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ORIGINAL ARTICLE

Established dietary estimates of net acid production do not predict measured net acid excretion in patients with Type 2 diabetes on Paleolithic–Hunter–Gatherer-type diets

LA Frassetto[1], L Shi[2], M Schloetter[1], A Sebastian[1] and T Remer[2]

BACKGROUND/OBJECTIVES: Formulas developed to estimate diet-dependent net acid excretion (NAE) generally agree with measured values for typical Western diets. Whether they can also appropriately predict NAE for 'Paleolithic-type' (Paleo) diets—which contain very high amounts of fruits and vegetables (F&V) and concurrent high amounts of protein is unknown. Here, we compare measured NAEs with established NAE estimates in subjects with Type 2 diabetes (T2D).
SUBJECTS/METHODS: Thirteen subjects with well-controlled T2D were randomized to either a Paleo or American Diabetes Association (ADA) diet for 14 days. Twenty-four hour urine collections were performed at baseline and end of the diet period, and analyzed for titratable acid, bicarbonate and ammonium to calculate measured NAE. Three formulas for estimating NAE from dietary intake were used; two ($NAE_{-diet\ R\ or\ L}$) that include dietary mineral intake and sulfate- and organic acid (OA) production, and one that is empirically derived ($NAE_{-diet\ F}$) only considering potassium and protein intake.
RESULTS: Measured NAE on the Paleo diet was significantly lower than on the ADA-diet ($+31 \pm 22$ vs 112 ± 52 mEq/day, $P = 0.002$). Although all formula estimates showed similar and reasonable correlations ($r = 0.52$–0.76) with measured NAE, each one underestimated measured values. The formula with the best correlation did not contain an estimate of dietary OA production.
CONCLUSIONS: Paleo-diets are lower in NAE than typical Western diets. However, commonly used formulas clearly underestimate NAE, especially for diets with very high F&V (as the Paleo diet), and in subjects with T2D. This may be due to an inappropriate estimation of proton loads stemming from OAs, underlining the necessity for improved measures of OA-related proton sources.

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INTRODUCTION

Paleolithic type diets (Paleo) are similar to those consumed by our preagricultural hunter–gatherer ancestors, characterized by high consumption of lean animal-source protein and uncultivated plant-source foods (mostly fruits, vegetables, and nuts, but no legumes or grains) and a low salt intake.[1] Numerous authors[2–4] have argued that a discordance between our contemporary diets and the paleolithic-type diets to which evolutionary forces adapted our core metabolism and physiology over a period of millions of years of hominin evolution, contributes in a major or critical way to the pathogenesis of the so-called diseases of civilization, for example, Type 2 diabetes (T2D), hypertension and cardiovascular disease. The somewhat higher blood acid levels induced by higher dietary acid loads, as well as the higher salt intake[5] of modern western diets have been discussed as one possible mechanism mediating the development of these metabolic abnormalities.

Net acid loads that result from consuming typical American and European diets average around $+60$ to $+70$ milliequivalents (mEq)/day;[6–8] those of vegetarians and/or fruits and vegetables (F&V) preferring people are usually lower.[8–10] Estimates of diet net acid load using current methodologies for Paleo-diets are almost all negative, that is, Paleo-diets on average would be expected to be net base producing.[11]

Total daily net proton loads produced by the body in response to both dietary intake and metabolic processes[12] can be directly quantified as net acid excretion (NAE) using a titration method in 24-h urine samples. In addition, two major approaches for estimating NAE based on dietary intake exist. One considers different dietary minerals, as well as sulfate generated from ingested protein and additionally includes an estimate for total endogenous organic acid (OA) production. The mineral- and sulfate-dependent diet acid load part is frequently expressed as potential renal acid load (PRAL),[13] and correspondingly the respective NAE is herein denoted: $NAE_{-diet\ R\ or\ L} = PRAL + OA$-estimate. The second simpler method relies on the two major dietary determinants of endogenous acid production, namely protein ('acid') intake and potassium ('base') intake [$NAE_{-diet\ F} = potassium + protein$] (for details, see Table 1). NAE estimates have been obtained in a number of epidemiological studies related to diet and acid-base balance using both methods.[14,15]

