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ORIGINAL RESEARCH

Spot-testing urine pH, a novel dietary biomarker? A randomised cross-over trial

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Aim: Spot-tests of urine pH are claimed to be an accessible biomarker of net acid excretion (NAE), and as such, they may be able to determine changes in an individual's intake of acid- or base-forming foods. To test this hypothesis, we aimed to determine if spot-tests of urine pH could index NAE and relay the consumption of a fruit and vegetable (F&V) concentrate whilst determining this concentrate's capacity to modulate NAE.

Methods: In a double blind, placebo-controlled, cross-over trial, healthy adults ($n = 13$) were allocated by simple randomisation to receive a F&V concentrate or placebo for three days each, with diet standardised throughout. Measurements of 24-hour NAE, 24-hour urine pH and spot-tests of urine pH were taken throughout the study.

Results: The 24-hour urine pH predicted 24-hour NAE ($P = <0.0001$). However, spot-tested urine pH displayed prediction intervals too wide to infer 24-hour NAE and inconsistent ability to reflect concentrate ingestion, despite 24-hour NAE and 24-hour urine pH decreasing (-25.8 mEq, 95% CI -44.3 to -7.4 , $P = 0.01$, $d = 0.94$) and increasing ($+0.51$, 95% CI 0.25 – 0.79 , $P = 0.002$, $d = 1.3$), respectively, following supplementation.

Conclusions: Spot-tests of urine pH are not a valid dietary biomarker of daily NAE and were unable to reliably track changes, despite a F&V concentrate clearly modulating the daily rate of NAE.

Key words: acid, base, diet, pH, urine.

Introduction

The search for dietary biomarkers that accurately assess dietary intake is an active area of investigation.¹ For example, both plasma vitamin C and carotenoids have been considered as biomarkers of usual fruit and vegetable (F&V) intake.² Effectively, such biomarkers could be useful to inform dietitians, patients and researchers alike of intake of specific foods and/or dietary constituents.¹ Indeed, biomarkers that are cost-effective, non-invasive, rapid and accurate may be useful to objectively assess dietary intake, confirm compliance of dietary interventions, help encourage the consumption of healthier food choices and aid researchers to clearly elucidate diet–disease relationships.¹ To this end, the rate of urinary net acid excretion (NAE), also termed the dietary acid load, is predominantly influenced by the intake of fixed acid (in meats and cereals) and fixed base

(in F&V), and consequently, it may be useful to inform an individual's intake or avoidance of acid- or base-forming foods. Yet NAE measurements are labour- and laboratory-intensive, and consequently, spot-testing urine pH was recently proposed as a surrogate cost-effective and accessible biomarker.^{3,4}

Spot-testing is based on the association between 24-hour urine pH and the rate of daily NAE.^{3,5} It has been suggested that by increasing the intake of base-forming foods, there is a decrease in NAE and consequently, an increase in urine pH, a metabolic effect with favourable outcomes associated with chronic degenerative disease.^{3,4,6} While many research groups have suggested or utilised spot-tests to reflect the dietary acid load or to track its modulation,^{7–15} data supporting their efficacy is limited to two studies with conflicting results.^{4,16} Moreover, as urine pH fluctuates over the course of a day,¹⁷ it is unlikely that spot-tests would accurately index NAE. Nonetheless, if NAE can be accurately determined, interventions that influence NAE may be easily monitored.

One purported intervention to facilitate a more basic pH is F&V concentrate, a strategy advocated by industry as a convenient alternative to addressing the low consumption rates of F&V within Australia.¹⁸ However, if applying interventions such as this, there is a need to monitor effectiveness and, moreover, their effect on NAE remains equivocal. As such, we aimed to determine the capacity of spot-tests

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to index NAE and relay the consumption of an F&V concentrate whilst determining this concentrate's capacity to modulate NAE. We hypothesise that spot-tested urine pH would not reasonably infer or track changes to NAE despite an F&V concentrate eliciting a corresponding reduction in NAE and an increase in 24-hour urine pH relative to a placebo.

