

Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents¹⁻³

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ABSTRACT Normal adult humans eating Western diets have chronic, low-grade metabolic acidosis, the severity of which is determined in part by the net rate of endogenous noncarbonic acid production (NEAP), which varies with diet. To prevent or reverse age-related sequelae of such diet-dependent acidosis (eg, bone and muscle loss), methods are needed for estimating and regulating NEAP. Because NEAP is difficult to measure directly, we sought a simple method to estimate it from diet-composition data. We focused on protein and potassium contents because the production of sulfuric acid from protein metabolism and bicarbonate from dietary potassium salts of organic acids are the major variable components of NEAP. Using steady state renal net acid excretion (RNAE) as an index of NEAP in 141 normal subjects eating 20 different diets, we found by multiple linear regression analysis that RNAE [mEq/d · 10460 kJ diet (mEq/d · 2500 kcal)] was predictable ($R^2 = 0.62$) from protein [g/d · 10460 kJ diet (g/d · 2500 kcal); positive regression coefficient, $P < 0.001$] and potassium [mEq/d · 10460 kJ diet (mEq/d · 2500 kcal); negative regression coefficient, $P = 0.001$] contents, which were not themselves correlated. Among diets, 71% of the variation in RNAE could be accounted for by the ratio of protein (Pro) to potassium (K) content: $\text{RNAE} = 62\text{Pro}/\text{K} - 17.9$ ($r = 0.84$, $R^2 = 0.71$, $P < 0.001$). Thus, by considering both the acidifying effect of protein and the alkalinizing effect of potassium (organic anions), NEAP can be predicted with confidence from the readily available contents of only 2 nutrients in foods. Provisionally, these findings allow estimation and regulation of NEAP through diet modification. *Am J Clin Nutr* 1998;68:576–83.

KEY WORDS Endogenous acid production, renal net acid excretion, potassium, protein, diet, metabolic acidosis

INTRODUCTION

Normal adult humans eating typical American diets characteristically have chronic, low-grade metabolic acidosis (1–4). That persisting perturbation of systemic acid-base equilibrium occurs because metabolism of the diet releases noncarbonic acids into the systemic circulation (eg, sulfuric acid from metabolism of protein) in amounts that exceed the amounts of base released concomitantly (eg, bicarbonate from combustion of organic acid salts of potassium in vegetable foods) (5, 6). The size of the discrepancy between acid and base production determines the net endogenous acid production rate (ie, the net acid load of the

diet), which in turn determines the degree of perturbation of systemic acid-base equilibrium (1, 2, 4). Under normal physiologic circumstances, the net endogenous acid production rate and the degree of the attendant low-grade metabolic acidosis are determined primarily by the composition of the diet (2, 4).

With advancing age, the severity of diet-dependent acidosis increases independently of diet (3, 4). That occurs because kidney function ordinarily declines substantially with age, resulting in a condition similar to that of chronic renal insufficiency (7). Renal insufficiency induces metabolic acidosis by reducing conservation of filtered bicarbonate and excretion of acid. Failure to recognize the respective and independent roles of age-related impaired renal acid-base regulatory capacity and diet net acid load has until recently prevented the recognition that low-grade metabolic acidosis is characteristically present and worsens with age in otherwise healthy adults (2, 4).

The pathophysiologic implications of this chronic, low-grade, diet-dependent, age-amplified metabolic acidosis have been examined by determining the effects of neutralizing the net acid load of the diet with a dietary supplement of base, namely potassium bicarbonate. Potassium bicarbonate is a natural base that the body generates from the metabolism of organic acid salts of potassium (eg, potassium citrate) (8), whose density (ie, mmol K/kJ food item) is greatest in fruit and vegetables. Long-term supplementation of the diet with potassium bicarbonate has numerous anabolic effects. In postmenopausal women for example, calcium and phosphorus balances improve (1), bone resorption markers decrease (1), bone formation markers increase (1), nitrogen balance improves (9), and serum growth hormone concentrations increase (10). These findings suggest that the adverse effects of chronic, low-grade, diet-dependent acidosis are not

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inconsequential and may contribute to such age-related disturbances as bone mass decline, osteoporosis, and muscle wasting.

One way to reduce or eliminate diet-dependent metabolic acidosis is by eating diets that impose little or no net acid load. Present methods for estimating the net acid load from the composition of the diet require a detailed inventory of nutrient composition and estimates of the gastrointestinal absorption rates of the nutrients; these methods have been validated for only a few diets (11, 12). In vivo methods for quantifying net endogenous acid production are complex and labor intensive (5) and are suitable only for specialized clinical research centers. Accordingly, simple dietary guidelines for quantifying and regulating endogenous acid production rates do not exist. In this paper we present a new method for estimating the net acid load of the diet from readily available information on diet composition, specifically total protein and potassium contents. We focused on these 2 components because the rate of sulfuric acid production from protein metabolism and the rate of bicarbonate generation from metabolism of intestinally absorbed potassium salts of organic acids are major and highly variable components of the net endogenous acid production rate (5, 6). This paper describes the empirically derived relations between these 2 components in different diets and how they can be used to predict net endogenous acid production.

