Acid Base Considerations in Stone-Age Farming Sweet Potato Eaters, Modern-Day Sweet Potato Eaters, and High-Protein Consumers

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Abstract: Net endogenous acid production (NEAP) can be estimated by a primarily anthropometry-dependent organic acid anion (OA) component and the particularly diet-dependent potential renal acid load (PRAL). However, there is evidence that certain foods may also impact on the OA component. Here, we discuss measurements of urinary 24-h OA excretion obtained in healthy subjects, against the background of relevant literature with a special focus on former Papuan New Guinea stone-age farmers eating predominantly highland sweet potatoes. Contrary to the reports in Papuans, we observed only modest increases of 5-12% in 24-h excretion rates of OAs (including the detoxification product of phenolic and benzoic acids, i.e., hippuric acid) in healthy adults consuming commonly available yellow-fleshed sweet potatoes. This and additional results on OA increases after higher protein intakes suggest that a specification of the NEAP=PRAL+OA model for estimating the diet-dependent acid load to the metabolic system might be useful regarding particular foods and their effects on the OA component.

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BACKGROUND

The net amount of endogenous acids produced each day, is determined to a considerable degree by the composition of the diet. Several approaches exist to estimate daily acid loads from diet. Recently, a standardization of the terminology regarding the procedures to estimate an acid load has been suggested [1]. Of these procedures, one uses only two nutrients, i.e., the diet's content of potassium and protein to allow a reasonable rapid calculation. The other two methods, specified in this standardizing terminology paper, consider additional dietary minerals as well as their average intestinal absorption rates. Also, both methods distinguish between two components to estimate net endogenous acid production (NEAP), namely a direct dietary component including minerals and proteins, which determines the potential renal acid load (PRAL) and a second indirect component, which corresponds to urinary organic acid (OA) anion excretion. While one method estimates the OA component as a function of body size [2, 3], the other estimates it as a function of dietary intakes of several mineral cations and anions [4, 5], whereby an excess of alkalizing cation intake over acidifying anion intake regularly yields a higher OA estimate from the unmeasured anions.

Several studies indicate that urinary OA excretion can particularly increase after enhanced ingestion of certain, especially phenol-containing plant foods [5-8], which can, but need not necessarily have a clear excess of alkalizing cation content (potassium, sodium, magnesium, calcium) over acidifying anions (phosphorus, chloride, sulfate from sulphur-containing amino acids). In addition to such food- specific influences on OA (related to phenolic compounds), a raised total urinary OA excretion has also been observed in

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humans with elevated intakes of dietary protein [9, 10]. Therefore, we aimed to examine exemplarily the consequences for renal excretion rates of total and selected single OAs after ingestion of one particular vegetable, for which clear increases in excretion of the phenolic acid-derived urinary OA hippuric acid have been described. Another focus was on the potential impact of stronger variations in protein intake on overall OA excretion and thus on a separate protein effect, independent of the acidifying potential of protein *via* sulphur-containing amino acids.

Altogether we aimed to raise the following question: Should the NEAP=PRAL+OA model be further specified in the future for selected phenol-rich plant foods and possibly for high protein intakes which both may add some acidity *via* the OA component, hitherto not taken into account in NEAP estimation models.

SWEET POTATOES AS A MAJOR SOURCE OF THE OA HIPPURIC ACID IN PAPUAN HIGHLAND STONE AGE FARMERS

In Papua New Guinea two contrasting communities exist: one coastal with a long interaction with external influences and cash-cropping and a highland community with a history of stone-age farming and less, more recent contacts (with other cultures) [11]. As reported in the sixties, the highland Papuans grow sweet potatoes and consume a low-protein vegetarian diet consisting predominantly of these tubers [6]. These ancient dietary habits persisted until the eighties, but at that time increased consumption of imported foods such as rice and canned fish began [12]. One metabolic characteristic of the aboriginal Papuan sweet potato eaters is an extremely high excretion of the OA hippuric acid [6]. Compared to people living in Europe, who excrete around 2 - 4 mmol of hippuric acid per day [6, 8], the Papuans excreted 31 mmol/d on average [6], which is almost the amount of total urinary

OA excretion that would be expected for healthy Caucasians of comparably small body size, but on normal western diets [3, 7]. Thus, the findings of Oomen [6] in the Papuan highland populations strongly suggest that sweet potatoes present a relevant source of dietary OAs which are ingested as phenols and phenolic acids and are then excreted mainly as hippuric acid as part of the total urinary OA fraction.

