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Insights about urinary hippuric and citric acid as biomarkers of fruit and vegetable intake in patients with kidney stones: The role of age and sex

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ABSTRACT

Objectives: Urinary hippuric acid (HA) and citrate can represent useful biomarkers of fruit and vegetable (FAV) intake in nephrolithiasis. However, their clinical significance across the life span has been poorly investigated. The aim of this study was to investigate the association between the two biomarkers with FAV intake across different age groups and sexes in a large group of stone formers (SFs).

Methods: SFs undergoing baseline 24-h urinary collection for metabolic profile of lithogenic risk at our institution were consecutively enrolled for a 6-y time span (N = 1185; 625 men). HA and citrate excretions were determined by ion chromatography and ultraviolet method, respectively. SFs completed a food frequency questionnaire on the intake of FAV. Stepwise logistic regression was applied to investigate factors associated with very low FAV ($\leq$1 servings/d) and analysis of covariance to compare citrate and HA excretion across age groups and sexes.

Results: Very low FAV intake prevalence declined with age ($P_{trend} < 0.001$), and was inversely associated with HA and citrate excretion ($P < 0.001$) in a stepwise logistic regression model. A significant increasing trend was verified for both biomarkers across age groups until the age of 65 for HA ($P < 0.001$) and 55 for citrate ($P < 0.001$). Citrate excretion significantly declined after the age of 65, and was higher in women than men in adult age groups, regardless of FAV intake.

Conclusions: Both urinary citrate and HA were positively associated with FAV intake in SFs. However, unlike HA, citrate excretion was significantly influenced by the female sex and by older age.

Introduction

Nephrolithiasis is a non-communicable disease with a high prevalence worldwide, especially in industrialized countries where a significant increasing trend has been detected [1]. It implies a significant health care cost burden, requiring frequent hospitalizations for urologic procedures and representing a significant risk factor for chronic kidney disease and end-stage renal disease [2]. Sedentary lifestyle and unhealthy dietary patterns have been claimed as important risk factors, leading to frequent overlapping with obesity and metabolic syndrome [3,4].

Namely, excessive intake of salt and animal proteins are the main factors promoting lithogenic salt supersaturation and precipitation in the urinary microenvironment [5,6]. A dietary approach limiting the intake of these nutrients, together with adequate fluid intake, is currently considered the mainstay for the prevention of stone recurrences [7–9].

Conversely, a high intake of fruit and vegetables (FAV) is associated with protection against stone formation [10], being associated with increased excretion of the lithogenesis inhibitors potassium,

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magnesium, and citrate [11]. This association is independent of the composition and physiopathology of stones because potassium, magnesium, and citrate exert their antilithogenic activity for both calcium and uric acid crystal precipitation. Because stone formers (SFs) generally have a very low consumption of FAV, especially those <30 y of age [12], the current consensus recommendation for prevention of stone recurrence in all types of idiopathic stones is to increase their intake to at least four portions per day [5,9].

Biomarkers of FAV intake are thus very useful in the management of kidney SFs, especially in settings where a comprehensive nutritional assessment is not feasible. Citrate excretion is positively correlated with FAV intake [11,13] but also depends on other factors significantly influencing the renal handling of this substance, such as systemic and dietary acid load, and drug treatments [14]. Even if citrate is a direct inhibitor of lithogenesis, these circumstances influence the interpretation of the dosage of citraturia in 24-h urine samples [15]. In fact, hypocitraturia may reflect both increased acid load and insufficient FAV intake, which do not necessarily coexist in the same patients [14].

Urinary excretion of hippuric acid (HA), a terminal metabolite resulting from the catabolism of polyphenols, and particularly flavonoids, which are largely represented in FAV [16], is significantly correlated with FAV intake in SFs [17]. HA excretion is not influenced by acid-base balance or drug treatments, and may represent a biomarker of FAV intake easier to interpret than citraturia even if not directly involved in lithogenesis. Interestingly, its use as nutritional marker has been recently validated in children and adolescents [18,19], but not yet extensively studied in the context of nephrolithiasis. In young SFs with very low FAV intake, 24-h excretion of HA and citrate is significantly decreased [17].