SUBJECTS AND METHODS

For this study of normal subjects, we used the steady state rate of renal net acid excretion (RNAE) as an index of the net rate of endogenous noncarbonic acid production (NEAP) (5, 6). We measured RNAE in 42 subjects, each of whom ate 1 of 6 different whole-food diets while residing in the University of California, San Francisco (UCSF) General Clinical Research Center. In addition, we obtained values of RNAE from the literature for 99 subjects eating ≥ 1 of 14 whole-food diets for which pertinent data on nutrient composition were reported (5, 11–15). In some cases the same subjects were restudied while ingesting a second or third diet (5, 11, 13–15). Data were accumulated for 20 different whole-food diets ingested by 141 different subjects, reflecting a total of 199 subject-diet combinations. All subjects ingested a diet for ≥ 1 wk, a period previously shown to be sufficient for establishing a steady state of acid-base equilibrium (2). Selected articles provided at minimum data on diet protein, potassium, and energy contents. We excluded articles in which diets were supplemented with mineral salts because such salts typically supply acid or base equivalents.

Subjects

The subjects were healthy men and women. Subjects participating in the UCSF General Clinical Research Center studies signed informed consent documents as specified by the university's committee on human research. The subjects ranged in age from 17 to 73 y [data on age are lacking for 8 subjects in one article (5)].

Diets

Values for protein, potassium, and energy contents were extracted from the available data for the 20 diets ingested by the 141 subjects included in the analysis. For the 6 diets ingested by the subjects studied in the UCSF General Clinical Research Center, we determined the content of those nutrients either by direct chemical analysis or from diet-composition tables (16). In some of the papers cited, the nutrient content was determined from

chemical analysis of the diet (5, 14, 15); in others, it was estimated by using specific diet-composition tables cited by the authors (11, 12).

For 13 diets sufficient information was available for separately estimating the protein contents of the animal and vegetable foods of the diet (5, 11, 14, 15) or by using *Agriculture Handbook no. 8* (16) as modified by chemical analyses of certain food items used by the UCSF General Clinical Research Center. For those 13 diets we also estimated sulfur content from the methionine and cystine contents of the listed food items (16). Sulfur content was computed from the formula

$$\text{Sulfur (mEq/diet)} = 2 \times [(\text{mg methionine}/149.2) + (2 \times \text{mg cystine}/240.3)] \quad (1)$$

where 149.2 is the molecular weight of methionine and 240.3 that of cystine. For 3 additional diets such estimation was not possible because a detailed listing of food items was lacking, but sulfur contents were specified by the investigators (13). Thus, sulfur contents were available for a total of 16 diets.

For 16 diets, sufficient data were available to calculate the difference between the inorganic cations and anions of the diet, namely $(\text{Na}^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}) - (\text{Cl}^- + \text{P}^{1.8-})$, expressed in milliequivalents. A positive value for the difference (ie, an excess of inorganic cations, or an anion gap) implies that the diet contains an excess of organic anions (eg, citrate) relative to organic cations (eg, lysine), which fills the anion gap. Because metabolism of organic anions yields base equivalents and that of organic cations yields acid equivalents, this difference can be taken as the potential base of the diet. Negative values of potential base are theoretically possible but are uncommon for ordinary diets. Because the Na^+ and Cl^- contents of table salt are equal and because the contents of the 2 ions in natural foods are equal on average (17), we omitted the mutually canceling terms Na^+ and Cl^- in the calculation. That allowed calculation of potential base for diets in which the Cl^- content was not specified.

Data analysis

For analysis of the relation of diet protein content, potassium content, and NEAP, the values for those variables were first collected into a master database comprising the individual values of those variables available for each subject. For the articles that failed to report values for individual subjects (11–13, 15), only the subject-group average was included. As a result, the master database contains individual subject data for 56 subjects and subject-group average data for 85 subjects, yielding a total of 73 data points. From the master database, a working database was developed comprising the average values of the selected variables for each group of subjects ingesting each diet, yielding a total of 20 data points, 1 for each of the 20 diets.

Because NEAP for subjects eating the same diet varies depending on the quantity ingested, most of the data analysis was carried out on values of NEAP adjusted to a standard quantity of diet ingested, expressed as energy intake, namely 10460 kJ/d (2500 kcal/d), a convenient reference value and one that was close to the average energy intake [10033 kJ/d (2398 kcal/d)] of the 141 subjects studied.

Laboratory analysis

RNAE, expressed as mEq/d, was determined as the sum of the excretion rates of titratable acid and ammonium minus that of

TABLE 1
Mean protein, potassium, and renal net acid excretion data for each diet¹

Diet code	Energy intake	Potassium intake	Protein intake	RNAE	Pro/K
	<i>kJ/d</i>	<i>mEq/d</i>	<i>g/d</i>	<i>mEq/d</i>	<i>g/mEq</i>
1	7113	80	75	12	0.94
2	9121	133	60	24	0.45
3	7113	74	75	26	1.01
4	9293	99	74	31	0.75
5	7657	40	39	32	0.96
6	8736	72	85	37	1.19
7	7113	72	75	39	1.04
8	13 104	56	60	40	1.06
9	13 598	75	90	45	1.21
10	10 962	75	78	47	1.04
11	13 765	93	114	53	1.23
12	15 803	73	93	62	1.27
13	10 343	85	99	64	1.17
14	10 209	101	95	70	0.94
15	12 636	98	100	70	1.02
16	8364	41	90	71	2.19
17	8527	58	96	75	1.64
18	9247	40	79	102	1.98
19	14 891	124	193	115	1.56
20	9037	54	120	136	2.21

¹RNAE, renal net acid excretion; Pro, protein in g/d; K, potassium in mEq/d; Pro/K, ratio of Pro to K.

bicarbonate. In our laboratory, the urine bicarbonate concentration was calculated from the measured values of urine pH and carbon dioxide content by use of the Henderson-Hasselbach equation, for which the solubility coefficient of carbon dioxide was taken as 0.0309 and pK' was corrected for ionic strength: $pK' = 6.33 - 0.5 \times ([Na^+] + [K^+])^{0.5}$, where Na^+ and K^+ concentrations are expressed in Eq/L. Urine total carbon dioxide content was determined by thermal conductivity. Titratable acid concentration was determined by titration, and urine ammonium concentration was determined by the phenol method (18). In the articles from the literature surveys, RNAE was calculated as described above from component assays as described in the respective articles (5, 11–15).