Methods

A convenience sample of apparently healthy men and women were recruited from the Sunshine Coast region, Queensland, Australia. Enrolment began in May, 2015 and was completed in August, 2015. Participants were included if they were between 18 and 65 years old and were excluded if they used medication (except birth control), alkaline water or mineral and herbal supplements. Furthermore, those with a diagnosed health issue, BMI of $<18 \text{ kg/m}^2$ or pregnant were excluded. Methods were approved by the University of the Sunshine Coast's Human Ethics Committee (reference number: S/14/70), and all participants provided informed consent. The trial was designed to adhere to the Consolidated Standards of Reporting Trials (CONSORT) guidelines and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12616000417482).

The study was a double blind, randomised, placebo-controlled, cross-over trial with a four-day wash out period between two phases (Figure 1). The wash out period was selected as large base loads are excreted within one to two

days following caseation.¹⁹ Calculation of an a priori power using G*power 3 (Düsseldorf University, Düsseldorf, Germany) identified a required sample size of 11 participants to observe an expected change of 0.45 pH units in the first morning fasting urine following a potential renal acid load (PRAL) modulation of -23 mEq/day .¹¹ Consequently, 16 adults were recruited and had their physique assessed using air displacement plethysmography, using protocols described elsewhere.²⁰

During phase one, participants were allocated by simple randomisation (computer-generated list) to receive an F&V concentrate or a relatively neutral concentrate, which acted as a placebo (both supplied by Morlife, Gold Coast, Australia). Participants consumed one serve (15 g/serve) at breakfast, lunch and dinner in water. The F&V concentrate had a total alkali load (PRAL = -43.7 mEq/day) equivalent to approximately 11 extra serves of F&V per day or (PRAL = -14.6 mEq/serve) approximately 3.5 serves per meal (assuming a standard serve of fresh F&V is 100 g, and the average alkalinity per 100 g fresh F&V is -3.7 mEq). The placebo (PRAL = -3.6 mEq/day) was identical in size and similar in appearance, taste and smell. Supplements were preweighed, sealed in silver bags and labelled A and B by a third party (Morlife). This concealed allocation to the investigator who generated the allocation sequence and enrolled the participants. Participants maintained an ad libitum intake and recorded all food and fluid consumed in a weighted food diary (Tanita Co., Tokyo, Japan). Meanwhile, participants completed an exercise record to enable

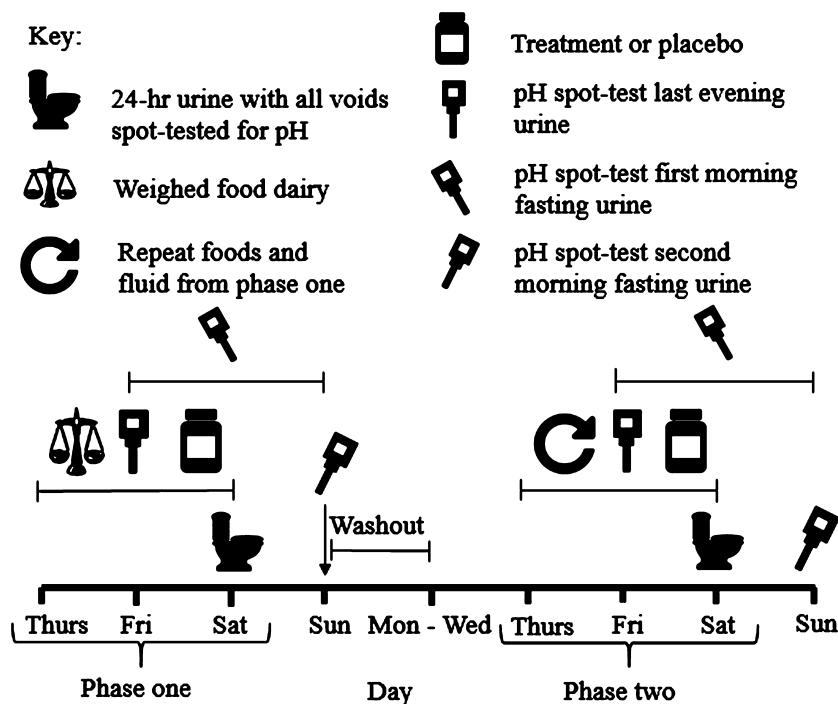


Figure 1 Experimental design during the 11-day trial highlighting the testing procedure. Twenty-four hour urinary net acid excretion (NAE) and pH comprised the primary and secondary outcomes, respectively, whereas spot-tests were incorporated to identify if they could index NAE and track changes to fruit and vegetable concentrate supplementation.

calculation of their ratio of energy intake (EI) to energy expenditure (EE) to screen for inaccurate diet reporters.^{21,22} Those with an EI:EE <0.50 or >1.50 were identified and questioned on their intake to confirm accuracy of records.