PHENOLS IN FOODS AND HIPPURIC ACID EXCRETION

Phenols or phenolic acids are common in human diet and high concentrations can be found in numerous plant products for example in tea, coffee, cranberries, and blueberries [8, 13]. In the last years, phenolic constituents have attracted increasing attention, since they did not only show significant antioxidant activities [13], but also anticancer effects in different *in vitro* test systems [14]. Additionally, a cardiovascular disease-preventive potential of dietary phenols is under discussion [8]. Phenolic and benzoic acids of fruits, vegetables and other sources are known to be (i) metabolically inactivated and detoxified after absorption from the gut and (ii) excreted at least partly as hippuric acid. Recent food chemistry research has shown that sweet potatoes and their leaves contain considerable, but varying amounts of various phenolic acids, of which – depending on the cultivars – cinnamic and chlorogenic (3-caffeoylquinic) acids play a major quantitative role [14, 15]. As demonstrated in a dietary controlled crossover study in which separate phenols were administered for 1 week to healthy subjects and subjects without colon, around half of the ingested chlorogenic acid was metabolized to hippuric acid and then renally excreted in normal subjects

with intact colon [8]. Accordingly, increases in both hippuric acid and total OA excretions should generally be expected to occur if the dietary intakes of fruit and vegetable products with relevant phenol contents rise. However, measurements of the contribution of specific foods to the urinary excretion rates of hippuric acid and other OAs are scanty. For the consumption of both black and green tea, significant increases in hippuric acid have been recently reported. These increases were in the order of magnitude of 2 mmol/d per 12 cups/d for both kinds of tea [16].

MODERN-DAY SWEET POTATO CONSUMPTION AND URINARY EXCRETIONS OF HIPPURIC ACID AND OTHER OAS – FINDINGS OF AN EXEMPLARY DIET STUDY

To examine the consequences of ingestion of a single meal of sweet potatoes for urinary hippuric acid excretion, we performed a controlled 4-d nutrition study. Eight healthy female adults [mean age: 32 y (range: 22 - 54 y); BMI: 24.5 ± 4,1 kg/m^2] participated and ingested a constant diet with all foods weighed and prepared in the institute's own kitchen. Energy and protein intake were 8.2 MJ/d and 50 g/d, respectively throughout the whole 4-d period. After a 3-d run-in period to metabolically adapt to the lacto-vegetarian whole food basal diet, the intervention meal, consisting of baked sweet potatoes (345 g fresh weight), was administered with lunch on day 4. Commonly available yellow-fleshed sweet potatoes were purchased in a local supermarket. To maintain a constant energy intake, 33.4 mL olive oil (300 kcal), included in the regular lunch, were isocalorically replaced by the sweet potato test food. Calculated PRAL [1] was 8.3
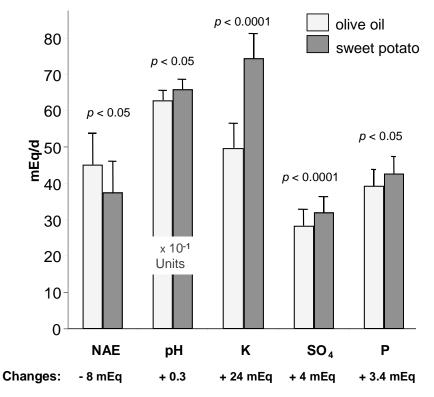

