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Meta-Analysis of the Effect of the Acid-Ash Hypothesis of Osteoporosis on Calcium Balance

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ABSTRACT: The acid-ash hypothesis posits that protein and grain foods, with a low potassium intake, produce a diet acid load, net acid excretion (NAE), increased urine calcium, and release of calcium from the skeleton, leading to osteoporosis. The objectives of this meta-analysis were to assess the effect of changes in NAE, by manipulation of healthy adult subjects’ acid-base intakes, on urine calcium, calcium balance, and a marker of bone metabolism, N-telopeptides. This meta-analysis was limited to studies that used superior methodological quality for the study of calcium metabolism. We systematically searched the literature and included studies if subjects were randomized to the interventions and followed the recommendations of the Institute of Medicine’s Panel on Calcium and Related Nutrients for calcium studies. Five of 16 studies met the inclusion criteria. The studies altered the amount and/or type of protein. Despite a significant linear relationship between an increase in NAE and urinary calcium (p < 0.0001), there was no relationship between a change of NAE and a change of calcium balance (p = 0.38; power = 94%). There was no relationship between a change of NAE and a change in the marker of bone metabolism, N-telopeptides (p = 0.95). In conclusion, this meta-analysis does not support the concept that the calciuria associated with higher NAE reflects a net loss of whole body calcium. There is no evidence from superior quality balance studies that increasing the diet acid load promotes skeletal bone mineral loss or osteoporosis. Changes of urine calcium do not accurately represent calcium balance. Promotion of the “alkaline diet” to prevent calcium loss is not justified.

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Key words: osteoporosis, nutrition, acid-base equilibrium, bone or bones, calcium, meta-analysis, systematic review

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INTRODUCTION

OSTEOPOROSIS HAS A substantial impact on the quality of life,1 and in some cases, the quantity of life.2 The acid-ash hypothesis attributes the etiology of osteoporosis to nutritional factors.3–6 This hypothesis states that the modern diet causes osteoporosis through the metabolic production of acid and that the process of buffering this acid requires mobilization of bone mineral, with the resultant calcium lost in the urine.7–10

The acid-ash hypothesis identifies protein and grain foods as those that cause release of calcium from the skeleton to buffer the acid load from the diet and increase urinary excretion of calcium. According to the hypothesis, this acid load causes gradual loss of skeletal calcium. The hypothesis also suggests fruit and vegetables provide a supply of organic molecules that are metabolized to bicarbonate and therefore protect skeletal mineral.5,7–10 Specifically, the hypothesis states that food or supplemental sources of anions (referred to as acids), phosphate (PO$_4^{3-}$), sulfate (SO$_4^{2-}$), chloride (Cl$^-$), and organic acids, reflect dietary acid intake, cause metabolic acidemia, and increase calciuria when consumed in excess, which is detrimental to bone health.4–6 In contrast, food sources or supplements of cations (referred to as alkaline or bases), sodium (Na$^+$), potassium (K$^+$), calcium (Ca$^{2+}$), and magnesium (Mg$^{2+}$), are considered under the hypothesis to reflect base intake, decrease calciuria, and exert a protective effect on bone.5,11 According to the acid-ash hypothesis, protein5,7,12–16 and grain7 foods are detrimental to bone health because of sulfate and phosphate production,7 whereas fruit and vegetables are bone protective because of their potassium-organic anion content.6,7,16

This acid-ash hypothesis has not been subjected to critical review. Despite no critical review, this hypothesis is promoted to the public as the “alkaline diet” through the internet as a cure to almost any disease.

A meta-analysis of the literature showed evidence of a linear relationship between urine net acid excretion (NAE) and the quantity of calcium excreted in the urine.17 The estimated excess urine calcium associated with the modern

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diet is 1.6 mmol/d (66 mg/d) calcium.\(^{(17)}\) Over a lifetime, this excess calciuria is of sufficient quantity that it could explain the progression of bone mineral loss of osteoporosis\(^{(17)}\); however, whether this urinary calcium is important for bone health depends on whether the interventions also cause a change of calcium balance.\(^{(18)}\)

It is assumed by the hypothesis that the excess urinary calcium is equal to the loss of bone calcium to the body. Some researchers have proposed that, if intestinal calcium absorption\(^{(18)}\) and/or endogenous secretion\(^{(19)}\) compensates for some or all of the increase of urinary calcium, calcium may or may not be lost from bone.

Trials that use calcium balance as an outcome need to be designed to differentiate between changes in calcium retention that are caused by an intervention and those caused by study design. For example, if the calcium intakes of the subjects are altered from their usual intakes at a similar time as an intervention is begun, the subjects may show both some adaption to the change of calcium intake and a response to the intervention itself. The Institute of Medicine’s Panel on Calcium and Related Nutrients recommended that rigorous methodology be followed in calcium balance studies\(^{(20)}\) to avoid confounding by changes in calcium absorption caused by changes in calcium intake.