Mounting evidence suggests that OA production is related to body size[8,16] and also increases if high amounts of particular F&V (containing ample amounts of OAs not combustible to bicarbonate) or proteins are ingested.[16] As long as the protein increase occurs in parallel with a reduction in F&V ingestion, the body size-dependent OA production will not vary greatly, and total NAE will rise according to the increase in the mineral- and

[1]University of California San Francisco, San Francisco, CA, USA and [2]Research Institute for Child Nutrition, Dortmund, Germany. Correspondence: Dr L Frassetto, University of California San Francisco, UCSF Campus box 0126 505 Parnassus Avenue, room 1202M, San Francisco, CA 94143, USA.
E-mail: frassett@gcrc.ucsf.edu
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sulfate-dependent acid load (that is, PRAL). However, if both protein and F&V rise, as is often the case in Paleo-diets, the PRAL reduction due to the marked elevation in F&V intake may no longer result in a corresponding NAE reduction due to a marked rise in OA production from increased ingestion of protein, as well as F&Vs.

Some data has suggested that subjects with T2D may be predisposed to have low urine pH and higher NAE.[17] Using data from an interventional diet study with a high protein, high alkali intake (the Paleo-diet), we tested whether the more sophisticated and specific dietary mineral-, sulfate- and OA-related estimation of total endogenous acid production ($NAE_{\_diet\ R\ or\ L}$) would correlate well with measured NAE after marked increases in F&V intakes. The control diet was an American Diabetic Association type diet (ADA-diet; also with high protein, but lower potassium intake). Participants were well-controlled Type 2 diabetic patients for whom the ADA-diet had been recommended *per se*.

MATERIALS AND METHODS

Subjects with well-controlled T2D who were recruited for this study completed the 24-h urine collections for NAE. Exclusion criteria included use of insulin, thiazolidinediones, thiazide diuretics, blockers of the renin-angiotensin system, medical conditions such as pregnancy, cardiac, pulmonary or gastroenterologic disease, anemia, thyroid dysfunction or inability to follow the diets. Subjects with any renal abnormalities were specifically excluded. The study was reviewed by the University of California San Francisco IRB, listed on clinicaltrails.gov (NCT00548782), and all subjects signed informed consent. Subjects were randomized to either the Paleo-Diet arm or ADA arm (see Table 2 for diet compositions). Two-day alternating menus were used to decrease subject boredom with the diet.

Subjects came to the University of California San Francisco Clinical Research Center daily for the blood, urine and physical function testing on days −2 to 0 and days 19–21 and for their meals on days 1-21. Subjects were weighed daily and calorie intake adjusted to maintain baseline weight.

On days −2 to 0, subjects brought in 24-h urine collections and had fasting blood samples and 24-h diet recalls on their usual diet. Subjects were then placed on 'ramp up' diet regimens for days 1–7 to gradually increase their intake of potassium and fiber, and then for the next 14 days (days 8–21) ate their assigned diet. The Paleo-diet contained no dairy products, no grains, no legumes, no added salt and no refined sugars; the ADA-diet contained minimal refined sugars. Diet composites were analyzed for calorie, fat and protein content, cysteine and methionine, sodium, potassium, chloride, magnesium, calcium and phosphate (Covance, Madison WI, USA). The tests done during days −2 to 0 were repeated during the last 3 days of the study (days 19–21).

Twenty-four hour urine collections were done under oil with thymol added as a preservative. Urines were analyzed for creatinine, sodium, potassium, chloride, phosphate, calcium, magnesium, pH, ammonium and titratable acid (TA). Ratios of urinary potassium to sodium, and potassium to creatinine, were calculated as a measure of compliance with the diet. All urine analytes were measured by Quest Diagnostics (San Jose, CA, USA) except ammonium, pH and TA. Analyses of ammonium were performed using the enzymatic method with glutamate dehydrogenase on Roche Cobas 6000 by Dr Jan Simoni at UT Southwestern. Glutamate dehydrogenase catalyzes the reductive amination of 2-oxyglutarate with $NH4^+$ and NADPH to form glutamate and $NADP^+$. The concentration of the $NADP^+$ formed was directly proportional to the $NH4^+$ concentration. It was determined by measuring the decrease in absorbance at 340 nm. The results were expressed in μmol/l. To measure level of $NH4^+$ in urine, the samples were diluted 100 × . TA and bicarbonate were measured using a three step titration.[18] Creatinine index was used as a measure of completeness of the collection and urine sodium and potassium content were used as a measure of compliance with the diet. NAE was calculated from the measured urine ammonium (NH_4) plus TA minus bicarbonate (HCO_3) (Table 1). HCO_3 was calculated from urine pH.[19]

