

# Estimated Net Acid Excretion Inversely Correlates With Urine pH in Vegans, Lacto-Ovo Vegetarians, and Omnivores

Lynne M. Ausman, DSc, RD,\*†‡§ Lauren M. Oliver, MS, RD,\*‡  
Barry R. Goldin, PhD,\*† Margo N. Woods, DSc,\*† Sherwood L. Gorbach, MD,\*†‡ and  
Johanna T. Dwyer, DSc, RD\*†‡§

**Objective:** Diet affects urine pH and acid-base balance. Both excess acid/alkaline ash (EAA) and estimated net acid excretion (NAE) calculations have been used to estimate the effects of diet on urine pH. This study's goal was to determine if free-living vegans, lacto-ovo vegetarians, and omnivores have increasingly acidic urine, and to assess the ability of EAA and estimated NAE calculations to predict urine pH.

**Design:** This study used a cross-sectional design.

**Setting and Participants:** This study assessed urine samples of 10 vegan, 16 lacto-ovo vegetarian, and 16 healthy omnivorous women in the Boston metropolitan area. Six 3-day food records from each dietary group were analyzed for EAA content and estimated NAE, and correlations with measured urine pH were calculated.

**Results:** The mean ( $\pm$  SD) urine pH was  $6.15 \pm 0.40$  for vegans,  $5.90 \pm 0.36$  for lacto-ovo vegetarians, and  $5.74 \pm 0.21$  for omnivores (analysis of variance,  $P = .013$ ). Calculated EAA values were not significantly different among the three groups, whereas mean estimated NAE values were significantly different:  $17.3 \pm 14.5$  mEq/day for vegans,  $31.3 \pm 8.5$  mEq/day for lacto-ovo vegetarians, and  $42.6 \pm 13.2$  mEq/day for omnivores (analysis of variance,  $P = .01$ ). The average deattenuated correlation between urine pH and EAA was 0.333; this value was  $-0.768$  for estimated NAE and urine pH, with a regression equation of  $\text{pH} = 6.33 - 0.014 \text{ NAE}$  ( $P = .02$ ,  $r = -0.54$ ).

**Conclusions:** Habitual diet and estimated NAE calculations indicate the probable ranking of urine pH by dietary groups, and may be used to determine the likely acid-base status of an individual; EAA calculations were not predictive of urine pH.

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\*Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy, Tufts University, Boston, Massachusetts.

†Department of Public Health and Family Medicine, Tufts University School of Medicine, Boston, Massachusetts.

‡Tufts Medical Center, Boston, Massachusetts.

§Jean Mayer United States Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts.

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Address reprint requests to Lynne Ausman, DSc, RD, Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy, Tufts University, 150 Harrison Ave., Boston, MA 02111. E-mail: [lynne.ausman@tufts.edu](mailto:lynne.ausman@tufts.edu)

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IT HAS BEEN KNOWN SINCE the early twentieth century that dietary composition influences the pH of urine.<sup>1-3</sup> Recent evidence suggests that higher protein intake results in more renal net acid excretion and more acidic urine.<sup>4-6</sup> Urine pH, as influenced by diet, affects the risk of formation of certain types of kidney stones.<sup>7,8</sup> High animal-protein diets were associated with increased urinary saturation of undissociated uric acid, and consequently an increased risk for uric acid kidney stone formation.<sup>4,9</sup> High protein intake is also associated with increased urinary calcium excretion.<sup>4,5,9,10</sup> Whereas the effect of protein intake on overall bone health remains controversial, higher protein intake, especially animal protein, was linked to an increased rate of bone loss and increased risk of hip and forearm fractures in women.<sup>11,12</sup> Renal net acid excretion (NAE) also correlates inversely with change in bone mineral density.<sup>13</sup> Experimental loading with acid increases endogenous acid production, and results in

increased calcium excretion as bone calcium is released to act as a buffer against metabolic acidosis.<sup>14–16</sup> Thus, dietary protein-induced hypercalciuria indicates calcium mobilization from bone to buffer the acidogenic properties of the protein, and over time, such negative calcium balance may lead to an increased risk for osteoporosis.<sup>4,9,17</sup> Conversely, some nutrients, including magnesium and potassium, are found abundantly in fruits and vegetables that are thought to have alkalizing properties, and such nutrients were positively associated with bone health.<sup>18–20</sup>

Because acid-base balance has significant implications for renal and bone health, it is important to understand the overall effect of diet on acid-base status and urine pH to help determine the risk of developing certain conditions and to introduce appropriate dietary modifications to prevent or treat the conditions, including kidney stones, bone loss and/or fractures, and possibly osteoporosis.

