 33–36, 39).

Although 5 of the 25 studies did not show greater calcium excretion with higher NAE (27, 29, 33, 35, 38), a significant relation was observed between NAE and calcium excretion for both acidic and alkaline urine for the studies once combined in the meta-analysis. The interventions in the studies that did not show the relation of interest included changes of food intake in well-controlled metabolic studies (33, 38), calcium carbonate compared with milk (27), a potassium citrate supplement (35), and a substitution of soy protein with lentils (29). In one study in which soy protein was substituted with lentils, urine calcium excretion significantly decreased (P < 0.01) despite a nonsignificant increase in NAE (29).

Whether the urine was acidic (pH < 6.5 or acid treated) significantly modified the relation between NAE and calcium excretion. A significant interaction was observed because the difference in the rates of increase in urinary calcium with the change in renal NAE depended on whether the urine was acidic (P < 0.001; Figure 1).

The change of calcium excretion with each milliequivalent change of NAE was 0.035 mmol/d of calcium (95% CI: 0.032, 0.038; P < 0.001) and was 0.023 (95% CI: 0.022, 0.025; P < 0.001) for alkaline urine. For a change of 47 mEq of NAE in acidic urine (in which calcium is more soluble and more readily absorbed salts (22, 32); or a combination of food and salts (24, 27). None of the non-English language studies met the criteria for acceptance (43, 44).

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DISCUSSION

The findings of this meta-analysis show that there is evidence of a linear association between average results for calcium excretion in response to the changes of NAE. The estimated NAE of the modern diet, based on a meta-analysis of the control arms
of the studies designed to represent the modern diet, was 47 mEq/d. Given the rate of change of urinary calcium in response to the change of NAE, if the 47 mEq/d of acid was neutralized, by diet or supplements, the predicted would be equal to 1.6 mmol/d change in urinary calcium. These findings alone are not evidence that the source of the extra calcium is from the bones or that this calciuria contributes to the development of osteoporosis.

This relation between calcium excretion and NAE was shown in the meta-analysis of 25 studies despite 5 studies that did not show this relation. The relations remained significant after removal of the 3 outlying study results, which indicated that the finding of a linear association was not due solely to the outlying cases.

The findings of relations between NAE and calcium excretion in both acidic and alkaline urine suggest that calcium insolubility does not explain all of the higher concentration of calcium in acidic urine. The significant difference seen between the acid-treated and non–acid-treated urine (P < 0.001) shows that some

| Study                                      | Intervention                          | Control of calcium intake | Change in NAE<sup>1</sup> | Change in urinary calcium | Acid treated<sup>2</sup> | Maximum urinary pH | Acidic urine<sup>3</sup> |
|--------------------------------------------|---------------------------------------|---------------------------|---------------------------|---------------------------|-------------------------|---------------------|------------------------|
| Weber et al (19)                           | Whole food diet ± NH₄Cl                | 6                         | Yes                       | 216                       | 9.1                     | No                  | 5.97                   |
| Schuette et al (20)                        | Amount protein                        | 11                        | Yes                       | 37                        | 2.15                    | Yes                 | —                      |
| Hegsted et al (21)                         | Amount protein                        | 6                         | Yes                       | 38.1                      | 2.48                    | Yes                 | —                      |
| Lutz and Linkswiler (22)                   | Amount protein                        | 8                         | Yes                       | 56.0                      | 2.05                    | Yes                 | —                      |
| Schuette et al (23)                        | Amount protein                        | 8                         | Yes                       | 32                        | 1.20                    | Yes                 | —                      |
| Schuette et al (18)                        | Amount protein                        | 8                         | Yes                       | 46.5                      | 3.56                    | Yes                 | —                      |
| Lutz (24)                                  | NaHCO₃                                | 6                         | No                        | 60                        | 1.5                     | Yes                 | 6.9                    |
| Lutz (24)                                  | Amount protein                        | 6                         | Yes                       | 39                        | 2.25                    | Yes                 | 6.1                    |
| Lemann et al (25)                          | NH₄Cl                                 | 5                         | Yes                       | 209                       | 7.3                     | No                  | 6.7                    |
| Breslau et al (26)                         | Vegetarian compared with carnivorous<sup>4</sup> | 10 | Yes | −27.1 | 1.1 | No | 6.55 | No |
| Breslau et al (26)                         | Ovo-vegetarian compared with carnivorous<sup>4</sup> | 15 | Yes | −13 | −0.7 | No | 6.32 | Yes |
| Lewis et al (27)                           | CaCO₃ compared with milk              | 8                         | Yes                       | 21.3                      | −0.6                    | Yes                 | 6.67                   |
| Lewis et al (27)                           | CaCO₃ compared with CaCl₂             | 8                         | Yes                       | 28.0                      | 0.6                     | Yes                 | 6.67                   |
| Trilok and Draper (28)                     | Amount protein                        | 8                         | Yes                       | 16.46                     | 1.39                    | No                  | 6.67                   |
| Remer and Manz (16)                        | Methionine                            | 6                         | No                        | 42.9                      | 0.9                     | No                  | 6.7                    |
| Remer and Manz (16)                        | Medium protein<sup>4</sup>            | 6                         | No                        | 45.6                      | 2.0                     | No                  | 6.7                    |
| Remer and Manz (16)                        | High protein                          | 6                         | No                        | 111.4                     | 2.4                     | No                  | 6.7                    |
| Sebastian et al (9)                        | Constant daily diet ± KHCO₃           | 18                        | Yes                       | −58.1                     | −1.6                    | Yes                 | —                      |
| Dahl et al (29)                            | Lentils<sup>1</sup> compared with soy protein | 10 | Yes | 3.1 | −0.9 | Yes | — | Yes |
| Frassetto et al (30)                       | KHCO₃ compared with placebo<sup>4</sup> | 19 | No | −38 | −0.7 | No | — | No |
| Sellmeyer et al (31)                       | Potassium citrate                     | 60                        | Yes                       | −53                       | −1.25                   | No                  | —                      |
| Maurer et al (32)                          | HCO₃<sup>−</sup> compared with Cl<sup>−</sup> | 9 | Yes | −71 | −0.6 | No | 7.07 | No |
| Roughhead et al (33)                       | High meat compared with low meat      | 15                        | Yes                       | 23.1                      | −0.08                   | No                  | 6.02                   |
| Ince et al (34)                            | High protein<sup>4</sup> compared with low protein | 42 | Yes | −21.5 | −1.1 | Yes | — | Yes |
| Marangella et al (35)                      | Self-selected diet ± potassium citrate | 52 | No | −21 | 0.275 | Yes | 6.33 | Yes |
| Gettman et al (36)                         | Slightly acid-ash metabolic diet<sup>4</sup> + water or cranberry juice | 12 | Yes | 8.6 | 0.4 | Yes | 5.97 | Yes |
| Kerstetter et al (37)                      | Amount protein                        | 13                        | Yes                       | 68.9                      | 1.66                    | No                  | —                      |
| Roughhead et al (38)                       | Meat<sup>4</sup> or soy               | 13                        | Yes                       | −11                       | 0.05                    | No                  | 6.33                   |
| Spence et al (39)                          | Milk protein<sup>4</sup> compared with soy protein | 15 | Yes | 1.6 | 1.03 | Yes | — | Yes |
| Jajoo et al (40)                           | Grains                                | 20                        | —                        | 17                        | 0.09                    | No                  | —                      |
| Jajoo et al (40)                           | Fruit or vegetables                   | 20                        | —                        | 7.8                       | 0.49                    | No                  | —                      |
| Kerstetter et al (41)                      | Meat or soy                           | 20                        | Yes                       | −24                       | −0.07                   | No                  | —                      |
| Kerstetter et al (41)                      | Amount soy                            | 20                        | Yes                       | 28.6                      | 0.83                    | No                  | —                      |
| Kerstetter et al (41)                      | Amount meat                           | 20                        | Yes                       | 18.4                      | 1.52                    | No                  | 6.41                   |