Based on dietary data, NAE was estimated using three different formulas (Table 1). Those developed by Remer and Manz[8] ($NAE_{\_diet\ R}$) and Lemann ($NAE_{\_diet\ L}$)[10] were both based on the equation, $NAE_{\_diet}$ = PRAL + OA-estimate. These two formulas differed between each other mainly with the estimation methods for OA (for details see Table 1). The formula developed by Frassetto *et al*. ($NAE_{\_diet\ F}$)[19] used the simpler equation, $NAE_{\_diet}$ = potassium + protein (Table 1), which does not include OA.

SAS procedures (version 9.1; SAS Institute Inc, Cary, NC, USA) were used for data analysis. A P-value < 0.05 was considered significant for the statistical tests. Descriptive data are given as mean (s.d.) and range. The Pearson coefficients were calculated for the correlations between measured urinary NAEs ($NAE_{\_urine\ M}$) and the different estimates of NAE ($NAE_{\_diet\ R}$, $NAE_{\_diet\ L,\ or}$ $NAE_{\_diet\ F}$) based on dietary records. The corresponding coefficient of determination (R^2) was also calculated. $R^2 \times 100$, indicates the percentage of the variation of $NAE_{\_urine\ M}$ being explained by the respective NAE_{diet}. All variables used were also checked for normality and where required, natural log transformation was performed.

Table 1. Calculation formulas for measured and estimated net acid excretion and estimates of organic acids used

Variable	*Calculation*
$NAE_{\_urine\ M}$ (mEq/day)[a]	= TA + NH_4 -HCO_3 [TA, NH_4, HCO_3 in mEq]
$NAE_{\_diet\ R}$ (mEq/day)[b]	= dietary PRAL + OA_est_Remer
$NAE_{\_diet\ L}$ (mEq/day)[c]	= dietary PRAL + OA_est_Lemann
Dietary PRAL (mEq/day)	= ($PO_4 \times 0.642$–0.355) × 1.8 + Protein × 0.49-K × 0.8 − (Ca × 0.17 + 1.496) × 2 − (Mg × 0.39–0.946) × 2 [PO4, K, Ca, Mg in mmol and protein in g]
$OA_{\_diet\ R}$	= BSA × 41/1.73; BSA (mm^2) = [(Height (ic) × Weight (lb)/3131] $^{0.5}$
$OA_{\_diet\ L}$	= 32.9 + 0.15 × (K + Cax2 + Mg × 2–PO_4 × 1.8)
$NAE_{\_diet\ F}$ (mEq/day)[d]	= 0.9 × protein − 0.57 × (K × 0.8) + 21 [K in mmol andprotein in g]

Abbreviations: BSA, body surface area; HCO_3, bicarbonate; NAE, net acid excretion; NH_4, ammonium; OA, organic acid; PRAL, potential renal acid load; TA, titratable acid. [a]Urine NAE was calculated from the measured urine ammonium plus titratable acid minus bicarbonate. [b]NAE was estimated using the methodology pioneered by Drs Remer and Manz;[8] organic acid component was estimated based on a function of body size. [c]NAE was estimated by Lemann,[10] which differentiated with Remer Method only in organic acid components. OA was estimated from a function of dietary intakes of several mineral cations and anions. [d]NAE was estimated by Frassetto *et al*.,[18] who used dietary protein as an estimated of diet acid intake and diet potassium as a measure of dietary base intake.

