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Authors

Parmenter, Benjamin H
Slater, Gary J
Frassetto, Lynda A

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ORIGINAL RESEARCH

Accuracy and precision of estimation equations to predict net endogenous acid excretion using the Australian food database

Benjamin H. PARMENTER,[1] Gary J. SLATER[1] and Lynda A. FRASSETTO[2]
[1]Faculty of Health, Science and Engineering, University of the Sunshine Coast, Queensland, Australia and [2]School of Medicine, University of California San Francisco (UCSF), California, USA

Abstract
Aim: The gold standard of measurement for net endogenous acid production (NEAP) is net acid excretion (NAE), a test that is not readily available, and consequently, estimative equations by Remer and Manz and Frassetto *et al.* are often used. These equations rely on nutrient databases and it is recommended that their validity be assessed using a country's database before their application in research in that country. We sought to delineate the accuracy and precision of these estimation equations using the Australian food database.
Methods: In a double blind, randomised, cross-over fashion, healthy participants (n = 13) residing in regional Australia were exposed to varying net acid loads while they collected weighted food diaries and 24-hour urine samples for measurement of NAE.
Results: In comparison to the Frassetto *et al.* equations (equation one bias = −57.1 mEq/day, equation two bias = −32.8 mEq/day), only the Remer and Manz equation was accurate (bias = −5.4 mEq/day); however, all equations were imprecise.
Conclusions: Using the Australian database, the performance of these equations to predict NEAP appears equal to other databases; however, caveats apply in their application. For future research, the equation by Remer and Manz is preferential for group estimates. None of the equations are recommended for individual estimates.

Key words: acid, base, diet, estimation.

Introduction

The homeostatic regulation of acid–base status is essential for physiological functioning, and the clinical consequences of acid–base disorders are well known. Another factor known to influence physiological acid–base systems is the dietary acid load.[1] The ratio of diet-derived fixed acid to base is estimated by the potential renal acid load (PRAL), and when endogenously produced organic acid is factored, cumulatively, this determines the net endogenous acid production (NEAP) for an individual.[1] When diet chronically releases fixed acid in excess of fixed base, the surplus acid has been hypothesised to be a contributing factor to chronic disease.[2,3] While an elevated (acid) NEAP does not result in clinical metabolic acidosis,[4] research is being conducted to determine its relationship to sarcopenia,[5,6] gout,[7,8] renal stones,[9,10] metabolic syndrome,[11] chronic renal insufficiency,[12] late metabolic acidosis in preterm infants,[13] hypertension,[14,15] insulin resistance,[16,17] non-alcoholic fatty liver disease,[18] impaired sports performance[19,20] and osteoporosis,[21,22] while the involvement of NEAP in obesity[23] and cancer[4] has been theorised.

For the research-focused investigation of NEAP, its accurate quantification is essential. To directly measure NEAP, 24-hour urinary net acid excretion (NAE) is the criterion method.[24,25] However, measurement of NAE is a labour- and laboratory-intensive technique and is not readily available.[26] As such, estimative equations from dietary intake were developed. Remer and Manz's equation ($NEAP_R$) factors for endogenously produced organic acid plus the PRAL, that is, the ratio of dietary conjugate bases of potassium, magnesium and calcium to conjugate acids of phosphorus- and sulphate-containing protein.[27] Conversely, the Frassetto *et al.* equations ($NEAP_F$) were developed to allow faster calculations and utilise the more easily obtained inputs of dietary protein and potassium intake.[28] However, world experts have recommended that before these equations are applied within a country, their accuracy is assessed with that country's food

B.H. Parmenter, BNut&Diet (Hons), Student
G.J. Slater, PhD, Senior Lecturer
L.A. Frassetto, MD, Non-Senate Acad Emeritus (WOS), Associate Clinical Professor of Medicine
Correspondence: B.H. Parmenter, Faculty of Health, Science and Engineering, University of the Sunshine Coast, 90 Sippy Downs Dr, Sippy Downs, QLD 4556, Australia. Tel: +61 4 2353 6798. Email: b_p026@student.usc.edu.au

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database.[24,29] To date, this has only been investigated using German[29] and American[28] databases. Consequently, for future research investigating NEAP within Australia, we sought to delineate the accuracy and precision of estimate $NEAP_R$ and $NEAP_F$ using the Australian food database. We hypothesise that estimate $NEAP_R$ and $NEAP_F$ would predict NAE to a similar degree when using the Australian food database as they do when using other databases.

