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Meta-analysis of the quantity of calcium excretion associated with the net acid excretion of the modern diet under the acid-ash diet hypothesis

Tanis R Fenton, Michael Eliasziw, Andrew W Lyon, Suzanne C Tough, and David A Hanley

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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 + NH_4^+ − 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

[1] From the Departments of Community Health Sciences (TRF, ME, SCT, and DAH), Medicine and Oncology (DAH), and Pathology and Laboratory Medicine (AWL), University of Calgary, Calgary, AB, Canada; the Calgary Health Region, Calgary, AB, Canada (TRF, AWL, SCT, and DAH); and the Calgary Laboratory Services, Calgary AB, Canada (AWL).

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[4] Address reprint requests to TR Fenton, Clinical Nutrition, 1403 29 Street NW, Calgary AB Canada T2N 2T9. E-mail: tanisfenton@shaw.ca.

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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, calciuria, 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 in healthy adult subjects. The included studies were limited to those with a manipulation of the subject's acid-base intake; therefore, the design was limited to clinical trials or crossover studies. Because the aim of this review was to study the potential for the acid-ash diet hypothesis to have a role in the development of osteoporosis in apparently healthy people, studies were not included if the subjects had chronic conditions such as renal diseases, diabetic ketoacidosis, or acute effects of drug abuse or poisoning. Studies of infants and children were not included, nor were studies in which the subjects were in conditions that could alter their calcium excretion, such as fasting, weight loss, or decreased ambulation. Studies of persons predisposed to renal stone formation were only included if there was a group without renal stones that could be included in the meta-analysis. To accurately estimate the quantity of calciuria, only studies that collected urine over a 24-h period were used. The meta-analysis was not limited to English language articles. Efforts were made to contact investigators for additional information or clarification when necessary.

Description of studies

The literature search identified 105 studies of which 25 met all the inclusion criteria (9, 16, 19–41), and these studies formed the database (**Table 1**). Of the 25 studies, 2 were randomized controlled trials (31, 40), 1 was a nonrandomized clinical trial (35), 21 studies had a crossover design ["a method of comparing 2 or more ... interventions in which the subjects ... on completion of the course of one treatment, are switched to another" (42)], 10 of which randomized the order of treatments (26, 29, 30, 33, 34, 36–39, 41), and 11 that did not randomize the order (9, 16,

TABLE 1
Studies included in the meta-analysis about change in net acid excretion and changes in calcium excretion or bone mineral density[1]

Study	Year	Intervention	Subjects	Design	Blinded study	Accounted for losses	Calcium treatment[2]	Calcium intake
			n				*d*	*mg/d*
Weber et al (19)	1976	NH_4Cl	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 $NaHCO_3$	6	CO	No	No	8	500
Lemann et al (25)	1986	NH_4Cl	5	CO	No	No	0	1300
Breslau et al (26)	1988	Type of protein	15/10	RCO	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	$KHCO_3$	18	CO	No	No	12	650
Dahl et al (29)	1995	Lentils	10	RCO	No	No	≥14	Usual
Frassetto et al (30)	2000	$KHCO_3$	19	CO	No	No	--	Usual
Sellmeyer et al (31)	2002	Potassium citrate	60	RCT	Yes	No	18	500
Maurer et al (32)	2003	HCO_3^-	9	CO	No	No	5	1000
Roughead 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	?	?
Gettman 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
Roughead 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.

19–25, 27, 28, 32). 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 urine calcium 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 these changes that could be achieved through diet of free-living adults who are not taking an acid supplement (NH_4Cl); 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 millieqivalent 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 measured), the estimated change of urine calcium was 1.6 mmol/d (66 mg/d) of calcium.

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

TABLE 2
Interventions of altered acid or base intake and change in net acid excretion (NAE) and the outcome of calcium excretion