During phase two, participants replicated food and fluid from phase one while the intervention crossed over. To ensure compliance, participants were given the meal plan of their previous intake, asked to record any changes and the time foods were eaten. Participants were met daily and questioned on their intake. The concentrates' elemental compositions were analytically determined before, proceeding to analyse the diet composition. The concentrates were analysed for nitrogen in duplicate by combustion analysis using a Leco TruMac N CNS analyzer (Leco Corporation, St. Joseph, MO, USA). Element composition was determined by 5:1 nitric-perchloric acid digests and Inductively Coupled Plasma Optical Emission Spectrophotometry using a 700-ES Series Axial (Aligent Corporation, Palo Alto, CA, USA) by the Analytical Services of the Land, Food and Crop Sciences Department (University of Queensland, Brisbane, Australia; Table 1). Diet compositions were determined by 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 net endogenous acid production (NEAP) by the Remer and Manz equation.²³

For the urinary parameters, two 24-hour urine samples were collected on the third day of each phase and analysed as described elsewhere.²⁰ To spot-test urine, participants were trained to evacuate their bladder, collect a sample mid-stream and immediately test the pH at different time points on different days throughout the study (Figure 1). During the 24-hour collections, samples were spot-tested prior to their addition to 24-hour vessels. Spot-tests were completed using electronic Hanna HI98103 Checker meters (Hanna Instruments, Woonsocket, RI, USA), which quantify pH within ± 0.1 ; these were calibrated daily.

To determine whether pH could index NAE, regression analyses with 95% prediction intervals were used. The 24-hour pH was compared to the same day's 24-hour NAE. Only the last evening pH on the days of 24-hour sampling was compared to 24-hour NAE. The first and second morning spot-tests were taken the morning following 24-hour collection and compared with the previous day's 24-hour NAE. Independent samples were assumed, and prior

checking occurred for linearity, independence of error terms (Durbin-Watson), normality of residuals, heteroscedasticity and outliers ($\pm >3$ SD). To assess the ability of spot-tests (last evening and first morning) to track changes following NEAP modulation, a two-way analysis of variance (ANOVA) with repeated measures (days X treatment) was used. Data were checked for normality, outliers ($\pm >3$ studentised residuals) and sphericity (Mauchly's test). In the instance a participant's food intake drifted resulting in a PRAL deviation of >3 mEq on any day during cross over, the participant was excluded from the ANOVA. Non-normal data was transformed by square root and, again, assumptions checked. Effects sizes were calculated as partial eta squared (η^2). Additionally, the average of the maximum and minimum spot-tests on the days where all voids were spot-tested is reported, as is the percent of participants who had maximum and minimum pH units >1.5 pH units apart.

To assess the concentrate's capacity to modulate NEAP, paired t-tests were computed for the primary (24-hour NAE) and secondary (24-hour pH) endpoints. In addition, such analysis was carried out on the second morning spot-test. For these analyses, only participants whose intake drifted (>3 mEq) on the day of the 24-hour collections were excluded. Outliers (by boxplot) and normality of difference scores was checked, whereas effects sizes were calculated as Cohen's *d*. All statistical analyses were completed using Microsoft Excel (XP professional edition; Microsoft Corp, Redmond, WA, USA), SPSS version 11 (SPSS Inc., Chicago, IL, USA) and MedCalc (MedCalc Software, MedCalc, Mariakerke, Belgium). Summary data are presented as mean \pm SD. Two-tailed significance was accepted at $P \leq 0.05$, and the Shapiro-Wilk test was used to accept normality at $P \geq 0.05$.