Fig. (1). Mean values (and standard deviations) of 24-h urinary net acid excretion, pH and further urine analyte excretion rates with the basal diet and after a sweet potato meal (n = 8 subjects).

mEq/d and –9.8 mEq/d for the basal diet and the sweet potato diet, respectively and mean OA excretion estimated from body surface area [1] was 41.7 mEq/d for the female participants. 24-h urine collections were performed on day 3 (basal diet) and day 4 (sweet potato meal). Net acid excretion (NAE), potassium, sulfate, phosphorus, and total OAs were analyzed as described elsewhere [2]. Individual OAs were quantified as follows: citrate and uric acid with commercial photometric assays (Citric acid, R-Biopharm AG, Darmstadt, Germany; Uric Acid plus, Roche Diagnostics GmbH, Mannheim, Germany), oxalate with ion exchange chromatography (Dionex), and hippuric acid with a direct colorimetric method [17].

As shown in Fig. (**1**), sweet potato consumption led to significant increases in 24-h urine pH and excretion rates of potassium, sulfate, and phosphorus. The elevation of potassium clearly exceeded the elevations of sulfate and phosphorus (Fig. **1**) and total OA (Fig. **2**) finally resulting in a significant drop in renal NAE. The changes in OAs were only modest. Sweet potato ingestion raised total and individual OAs by not more than 5-12 % (Fig. **2**). Although significant, the increase especially of hippuric acid was almost negligible when compared to that reported for the aboriginal Papuan sweet potato eaters. Even though a regular daily consumption of the tested sweet potato type may result in a higher excretion level of hippuric acid in the long run, it appears that this commonly available yellow-fleshed tuber does not contain such high amounts of hippuric acid-yielding plant phenols as the sweet potato cultivars of the highlands of Papua New Guinea.

A part of the total OA increase of 3 mEq (Fig. **2**) in our sweet potato test meal study may have been due to phenol metabolites which are not metabolized to hippuric acid. A considerable quantitative variation has been reported for such differently metabolized phenolic fractions in a number of sweet potato varieties [15]. It remains to be examined in further dietary tests whether the observed increase in total OA of < 1mEq/100 g food (3 mEq/345 g intake) is a realistic level that also applies to other commercially available sweet potato varieties. Not till then a conclusion is possible whether such separate, small acidifying effects (contributing to a diminution of the alkalizing impact as inferred from PRAL) should also be considered as an acid-base relevant food peculiarity apart from the established dietary PRAL.

METABOLIC ASPECTS OF ENDOGENOUS OA PRODUCTION

Hundreds of different OAs are endogenously produced and finally excreted in urine each day. Table **1** presents an overview of relevant metabolic sources and gives examples of corresponding OAs that can be measured in urine samples. These OAs require buffering in systemic circulation and in urine. However, of the huge amounts actually produced in metabolism, only a relatively small portion which is not combusted, i.e., not oxidized to carbon dioxide and water, sustainably influences acid base status. These non-combusted organic acid anions are renally excreted and they can burden acid base balance with a reduction in buffer capacity since their renal elimination requires a corresponding cation excretion which implies losses in alkali equivalents too. The overall acidifying potential of OAs varies between average values of 40 mEq/d in young women and 55 mEq/d in young men [3].
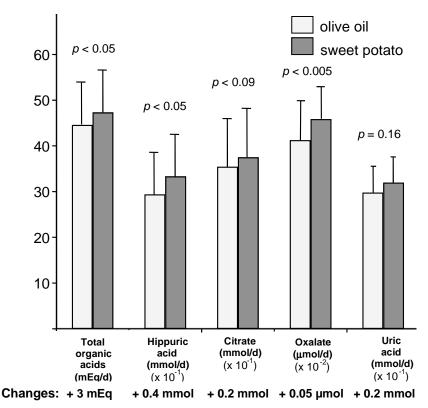

Fig. (2). Mean values (and standard deviations) of 24-h urinary excretion rates of selected organic acids with the basal diet and after a sweet potato meal (n = 8 subjects).