Methods

The design of the trial is described in detail in our companion paper.[30] In summary, in a double blind, randomised, cross-over fashion, healthy participants (n = 13) residing in regional Australia were exposed to varying NEAP loads while they collected weighted food diaries and 24-hour urine samples. To vary the NEAP loads, the participants consumed a fruit and vegetable concentrate or placebo for three days each, with diet standardised throughout. The urine samples were collected under thymol and paraffin oil in portable cooler boxes with ice packs for the subsequent analysis of NAE, pH and creatinine. Compliance to 24-hour urine collections was measured by creatinine index; those with a daily excretion <0.1 mmol/kg body weight were excluded.[31] Diet compositions were determined using Foodworks Professional (Xyris, Brisbane, Australia) using the Australian food database (NUTTAB 2010 Australian Government Nutrient Database, Canberra, Australia). When food items were missing, nutrients were entered according to nutrient information on the food label before proceeding to estimate $NEAP_R$ and two $NEAP_F$ equations (Table 1). The methods were approved by the University of the Sunshine Coast's Human Ethics Committee (reference number: S/14/70), and all participants signed informed consent. The trial was designed to adhere to the Consolidated Standards of Reporting Trials (CONSORT) guidelines, and the trial protocol was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12616000417482).

For the assessment of physique, in the week prior to commencing the study, volunteers presented to the laboratory in a rested state after an overnight fast. After bladder evacuation, participants had their stretch stature measured to the nearest 0.1 cm using a technique previously described,[32] with a Harpenden stadiometer (Holtain Limited, Crymych, UK) and undertook air-displacement plethysmography for assessment of body composition (BODPOD®; COSMED USA, Inc., Concord, CA, USA). The manufacturer's recommended procedure was followed,[33] and a predicted thoracic lung volume was used.[34] During the procedure, the participants wore skin-tight clothing, a silicone cap and removed all metal objects. Following the procedure, body mass index (BMI) was calculated conventionally, while the system's software (Version 5.3.2; COSMED USA, Inc., Concord, CA, USA) estimated fat mass and fat-free mass using the model defined by the Siri equation.[35]

The 24-hour urine samples were analysed on the day of their return for urine pH, creatinine and NAE. For the determination of pH, samples were analysed by a calibrated labChem pH meter (TPS Pty Ltd, Melbourne, Australia) at ambient temperature and 36.5 ± 0.5 °C. Creatinine was measured spectrophotometrically by a colorimetric detection kit Cat no. 09151410 (Enzo Life Sciences, Farmingdale NY, USA) using a multi-scan GO plate reader (Thermofisher Scientific, Waltham, MA, USA). Ammonium was measured using methods of Rice *et al.*[36] by flow injection analysis using a Lachat QuikChem 8000 (Lachat Instruments, Milwaukee, WI, USA). Titratable acids and bicarbonate were measured in duplicate by using the methods of Litkowski and Wilson[37] with the following modifications. Briefly, after addition of 0.05 M hydrogen chloride to 20 mL of urine samples, simmering occurred for 20 minutes at 90 °C. Concurrently, a blank was run in the same manner using MilliQ water (Milliport Corp., Bedford, MA, USA). After samples cooled, bicarbonate was determined as the amount of 0.05 M sodium hydroxide necessary to titrate back to the initial pH at ambient temperature less the corresponding value for the blank. Thereafter, titratable acid was determined as the amount of 0.05 M sodium hydroxide necessary to titrate to pH 7.4 from the bicarbonate endpoint. The mean of the duplicates was used for the calculation of NAE (Table 1), and the intra-assay CV was 8.0%.