Units of measure

Charged species are expressed in milliequivalents to allow calculation (by algebraic summation) of charge balances (ie, estimation of cation or anion gaps) necessary for conclusions about dietary potential acid or base content. The number of milliequivalents of a charged species is equal to the number of millimoles multiplied by the charge valance of the species. There is no SI unit for net acid; the standard units of milliequivalents are used throughout.

Statistical analysis

Statistical analyses were carried out with SIGMASTAT (Jandel Corp, San Rafael, CA).

RESULTS

The subject-group averages for the 20 diets analyzed are summarized in **Table 1**. In the 20 diets, protein content ranged from 39 to 193 g/d, potassium content from 40 to 133 mEq/d, energy

TABLE 2
Regression analyses for 20 diets¹

	Potassium	Protein	Pro/K	R^2	r	P
RNAE						
b_i	-0.440	—	—	—	—	—
B_i	-0.374	—	—	0.14	-0.37	NS
P	NS	—	—	—	—	—
RNAE						
b_i	—	0.792	—	—	—	—
B_i	—	0.597	—	0.36	0.69	0.006
P	—	<0.01	—	—	—	—
RNAE						
b_i	-0.614	-0.937	—	—	—	—
B_i	-0.522	-0.707	—	0.62	—	<0.001
P	<0.005	<0.001	—	—	—	—
RNAE						
b_i	—	—	62.1	0.71	0.84	<0.001
P	—	—	<0.001	—	—	—

¹ b_i , nonstandardized regression coefficient; B_i , standardized regression coefficient; horizontal rows of P values indicate the levels of significance of the regression coefficients. Protein (Pro, in g) and potassium (mEq) are in units/d per 10 460 kJ (2500 kcal) diet. RNAE, renal net acid excretion.

content from 7113 to 15 803 kJ/d (1700 to 3777 kcal/d), and RNAE from 12 to 136 mEq/d. Expressed in units per day per 10 460 kJ diet ingested, the corresponding ranges were similar: protein content, 48–139 g; potassium content, 45–153 mEq; and RNAE, 18–157 mEq. The energy-adjusted values of both protein and potassium contents were normally distributed (Kolmogorov-Smirnov test). The ratio of protein to potassium content varied over a 5-fold range, from 0.45 to 2.21 g/mEq. There was no significant correlation between protein content and potassium content among diets.

By multiple linear regression analysis of the energy-adjusted variables (**Table 2**), protein content and potassium content were independent predictors of RNAE (**Figure 1**; $R^2 = 0.62$, $P < 0.001$):

$$\text{RNAE} = 0.94\text{Pro} - 0.61\text{K} + 22 \quad (2)$$

where Pro is protein.

That is, the regression coefficients of both protein and potassium were significantly different from zero, $P < 0.001$ and $P < 0.003$, respectively. The regression coefficient of protein was positive and that of potassium was negative, suggesting that increasing protein content increases RNAE and increasing potassium content decreases it. Differences in protein content had a slightly greater (1.4-fold) effect on RNAE than did differences in potassium content, as indicated by the values of their respective standardized regression coefficients, namely, 0.71 compared with -0.52 (Table 2).

Similar results were obtained when the regression was performed without adjusting the 3 variables to a constant energy content ($R^2 = 0.67$, $P < 0.001$):

$$\text{RNAE} = 0.91\text{Pro} - 0.57\text{K} + 21 \quad (3)$$

Both regression coefficients were significantly different from zero, $P < 0.001$ and $P < 0.007$, respectively. Differences in protein content had a 1.9-fold greater effect on RNAE than did differences in potassium content, as indicated by the values of their respective standardized regression coefficients, 0.88 compared with -0.46.