Table 2. Composition of the diets (by composite analysis) per 2500 kcal; each diet had a 2-day rotating menu

Diet name	*Total energy (Kcal)*	*Protein (g)*	*Fat (g)*	*Carbohydrate (g)*	*Phosphate (mg (mmol))*	*Magnesium (mg (mmol))*	*Sodium (mg (mmol))*	*Potassium (mg (mmol))*	*Calcium (mg (mmol))*
Paleo (day 1)	2502	122	83	338	1860 (60)	686 (28)	1240 (54)	10946 (280)	814 (20)
Paleo (day 2)	2501	109	68	391	1490 (40)	745 (31)	1393 (61)	9517 (243)	739 (18)
Average	2502	116	75	364	1861 (50)	716 (29)	1336 (58)	10127 (259)	722 (18)
ADA (day 1)	2504	103	85	352	1989 (64)	496 (20)	3407 (148)	5478 (140)	1703 (42)
ADA (day 2)	2497	152	75	328	2273 (73)	556 (23)	3447 (150)	5083 (130)	1627 (41)
Average	2501	128	80	340	2144 (69)	526 (22)	3427 (149)	5281 (135)	1665 (42)

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RESULTS

Twenty-two subjects were recruited for this study. Thirteen subjects (7 females: 6 males), whose mean age was 56 ± 12 years and an average BMI of 32.8 ± 7.1 kg/m^2 completed urine collections for NAE during days −2 to 0 and 19–21 and were included in the analyses. Five of the subjects were randomized to the ADA-diet and eight were randomized to the Paleo-diet. At baseline, there was no significant difference in protein or calorie intake between the two groups (protein (g): Paleo 106 ± 63 vs ADA 136 ± 45, $P = 0.4$; calories (kcal): Paleo 2047 ± 602 vs ADA 2729 ± 787). The average weight change over the course of the study for the Paleo diet was −2.7 ± 1.1 kg and for the ADA-diet was −2.3 ± 1.3 kg, with no significant difference between groups ($P > 0.5$).

Formulas for measured and estimated NAEs, as well as diet compositions are shown in Tables 1 and 2. The potassium and protein intakes from the study diet interventions were compared with the baseline (subject's usual diet) data and corresponding NAE-determining urinary measurements are given in Table 3. In the Paleo group, the 24-h urine potassium to sodium ratio increased from 0.5 ± 0.3 to 2.3 ± 0.7 ($P < 0.001$), while in the ADA group, the 24-h urine potassium to sodium ratio increased from 0.4 to 0.9 ($P = 0.01$). Creatinine clearance was unchanged.

Table 4 demonstrates the results for the measured NAE ($NAE_{\_urine\ M}$) and estimated NAE ($NAE_{\_diet}$) derived from the dietary data. Subjects on the Paleo diet demonstrated a 63-mEq decrease in total daily acid load using NAE_urine ($P = 0.01$), while those on the ADA-diet had essentially no change ($P = 0.8$). Correspondingly, at the end of interventions, subjects on Paleo-diet had significantly ($P = 0.002$) lower $NAE_{\_urine\ M}$ than those on ADA-diet.

The measured $NAE_{\_urine\ M}$ on Paleo diet was positive, although the formulas for $NAE_{\_diet\text{-}R\ or\ L}$ predicted negative net acid production for the Paleo diet. As expected, the analyzed $NAE_{\_urine\ M}$ for the ADA-diet was positive, as were all of $NAE_{\_diet}$ predictions. The means of the absolute differences between $NAE_{\_urine\ M}$ and $NAE_{\_diet\ R\ or\ L}$ were −26 mEq for the subject's baseline (usual) diet, −50 mEq for the ADA-diet, and −68 mEq for Paleo diet, respectively. All results using the formula for $NAE_{\_diet\ F}$ were positive. Not surprisingly, the absolute difference between $NAE_{\_urine\ M}$ and $NAE_{\_diet\ F}$ was therefore lower, between 4 and 20 mEq.

For all subjects, Pearson correlation coefficients demonstrated similar and relatively strong correlations. For the baseline data, $NAE_{\_diet\ L}$ correlated the least ($r = 0.53$, $P = 0.07$) and $NAE_{\_diet\ R}$ correlated the best ($r = 0.62$, $P = 0.02$). For the invention diets, $NAE_{\_diet\ R}$ correlated the least ($r = 0.67$, $P = 0.01$) and $NAE_{\_diet\ F}$ correlated the best ($r = 0.76$, $P = 0.003$). The corresponding R^2 ranged 0.29–0.40 for baseline and 0.44–0.58 for intervention diet. In average $NAE_{\_diet}$ predicted about 40% of the variability found in the measured values.