However, the age- and sex-specific variations of urinary citrate and HA, especially in the light of the different hormonal milieu and variations in renal function, are still poorly known. Thus, the objective of this cross-sectional study was to verify the correlations of urinary citrate and HA excretion with FAV intake across different age ranges and sex in a large population of Italian SFs, to better understand their possible role as biomarkers of FAV intake.

Methods

Study population and procedures

Enrolled in this cross-sectional study were SFs >10 y of age who were consecutively evaluated at Parma University Kidney Stone Clinic for the first time from 2011 to 2016 and prescribed a 24-h urinary collection for determination of the profile of lithogenic risk. All the SFs were considered for inclusion in the study, regardless of the composition of calculi. In fact, the role of both HA and citric acid excretion as possible biomarkers of FAV intake is independent of the type of stone disease [17].

We excluded SFs with a documented condition able to influence citrate excretion, such as enteric malabsorption syndromes (Crohn's disease, ulcerative colitis, chronic diarrhea, bariatric surgery, major intestinal surgery), renal tubular acidosis, and renal failure with glomerular filtration rate (GFR) <70 mL/min [14]. We also excluded those individuals who were chronically taking treatment with alkali, such as citrate or magnesium salts, at the moment of the first evaluation in the center, and those with infected nephrolithiasis (struvite or calcium phosphate stones associated with a documented urinary tract infection).

All the participants collected a 24-h urine sample on Sunday at least 1 mo after the latest episode of renal colic, as a routine procedure performed during the diagnostic workup of stone disease in the center [20]. Antihypertensive drugs and other medications known to affect urinary excretion of pro- and antilithogenic factors were suspended 1 wk before the exam. Details of urine collection procedures are given elsewhere [17]. Several parameters were evaluated on 24-h urine samples, including calcium, magnesium, potassium, sodium (by atomic absorption spectrophotometry), sulphate, chloride, phosphorus, oxalate, HA (by ion chromatography), citrate, uric acid (by ultraviolet method), urea, creatinine (by enzymatic colorimetric method), ammonium (by colorimetric method), pH (by potentiometric method), and volume [20]. For the purposes of this study, 24-h excretion of citrate and HA was particularly considered.

During the baseline visit predating 24-h urine collection, every patient was advised not to modify dietary and lifestyle habits until the end of the procedure, to limit the possible "stone clinic effect" [21]. This is a particular type of Hawthorne effect [22] involving SFs who unconsciously adopt healthier nutritional behaviors after an episode of renal colic and the following medical evaluation. According to international consensus, the results of the 24-h urinary profile of lithogenic risk are the mainstay for guiding the dietary and pharmacologic approach for secondary prevention of nephrolithiasis [5], provided that the urine sample has been obtained before the patient has significantly modified his or her habits.

A simple food frequency questionnaire (FFQ) investing FAV intake, routinely used in the center [12,17], was administered during the baseline visit before 24-h urine sample collection. FAV intake amounts were estimated by counting servings, according to recommendations by the Italian Society of Human Nutrition (LARN 2014). The results of the FFQ were interpreted and discussed with the patient by an expert physician.

Height and weight were also measured in every participant and used to calculate the body mass index and the body surface area (BSA). Body mass index was calculated as weight (kg) divided per square of height in meters (m^2). BSA was calculated with the DuBois and DuBois equation, as follows:

$$BSA\,(m^2) = 0.007184 \times height\,(cm)^{0.725} \times weight\,(kg)^{0.425}$$

GFR was calculated according to the creatinine clearance formula.

Statistical analyses

Data were expressed as mean ± standard deviation, median and interquartile range, geometric mean and 95% confidence interval (CI), or percentages as appropriate. To identify the factors associated with a very low intake of FAV, participants were categorized as one of the following: ≤1 servings/d (very low intake) and >1 servings/d. Stepwise logistic regression analysis was then applied to assess the relationship between urinary citrate and HA excretion and very low FAV intake.

Participants were then stratified by sex and age groups, as highlighted in Table 1. The average number of FAV servings per day was calculated for each sex and age group. The trends of FAV intake, citrate acid, and HA excretion, and their ratios, were explored across different age groups with analysis of covariance and adjusted for BSA as suggested by other authors [23]. Levels of 24-h urinary excretion of citrate acid and HA, together with other urinary pro- and antilithogenic risk factors, also were compared after stratification of the population according to the number of daily FAV servings.