Calculations of excess acid/alkaline ash (EAA) give increasingly more excess alkalinity values for typical omnivore, lacto-ovo vegetarian, and vegan menus.<sup>21</sup> Whether this difference is reflected in urine pH among free-living adults has not been determined using actual food records. Another method of assessing acid-base status is by estimating urinary NAE, which Remer and Manz found to correlate well with measured NAE and urine pH.<sup>22</sup> Urine pH decreased with increasingly higher protein intake among subjects eating three different experimental diets for 5 days each.<sup>22</sup> To calculate the estimated NAE, the authors also introduced the concept of potential renal acid load (PRAL) of foods, an indication of the acid-forming or base-forming potential of foods based on the average intestinal absorption rates of cations and anions present in the foods.<sup>22,23</sup> The method of Remer et al.<sup>23</sup> also takes into account the excretion of organic acids, a diet-independent determinant of NAE that varies with body surface area. Thus, the model addresses some of the apparent weaknesses of the EAA method.<sup>21</sup> Estimated NAE using PRAL correlates closely with measured NAE for children and adolescents as well as adults.<sup>24</sup> Michaud et al. found that NAE estimates from food frequency questionnaires and 24-hour diet recalls correlated well with urine pH, suggesting that the estimation of NAE could substitute for measuring urine pH in epidemiologic studies.<sup>25</sup>

This study had two objectives: (1) to test the hypothesis that free-living vegans, lacto-ovo vegetarians, and omnivores eating their typical diets have increasingly acidic urine pH; and (2) to assess the potential ability to predict urine pH using the excess acid/alkaline ash (EAA) and estimated net acid excretion (NAE) calculation methods, with data from 3-day food records.

## Materials and Methods

### Subject Recruitment and Urine Collection

For this cross-sectional study, dietary records and urine samples were obtained from a larger study that examined the effects of diet on osteoporosis in women.<sup>26</sup> The 42 Caucasian women participants in this study ranged in age from 23 to 60 years, habitually consumed vegan ( $n = 10$ ), lacto-ovo vegetarian ( $n = 16$ ), or omnivorous ( $n = 16$ ) diets, and provided two separate 72-hour urine samples at least 3 months apart. The majority of omnivores were healthy volunteers on the staff of the institution where the study took place, and who ate typical nonvegetarian diets. Lacto-ovo vegetarians were recruited through advertisements in local newspapers and by word of mouth. Vegans were recruited primarily through macrobiotic groups in the Boston area. Subjects were excluded if they had a history of heart disease, hypertension, diabetes mellitus, arthritis, bone fractures, thyroid problems, renal or liver disease, irregular menstrual cycles, or regular consumption of alcohol. The use of oral contraceptives, corticosteroids, or antibiotics also resulted in exclusion. In addition, women who were over 120% or under 90% of their ideal body weight, and those who had evidence of “crash dieting” in their history, failure to consume a consistent diet, or inability to keep a 3-day dietary record, were also excluded. Informed consent was obtained from all women, and the study was approved by the Tufts Medical Center and Tufts University Health Sciences Institutional Review Board.

Subjects' age, height, weight, collection period, and identifying numbers were abstracted from study records. The frozen urine samples for each subject had been stored at  $-70^{\circ}\text{C}$  for a few months to 2 years. As a preservative, 1.0 g of ascorbic acid had been added to each 1000-mL urine-collection bottle before the urine had been pooled into a single 72-hour specimen. Immediately before frozen storage, sodium azide solution had also been added

to each sample as an antibacterial agent, to make a final concentration of 15.4 mmol/L (1 g/L).

### Urinary Creatinine Excretion

Total urine volume data were obtained for each 72-hour pooled urine collection. Urine creatinine concentrations were available for 80 of 84 urine samples. Combined with the urine volume data, the total urinary creatinine excretion (mg/kg body weight per day) was calculated to estimate the completeness of urine collection.