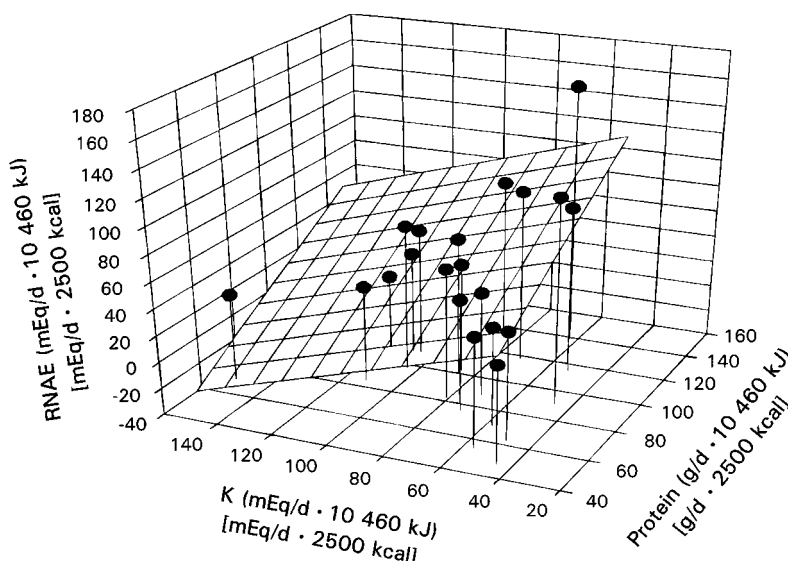


FIGURE 1. The relation between steady state renal net acid excretion (RNAE) and dietary contents of protein (Pro) and potassium for 20 different whole-food diets. $\text{RNAE} = 0.94\text{Pro} - 0.61\text{K} + 22$ ($R^2 = 0.62$, $P < 0.001$). The regression coefficients of both Pro and K were significantly different from zero, $P < 0.001$ and $P < 0.003$, respectively.

Because protein and potassium contents were directionally opposing independent predictors of RNAE by multiple linear regression analysis, the ratio of protein to potassium content in the diet should provide a good linear index of RNAE. Thus, by linear regression analysis, RNAE (mEq/d · 10460 kJ diet) correlated significantly with Pro/K (g/mEq) (Table 2, **Figure 2**; $r = 0.84$, $R^2 = 0.71$, $P < 0.001$):

$$\text{RNAE} = -17.9 + 62.1(\text{Pro}/\text{K}) \tag{4}$$

Similar results were obtained when the variables were not energy adjusted ($r = 0.79$, $R^2 = 0.62$, $P < 0.001$):

$$\text{RNAE (mEq/d)} = -10.2 + 54.5(\text{Pro}/\text{K}) \tag{5}$$

For the subset of 13 diets for which data for both animal and vegetable foods were available, RNAE was highly correlated with animal protein content ($r = 0.84$, $P < 0.0005$) but not with vegetable protein content. The range of variation of vegetable protein content (38 g/10460 kJ diet; minimum and maximum: 18 and 57 g/10460 kJ) was much less than that of animal protein content (104 g/10460 kJ of diet; minimum and maximum: 13 and 117 g/ 10460 kJ), a nearly 3-fold difference. Expressing vegetable food intake in terms of energy ingested likewise did not give a significant correlation with RNAE. The ratio of animal to vegetable protein content of the 13 diets varied from 0.23 to 4.31, a nearly 20-fold range, and the individual values for animal and vegetable protein contents varied independently for the diets, as did animal and vegetable energy ingested.

In the subset of 16 diets for which data for sulfur content were available, RNAE correlated directly with sulfur content ($r = 0.75$, $P < 0.001$; **Table 3**). Sulfur content in turn correlated directly with total protein ($r = 0.78$, $P < 0.001$, $n = 16$ diets; $r = 0.84$, $P < 0.001$, $n = 13$ diets) and with animal protein ($r = 0.88$, $P < 0.001$, $n = 13$ diets). (Correlations for both $n = 16$ and $n = 13$ diets are reported to allow comparison of the relation of sulfur content with both total and animal protein contents

because total protein content was known for all diets but animal protein content for only 13 diets.)

In the subset of 16 diets for which data were available to calculate potential base content, potential base varied from -2 to 141 mEq/d · 10460 kJ diet. RNAE correlated inversely with potential base ($r = -0.57$, $P < 0.003$), and potential base in turn correlated directly with potassium content ($r = 0.89$, $P < 0.001$). By multiple regression analysis, potential base and protein content together accounted for 69% of the variation in RNAE ($P < 0.001$, $n = 16$ diets; **Table 3**). By comparison, for this same subset of diets, protein and potassium content accounted for 64% of

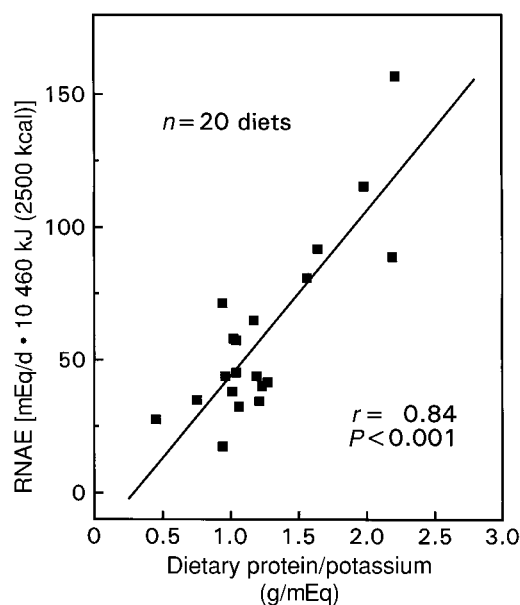


FIGURE 2. The relation between steady state renal net acid excretion (RNAE) and the ratio of dietary protein (Pro, g/d · 10460 kJ) to potassium (mEq/d · 10460 kJ) content for 20 different whole-food diets. $\text{RNAE} = -17.9 + 62.1(\text{Pro}/\text{K})$ ($r = 0.84$, $R^2 = 0.71$, $P < 0.001$).