Study	Intervention	Subjects	Control of calcium intake	Change in NAE[1]	Change in urinary calcium	Acid treated[2]	Maximum urinary pH	Acidic urine[3]
		n		*mEq/d*	*mmol/d*			
Weber et al (19)	Whole food diet[4] ± NH_4Cl	6	Yes	216	9.1	No	5.97	Yes
Schuette et al (20)	Amount protein	11	Yes	37	2.15	Yes	—	Yes
Hegsted et al (21)	Amount protein	6	Yes	38.1	2.48	Yes	—	Yes
Lutz and Linkswiler (22)	Amount protein	8	Yes	56.0	2.05	Yes	—	Yes
Schuette et al (23)	Amount protein	8	Yes	32	1.20	Yes	—	Yes
Schuette et al (18)	Amount protein	8	Yes	46.5	3.56	Yes	—	Yes
Lutz (24)	$NaHCO_3$	6	Yes	−60	−1.5	Yes	6.9	Yes
Lutz (24)	Amount protein	6	Yes	39	2.25	Yes	6.1	Yes
Lemann et al (25)	NH_4Cl	5	Yes	209	7.3	No	6.7	No
Breslau et al (26)	Vegetarian compared with carnivorous[4]	10	Yes	−27.1	−1.1	No	6.55	No
Breslau et al (26)	Ovo-vegetarian compared with carnivorous[4]	15	Yes	−13	−0.7	No	6.32	Yes
Lewis et al (27)	$CaCO_3$ compared with milk	8	Yes	21.3	−0.6	Yes	6.67	Yes
Lewis et al (27)	$CaCO_3$ compared with $CaCl_2$	8	Yes	28.0	0.6	Yes	6.67	Yes
Trilok and Draper (28)	Amount protein	8	Yes	16.46	1.39	No	6.67	No
Remer and Manz (16)	Methionine	6	No	42.9	0.9	No	6.7	No
Remer and Manz (16)	Medium protein[5]	6	No	45.6	2.0	No	6.7	No
Remer and Manz (16)	High protein	6	No	111.4	2.4	No	6.7	No
Sebastian et al (9)	Constant daily diet[4] ± $KHCO_3$	18	Yes	−58.1	−1.6	Yes	—	Yes
Dahl et al (29)	Lentils[6] compared with soy protein	10	Yes	3.1	−0.9	Yes	—	Yes
Frassetto et al (30)	$KHCO_3$ compared with placebo[4]	19	No	−38	−0.7	No	—	No
Sellmeyer et al (31)	Potassium citrate	60	Yes	−53	−1.25	No	—	No
Maurer et al (32)	HCO_3^- compared with Cl	9	Yes	−71	−0.6	No	7.07	No
Roughead et al (33)	High meat compared with low meat	15	Yes	23.1	−0.08	No	6.02	Yes
Ince et al (34)	High protein[7] compared with low protein	42	Yes	−21.5	−1.1	Yes	—	Yes
Marangella et al (35)	Self-selected diet[4] ± potassium citrate	52	No	−21	0.275	Yes	6.33	Yes
Gettman et al (36)	Slightly acid-ash metabolic diet[4] + 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	—	No
Roughead et al (38)	Meat[8] or soy	13	Yes	−11	0.05	No	6.33	Yes
Spence et al (39)	Milk protein[9] compared with soy protein	15	Yes	1.6	1.03	Yes	—	Yes
Jajoo et al (40)	Grains	20	—	17	0.09	No	—	No
Jajoo et al (40)	Fruit or vegetables	20		7.8	0.49	No	—	No
Kerstetter et al (41)	Meat or soy	20	Yes	−24	−0.07	No	—	No
Kerstetter et al (41)	Amount soy	20	Yes	28.6	0.83	No	—	No
Kerstetter et al (41)	Amount meat	20	Yes	18.4	1.52	No	6.41	Yes

[1] Refers to experimental NAE − control NAE.
[2] Refers to whether the urine was acid treated.
[3] Refers to whether the urine was acidic because of treatment with acid or naturally acidic in both arms of the study.
[4] Control arms used to calculate the NAE, which may represent the modern diet.

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

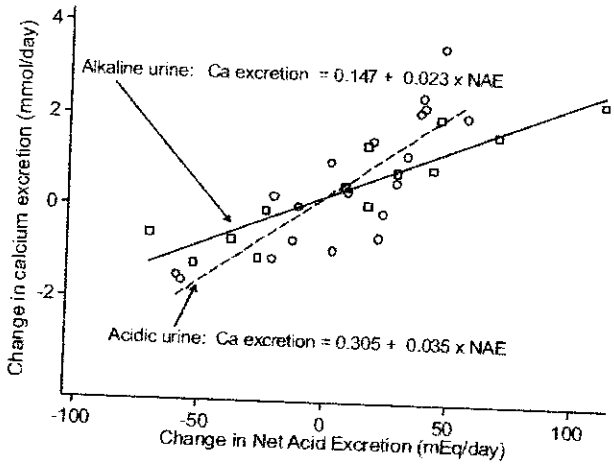

FIGURE 1. The effect of the change in net acid excretion (NAE) on the change in calcium excretion, which depended on whether the urine was acidic ($P < 0.001$). For acidic urine (○; dashed line), $R^2 = 0.6458$ ($P < 0.001$); for alkaline urine (□; solid line), $R^2 = 0.8787$ ($P < 0.001$).

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 diet 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 calciuria 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 calciuria 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 calciuria 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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