Results

Of the 16 enrolled individuals, 13 completed the study (Figure 2); their characteristics are described in our companion paper.²⁰ Two participants were identified as potential inaccurate diet reporters, yet both revealed a reason why their intake was low. One participant was time restriction fasting and consequently consuming a hypocaloric diet, whereas the other reported limited food access that week. As their EI:EE ratio was not impacted by misreporting, both were included in the analysis. However, one participant misplaced their written recipes following phase one, resulting in a change of meals, and another returned an incomplete 24-hour urine sample in phase two. The two aforementioned, along with a third, were identified as having drifted >3 mEq in their food intake on the second day of phase two. For participants who successfully completed all requirements, their dietary intake is presented in Table 2. One participant reported slight occasional bloating when consuming the F&V supplement; however, no other adverse events occurred.

Concerning the capacity of spot-tests to index NAE, only the single-incomplete 24-hour urine was excluded in the

Table 1 Nutrient analysis of the powered fruit and vegetable supplement and the placebo

	Supplement	Placebo
Protein (g/45 g)	5.57	1.85
Phosphorus (mg/45 g)	70	42
Potassium (mg/45 g)	880	237
Magnesium (mg/45 g)	360	21
Calcium (mg/45 g)	1720	54

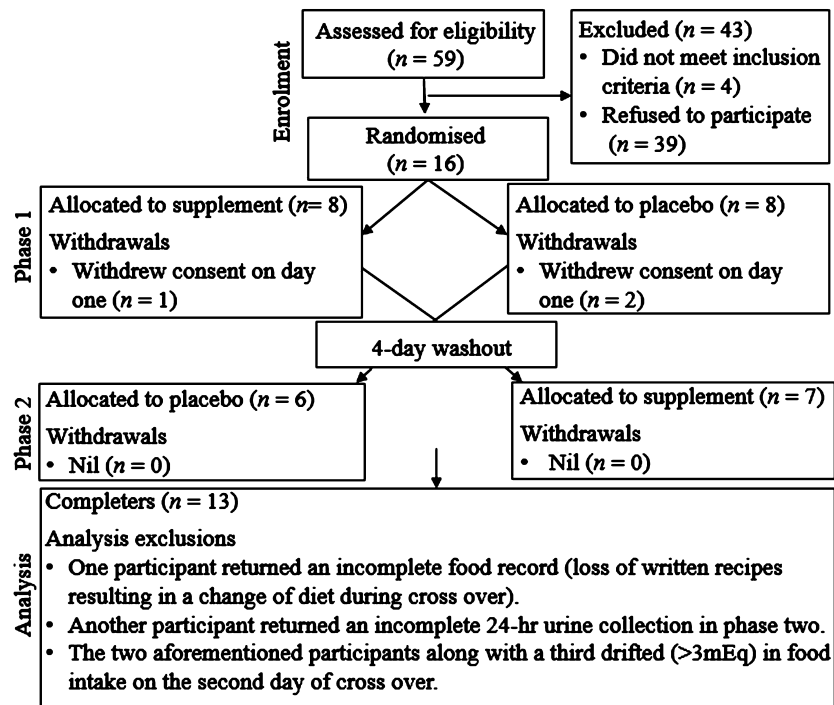


Figure 2 Consolidated Standards of Reporting Trials (CONSORT) diagram describing the flow of participants through the trial.

Table 2 Daily nutrient intake (including supplementation) for participants. Nutrient data for participants identified as altering their food intake during phase two or returning an incomplete urine collection are omitted from that day for both phases

	Supplement			Placebo		
	Thursday (n = 11)	Friday (n = 10)	Saturday (n = 11)	Thursday	Friday	Saturday
Energy (MJ)	9.0 ± 3.6	9.8 ± 3.5	9.6 ± 3.3	8.9 ± 3.6	9.8 ± 3.5	9.6 ± 3.3
Protein (g)	141 ± 58	143 ± 63	143 ± 67	138 ± 58	139 ± 63	139 ± 67
Phosphorous (g)	1.7 ± 0.8	1.9 ± 0.9	1.7 ± 0.7	1.7 ± 0.8	1.9 ± 1.0	1.6 ± 0.7
Potassium (g)	5.6 ± 2.0	5.3 ± 1.6	4.9 ± 1.5	4.9 ± 2.0	4.7 ± 1.6	4.3 ± 1.5
Magnesium (g)	0.9 ± 0.3	0.9 ± 0.3	0.8 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.5 ± 0.3
Calcium (g)	2.6 ± 0.6	2.7 ± 0.5	2.7 ± 0.5	0.9 ± 0.6	1.1 ± 0.6	1.0 ± 0.5
NEAP _R (mEq)	-39 ± 39	-29 ± 45	18 ± 40	1 ± 39	12 ± 45	58 ± 40
NEAP _R _{supp} - NEAP _R _{placebo} (mEq)	-40	-40	-40			

Values are means ± SD.