Table 1 Equations to estimate the potential renal acid load, the net endogenous acid production and to calculate net acid excretion

Equation	*Formula*
$NEAP_F$ (mEq/day)	Equation 1 = (0.91 × protein (g/day)) – (0.57 × potassium (mEq/day)) + 21 Equation 2 = (54.5 × protein (g/day)/potassium (mEq/day)) – 10.2
$NEAP_R$ (mEq/day)	=PRAL (mEq/day) + organic $acids_{anthro}$
PRAL (mEq)	=0.488 × protein (g/day) + 0.0366 × phosphorus (mg/day) – 0.0205 × potassium (mg/day) – 0.0263 × magnesium (mg/day) – 0.0125 × calcium (mg/day)
Organic $acids_{anthro}$, (mEq/day)	=body surface area × 41/1.73
Body surface area (m^2)	=0.007184 – height $(cm)^{0.725}$ – weight $(kg)^{0.425}$
NAE (mEq)	=titratable acids (mEq) + ammonium (mEq) – bicarbonate (mEq)

Anthro, anthropometrical; NAE, net acid excretion; NEAP, estimate net endogenous acid production where subscript R pertains to the equation by Remer and Manz[27] and subscript F pertains to the equations by Frassetto *et al.*[28]; PRAL, potential renal acid load.

Table 2 Characteristics of participants completing the study

	All participants	*Males*	*Females*
n	13	6	7
Age (years)	35 ± 13	30 ± 9	39 ± 16
Height (cm)	172 ± 7	177 ± 6	166 ± 3
Weight (kg)	73 ± 10	77 ± 7	69 ± 11
Fat mass (kg)	19 ± 10	12 ± 7	25 ± 9
Fat-free mass (kg)	54 ± 11	65 ± 4	44 ± 4
BMI (kg/m^2)	25 ± 3	24 ± 1	25 ± 5

Values are means ± SD.

Table 3 Net acid excretion and estimation of the net endogenous acid production using the Australian food database during the placebo period, supplement period and for all observations

	n	*Placebo*	*Supplement*	*Combined placebo and supplement*
NAE (mEq/day)	10	40 ± 19	7 ± 25	23 ± 27
$NEAP_R$ (mEq/day)	10	48 ± 26	8 ± 26	28 ± 33
NAE (mEq/day)	11	38 ± 18	12 ± 30	25 ± 27
$NEAP_{F^1}$ (mEq/day)	11	85 ± 55	79 ± 55	82 ± 53
$NEAP_{F^2}$ (mEq/day)	11	63 ± 34	53 ± 28	57 ± 30

Values are means ± SD.
NAE, net acid excretion; NEAP, estimate net endogenous acid production where subscript R pertains to the equation by Remer and Manz[27] and subscripts F^1 and F^2 pertain to equations one and two by Frassetto *et al*.[28].

To statistically assess the agreement between estimated and analysed NEAP, the Bland–Altman method for repeat non-constant observations was used (i.e. one observation during the placebo period and one observation during the supplement period on the same participant).[38] Equations were computed using dietary data from the concurrent 24-hour urine collection periods. The distribution of the differences was checked and outliers (±>3.0 SD of the difference) excluded.[39] Those with missing data were excluded.[38] A priori limits of agreement were set at ±15 mEq/day as this would permit a reasonable estimation of NEAP in individuals and also account for imprecision in the criterion method. All statistical analyses were completed using MedCalc (MedCalc Software; MedCalc, Mariakerke, Belgium).

Results

Of the 16 enrolled individuals, 13 completed the study; their characteristics are described in Table 2. Conversely, their dietary intake and a CONSORT diagram are presented in our companion paper.[30] During phase two, one participant returned an incomplete 24-hour urine sample, while another failed to replicate their food and fluid from phase one; both were excluded from analysis. One outlier was identified in the dataset of $NEAP_R$, while none where identified in either $NEAP_F$. The mean ± SD of NAE, $NEAP_R$ and $NEAP_F$ during the placebo period, supplement period and for all observations is presented in Table 3, while Table 4 presents the Bland–Altman bias and limits of agreement for all observations. $NEAP_R$ showed the best agreement with an acceptable accuracy; however, all equations were beyond the a priori limits of agreement to predict NAE in individuals.

Discussion

To our knowledge, this is the first time the estimate NEAP equations have been assessed using the Australian food database. This is important because it impacts the choice of equation potentially used in future research conducted in Australia. Using the Australian database, the performance of these estimate NEAP equations appears similar to their performance reported using other databases. However, known imprecisions within the equations themselves are apparent, which precludes their application to individuals. Of the equations investigated, $NEAP_R$ appears more accurate for group estimates, and its use is, therefore, preferentially encouraged.