TABLE 3
Regression analyses for 16 diets¹

	Potassium	Protein	Pro/K	Potential base	Sulfur	S – PB	R ²	r	P
RNAE									
b _i	-0.519	—	—	—	—	—	0.19	-0.43	NS
P	NS	—	—	—	—	—	—	—	—
RNAE									
b _i	—	0.904	—	—	—	—	0.37	0.61	0.013
P	—	0.013	—	—	—	—	—	—	—
RNAE									
b _i	-0.632	1.014	—	—	—	—	—	—	—
B _i	-0.530	0.681	—	—	—	—	0.64	—	<0.002
P	<0.01	<0.002	—	—	—	—	—	—	—
RNAE									
b _i	—	0.794	—	-0.557	—	—	—	—	—
B _i	—	0.534	—	-0.574	—	—	0.69	—	<0.001
P	—	<0.005	—	<0.003	—	—	—	—	—
RNAE									
b _i	—	—	62.7	—	—	—	0.73	0.85	<0.001
P	—	—	<0.001	—	—	—	—	—	—
RNAE									
b _i	—	—	—	-0.624	—	—	0.41	-0.64	<0.01
P	—	—	—	<0.01	—	—	—	—	—
RNAE									
b _i	—	—	—	—	1.24	—	0.56	0.75	<0.001
P	—	—	—	—	<0.001	—	—	—	—
RNAE									
b _i	—	—	—	—	—	0.605	0.68	0.82	<0.001
P	—	—	—	—	—	<0.001	—	—	—
RNAE									
b _i	—	—	—	-0.429	0.994	—	—	—	—
B _i	—	—	—	-0.442	0.602	—	0.74	—	<0.001
P	—	—	—	<0.02	0.002	—	—	—	—

¹b, nonstandardized regression coefficient; B, standardized regression coefficient; horizontal rows of P values indicate the levels of significance of the regression coefficients. PB, potential base, or K+Ca+Mg–P; S, sulfur; RNAE, renal net acid excretion. Protein (Pro, in g), potassium (mEq), PB (mEq), S (mEq), and S–PB (mEq) are in units/d per 10460 kJ (2500 kcal) diet.

the variation in RNAE ($P < 0.001$). Both potassium and potential base contents varied independently of protein content in diets.

By multiple regression analyses, sulfur and potential base contents together accounted for 74% of the variation in RNAE among diets ($P < 0.001$, $n = 16$ diets; Table 3). By comparison, for those same 16 diets, protein and potassium contents together accounted for 64% of the variation in RNAE ($P < 0.001$). Differences in sulfur content had a slightly greater (1.4-fold) effect on RNAE than did differences in potential base content, as indicated by the ratio of their respective standardized regression coefficients, 0.60 and -0.44 , respectively. That 1.4-fold greater effect of sulfur compared with potential base on RNAE is in accord with a 1.3-fold greater effect of total protein compared with potassium for the same diets (standardized regression coefficients, 0.68 and -0.53 , respectively; Table 3). Sulfur and potential base contents varied independently among diets.

By simple regression analysis, we tested whether the difference between sulfur (acid precursors) and potential base (base precursors) predicted RNAE. Sulfur minus potential base correlated directly with RNAE and accounted for 68% of the variation in RNAE among diets ($r = 0.82$, $P < 0.001$, $n = 16$ diets; Table 3, **Figure 3**). For those same 16 diets, protein and potassium correlated directly with RNAE and accounted for 73% of the variation in RNAE among diets ($r = 0.85$, $P < 0.001$). Pro/K and sulfur minus potential base were highly correlated ($r = 0.87$, $P < 0.001$).

Of the 20 diets studied, magnesium content was known for 18 diets. For those diets, in mEq/10460 kJ diet, magnesium content was 27.5 ± 11.9 , potassium content was 82.4 ± 29.3 , and the sum of magnesium plus potassium was 109.9 ± 37.2 ; magnesium content represented $\approx 25\%$ of the total of magnesium plus potassium. Incorporating magnesium content into the data analysis did not improve the overall ability to predict RNAE. By multiple regression analysis with protein content and total potassium and magnesium content as the 2 independent variables, 64% of the variation in RNAE among diets was accounted for ($P < 0.001$, $n = 18$ diets). For those same 18 diets, protein and potassium contents alone accounted for 63% of the variation in RNAE ($P < 0.001$). The ratio of protein content to total content of potassium plus magnesium (Pro/[K + Mg]) correlated less well with RNAE ($r = 0.79$) than did Pro/K alone ($r = 0.85$) ($n = 18$ diets). Substitution of magnesium for potassium in the multiple regression model with protein did not improve R^2 (0.57 compared with 0.63), and the ratio of protein to magnesium did not correlate significantly with RNAE ($r = 0.46$, $P > 0.05$).

DISCUSSION

The results here indicate that in normal subjects steady state RNAE is highly correlated with the ratio of the dietary content of total protein to potassium ($r = 0.84$, $P < 0.001$), 2 components of the diet for which quantitative information is widely available