Urinary calcium excretion is not a direct measure of osteoporosis or calcium balance, but rather a surrogate measure, because it is possible that there are differences in calcium absorption that offset any change in excretion.\(^{(18,21)}\) Measures of calcium balance are superior measures of calcium status because they assess the effect of an intervention on the whole body retention of calcium and take alterations of absorption and/or endogenous secretion into account. The research question for this study was as follows: among adults, is there a dose-response relationship between NAE and calcium balance? Specifically, the objective of this study was to use the techniques of meta-analysis to assess the effect of changes in NAE on both urine calcium and calcium balance among studies with superior methodological quality for the study of calcium metabolism. As well, we examined the relationship between changes in NAE and the marker of bone metabolism, N-terminal telopeptide of collagen from these studies.

**MATERIALS AND METHODS**

*Literature search for the systematic review*

Literature relating to the acid-ash diet hypothesis was identified through computerized searching using, but not limited to, the keywords/textwords acid-base equilibrium, fruit, vegetables, net acid excretion, acid excretion, hydrogen ion concentration, bone or bones, and bone density, calciuria, calcium, phosphorus, excretion, balance, retention, biopsy, fracture(s), and bone mineral density. Reference lists were reviewed for additional relevant studies. 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 January 2009. The literature was not limited to English language articles.

**Selection criteria for the literature**

Studies were eligible to be included in this meta-analysis of calcium balance studies if they (1) were intervention studies that examined the acid-ash diet hypothesis by manipulating subjects’ acid-base intake through foods or supplemental salts (such as potassium bicarbonate), (2) reported the change of NAE (intervention) and calcium balance (outcome) in healthy adult subjects, (3) randomized subjects to the order of treatments,\(^{(22–24)}\) and (4) followed the recommendations for calcium balance studies.\(^{(20)}\) Calcium balance was defined as calcium intake minus excretion (urinary plus fecal).\(^{(25)}\) The recommendations for quality calcium balance studies include controlling the subjects’ calcium intakes for the recommended 7 or more days before measurement of the outcomes, provision of all the food to subjects, accurate measurement of the amounts consumed, and laboratory analysis to determine the nutrient composition of the food.\(^{(20)}\)

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 adults, studies were excluded if the subjects were not adults or had chronic conditions such as renal diseases, diabetic keto-acidosis, or were in states that could alter their calcium excretion such as acute effects of drug abuse, poisoning, fasting, weight loss, or decreased ambulation. Investigators were contacted for additional information clarification when necessary.

**Regression analysis**

Regression analyses, weighted by study sample size, were used to assess the dose-response relationship across the studies of the effect of changes of NAE on change of urinary calcium, calcium balance, and the marker of bone metabolism, N-terminal telopeptides, using Stata 10 (College Station, TX, USA).

We calculated the power of the balance study meta-analysis using a two-sided test and a of 0.05. First, we separated the variance into that between NAE levels from that within the subjects; we used the raw data reported in four studies of NAE and protein intakes,\(^{(12,14,15,26)}\) because none of the included studies reported individual subject data. Next, we calculated the weighted difference in calcium balance between the lower and higher NAE arms of the included studies.

**RESULTS**

The literature search identified five studies that met all the inclusion criteria and reported calcium balance as an outcome\(^{(19,25,27–29)}\) (Table 1) from a total of 16 balance studies of the acid-ash hypothesis.\(^{(4,12,14–16,18,19,25–33)}\) All of the included balance studies had cross-over study designs. The manipulations to alter diet acid load in the studies included changes in the amount\(^{(27,29)}\) and/or type\(^{(19,25,28,29)}\) of protein (Table 1). The included studies that assessed the effect of protein amount on changes of urine calcium and calcium balance all compared adequate intakes\(^{(33)}\) with higher intakes, and none of the studies compared the effect of lower protein intakes. No non-English language papers met the criteria for acceptance.
The reasons for study exclusion included lack of randomization of subjects to the order of interventions in nine studies, \(^{(4,12,14–16,30–33)}\) insufficient control of calcium intake in eight studies, \(^{(12,14,16,26,30–33)}\) and lack of laboratory chemical analysis of identical food portions in one study. \(^{(18)}\)

The change of NAE in the included studies ranged from a decrease of 24 mEq/d to an increase of 29 mEq/d, which is a range of 54 mEq/d (Table 2). Frequently cited balance studies of bicarbonate salts to alter NAE did not qualify for inclusion because neither study randomized the subjects to the interventions. \(^{(4,15)}\)

The included studies made 133 cross-over comparisons in eight interventions (Table 1). Each cross-over comparison represented a cross-over of two periods. The adaption periods during which the calcium intakes were controlled before the beginning of the outcome measurements ranged from 7 to 28 days, and the subsequent duration of the balance studies ranged from 16 to 56 days for each intervention (Table 1). Not all subjects contributed balance data in one study, \(^{(29)}\) so the total number of balance study cross-over comparisons was 77.