### Urine pH Measurement

Urine samples were thawed at room temperature for 2 to 4 hours, and then analyzed for pH using a Corning Model 12 Research pH Meter (Corning Inc., Life Sciences, Lowell, MA) by a technician who was blinded to subject names and diets. A 2-point standardization at pH 4.0 and pH 7.0 was carried out on every 5 samples on the pH meter, to minimize the potential for drift of the instrument. Urine pH was measured in 42 duplicate pairs of urine samples, or 84 samples in total.

### Dietary Data

Subjects had been instructed on measuring portions and on procedures for keeping accurate food records using household measurements, and submitted a practice record, which was reviewed with a nutritionist before the food records were collected. Three-day food records that had been simultaneously collected with the urine were available for 6 women from each dietary group, and EAA and estimated NAE calculations were performed on these 18 records, for 108 record-days in total.

A software program and an abbreviated 730-item database were used to determine values of dietary sodium, calcium, phosphorous, and potassium, based on the food records.<sup>27,28</sup> The database was supplemented from various other sources to include 180 foods commonly eaten by vegetarians.<sup>29-34</sup> Sodium, calcium, phosphorous, and potassium values were available for most foods, and when they were not, estimates were imputed using a similar food item.

The EAA calculations also require chloride, magnesium, and sulfur, elements not provided in the food-composition tables and databases commonly used in the United States. Chloride values were imputed by multiplying sodium by a factor

of 1.5, based on the assumptions that most sodium occurs as sodium chloride, and that the molar ratio is 1:1. Magnesium values were calculated by hand from available existing tables.<sup>31,32,34-36</sup> Sulfur values were obtained from published sulfur values,<sup>35-37</sup> or estimated as based on the methionine (21.5% sulfur) and cysteine (31.4% sulfur) content of foods, as these amino acids provide the largest sulfur contributions among proteins.<sup>36,38,39</sup>

### Calculations

#### Excess Acid/Alkaline Ash

The excess acid or base-forming potential of the diets consumed and recorded in the food records was calculated by the methods of Sherman and Gettler,<sup>1</sup> and as more recently described by Dwyer et al.<sup>21</sup> Because tables of food composition and nutrient databases present nutrients by weight, milligrams of the elements were converted to milliequivalents before calculation. The sums of the acid and base-forming elements were calculated, and then the difference between the two was found, according to the formula:

$$\text{EAA} = [\text{mEq}(\text{Na} + \text{K} + \text{Ca} + \text{Mg}) - \text{mEq}(\text{PO}_4 + \text{SO}_4 + \text{Cl})] / \text{day}$$

#### Net Acid Excretion

To calculate estimated NAE, the PRAL of foods eaten per day was added to an estimate of organic acid (OA) excretion, as adapted from Remer and Manz<sup>22,23</sup> by Michaud et al.<sup>25</sup>

$$\text{NAE} = \text{PRAL} + \text{OA}$$

where PRAL = mEq (PO<sub>4</sub> + SO<sub>4</sub> - K - Ca - Mg)/day, and OA (mEq/day) = body weight × 0.66.

Sodium and chloride electrolytes were excluded from this equation because the two are assumed to have equal absorption rates and similar excretion levels, and because incomplete food table values for chloride are thought to result in estimation errors.<sup>22,24,25</sup>

### Statistical Methods

For these analyses, SAS (SAS, Inc., Cary, NC) was the primary statistical package used.<sup>40</sup> Before

analysis of the data, descriptive statistics and graphs (PROC UNIVARIATE and PROC MEANS) were used to summarize the overall effects of diet. When violations of the basic testing assumptions were noted, appropriate transformations of the data were used (square root, logarithmic, or inverse). A two-way analysis of variance (PROC GLM), with main effects of DIET (omnivorous, lacto-ovo vegetarian, or vegan) and COLLECTION, and with SUBJECT as a repeated measure, was performed for each outcome measure. The error term for DIET was the subject nested within diet [SUBJECT(DIET)], and the error term for COLLECTION and DIET \* COLLECTION interaction was COLLECTION \* SUBJECT (DIET). Tukey's *t* test for multiple comparisons was used to test differences among group means, using an  $\alpha$  level of  $\leq 0.05$ . We used PROC CORR to examine correlations between urinary pH and both of the methods of calculating acid/alkaline urine excretion (EAA and NAE). The procedure of Willett (1990) was used to compute deattenuated correlation coefficients,<sup>41</sup> and PROC GLM was used to calculate the regression of urine pH on NAE.