Using the Australian food database, the performance of the estimate NEAP equations appears similar to their performance reported using other databases. Similar to the German database, our results show a reasonable group estimate using $NEAP_R$, evident by the small bias (–5.4 mEq).[29] Compared to the American database, investigations were completed under steady-state conditions, which are known to increase the magnitude of accuracy.[28,40] Taken together, there is no reason to suggest that the equations performance is altered when using the Australian database.

Table 4 Bland-Altman agreement between measured 24-hour net acid excretion and net endogenous acid estimation equations in repeat non-constant observations in participants consuming non-steady-state diets using the Australian food database

	n	*Bias*	*95% CI for the bias*	*Limits of agreement*	*95% CI for the upper limit of agreement*	*95% CI for the lower limit of agreement*
$NEAP_R$ (mEq/day)	10	–5.4	–19.8, 9.0	–54.2, 43.3	18.3, 68.3	–79.1, –29.2
$NEAP_{F^1}$ (mEq/day)	11	–57.1	–82.0, –32.2	–146.6, 32.4	10.7, 75.5	–189.7, –103.5
$NEAP_{F^2}$ (mEq/day)	11	–32.8	–48.6, –16.9	–90.7, 25.1	–2.3, 52.5	–118.1, –63.3

NEAP, estimate net endogenous acid production where subscript R pertains to the equation by Remer and Manz[27] and subscripts F^1 and F^2 pertain to equations one and two by Frassetto *et al*.[28]

However, $NEAP_R$ was imprecise, which precludes its application to individuals. This may be because of known errors within the equation surrounding dietary protein and exogenous organic acids.[29] To account for this, Sebastian *et al.*[41] developed another equation that incorporates dietary cystine and methionine intake. Yet, as the Australian database has limited amino acid records, this equation was not computed. That said, no equations currently factor for exogenous organic acids. As such, researchers may wish to revise the equations by factoring for the variability in protein composition as well as exogenous organic acids, perhaps based on food groups.[29]

The inaccuracy of the $NEAF_F$ equations may have been caused by the experimental conditions. That is, the predominance of the supplements base was delivered by a large calcium dose (1.7 g/day); however, both $NEAP_F$ equations do not factor for calcium. Consequently, it appears that these equations are less robust under such circumstances, and $NEAP_F$ is not universally applicable. Moreover, negative-feedback control of endogenous acid production may have influenced NAE in some individuals, further contributing to the equations' imprecision.[42] To this end, it may be most beneficial for future investigations to collect not only urinary PRAL (electrolytes measured in 24-hour urines) but also total urinary organic acids alongside dietary data so as to aid in the elucidation of the issues associated with endogenous and exogenous organic acids and NAE prediction. This may be pivotal in the interpretation of investigations delineating the relationship between NAE and various chronic degenerative diseases as NAE includes both organic acid components, wherein the exogenous component may provide an acid-forming yet health-supporting effect in vivo.[43]

The limitations of the present study include measurement error within the NAE technique, the use of weighted food diaries and small sample size. However, our CV (8.0%) for NAE was below all other studies that reported a CV (10.1–10.9%).[44,45] Consequently, this appears to be a common limitation. The use of free-living weighted food diaries may have introduced error (e.g. under-reporting); however, it may also provide re-assurance that the $NEAP_R$ equation has a reasonable capacity to perform group estimates under free-living conditions. Finally, the small sample size impacts the analysis by the creation of large confidence intervals around the upper and lower limits of agreement. Yet, given that estimations of NEAP are not utilised in a critical clinical setting, the data adequately serves to illustrate which method is preferential for research purposes in Australia. In conclusion, the estimate NEAP equations studied should not be applied to individuals, and $NEAP_R$ is preferential for group estimates.

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Conflict of interest

The authors have no conflicts of interest to declare.

Authorship

We thank GS and BP for the project conception; BP for conducting the research and obtaining donations, Dr Peter Brooks for developing the analytical methods, Daryle Sullivan for the measurement of ammonium and LF for methodological contributions; Michael Nielsen for sourcing essential reagents and materials; BP for performing the statistics and writing the paper; and GS and LF for critical revision of the manuscript and study supervision. All authors have read and approved the final manuscript. The content has not been published or submitted for publication elsewhere.

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