### Results of individual studies

The urinary calcium results from the individual studies showed statistically significant differences in four of the interventions involving the type \(^{(19,25)}\) and amount \(^{(29)}\) of protein, whereas four other interventions did not show significant differences for the type \(^{(28,29)}\) or amount of protein. \(^{(27)}\) None of the individual studies showed significant changes in calcium balance.

#### Regression meta-analyses

**Meta-analyses of studies of superior methodology:** The regression analysis of the effect of NAE on urine calcium showed a statistically significant linear relationship (Table 3; Fig. 1). For every 10-mEq increase of NAE, urine calcium increased by 0.3 mmol/d. The regression analysis of the effect of NAE on calcium balance showed no relationship between change in NAE and change in calcium balance (Table 3; Fig. 2). In response to changes in NAE, calcium balance equally increased and decreased. The data regarding the relationship between NAE and calcium balance did not fit a straight line relationship, as is evident because the low proportion of explained variance was 0.003 \((R^2;\) Table 3; Fig. 2). The estimated power for the calcium balance meta-analysis was 94%; the probability of a type II error was 6%.

The results for the effect of a change of NAE on the change of the marker of bone metabolism, N-terminal telopeptides, did not appear to have a straight line relationship (Fig. 3), and the regression analysis found no relationship (Table 3).
This meta-analysis, based on studies with superior methodology, showed that, despite a linear relationship between NAE and urine calcium, there was no relationship between NAE with either calcium balance or N-terminal telopeptides, a marker of bone metabolism. The assertions that diets that produce high levels of NAE cause osteoporosis have been primarily based on changes in urine calcium (7–10); however, the findings from this meta-analysis do not support the concept that the increased urine calcium associated with altered NAE represents the loss of whole body calcium.

This meta-analysis has three strengths. First, this study systematically assessed the calcium balance literature in response to changes of NAE. Second, we decreased the risk of bias by both including only studies that followed the recommended practices for calcium balance studies (20) and the subjects were randomized to the interventions. By avoiding major potential sources of bias, this meta-analysis provides a more accurate estimate of the effect of changes of NAE on calcium balance. Third, the power of the balance meta-analyses was sufficient to provide a respectable power and an acceptable risk of a type II error. The study power was increased by the use of cross-over designs by the included studies because the variability between subjects was eliminated.

This meta-analysis had two limitations. First, although calcium balance is a better measure of what is going on in terms of whole body calcium metabolism relative to changes in urine calcium, calcium balance is neither a direct measure of bone health nor of the progression of osteoporosis, but rather it is a surrogate measure of disease progression. Second, the studies that met the inclusion criteria for this meta-analysis compared adequate versus high protein intakes. None of the included studies examined the effect of changes of NAE from either bicarbonate salts or altered intakes of fruit and vegetables or grain foods on calcium balance and so it does not provide information on these other interventions.

Because calcium balance did not change with respect to changes in NAE, factors that alter NAE must also increase

TABLE 3. Regression Analysis Results

<table>
<thead>
<tr>
<th>Meta-analysis</th>
<th>Regression results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change of NAE on urinary calcium</td>
<td>$B_1 = 0.029$</td>
</tr>
<tr>
<td></td>
<td>$95%\ CI$ for $B_1 = 0.023–0.035$</td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.0001$</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.406$</td>
</tr>
<tr>
<td></td>
<td>Number of subjects: 133</td>
</tr>
<tr>
<td>Change of NAE on calcium balance</td>
<td>$B_1 = -0.006$</td>
</tr>
<tr>
<td></td>
<td>$95%\ CI$ for $B_1 = -0.021–0.008$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.38$</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.003$</td>
</tr>
<tr>
<td></td>
<td>Number of subjects: 77</td>
</tr>
<tr>
<td>Change of N-telopeptides of type I collagen</td>
<td>$B_1 = 0.00003$</td>
</tr>
<tr>
<td></td>
<td>$95%\ CI$ for $B_1 = -0.001–0.001$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.95$</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.000$</td>
</tr>
<tr>
<td></td>
<td>Number of subjects: 123</td>
</tr>
</tbody>
</table>

$B_1$, slope coefficient for regression analysis; $R^2$, the proportion of variance explained by the regression analysis.