## Results

### Characteristics of Study Subjects

Table 1 describes the characteristics of women studied. Among 42 women, those consuming the lacto-ovo vegetarian and omnivore diets were comparable in age and body weight, whereas those consuming the vegan diet appeared somewhat younger and lighter than the other two groups, although these differences were not statistically

significant because of the range of variability. In addition, height was similar among groups. The vegans had a significantly smaller average body mass index (BMI) than the lacto-ovo vegetarians, with the omnivores at an intermediate position. The characteristics of the sample of 18 women whose diets were analyzed were similar to those of the larger group, although in the smaller sample, the vegan group was significantly younger and had a significantly lower average BMI than the other two groups.

### Completeness of Urine Collection

Table 2 presents the means for urinary creatinine excretion per kilogram of body weight per day for each of the three groups, along with the results of the analysis of variance. The means for the lacto-ovo vegetarian and vegan groups were similar and within the usual range of variability for individuals consuming a meat-free diet, suggesting that these collections were complete.<sup>42</sup> The creatinine excretion for the omnivores was significantly greater than for both other groups, reflecting the likely greater dietary creatine consumption in this group, but still within the usual range for those consuming an omnivorous diet.<sup>42</sup> We also calculated the coefficient of variation (CV) of urinary creatinine excretion within duplicate pairs collected on each individual. The CV was 13.1% for vegans, 16.1% for lacto-ovo vegetarians, and 15.6% for omnivores. Because variations within each duplicate pair were similar in all three groups and the urinary excretion of creatinine was normal, these findings suggest that the urine collections were complete.

**Table 1.** Characteristics of 42 Female Study Participants and the Subsample of 18 Participants

	Vegans	Lacto-Ovo Vegetarians	Omnivores
Complete study	10	16	16
Age (y)	30.9 ± 10.7	43.2 ± 16.5	43.3 ± 15.2
Body weight (kg)	51.3 ± 7.6	57.5 ± 5.9	56.8 ± 6.3
Height (m)	1.64 ± 0.08	1.63 ± 0.07	1.65 ± 0.05
Body mass index	18.9 ± 1.2*	21.8 ± 2.5†	20.8 ± 2.1*†
Subsample	6	6	6
Age (y)	29.0 ± 10.8*	47.7 ± 18.0†	54.7 ± 1.5†
Body weight (kg)	49.3 ± 5.8*	59.0 ± 6.7*†	57.5 ± 4.9†
Height (m)	1.63 ± 0.07	1.66 ± 0.07	1.62 ± 0.06
Body mass index	18.5 ± 0.9*	21.6 ± 4.0†	21.8 ± 1.8†

Values represent mean ± standard deviation.

\*†Means in rows without common superscripts differ significantly at  $P < .05$ .

**Table 2.** Urinary Creatinine and Urine pH for 42 Vegan, Lacto-Ovo Vegetarian, and Omnivorous Women

Constituent	Period	Vegan	Lacto-Ovo Vegetarian	Omnivore	Analysis of Variance <i>P</i> Value		
		(n = 10)	(n = 16)	(n = 16)	Diet	Collection	Interaction
Urinary creatinine (mg · kg <sup>-1</sup> · d <sup>-1</sup> )	Average	17.6 ± 2.3*	16.5 ± 2.7*	19.6 ± 3.1†	.011	.897	.511
	1	17.3 ± 2.9	16.8 ± 2.9	19.5 ± 3.0			
	2	17.5 ± 2.1	16.2 ± 3.3	19.7 ± 3.5			
Urine pH	Average	6.15 ± 0.40*	5.90 ± 0.36*†	5.74 ± 0.21†	.013	.723	.830
	1	6.17 ± 0.35	5.85 ± 0.48	5.73 ± 0.30			
	2	6.14 ± 0.65	5.95 ± 0.53	5.77 ± 0.18			

Values are daily means ± SD. Two separate urine collections were obtained for each person. The means for each diet and collection are given, as well as the overall average for each diet (using one average value per subject). A two-way analysis of variance with main effects of diet and collection period was used to analyze the data, followed by Tukey's *t* test for multiple comparisons when diet effects were significant.