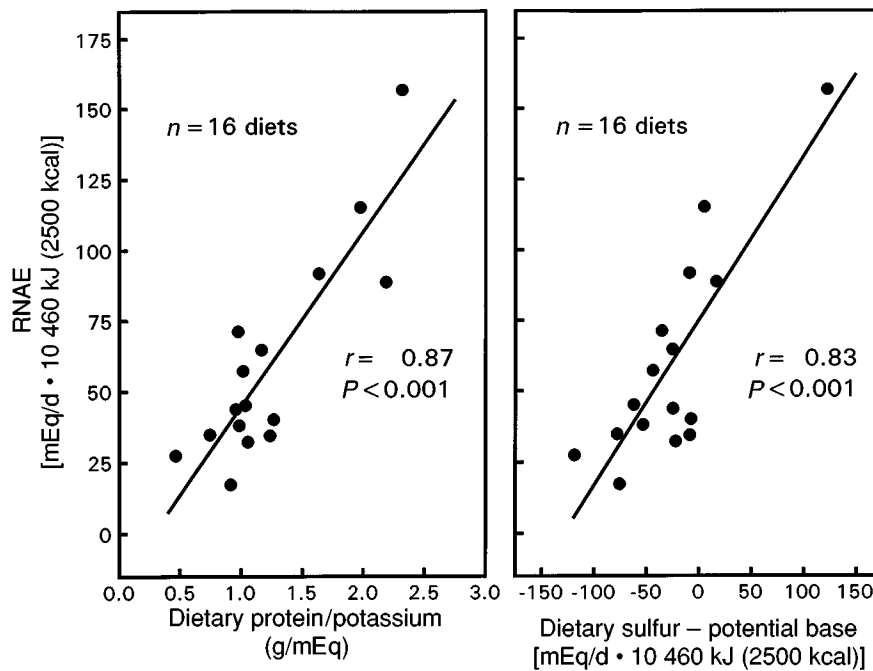


FIGURE 3. Comparison of the predictive ability of dietary sulfur minus potential base and the ratio of protein to potassium on steady state renal net acid excretion (RNAE) for the 16 of 20 diets studied for which sulfur and potential base contents were known. Protein is expressed as g/d · 10 460 kJ; RNAE, potassium, sulfur, and potential base are expressed as mEq/d · 10 460 kJ.

in standard food-composition tables. Given that steady state RNAE in normal subjects corresponds closely with the diet-dependent rate of endogenous acid production (5), these results provide a relatively simple and reliable method for determining and controlling the net acid load of the diet.

In studying the acid-base effects of diet, most workers use steady state RNAE to estimate the net acid load of the diet (ie, the net rate of NEAP) (11–13, 19). In fact, when endogenous acid production is measured in normal subjects by methods that are independent of measurement of RNAE, concomitantly measured RNAE and endogenous acid production are strongly and directly correlated ($r = 0.94$, $P < 0.01$) (5, 6). Accordingly, in normal subjects in a steady state, RNAE is a reliable predictor of the diet net acid load. Indeed, RNAE may be a better index of diet net acid load than that provided by independent measurement of net endogenous acid production because the latter inherits the cumulative errors of the measurement of multiple inorganic constituents of the diet and stool and of organic anions excreted in the urine (5, 6).

In the present study, using RNAE as an index of endogenous acid production, we tested whether the net acid load of the diet could be predicted simply from the nutrient composition of the diet. We focused on dietary protein and potassium contents because the rate of sulfuric acid production from protein metabolism and the rate of bicarbonate generation from metabolism of intestinally absorbed potassium salts of organic acids are major and highly variable components of the net endogenous acid production rate (6). The only other quantitatively significant component of the endogenous acid production rate is organic acid production (eg, lactic acid), reflecting incomplete combustion of carbohydrate and fat to carbon dioxide and water (6). Accordingly, differences in organic acid production resulting from the different diets studied might account for some of the unexplained variation

in RNAE among diets with similar ratios of protein to potassium content (Figure 2). That is, at any given protein-to-potassium ratio, the observed differences in RNAE might be due in part to differences in diet-induced organic acid production. In normal subjects, however, organic acid production varies little among diets, even when the diets are otherwise sufficiently different to yield a 17-fold difference in endogenous acid production over a wide range (7–122 mEq/d) similar to that observed here (11).

The validity of using diet protein content as a surrogate for sulfuric acid production is supported by the finding that urinary sulfate excretion correlated strongly and directly with total protein content ($r = 0.78$, $P < 0.001$, $n = 16$ diets; $r = 0.84$, $P < 0.001$, $n = 13$ diets) and with animal protein content ($r = 0.88$, $P < 0.001$, $n = 13$ diets). The correlation with animal protein content was only marginally stronger than with total protein content ($r = 0.84$ compared with 0.88), and the slopes of the regression lines were identical ($b_1 = 0.81$). Accordingly, for the purpose of predicting the net acid load of the diet from the protein-to-potassium ratio of the diet, it seems that either total protein content or animal protein content can be used. Indeed, for the subset of 13 diets in which animal protein content was available, the correlation of RNAE with the ratio of animal protein to potassium was only marginally greater than that with the ratio of total protein to potassium ($r = 0.90$ compared with 0.86).

Although urinary sulfur excretion correlated both with animal protein content and with total protein content, it did not correlate significantly with vegetable protein content. That apparent lack of correlation may partly reflect the substantially narrower range of variation of vegetable protein content compared with animal protein content in the diets analyzed in this study (range of protein contents for vegetable and animal content, respectively, 18–57 and 13–117 g/10 460 kJ of diet; $n = 13$ diets). It may also partly reflect the greater variation in the sulfur content of veg-

etable proteins compared with that of animal proteins (ie, sulfur content per unit protein content), a finding that is evident from food-composition tables that report sulfur content (20).