Table 1.   Sources of Endogenous Organic Acid (OA) Production in Human Metabolism and Examples for OAs in Urine[a]

Energy production	Citrate, α-Ketoglutarate, Malate, Succinate, Fumarate, Hydroxymethylglutarate
Fatty acid oxidation	Adipate, Suberate, Ethylmalonate
Carbohydrate metabolism	Pyruvate, L-Lactate, α-Hydroxybutyrate, β-Hydroxybutyrate an/aerobic energy production
Amino acid metabolism	α-Ketoisovalerate, α-Ketoisocaproate, α-Keto-β-methylvalerate, β-Hydroxyisovalerate; Methylmalonate, Formiminoglutamate
Hepatic Phase II conjugation Gut microflora products	Benzoate, Hippurate, Phenylacetate, Phenylpropionate p-Hydroxybenzoate, p-Hydroxy-phenylacetate, Indican, D-lactate, Tricarballylate, D-Arabinitol
Detoxification processes	Orotate, 2-Methylhippurate, Glucarate, Pyroglutamate
Neurotransmitter metabolism	Vanilmandelate, Homovanillate, 5-Hydroxy-indoleacetate, Kynurenate, Quinolinate
DNA oxidation	p-Hydroxyphenyllactate, 8-Hydroxy-2´-deoxyguanosine

[a]Adapted from Bralley and Lord [24].

As is discernible in Table **1**, among those OAs that potentially contribute to a reduction of the renal buffer capacity, are numerous which originate from diet-dependent metabolic processes. Accordingly, OAs from carbohydrate and fatty acid metabolism may vary with changes in the dietary intakes of these major nutrients. However, fat and carbohydrate intakes are closely related to energy production and/or energy requirement which is less explicitly the case for protein intake. Thus, if measured urinary total OA excretions are adjusted for energy requirement, e.g., by using individual body surface area as a proxy variable, then especially protein intake variation may still explain an additional portion of total OAs' variability. Actually, higher urinary OA excretion rates have been observed in bodybuilders on high protein diets compared to control subjects ingesting significantly less protein [9]. Also, prematures receiving formulas, which have a higher protein content than mothers milk, have shown elevated urinary total OA excretion rates [10].

PROTEIN INTAKE AND OA EXCRETION – FINDINGS IN ADOLESCENTS AND ELDERLY

In recent papers, we studied total OA excretion rates in children and adolescents [3] as well as associations between anthropometry and acid-base parameters in young adults and elderly [18]. Based on the available OA measurements in adolescents [3] and complementary OA analyses in a group of elderly [18], we additionally examined whether higher protein intakes may have significant associations with urinary total OA excretion rates. In all subjects, protein intake was estimated from urinary total nitrogen excretion after allowing for potential correlated measurement errors as described elsewhere [19]. For the protein intake estimate, total urinary nitrogen excretion was measured by the Kjeldahl technique (Buechi 430 Digestor and Buechi Distillation Unit B-324; Flawil, Switzerland) and the corresponding 24-h nitrogen measurement was multiplied by 6.25. Compared to the original sample size of the published OA excretion paper [3], the present analysis had additional measurements of OA and nitrogen available from the DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study, hence the sample size increased to 112 adolescents (68 boys, 44 girls; mean age: 14 ± 0.5). Mean age of the healthy elderly was 64±4.7 y (41 males, 44 females).

As shown in (Fig. **3A** and **B**), highly significant correlations exist between protein intake and absolute daily urinary total OA excretion rates in adolescents and elderly. It is clear that an important part of this association is due to the fact that subjects with a higher body size and a correspondingly higher body size-related OA excretion also have a higher dietary protein requirement and higher protein intake. In order to control for this body size effect, 24-h urinary OA excretions were adjusted for body surface area (BSA). Also after BSA adjustment, protein remained a significant determinant of 24-h urinary OAs (Fig. **3C** and **D**), indicating that higher protein intakes contribute to higher total OA excretion rates.