\*†Means in rows without common superscripts differ significantly at *P* < .05.

## Urine pH

Mean urinary pH values by diet, and results of the analysis of variance and Tukey's *t* test for each collection period and by average, are presented in Table 2. Diet had a significant effect on urinary pH (*P* = .013). Vegans had a mean (± SD) urinary pH of 6.15 ± 0.40, which was significantly higher than that of the omnivores (5.74 ± 0.21). The urinary pH of the lacto-ovo vegetarians (5.90 ± 0.36, *n* = 16) was intermediate between the two, and did not vary significantly from either of the other groups. There was no significant effect of either collection period or interaction term.

## Dietary Analysis

Table 3 presents the means of selected dietary constituents for 6 individuals from each of the vegan, lacto-ovo vegetarian, and omnivore groups for both collection periods, as well as the overall average. The analysis of variance indicated significant effects of diet for dietary protein and magnesium. For protein, the value of 72.0 ± 7.1 g/day among omnivores was significantly greater than for the lacto-ovo vegetarians (57.5 ± 8.8) and vegans (49.4 ± 11.0). The greater protein intake for the omnivores and lacto-ovo vegetarians is consistent with their consumption of meat or animal products. For dietary magnesium, the vegans had a significantly increased intake (45.0 ± 16.2 mEq/day) compared with the omnivores (26.4 ± 5.9), and the value for lacto-ovo vegetarians (29.9 ± 5.1) was intermediate. These results are consistent with the increased plant consumption of the lacto-ovo vegetarian and vegan groups. Interestingly, there was a significant effect of collec-

tion period for magnesium, as evidenced by the wide difference in consumption between the two collection periods for the lacto-ovo vegetarian group. There was high variability in the sodium, potassium, calcium, phosphorus, sulfur, and chloride consumption within each group, with no statistically significant effect of diet or collection period. At least some of this variability in sodium and chloride values may be due to incomplete or inaccurate food-data tables.

## Urine pH and Calculation Methods by Diet Group

As in the 42-person total sample, urine pH among the 18-person subsample decreased from vegans (6.18 ± 0.41), to lacto-ovo vegetarians (5.92 ± 0.42), to omnivores (5.61 ± 0.19) with borderline statistical significance (*P* = .066), as shown in Table 4. Urinary creatinine excretion was similar in all groups, and suggested complete urine collection (data not shown). The calculations for EAA and NAE by diet group are also shown in Table 4. The EAA calculation showed a trend opposite to that of NAE. Calculated EAA values were 45.1 ± 17.9 mEq/day for the vegan group, and decreased to 35.5 ± 16.8 mEq/day and 21.1 ± 19.9 mEq/day in the lacto-ovo vegetarian and omnivorous groups, respectively. The analysis of variance showed that diet was not a significant predictor of EAA (*P* = .17), and neither the collection-period term nor the interaction term was statistically significant. The NAE was 17.3 ± 14.5 mEq/day in the vegan group, and increased significantly to 42.6 ± 13.2 mEq/day for the omnivore

**Table 3.** Average Daily Dietary Intakes for 18 Vegan, Lacto-Ovo Vegetarian, and Omnivorous Women, Based on 72-Hour Food Records