Because the sulfur content of vegetable proteins is much more variable than that of animal proteins (20), it may seem surprising that the ratio of total protein (animal plus vegetable) to potassium was such a good predictor of the diet net acid load (Figure 2). Indeed, the greater variability of sulfur content in vegetable compared with animal protein is evident specifically when sulfur content is computed per unit potassium content (20). As noted above, however, differences in vegetable protein content among the diets considered here were substantially less than the corresponding differences in animal protein content, so vegetable protein content would presumably have less weight as a determinant of the overall variability in the diet net acid load. Most important, however, for the estimation of RNAE, the data clearly show that RNAE is highly predictable from the ratio of total protein to potassium content over a wide range of ratios of animal to vegetable protein content. In fact, the ratio of animal to vegetable protein content of the studied diets varied over a nearly 20-fold range, from 0.23 to 4.31; Nevertheless, additional studies with larger numbers of different diets will be needed to determine whether total protein-to-potassium ratio remains generally predictive of the diet net acid load.

Because the net rate of endogenous acid production is the difference between the rates of acid and base production, we also sought a marker for the content of base precursors in the diet. Dietary base precursors are predominantly organic anions, such as citrate, succinate, and other conjugate bases of carboxylic acids, which are predominantly intracellular constituents that the body metabolizes to bicarbonate (6, 8). Organic and inorganic cations both balance the charge of these anions. Organic cations, such as lysine and arginine, however, yield acid equivalents on metabolism and thereby reduce the net base load resulting from metabolism of organic anions (6, 8). [In ordinary diets, the content of organic anions exceeds that of organic cations (5, 6).] Accordingly, the effective base load from organic anions is from the metabolism of that quantity of organic anion with charge balanced by inorganic cations (6). As the predominant intracellular inorganic cation, potassium is the major source of inorganic counter-ion for organic anions. Therefore, we used dietary potassium content as an index of the content of base precursors in the diet.

Indeed, with protein content held constant, potassium content was a significant inverse predictor of RNAE ($P < 0.003$) (Table 2, Figure 1). That is, as potassium content increased for any given protein content, RNAE decreased (Figure 1). Over their respective ranges of variation among diets, potassium content had about one-third less quantitative effect on RNAE than did protein content (the protein effect was 1.4 times greater than the potassium effect). However, together, as independent predictors in a regression model, protein and potassium content accounted for $\approx 60\%$ (Table 2, Figure 1) and as the ratio of protein to potassium content accounted for $\approx 70\%$ of the variation in RNAE among diets (Table 2, Figure 2).

Because cells are rich in magnesium as well as potassium, we tested whether inclusion of diet magnesium content in the analysis would improve the ability to predict RNAE from protein and potassium content. Magnesium content averaged $\approx 25\%$ of potassium content, and the 2 were highly correlated ($r = 0.77$, $P < 0.001$). Inclusion of magnesium content in the mul-

tiple regression model with protein and potassium decreased the unaccounted variability in RNAE by 4%; in the simple regression model of RNAE against Pro/K, inclusion of magnesium, by using Pro/[K + Mg] instead of Pro/K, actually increased the unaccounted variability in RNAE (by 10%). Thus, consideration of diet magnesium content little improved, or worsened, the ability to predict RNAE from protein and potassium content. Substitution of magnesium for potassium in the regression models (ie, using protein and magnesium only) also worsened the ability to predict RNAE.

Several methods for predicting or estimating the net acid load of the diet from its nutrient composition have been reported. Remer and Manz (11) and their colleagues developed a physiologically based calculation model based on 1) the estimated contents of all of the major inorganic components of the diet (sodium, potassium, calcium, magnesium, chloride, and phosphorus) and reference values for their fractional intestinal absorption; 2) the estimated total protein content of the diet, its estimated fractional intestinal absorption, and the estimated average sulfur content of protein from methionine and cystine; and 3) the assumption of a diet-independent rate of organic acid production. Remer and Manz (11) and Manz et al (12) tested the predictive ability of this model for several synthetic and formula diets and for 3 whole-food diets differing in protein and other nutrients. For the 3 whole-food diets, the predicted diet acid loads underestimated measured RNAE, the differences ranging from 8 to 20 mEq/d, or from 11% to 85%, depending on the size of the net acid load (11).

We compared the methods for estimating RNAE by using the simpler models that we developed in this report, which depend on knowing only the protein and potassium content of the diet, with the more complex model of Remer and Manz (11), which requires a more detailed inventory of diet composition. Specifically, to compare the 2 predictors, Pro/K and the Remer-Manz multivariable-calculated RNAE, we computed RNAE according to the Remer-Manz model for all of the diets used in the present study for which sufficient data were available in the published reports to permit that computation (11 diets), and we also computed Pro/K for those diets. Pro/K and the Remer-Manz calculated RNAE were highly correlated ($r = 0.90$, $P < 0.001$, $n = 11$). With protein and potassium used as independent variables in a multiple regression analysis, the RNAE calculated by the Remer-Manz model varied directly with protein content ($P < 0.001$) and inversely with potassium content ($P = 0.006$), and the multiple correlation coefficient was 0.93. Thus, most of the RNAE-predictive power of the Remer-Manz model resides in the protein and potassium contents of the diets.