DISCUSSION

The high alkali load from eating a high F&V Paleo-diet would be expected to result in a clear net base load to the body, leading to a negative NAE. In the present study, although we found a clear drop in urinary NAE of about 60 mEq after the Paleo-diet intervention in our Type 2 diabetic patients, the measured NAE was unexpectedly still in the lower positive range (+31 mEq). The more sophisticated and specific dietary mineral-, sulfate- and OA-related estimation of total endogenous acid production ($NAE_{\_diet\ R\ or\ L}$) underestimated measured urinary NAE for all diet forms including the baseline diet. The magnitude of underestimation was lowest in the Type 2 diabetic patients eating the American or Westernized baseline diets and increased when subjects increased their protein and their F&V-based potassium intakes (the extent of underestimation highest with the Paleo diet and less marked with the ADA-diet providing less F&V). These findings suggest that the $NAE_{\_diet\ R\ or\ L}$ methodology is less

Table 3. Urinary and dietary measurements for NAE in the study subjects[a]

	Paleo diet (n = 8)			*ADA-diet* (n = 5)		
	Baseline	*Intervention*	P-*value*	*Baseline*	*Intervention*	P-*value*
Urinary variables						
NH_4 (mmol)	73 ± 26	30 ± 9	0.004	86 ± 38	95 ± 42	>0.1
TA (mmol)[b]	23 (20, 24)	4.8 (3.7, 17)	0.02	31 (29, 45)	24 (18, 18)	>0.1
HCO_3 (mmol)[b]	−0.4 (−3.2, 9.9)	−4.3 (−5.1, 21.5)	>0.1	7.3 (1.7, 8.9)	−1.9 (−2.0, −0.9)	>0.1
pH	5.8 ± 0.4	6.7 ± 0.5	0.003	5.8 ± 0.3	5.9 ± 0.5	>0.1
Sodium (mmol)	148 ± 37	59 ± 27	0.0005	204 ± 82	123 ± 30	0.04
Chloride (mmol)	170 ± 32	92 ± 41	<0.0001	203 ± 74	145 ± 38	0.07
Chloride - Sodium (mmol)[b]	8.2 (5.3, 32)	26 (22, 32)	0.06	2.6 (−10, 9.9)	25 (19, 30)	0.1
Potassium (mmol)	70 ± 36	139 ± 68	0.009	73 ± 29	117 ± 52	0.04
Calcium (mmol)	3.6 ± 1.9	1.5 ± 1.0	0.009	3.7 ± 1.9	3.0 ± 1.5	0.4
Dietary variables						
Total energy intake/kg body weight	23 ± 4.2	21 ± 4.1	>0.1	27 ± 9.9	26 ± 10	>0.1
Protein (g)[b]	92 (72, 110)	133 (112, 159)	0.045	134 (102, 159)	173 (140, 179)	0.08
Potassium (mmol)	66 ± 33	254 ± 49	<0.0001	90 ± 26	156 ± 36	0.01
Calcium (mmol)	19 ± 6.5	21 ± 3.8	0.6	25 ± 8.0	48 ± 11	0.002
Magnesium (mmol)	12 ± 6.1	32 ± 5.8	<0.0001	17 ± 4.8	26 ± 6.0	0.05
$OA_{\_diet\ R}$	48 ± 5.7	48 ± 5.7	> 0.1	53 ± 12	53 ± 12	> 0.1
$OA_{\_diet\ L}$	41 ± 3.9	69 ± 7.1	<0.0001	43 ± 4.5	56 ± 5.4	0.005
PRAL (mEq)	28 ± 24	−96 ± 21	<0.0001	39 ± 29	7.7 ± 4.2	0.07

Abbreviations: HCO_3, bicarbonate; NAE, net acid excretion; NH_4, ammonium; OA, organic acid; PRAL, potential renal acid load; TA, titratable acid. [a]Data are given as mean ± s.d.; *P* for difference between baseline and intervention, examined using paired *t*-test. [b]Non-normal distributed variables are given as median (25 percentile, 75 percentile); differences between baseline and intervention were examined using Wilcoxon-test.