Constituent	Period	Vegan	Lacto-Ovo Vegetarian	Omnivore	Analysis of Variance <i>P</i> Value		
		(n = 6)	(n = 6)	(n = 6)	Diet	Period	Interaction
Protein (g · d <sup>-1</sup> )	Average	49.4 ± 11.0*	57.5 ± 8.8*	72.0 ± 7.1†	.002	.217	.026
	1	43.9 ± 13.6	67.5 ± 12.2	75.5 ± 8.1			
	2	54.9 ± 17.6	47.4 ± 12.0	68.6 ± 10.2			
Sodium (mEq · d <sup>-1</sup> )	Average	100.6 ± 52.7	80.7 ± 15.7	108.7 ± 64.0	.675	.709	.772
	1	118.5 ± 107.0	81.6 ± 15.6	109.6 ± 60.6			
	2	82.6 ± 18.4	79.8 ± 19.0	107.8 ± 72.0			
Potassium (mEq · d <sup>-1</sup> )	Average	74.5 ± 14.2	75.9 ± 16.7	71.7 ± 14.7	.919	.381	.206
	1	70.8 ± 19.1	86.1 ± 23.5	73.8 ± 18.9			
	2	78.2 ± 20.4	65.6 ± 14.7	69.6 ± 16.4			
Calcium (mEq · d <sup>-1</sup> )	Average	32.2 ± 14.2	43.0 ± 12.6	38.2 ± 5.1	.284	.932	.005
	1	22.9 ± 6.6	48.1 ± 15.6	42.0 ± 9.3			
	2	41.4 ± 22.0	37.9 ± 11.8	34.6 ± 7.9			
Magnesium (mEq · d <sup>-1</sup> )	Average	45.0 ± 16.2*	29.9 ± 5.1*†	26.4 ± 5.9†	.014	.002	.055
	1	48.7 ± 16.6	37.7 ± 10.0	27.4 ± 6.0			
	2	41.2 ± 19.1	22.2 ± 3.3	25.5 ± 6.5			
Phosphorus (mEq · d <sup>-1</sup> )	Average	68.0 ± 18.5	72.1 ± 12.1	71.4 ± 7.8	.856	.092	.018
	1	61.6 ± 13.8	84.4 ± 16.8	78.4 ± 13.5			
	2	74.3 ± 27.4	59.7 ± 15.1	64.4 ± 9.8			
Sulfur (mEq · d <sup>-1</sup> )	Average	41.4 ± 13.1	43.4 ± 9.2	46.8 ± 4.5	.618	.055	.053
	1	40.4 ± 15.7	50.9 ± 11.5	48.5 ± 8.6			
	2	42.4 ± 12.3	35.9 ± 10.3	45.2 ± 5.4			
Chloride (mEq · d <sup>-1</sup> )	Average	97.8 ± 15.3	78.5 ± 15.3	105.7 ± 62.2	.675	.709	.772
	1	115.2 ± 104.0	79.3 ± 15.1	106.6 ± 59.0			
	2	80.4 ± 17.9	77.7 ± 18.4	104.9 ± 70.0			

Two separate urine collections were obtained for each person, each over a 72-hour period corresponding to 3-day food intake. The daily means for each diet and collection are given, as well as the overall average for each diet (using one average value per subject). Data for magnesium were log<sub>10</sub>-transformed, and those for sodium, potassium, and chloride were inverse-transformed, before statistical analysis. A two-way analysis of variance with main effects of diet and period was used to analyze the data, followed by Tukey's *t* test for multiple comparisons of diet effects.

\*†Means in rows without common superscripts differ significantly at *P* < .05.

group. The value of 31.3 ± 8.5 mEq/day for the lacto-ovo vegetarian group was intermediate between the other two. The effect of diet on NAE was highly significant (*P* = .01), but neither the collection period nor the interaction term was significant.

### Correlations Between Calculation Methods and Urine pH

To investigate further the relationship between urinary pH and urinary mineral excretion, we calculated the correlation between pH and EAA or NAE. This was done for each collection period, as well as for an average of the two collection periods. Table 5 shows the results of these correlations. The correlation for the relationship between NAE and urinary pH was -0.540 (*P* = .02). The correlation with excess alkalinity was not significant, at *P* = .23.

### Discussion

This study confirms the hypothesis that the urine of vegans, lacto-ovo vegetarians, and omnivores is increasingly acidic. The mean urinary pH of individuals in these 3 dietary groups varied, and the difference between vegans and omnivores was statistically significant in spite of very large intra-individual variations. The urine pH values for all groups were within the normal range of 4.6 to 8.0.<sup>43</sup> Although the added urine preservative decreased the urine pH by approximately 0.45 units in all groups (data not shown), the finding of increasingly acidic urine pH from the vegan to the omnivore groups remains, because the correction factor would be a constant.