NEAP, estimate net endogenous non-carbonic acid production where subscript R pertains to the equation by Remer and Manz.²³

analyses. It was found that 24-hour pH predicted 24-hour NAE ($F(1,23) = 108.7$, $P = <0.0001$), where 82.5% of the variability in 24-hour NAE was explained by the variability in 24-hour pH. Conversely, both the first ($F(1,23) = 2.8$, $P = 0.11$) and second ($F(1,23) = 0.83$, $P = 0.37$) morning fasting pH could not significantly predict NAE, and their variabilities explained 10.9 and 3.5% of NAE variability, respectively. While the last evening spot-tests predicted NAE ($F(1,23) = 10.2$, $P = 0.004$), the variability in spot-tests accounted for 30.8% of the variability in NAE, and the 95% prediction intervals are markedly wide (Figure 3). The mean maximum and minimum of all spot-tested voids

captured during the 24-hour collection days was 7.0 ± 0.6 to 5.4 ± 0.4 , where 64% of participants had maximum and minimum pH units >1.5 units apart.

Concerning the effects of the F&V concentrate on the urinary indices, in the last evening void, two outliers were identified and removed. There was neither two-way interaction ($F(2, 14) = 1.5$, $P = 0.27$, partial $\eta^2 = 0.17$) nor was there a main effect of time ($F(1,7) = 0.19$, $P = 0.83$, partial $\eta^2 = 0.03$); however, there was a main effect of treatment ($F(1,7) = 8.5$, $P = 0.02$, partial $\eta^2 = 0.55$), where on average the pH in the supplement group was +0.31 (95% CI, 0.06–0.56) higher than the placebo. Conversely, in the