All *P*-values were two-tailed and considered significant for $P < 0.05$. Analyses were carried out using the SPSS software version 23 (SPSS Inc., Chiacago, IL, USA).

Ethical standards

All data were obtained and handled anonymously. This study was conducted in compliance with the principles of the Declaration of Helsinki and its later amendments. The study protocol was approved by the local Ethics Committee. Informed consent for participation was obtained in compliance with the Italian law.

Results

The number of SFs included in the study was 1185 (625 men, 560 women). Their age range was from 10 to 82 y old (mean age 45 ± 16 y). The age and sex distribution of the population is depicted in Table 1. The most prevalent forms of kidney stones were idiopathic calcium nephrolithiasis (54%), gravel-like urolithiasis (21%), uric acid (11%), and mixed uric acid-calcium oxalate stones (6%). The percentage of those who had more than one renal colic episode in their personal history before the baseline evaluation was 56%. All the participants had GFR ≥70 mL/min. A significant age-related subclinical decline in GFR was detected only after the age of 55 (mean GFR 119 ± 32 mL/min in the 10–55 y age

Table 1
Study participants (N = 1185 stone formers), stratified by sex and age groups

Age groups (y)	Participants, n (Female/Male)
10–16	52 (24/28)
17–25	126 (78/48)
26–35	159 (79/80)
36–45	265 (118/147)
46–55	254 (109/145)
56–65	212 (106/106)
>65 y	117 (46/71)
Total	1185 (560/625)

Table 2
Stepwise logistic regression model* exploring factors associated with very low fruit and vegetable intake (≤1 serving/d) in 1185 stone formers

	OR†	95% CI†	*P*-value
Age (y)	0.973	0.961–0.984	<0.00001
Hippuric acid, mg/24 h	0.998	0.997–0.999	<0.00001
Citric acid, mg/24 h	0.999	0.998–0.999	<0.00001

CI, confidence interval; OR, odds ratio
*Model also adjusted for glomerular filtration rate, body mass index and body surface area.
†OR and 95% CI are referred to unitary variations of the variable of interest.

range, 111 ± 38 mL/min in the 56–65 y age range, 94 ± 27 mL/min after the age of 65; P_{trend} <0.001).

The FFQ analysis showed that 225 participants (19%) had a very low FAV intake (≤1 serving/d): 94 women (17%) and 131 men (21%; *P* = 0.07). Stepwise logistic regression analysis, exploring factors associated with a very low FAV intake (≤1 serving/d), showed that age, citrate excretion, and HA excretion were all inversely associated with the condition ($P < 0.0001$; Table 2). A comparison of 24-h urinary profile of lithogenic risk parameters between SFs with very low FAV intake and SFs with other levels of FAV intake (>1 servings/d) is shown in the supplementary material.

The stratification of the population in different age groups, according to Table 1, showed a significant decreasing trend ($P < 0.001$) in the prevalence of a very low FAV intake (Fig. 1A), with a peak in adolescents and young adults (40%) and a nadir in SFs >65 y of age (10%). No sex-specific differences could be detected in the prevalence of a very low FAV intake in SFs (Fig. 1B).

Levels of FAV intake across the considered age groups are shown in Table 3. The mean and 95% CI of FAV servings per day showed a significant increasing trend from adolescents (1.2 servings/d; 95% CI, 1–1.4) to adults ages 56 to 65 y (2.6 servings/d, 95% CI, 2.5–2.7; $P < 0.001$ Fig. 2). No association could be detected between sex and number of FAV servings per day (*P* = 0.116; Fig. 2).

The mean values of BSA-adjusted 24-h urinary HA excretion across different age groups and sexes are shown in Figure 3 and in the supplementary material. A significant increasing trend in HA excretion (+127%) was found in the adolescent age group (mean 222 mg/24 h, 95% CI, 185–266) to the 56 to 65 y age group (mean 507 mg/24 h, 95% CI, 465–553; $P < 0.001$). No association was found between HA excretion and sex (*P* = 0.247). However, the comparison of HA excretion between men and women in single age groups showed a significantly higher excretion in women in the 36 to 45 y age group (*P* = 0.027, Fig. 3B).