To our knowledge, this is the first study to present calculations of both EAA and NAE for vegans and vegetarians as well as omnivores, using data

**Table 4.** Calculated Net Acid Excretion, Calculated Excess Alkaline/Acid, and Urine pH for 18 Vegan, Lacto-Ovo Vegetarian, and Omnivorous Women, Based on 72-Hour Food Records and 72-Hour Urine Samples

Constituent	Period	Vegan	Lacto-Ovo Vegetarian	Omnivore	Analysis of Variance <i>P</i> Value		
		(n = 6)	(n = 6)	(n = 60)	Diet	Period	Interaction
NAE (mEq · d <sup>-1</sup> )	Average	17.3 ± 14.5*	31.3 ± 8.5†*	42.6 ± 13.2†	0.010	0.863	0.614
	1	14.7 ± 21.7	31.9 ± 11.2	45.8 ± 16.9			
	2	19.9 ± 19.5	30.6 ± 8.2	39.4 ± 13.6			
EAA (mEq · d <sup>-1</sup> )	Average	45.1 ± 17.0	35.5 ± 16.8	21.1 ± 19.9	0.168	0.562	0.679
	1	43.7 ± 26.8	38.8 ± 24.9	19.3 ± 24.8			
	2	46.4 ± 18.7	32.2 ± 11.5	23.0 ± 21.0			
Urine pH	Average	6.18 ± 0.41*	5.92 ± 0.42*†	5.61 ± 0.19†	0.061	0.024	0.340
	1	6.05 ± 0.34	5.65 ± 0.48	5.67 ± 0.27			
	2	6.30 ± 0.65	6.18 ± 0.46	5.70 ± 0.14			

EAA = (Na + K + Ca + Mg) – (PO<sub>4</sub> + SO<sub>4</sub> + Cl); NAE = PRAL + OA; PRAL = PO<sub>4</sub> + SO<sub>4</sub> – K – Ca – Mg; OA = body weight (in kilograms) × 0.66.

NAE, net acid excretion; EAA, excess alkaline/acid ash; OA, organic acid; PRAL, potential renal acid load.

All values are in mEq = [(mg/atomic weight) × valence]. Values are arithmetic means ± SDs of a 3-day period. Two separate urine collections were obtained for each person. The means for each diet and collection are given, as well as the overall average for each diet (using one average value per subject). Data for EAA were log<sub>10</sub>-transformed before statistical analysis. A two-way analysis of variance with main effects of diet and period was used to analyze the data, followed by Tukey's *t* test for multiple comparisons of diet effects.

\*†Means in rows without common superscripts differ significantly at *P* < .05.

from food records. Our finding of a significant relationship between NAE and urinary pH was consistent with the observations of Remer and Manz,<sup>23</sup> who cited a regression equation for adult males of:

$$\text{Urinary pH} = 6.9 - 0.01 \text{ NAE}$$

We found a similar equation for adult females (Figure 1):

$$\text{Urinary pH} = 6.3 - 0.014 \text{ NAE}$$

Indeed, if the pH values presented here were increased approximately 0.45 pH units to com-

pensate for the effect of the urine preservative, the y-intercept would have been similar to that in the equation of Remer and Manz.<sup>23</sup> It is not known whether any male/female differences would have influenced these comparisons.

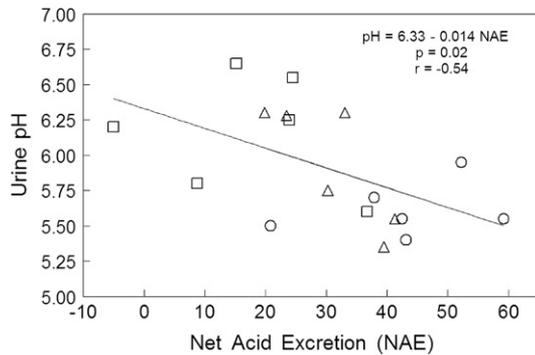
One limitation of our study involves its small size, with only 18 females. Larger studies with both sexes may be necessary for the further refinement of these observations. Another study limitation is that the lacto-ovo vegetarian and omnivorous women in the subset of 18 were older than the vegan women. Renal function, as assessed by glomerular filtration rate (GFR), was shown to decline with age, but studies disagree on the rate of such decline, and

**Table 5.** Correlations Between Average Urine pH and Net Acid Excretion and Excess Acid/Alkaline Ash for 18 Vegan (n = 6), Lacto-Ovo Vegetarian (n = 6), and Omnivorous (n = 6) Women

Calculation	Urine pH			
	First Collection	Second Collection	Average	Average Deattenuated*
NAE				
<i>r</i>	–0.301	–0.660	–0.540	–0.768
<i>P</i> value	.224	.004	.020	
Protein/potassium ratio				
<i>r</i>	–0.260	–0.590	–0.513	–0.730
<i>P</i> value	.297	.013	.029	
EAA				
<i>r</i>	0.028	0.460	0.234	0.333
<i>P</i> value	.912	.063	.349	

NAE, net acid excretion; EAA, excess alkaline/acid ash.