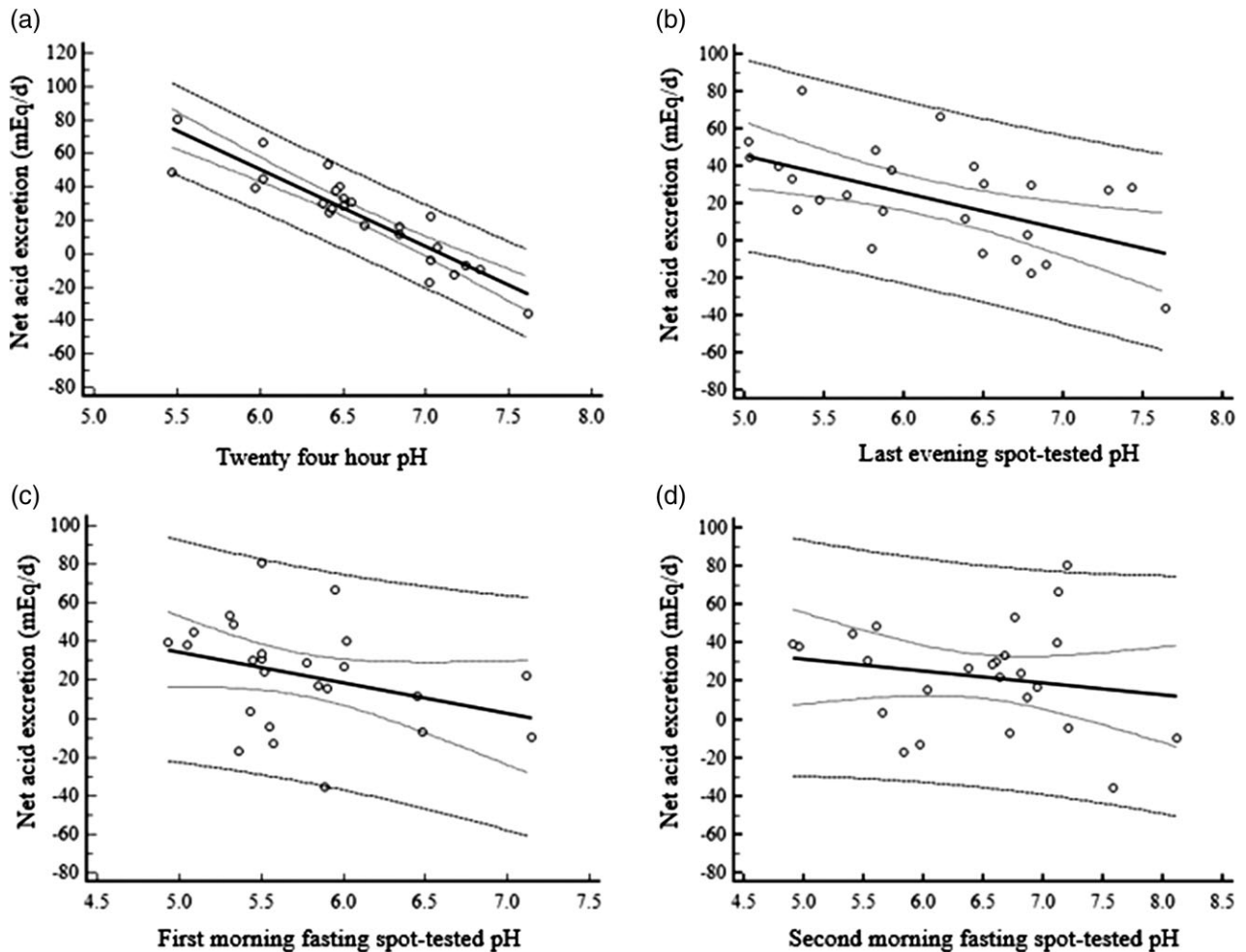


Figure 3 Linear regression with 95% confidence intervals for the regression line and 95% prediction intervals of urinary pH to predict 24-hour net acid excretion in participants ($n = 25$ (assuming independent observations)) for (a) pH measured in the same day's 24-hour urine collections at 36.5 ± 0.5 °C, (b) pH spot-tested in the last void of that evening, (c) pH spot-tested in the following morning's first void in the fasted state and (d) pH spot-tested in following morning's second void in the fasted state.

following morning's first spot-tested pH, after transformation of the data, there was neither two-way interaction between treatment and time ($F(2,18) = 0.263$, $P = 0.77$, partial $\eta^2 = 0.03$) nor was there a main effect of treatment ($F(1,9) = 2.30$, $P = 0.12$, partial $\eta^2 = 0.25$) or time ($F(1,9) = 0.20$, $P = 0.82$, partial $\eta^2 = 0.02$). Again, the change in the second morning fasting spot-test of $+0.41$ (95% CI, -0.09 – 0.91 , $t(10) = 1.814$, $P = 0.1$, $d = 0.55$) was not significant; however, in the 24-hour urine, there was a significant decrease in NAE of -25.8 mEq/day (95% CI, -44.3 to -7.4 , $t(10) = -3.12$, $P = 0.01$, $d = 0.94$) and increase in pH of $+0.51$ (95% CI, 0.25 – 0.79 , $t(10) = 4.23$, $P = 0.002$, $d = 1.3$) following supplementation.

Discussion

Spot-testing urine pH has been considered a potential biomarker for NAE. The major finding of our study is that spot-testing urine pH is not an efficacious measure of NAE.

Secondly, while an F&V concentrate modulated the rate of NAE, spot-tested urine pH was unable to reliably identify this change. Taken together, spot-testing urine pH is unreliable to inform dietitians and researchers of NAE and, seemingly, changes in alkali food intake; spot-testing urine pH to do either is not recommended.