The mean values of BSA-adjusted 24-h urinary citrate excretion across different age groups and sexes are shown in Figure 4 and in the supplementary material. A significant increasing trend (+35%) was found in the adolescent age group (mean 431 mg/24 h, 95% CI, 370–501) to the 46 to 55 y age group (mean 578 mg/24 h, 95% CI, 541–619; $P < 0.001$). Conversely, a significant decline of citrate excretion was found after 65 y of age (*P* = 0.021; Fig. 4). Female sex was significantly associated with a higher citrate excretion ($P < 0.001$). A comparison between the sexes yielded significantly higher levels of citrate excretion for all age groups ($P < 0.05$), except for adolescents (10–16 y) and SFs >65 y of age (Fig. 4B).

Age-related trends for citrate acid and HA excretion in men and women are shown in Figure 5A and B, respectively. In both sexes, adolescents and young adults (10–25 y) experienced a higher excretion of citrate than HA, and this trend was more evident in women (Fig. 5A). In the older age groups, this difference attenuated in both sexes and, in men, HA excretion was on average higher than citrate excretion after the age of 55 (Fig. 5B). Consistently with these trends, a significant age-related decline of the ratio of citrate acid to HA was found in both sexes (in women, from 2.2, 95% CI, 1.6-3, in adolescents to 1.1, 95% CI, 0.9–1.4, in the >65 age group, $P_{trend} < 0.001$; in men, from 1.7, 95% CI 1.3–2.2, in adolescents to 0.9, 95% CI, 0.8–1.1, in the >65 age-group, $P_{trend} < 0.001$).

The levels of 24-h urinary excretion of HA and citrate after stratification of participants for number of FAV servings per day is reported in Table 4. HA excretion significantly increased with increasing numbers of FAV servings per day, whereas citrate excretion was significantly different only when comparing very low FAV intake (≤1 servings/d) with other intake levels (Table 4).

Discussion

The present study demonstrated that in a large group of Italian SFs, a significant age-related increase in FAV intake was associated with an increase in the urinary excretion of HA in both sexes until the age of 65 y. A mild decline in HA excretion was shown after the age of 65 despite high FAV consumption.