\*Within-person variance = 0.158; between-person variance = 0.324. Correcting for the attenuating effect of within-person error in urine pH, true correlation = observed correlation × √(1 + (0.324/0.158)/2).<sup>42</sup>



**Figure 1.** Regression of urine pH on net acid excretion (NAE) for 18 women eating vegan (□), lacto-ovo vegetarian (Δ), or omnivorous (○) diets.

showed a conflicting influence of sex on the changes.<sup>44-47</sup> Frassetto et al. noted an age-related decline in GFR, and asserted that it contributed to an 8% increase in blood acidity from ages 17 to 74 years.<sup>45</sup> They did not, however, stratify by sex or show a change in urine pH with age. They also held the dietary net acid load constantly positive, so it is not clear that women consuming different diets, as in our study, would experience such changes in GFR. Berg found a significant decrease in GFR in males between ages 20 and 50 years, but found no significant decline in females, even when including subjects aged 50 years or over.<sup>47</sup> As the women studied here were all under age 60 years and did not have any history of renal insufficiency, it is reasonable to assume that the slight age difference was not enough to affect renal function significantly. To our knowledge, there is no evidence that age independently and significantly affects urine pH. Future studies with women and men of various age groups are necessary to investigate such potential differences by age and sex.

Correlations between EAA and urinary pH were low and not statistically significant. As noted previously, the EAA calculations were onerous, time-consuming, and imprecise, because the available food tables lacked compositional information on many of the elements.<sup>21</sup> Our results support the conclusion that calculations of EAA hold little promise for the precise quantitative prediction of urine pH, although they indicate the likely direction of changes when diets shift from vegan to lacto-ovo vegetarian to omnivorous.

The lack of complete, accurate food-composition data could also negatively affect the predictive ability of the estimated NAE equation. Incomplete

or inaccurate data for chloride, in particular, make the omission of sodium and chloride from the NAE equation necessary for a more accurate estimation.<sup>24</sup> Frassetto et al. proposed that a simpler calculation, using the ratio of dietary protein to potassium (protein/K), would provide a valid estimate of effect of diet on acid-base status without the need for accurate data for a complete NAE calculation.<sup>48</sup> Among 141 subjects eating 20 different diets, they found that 71% of the variation of renal NAE was accounted for by the dietary protein-to-potassium ratio ( $r = 0.84$ ,  $R^2 = 0.71$ ).<sup>48</sup> Remer et al., however, in a study of 238 children and adolescents, found that although both estimated NAE and protein/K correlated significantly with measured NAE, the protein/K correlation coefficient was much lower than that for estimated NAE ( $r = 0.36$  vs.  $r = 0.62$ , respectively).<sup>24</sup> Moreover, the standard deviations of the residuals were much smaller for the regression of estimated NAE on measured NAE than for the regression of protein/K on measured NAE, suggesting that estimated NAE may be a more precise predictive calculation.<sup>24</sup> We found that the correlation between estimated NAE and urine pH ( $r = -0.77$ ,  $P = .02$ ) was slightly stronger than that between protein/K and urine pH ( $r = -0.73$ ,  $P = .03$ ). Thus, the estimated NAE calculation seems better able to predict actual NAE and urine pH than protein/K, but protein/K may provide a general indication of urine pH and acid-base status when data on other nutrients are not available for calculating the estimated NAE. More complete and accurate food-composition data in the future may allow for an even more accurate estimation of NAE and prediction of urine pH using diet record data.

## Conclusions

In this study of free-living females, vegans, lacto-ovo vegetarians, and omnivores were found to have decreasing urine pH values, reflecting differences in the diets they consumed. Our findings suggest that the EAA method does not predict urine pH, whereas estimated NAE (and protein/K) correlates with urine pH and therefore may permit general assessments of the effects of diet on acid-base balance. In a research setting, estimated NAE may be used in conjunction with dietary records to provide a better idea of a group's or individual's usual acid-base status, whereas in

the clinical setting, urine pH may be a more easily obtainable measurement. In either case, the assessment of typical diet and resulting acid-base status would help assess associations with disease risk and potentially appropriate dietary modifications.

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