CONSEQUENCES FOR THE ESTIMATION OF NET ENDOGENOUS ACID PRODUCTION (NEAP)

Already in 1923 it was reported that ingestion of some fruits, i.e., prunes and cranberries, can decrease urine pH [20]. This elevation of dietary acidity has recently been reproduced for cranberry and in part for plum ingestion [21]. The authors found a significant fall in urine pH only after cranberry juice, but not after plum juice ingestion [21] which can be explained by a lower dietary PRAL (higher alkalizing potential) of plums compared to cranberries. Various phenolic acids have been identified in these and other fruits [22, 23] which are partly absorbed [8] and further metabolized to biochemically altered phenolic acids, e.g., hippuric acid. These data along with (i) the study results in the Papuan stone-age farming sweet potato eaters and (ii) our exemplary findings in modern-day sweet potato eaters (increase of 3 mEq of OA per meal of 345 g of sweet potatoes), confirm that certain fruits and vegetables (which are mostly alkalizing and yield a negative PRAL) can have substantial OA increasing effects. It is clear that particular plant foods with higher phenolic acid contents will be relevant in this respect. Data on urinary hippuric acid responses to black and green tea consumption have been recently published [16]. However, reasonable data on the contribution of particular plant foods to the total OA component of NEAP is completely lacking. Our findings of a significant association between total OA excretion rate and protein intake indicate that such a relationship – hitherto observed in specific population groups, i.e., bodybuilder with excess protein intake [9] and preterm infants on protein-rich formulas [10] – may exist in
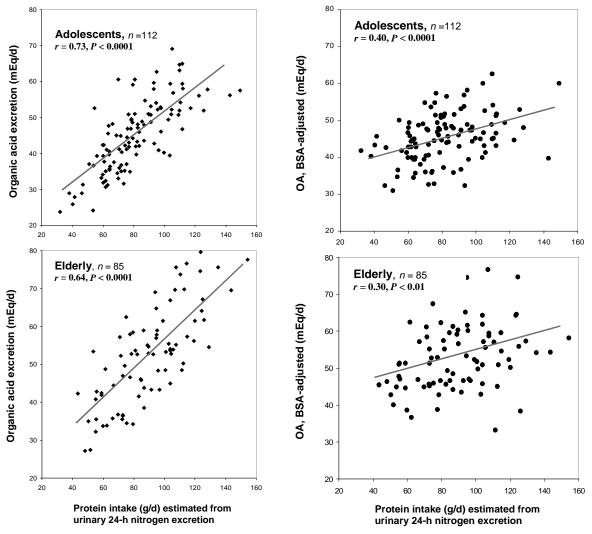
Fig. (3). Urinary total organic acid excretion in healthy adolescents and elderly; **A, B** unadjusted daily excretion rates; **C, D** daily excretion rates adjusted for individual body surface area (using the residual method).

healthy young (adolescents) and elderly subjects on their normal diets too. Possibly, parts of the non-combusted degradation products of amino acid metabolism (Table **1**) may relevantly contribute to total OA excretion and may thus alter the total body's acid load with stronger changes in protein intake, independently of the PRAL-specific protein-related dietary acidity.

In conclusion, in addition to the PRAL-specific diet effects on NEAP [1-4], ingestion of particular plant foods and/or larger changes in consumption of protein may additionally add to the body's total acid load *via* increases in OAs. Future research is required to identify those fruits and vegetables for which apart from the established NEAP=PRAL+OA model, an additional – quantitatively relevant – plant food-specific OA contribution exists. Also the extent to which increases in protein intake may further enhance the OA component needs to be determined more precisely.

ABBREVIATIONS

BSA   =   Body surface area

DONALD Study	=	Dortmund Nutritional and Anthropometric Longitudinally Designed Study
NAE	=	Net acid excretion
NEAP	=	Net endogenous acid production
OA	=	Organic acids
PRAL	=	Potential renal acid load

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