DISCUSSION

This meta-analysis, based on studies with superior methodology, showed that, despite a linear relationship between NAE and urine calcium, there was no relationship between NAE with either calcium balance or N-terminal telopeptides, a marker of bone metabolism. The assertions that diets that produce high levels of NAE cause osteoporosis have been primarily based on changes in urine calcium (7–10); however, the findings from this meta-analysis do not support the concept that the increased urine calcium associated with altered NAE represents the loss of whole body calcium.

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Because calcium balance did not change with respect to changes in NAE, factors that alter NAE must also increase
calcium absorption\(^{(18)}\) and/or decrease the secretion of endogenous calcium into the gastrointestinal tract.\(^{(15)}\) At this point, there is evidence from isotope studies for the effect of both of these mechanisms.\(^{(18,19)}\)

The Institute of Medicine’s Panel on Calcium and Related Nutrients recommends subjects in calcium balance studies consume either their usual calcium intake or else consume the defined study calcium intake for at least 7 days before measurement of calcium balance.\(^{(20)}\) The percent of subjects’ calcium absorption depends on their habitual calcium intakes, and any change from their usual intakes causes an adaption to the new intake.\(^{(20)}\) To accurately measure differences in calcium balance between study interventions, it is necessary for the subjects to have had equal adaption to the study calcium intake in all study arms. Adaption to the study calcium intakes is of most importance in studies without randomization (in which the subjects receive the interventions in the same order) as was done by one half \((8/16)\) of the published calcium balance studies of the acid ash hypothesis. In nonrandomized comparison studies, the amount of adaption to the study calcium intake would differ between treatment 1 and treatment 2, thus possibly confounding or biasing the study results. The direction of the effect would vary for the individual subjects, depending on whether the study calcium intakes were greater or less than their usual calcium intakes.

Recent studies of the acid-ash hypothesis, with superior designs (randomized controlled trial\(^{(36)}\) and prospective cohort studies\(^{(37,38)}\)) and superior outcome measures of osteoporosis (fractures\(^{(37,38)}\) and changes of BMD\(^{(36)}\)) have shown results consistent with this meta-analysis. Two recent cohort studies that examined the acid-ash hypothesis using fracture risk (a direct measure of bone strength\(^{(39)}\)) did not confirm the acid-ash hypothesis.\(^{(37,38)}\) First, during 8 yr of follow-up, fracture risk was not reduced among a cohort of 36,217 postmenopausal women who consumed either lower protein or lower NAE diets.\(^{(37)}\) Second, wrist fracture risk was highest among 1865 peri- and postmenopausal women who consumed the lowest protein intakes over 25 yr of follow-up.\(^{(38)}\) As well, a recent 2-yr trial in 276 postmenopausal women either supplemented with potassium citrate (expected to neutralize the acid of the Western diet) or encouraged to consume increased fruit and vegetables showed that these interventions did not reduce bone turnover or decrease bone loss.\(^{(36)}\) These three recent studies have not supported the acid-ash hypothesis and are in agreement with the findings of the present calcium balance meta-analysis.

There is evidence that protein may be supportive of bone health, from a prospective cohort study and a randomized controlled trial.\(^{(40,41)}\) The cohort study found lower risks of hip fracture among those with higher protein intakes,\(^{(40)}\) and the randomized trial of a protein supplement for elderly patients found attenuation of proximal femur bone loss and shorter length of stay in hospital after hip fractures in the group randomized to the protein supplement.\(^{(41)}\) This current meta-analysis assessed the effect of the change of protein intake between adequate intakes\(^{(34)}\) and higher intakes and did not compare adequate intakes compared with low intakes, because no studies that address this latter comparison met the inclusion criteria. Because protein may be a protective factor for the maintenance of bone health,\(^{(37,38,40,41)}\) the advice under the acid-ash hypothesis that protein is detrimental\(^{(4,7,12,15)}\) could be harmful.

In conclusion, this meta-analysis does not support the concept\(^{(4,7,12,15)}\) that the calciuria associated with higher NAE reflects lower calcium retention and skeletal bone mineral loss. It should be emphasized that changes in calcium excretion do not accurately represent changes in calcium balance. Promotion of the acid-ash hypothesis or “alkaline diet” to the public to prevent calcium loss is not justified.

A definitive well-designed adequately powered study is needed to determine whether there is an association between NAE and osteoporosis. To be definitive, this study must use direct measures of bone strength\(^{(39)}\) such as biomechanical testing of bone or the incidence of fragility fractures, and follow recommendations for good methodological study quality.\(^{(20,23,42)}\)

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