<sup>1</sup> Refers to experimental NAE – control NAE.
<sup>2</sup> Refers to whether the urine was acid treated.
<sup>3</sup> Refers to whether the urine was acidic because of treatment with acid or naturally acidic in both arms of the study.
<sup>4</sup> Control arms used to calculate the NAE, which may represent the modern diet.
of the difference in calcium concentration between acidic and alkaline urine was due to preanalytic bias (eg, the lower solubility of calcium in alkaline urine). It is possible that the addition of acid to the urine after collection was insufficient to make all of the calcium soluble and that some measurement error remained. Because there was a difference in measurable calcium between the urine treated with acid compared with urine not so treated, we recommend that future studies of urine calcium acidify the urine to assure analytic consistency (17).

The measurement of calcium in urine is influenced by additional factors such as the concentration of calcium and other constituents, the timing of the acidification, and how long the samples are stored before analysis (18). An important methodologic consideration for future studies is that the measurement of urine pH and NAE must be conducted in urine samples that are not acidified. Therefore, future studies of the acid-ash hypothesis require use of both acidified samples, to improve the measurement of calcium, and nonacidified samples, for the measurement of pH. It would therefore be necessary to either divide the urine sample into aliquots of acidified and unaltered samples or collect acidified and nonacidified samples on different days. Because the timing of the acidification of the samples may influence the final solubility of the urine, the estimates may be influenced if this acidification is done only after all of the samples are collected. These factors contribute sources of error in the estimates of calcium excretion.

The quantity of excess calcium in the urine associated with the modern diet is sufficient in quantity that the acid-ash hypothesis could more than explain the bone loss that results in osteoporosis. Specifically, if this calcium loss estimated from short-term studies were extrapolated over time without adaption, a continuous loss of 66 mg/d (1.6 mmol/d) would lead to 24 g/y or 480 g over 20 y. Adult humans have ≈1150 g of calcium in their skeletons (135). A loss of 480 g is almost half of the skeletal calcium and consistent with severe osteoporosis. However, this observation is not evidence that the source of the extra calcium is from bone or that this calciciuria contributes to the development of osteoporosis because changes in the excretion of calcium are not a direct measure of osteoporosis as are changes in bone strength as measured by fragility fractures or bone biopsy. It is possible that the cause of changes in NAE and calciciuria also alter intestinal absorption of calcium, and there may be little or no bone calcium loss affected by these processes (37). Our study shows that the quantity of calcium excreted in the urine is of sufficient quantity that the acid-ash hypothesis could explain the cause of osteoporosis; further research is needed to determine the exact fluxes of calcium between intestinal absorption, bone mineralization, and urinary excretion. These findings are not evidence that the source of the excreted calcium is bone or that this calciciuria contributes to the development of osteoporosis.

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The author’s responsibilities were as follows—TRF and AWL: designed the study; TRF: searched the literature, extracted the data, performed the statistical analysis and graphic representation, and wrote the manuscript; ME: directed the study’s statistical analysis and graphic representation; AWL: contributed to data analysis and writing of the manuscript; SCT and DAH: helped design the study and interpret the findings. None of the authors had a personal or financial conflict of interest.

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