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Table 4. Net acid calculations from measured net acid excretions and from dietary formulary estimates

NAE calculations	*Paleo diet (n = 8)*					*ADA-diet (n = 5)*					P-*value*[a]
	Baseline		*Intervention*		P-*value*[b]	*Baseline*		*Intervention*		P-*value*[b]	
	Mean (s.d.)	*Range*	*Mean (s.d.)*	*Range*		*Mean (s.d.)*	*Range*	*Mean (s.d.)*	*Range*		
Urine measurements											
$NAE_{-urine\ M}$ (mEq/day)	94 (32)	(52,155)	31 (22)	(−11, 57)	0.01	118 (48)	(47,173)	112 (52)	(47, 161)	0.8	0.002
Dietary estimates											
$NAE_{-diet\ R}$ (mEq/day)	76 (28)	(50,133)	−47 (18)	(−67, −25)	<0.0001	92 (36)	(55,135)	61 (13)	(38, 73)	0.07	<0.0001
$NAE_{-diet\ L}$ (mEq/day)	69 (25)	(46,124)	−26 (14)	(−41, −9)	0.0001	82 (28)	(60, 115)	64 (8.5)	(53, 72)	0.15	<0.0001
$NAE_{-diet\ F}$ (mEq/day)	87 (45)	(48,191)	27 (5)	(20, 35)	0.008	102 (40)	(65, 160)	92 (18)	(66, 108)	0.5	0.001

Mean differences between measured urinary and estimated dietary NAEs.

	Baseline	*Intervention*	P-*value*[b]	*Baseline*	*Intervention*	P-*value*[b]
	Mean (s.d.)	*Mean (s.d.)*		*Mean (s.d.)*	*Mean (s.d.)*	
$NAE_{-urine\ M} - NAE_{diet\ (mean\ of\ R\ and\ L)}$	21 (25)	68 (26)	0.02	31 (36)	50 (48)	0.3
$NAE_{-urine\ M} - NAE_{-diet\ F}$	7 (33)	4 (23)	0.8	16 (30)	21 (47)	0.8

Abbreviation: ADA, American Diabetes Association; NAE, net acid excretion. [a]*P*-value for difference between Paleo diet and ADA-diet, examined using unpaired *t*-test. [b]*P*-value for difference between baseline and intervention examined using paired *t*-test.

accurate when marked increases in protein and F&V-based potassium intakes occur in parallel in these subjects with T2D; however, the magnitude of underestimation of the respective plant food- and protein-related OA protons was larger than we expected.

$NAE_{-diet\ R\ or\ L}$ was about 20–30 mEq lower than the measured urinary NAE for the baseline diet; these results are similar to earlier diet intervention observations in healthy subjects on different diets, where underestimation ranged from 8–20 mEq.[8] In the latter diet study, varying protein was administered along with oppositely varying F&V, but even the most extreme F&V intake (1600 g/day) was only associated with the highest discrepancy in NAE estimation (20 mEq/day). The fact that the discrepancies between the estimated and measured NAEs were so exceptionally high in the present study may not only be due to the quite unusual and extreme diet composition of raising both protein and F&V intake, but might also be due to the special metabolic state of patients with T2D, who tend to have a greater NAE than healthy subjects independent of their diet-dependent acid load.[20] Maalouf *et al.*[17] demonstrated higher sulfate excretion and relatively low ammonium excretion in Type 2 diabetics compared with normal subjects when given a typical Western diet. Tannen and Hood demonstrated that adding sodium bicarbonate in subjects who were fasting increased the production of ketoacids, while adding ammonium chloride decreased the production of ketoacids.[21] Possibly a similar mechanism may also work in diabetic patients. Conceivably, insulin resistance may have a role in increased production of endogenous OAs.[20] Lactate and pyruvate levels were higher in the urine of individuals with greater degrees of insulin resistance.[22] Accordingly, it would be interesting to specify whether the OAs stemming from diet-related metabolism show a particular amplification in patients with T2D compared with healthy controls.

Measuring OAs is difficult, and what the 'best' methodology is, is unclear. In the past, titration methods have been used to estimate OAs.[23] More recently, urine OA analysis has been performed with gas chromatography/mass spectrometry, utilizing a capillary column. However, even with this methodology only a part of the extremely heterogeneous total OA pool can be quantitatively profiled. Therefore, to date only rough estimates for actual urinary OA production is used in NAE estimation formulas. As is evident from the present study, this commonly not-analyzed fraction of total renally excreted acidity is not satisfactorily estimated, especially for those diets with rather unusual patterns (for example, the Paleo diet).