Spot-tests of urine pH have been considered a potential biomarker for dietary acid load. While we found that 82.5% of the variability in 24-hour pH explained the variability in 24-hour NAE, the variability in spot-tests did not reasonably explain the variability in 24-hour NAE, and their prediction intervals were too wide to reasonably infer 24-hour NAE. Our data also captured the known fluctuation in urine pH over a day where 64% of participants had maximum and minimum pH >1.5 units apart. In light of these findings, we agree with Remer *et al.*²⁴ that spot-tested urine pH does not predict the dietary acid load. Consequently, it may be necessary to interpret data from groups that have utilised spot-tests to reflect the dietary acid load or to track

its modulation with caution.^{7–15} While future research groups may suggest averaging multiple spot-tests throughout the day to index NAE, it is advised that urine is a complex buffered solution that precludes averaging.²⁵ In summary, 24-hour collections are required for NAE measurement.²⁴

As spot-tests did not reasonably index 24-hour NAE, not surprisingly, their efficacy to track consumption of the F&V concentrate was inconsistent. Yet, other studies tracking modulation of the diet's acid load with spot-tests all reported significant changes.^{9–12} While we found that the last evening spot-test significantly increased during supplementation, no significant change occurred in the following morning's spot-test despite a reasonable effects size. The most plausible explanation is that following supplementation at dinner, the excess base was mostly excreted that evening. This elevated the pH of the last evening void, and consequently, as the excess base was excreted, this removed the effect on the following morning's spot-tests. This explanation concurs with the findings of other studies that have measured NAE pre- and post-prandial.^{26–28} These studies report NAE significantly altered following a meal when the base load was modulated. Given the relationship between pH and NAE of the same sample, it appears reasonable to suggest that pH also alters. Indeed, if nutrient transition times impact urine pH, this may explain the findings of other studies. For example, Anton *et al.*⁹ found significant changes when they supplemented with powdered F&V pre-bed and spot-tested the following morning's void, whereas others have reported high variability in day-to-day spot-tests.^{10,12} As a result, the data suggest spot-tests are unreliable to track NAE modulation, yet they may crudely reflect the acid–base constituents of the last meal.

Following supplementation at dinner, we found that the last evening's spot-test significantly changed. As such, it may be that spot-tests following a meal have the capacity to inform dietitians of the acid–base constituents of that meal. However, in the context of the real world, this is unlikely as NAE is reflective of a ratio of dietary acid to base intake. Consequently, if there is an increase in base intake and a concurrent equivalent increase in acid intake, the NAE and thus pH remains unaltered. Likewise, simply lowering meat intake may result in an alkalinised pH, which may be mistaken as an increase in F&V intake. In this light, the capacity for spot-tests to inform dietitians of the relative constituents of the last meal becomes non-sensical. Indeed, the reason why we found a significant change in the last evening pH is likely because we standardised dietary intake. Moreover, spot-tests are likely to be further confounded by the consumption of multiple meals throughout the day and the potential for overlapping in the excretion of their respective metabolites, coupled with variations in urination times, on top of differing rates of endogenous organic acid production.

This is the first study to examine the effect of short-term F&V concentrate ingestion on NAE, and we found that this supplement significantly alkalinised NAE and 24-hour pH. Given that the intervention was implemented for both

genders with a wide age bracket, this appears reasonably generalisable. Although, investigators should be aware that different concentrates likely differ in effect because of their different quantities of bases. Moreover, the decrease in NAE was -25.8 mEq, whereas the expected change was -40.1 mEq. This may be because of limitations in NEAP calculation models. That is, the supplement contained powdered F&V, and consequently, it also contained an unknown concentrated quantity of plant-derived organic acids. As models do not factor for plant-derived organic acids, there may have been an over-estimation in the dose.²³ In brief, our findings show that this supplement tangibly modulates NAE, although we are unsure of any impact on clinical outcomes.

The study is limited by the use of ad libitum diets and temperature issues in the measurement of NAE. That is, participants were responsible for replicating their food and fluid intake during phase two and it is possible some may have deviated. However, the dietitian met daily with the participants to ensure compliance, and the urine data appears to suggest that for the most part, the participants were compliant. Finally, titration of urine at ambient temperature theoretically introduces a slight error; however, the magnitude would be too small to markedly alter the results, and the 24-hour pH was standardised to the temperature of spot-tested pH. In conclusion, spot-tests of urine pH are not a valid biomarker of the dietary acid load and were unable to reliably track changes to it, despite an F&V concentrate clearly modulating 24-hour NAE.

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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 ammonia and L.F. 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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