If the net acid load of whole-food diets can be predicted from their protein and potassium content, then for any large number and variety of individual food items composing such diets, the potential net acid load of the individual food items should correlate highly with their protein and potassium content. That follows because whole-food diets are simply mixtures of a variety of individual food items. Fortunately, it is possible to test for this, because Remer and Manz (19) presented tabular data on the potential net acid load of a large number and variety of individual food items, calculated according to their model, which incorporates all of the major inorganic constituents of the diet plus an estimate of potential sulfuric acid production from protein content. They refer to the potential net acid load of a food item as the PRAL, or potential renal acid load. Using their data for 112 different food items in 10 different categories of foods, we found

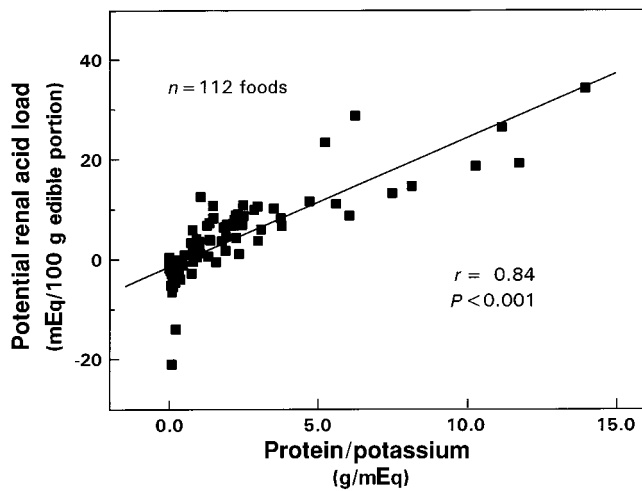



FIGURE 4. The relation between the potential renal acid load and the ratio of protein to potassium content of 112 of 114 food items for which potassium content was not zero. Tabulated by Remer and Manz (19).

that PRAL and Pro/K were highly correlated ($r = 0.84$, $P < 0.001$; **Figure 4**). (Two of 114 tabulated food items were omitted because the listed potassium content was zero, which would have yielded infinite values for Pro/K.) As was the case for the 11 whole-food diets discussed in the preceding paragraph, using protein and potassium as independent variables in a multiple regression analysis, RNAE, which is equivalent to PRAL, varied directly with protein content ($P < 0.001$) and inversely with potassium content ($P < 0.01$), and the multiple correlation coefficient was 0.93. Thus, again, most of the RNAE- or PRAL-predictive power of the Remer-Manz multiple-variable model resides in only 2 diet constituents, protein and potassium, taken together. In summary, the results of this study indicate that in normal humans eating ordinary whole-food diets, the major determinants of differences in NEAP rate among subjects are differences in the protein and potassium content of the diet and that the absolute rate of net endogenous acid production for a given diet can be predicted simply from knowledge of the diet's protein and potassium content. 

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REFERENCES

1. Sebastian A, Harris ST, Ottaway JH, Todd KM, Morris RC Jr. Improved mineral balance and skeletal metabolism in post-

- menopausal women treated with potassium bicarbonate. *N Engl J Med* 1994;330:1776–81.
2. Kurtz I, Maher T, Hulter HN, Schambelan M, Sebastian A. Effect of diet on plasma acid-base composition in normal humans. *Kidney Int* 1983;24:670–80.
3. Frassetto L, Sebastian A. Age and systemic acid-base equilibrium: analysis of published data. *J Gerontol* 1996;51A:B91–9.
4. Frassetto L, Morris RC Jr, Sebastian A. Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. *Am J Physiol* 1996;271:1114–22.
5. Lennon EJ, Lemann J Jr, Litzow JR. The effect of diet and stool composition on the net external acid balance of normal subjects. *J Clin Invest* 1966;45:1601–7.
6. Kleinman JG, Lemann J Jr. Acid production. In: Maxwell MH, Kleeman CR, Narins RG, eds. *Clinical disorders of fluid and electrolyte metabolism*. 4th ed. New York: McGraw Hill, 1987:159–73.
7. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc* 1985; 33:278–85.
8. Halperin ML. Metabolism and acid-base physiology. *Artif Organs* 1982;6:357–62.
9. Frassetto L, Morris RC Jr, Sebastian A. Potassium bicarbonate reduces urinary nitrogen excretion in postmenopausal women. *J Clin Endocrinol Metab* 1997;82:254–9.
10. Frassetto L, Morris RC Jr, Sebastian A. Potassium bicarbonate increases serum growth hormone concentrations in postmenopausal women. *J Am Soc Nephrol* 1996;10:1349 (abstr).
11. Remer T, Manz F. Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. *Am J Clin Nutr* 1994;59:1356–61.
12. Manz F, Remer T, Decher-Splithoff E, et al. Effects of a high protein intake on renal acid excretion in body builders. *Z Ernährungswiss* 1995;34:10–5.
13. Breslau NA, Brinkley L, Hill KD, Pak CYC. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. *J Clin Endocrinol Metab* 1988;66:140–6.
14. Lutz J. Calcium balance and acid-base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. *Am J Clin Nutr* 1984;39:281–8.
15. Lewis NM, Marcus MSK, Behling AR, Greger JL. Calcium supplements and milk: effects on acid-base balance and on retention of calcium, magnesium, and phosphorus. *Am J Clin Nutr* 1989;49:527–33.
16. US Department of Agriculture. *Agricultural handbook no. 8: composition of foods*. Washington, DC: US Government Printing Office, 1997.
17. Yarbrough S, Nix L, Katz R, Korn M, Sebastian A. The quantitative distribution of chloride in food and relation to sodium content. *J Am Diet Assoc* 1988;4:472–5.
18. De Giorgio J. Nonprotein nitrogenous constituents. In: Henry RJ, Cannon DC, Winkelman JW, eds. *Clinical chemistry. Principles and technics*. New York: Harper and Row, 1974:522–6.
19. Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. *J Am Diet Assoc* 1995;95:791–7.
20. Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT. *McCance and Widdowson's the composition of foods*. 5th ed. Cambridge, UK: The Royal Society of Chemistry, 1991.