Our present data show that the difference between ADA- and Paleo-diet is primarily induced by F&V intakes (as the increase of protein intake is nearly identical). This suggests that the difference of the underestimation between the ADA- and Paleo-diets of ~20 mEq may be due to an increased production of OAs in response to the high phenol-containing plant foods in the Paleo-diet. One prominent component of these OAs is hippuric acid.[24,25] Typical fruits for which clear increases in hippuric acid excretion have been documented after ingestion are prunes and cranberries,[24,26] and consumption of both fruits under standardized experimental diet conditions results in a clear increase in urinary acidity, as discernible by significant decreases in 24-h urine pH. Another source of excess food-borne OAs has been shown to be excess protein intake.[16,27] In accord herewith, protein intake was much higher in both the ADA and Paleo than in the baseline diets. Therefore, it seems that elevations in protein degradation-derived OAs (due to high protein intake) and non-combustible OAs (due to high F&V intake) can be too large to be ignored. Accordingly, appropriate more food-specific OA estimation approaches are required.

As sodium (Na) and chloride (Cl) content can not be reliably estimated from dietary records, they were not included in the calculation formula $NAE_{-diet\ R\ or\ L}$. Although NaCl intake stems primarily from added salt (table salt), and is generally considered to be present on an equimolar basis, a controlled nutrition study with lower NaCl intakes[28] on different diets demonstrated that Cl content can frequently be in excess over Na. Both of our intervention diets in patients with T2D decreased salt intake. Based on urinary Na and Cl excretion data, we found that the (Cl-Na) difference was very low for baseline diet, on average 6 mmol/day, but was 25 mmol/day for both the ADA and Paleo-diets. Thus, an additional factor in the underestimation of $NAE_{-urine\ M}$ by about 20 mEq/day after the ADA or Paleo intervention may be related to the omission of Na and Cl from the estimates. If appropriate Na and Cl data for a given diet is available, use of both electrolytes should be considered in the calculation.[29]

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Although the present diet study confirms that the current $NAE_{-diet\ R\ or\ L}$ cannot reliably estimate total daily net acid production in adults (patients with T2D) on high protein and F&V diets, this estimation method does provide appropriate NAE calculations in children and adults on usual nutrition including lactovegetarian, medium and high protein diets.[8,30] In addition $NAE_{-diet\ R\ or\ L}$ also allows one to specify particular dietary alkali or proton load sources. For example, the alkalizing potential of calcium and/or magnesium supplements (if not ingested as chloride, sulfate, or phosphate salts), sodium bicarbonate-rich foods or beverages (for example, mineral waters), low phosphorus intakes, and—if required—of specific protein sources (for example, with low methionine and cysteine content) can be reliably distinguished by using $NAE_{-diet\ R\ or\ L}$.[31,32] As total NAE can obviously encompass a considerable amount of plant-based (for example, polyphenol-derived) OAs with clear positive health effect, examination of PRAL independent of OAs may be advantageous for certain preventive nutritional research question in the future.[28]

One limitation of the present study is the relative small sample size. Another limitation is that we could not fully guarantee the presence of a full steady state, especially for the ADA-diet because of the alternating diet regimen with mild differences in protein and potassium intake between each of the two varying diet days. Doing repeated 24-h urine collections and averaging the data was used to mitigate the effects of the 2-day diet regimens.

In conclusion, net acid production in Type 2 diabetic patients decreased about 60 mEq on our Paleo-diet intervention, despite the dietary models predicted a much greater decrease in NAE. This underestimation of net acid production occurs to a greater degree when using the more sophisticated and specific dietary mineral-, sulfate- and OA-related estimates of NAE, especially if high amounts of protein and F&V are concurrently ingested, as is the case with Paleolithic–Hunter–Gatherer-type diets. We believe this underestimation is due to an inappropriate calculation of the proton load stemming from OAs, underlining the necessity to improve methods to measure OAs and to more clearly specify the dietary OA-related proton sources for diets of different F&V items and of high protein intakes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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