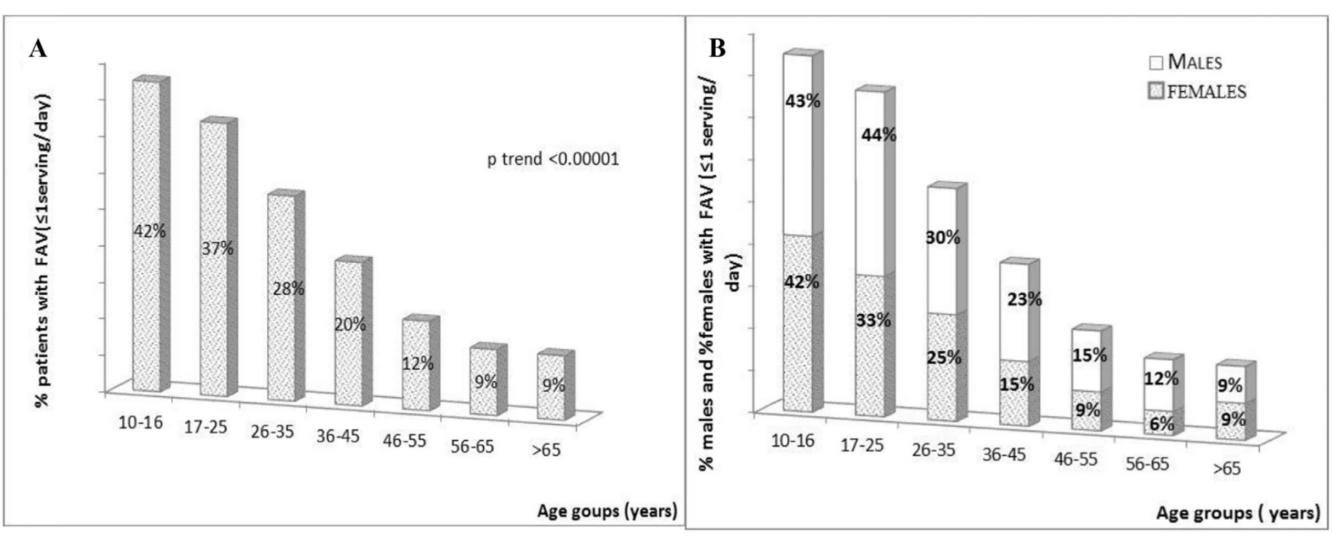

Fig. 1. Prevalence of stone formers with very low FAV intake (≤1 serving/d) in different age groups: Overall (**A**) and stratified by sex (**B**). No sex differences were detected in any of the age groups. FAV, fruit and vegetable.

Table 3
Prevalence (and absolute number) of different levels of FAV habitual intake across different age groups in the studied population (N = 1185 stone formers)*

Age groups (y)	Stone formers, n	FAV servings per day, % (n)					Bonferroni test significant *P*-values
		≤1	2	3	≥4	Mean (95% CI)	
(1) 10–16	52	42 (22)	54% (28)	4% (2)	0% (0)	1.2 (1–1.4)	(1) and (2) vs all the others, $P < 0.05$
(2) 17–25	126	37 (47)	57 (72)	6 (7)	0 (0)	1.5 (1.4–1.6)	
(3) 26–35	159	28 (45)	60 (95)	10 (16)	2 (3)	1.8 (1.7–1.9)	(3) and (4) vs all the others, $P < 0.05$
(4) 36–45	265	20 (53)	60 (158)	18 (48)	2 (6)	2.0 (1.9–2.1)	
(5) 46–55	254	12 (30)	52 (133)	30 (76)	6 (15)	2.3 (2.2–2.4)	(5) vs all the others, $P < 0.05$
(6) 56–65	212	9 (18)	38 (81)	40 (85)	13 (28)	2.6 (2.5–2.7)	(6) and (7) vs all the others, $P < 0.05$
(7) >65 y	117	9 (10)	35 (40)	48 (57)	8 (10)	2.6 (2.4–2.7)	
Total	1185	19 (225)	51 (607)	25 (291)	5 (62)	2.1 (2–2.2)	

FAV, fruit and vegetable
*Comparison of FAV intake distributions across different age groups is provided with Bonferroni test.

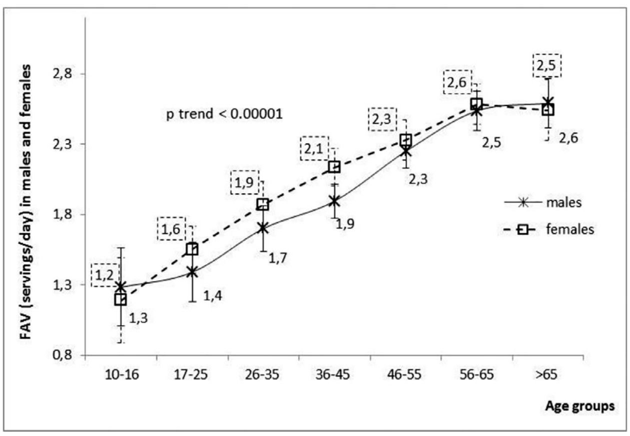

Fig. 2. Mean and 95% confidence interval of intake of FAV (servings/d) in 1185 stone formers stratified by age groups and sex. FAV, fruit and vegetable.

The age-related increase in HA excretion is related to the catabolism of polyphenol compounds, whose main dietary sources in countries following a Mediterranean-like diet, such as Italy, are FAV. Other foods containing high polyphenol amounts, like coffee, tea, red wine, and chocolate, instead give lower contributions to total dietary polyphenol load [16,24,25].

Results from the present study found that HA excretion was positively associated with both age and FAV intake. FAV intake itself increased with age. Interestingly, HA excretion was lower in young adults with ≤1 servings/d than in other age ranges with similar FAV intake. In fact, in participants <25 y of age, the prevalence of those reporting no consumption of FAV at all was significantly higher than in all other age groups. These data reinforce the role of urinary HA as a biomarker of FAV intake, which has already been studied in the context of nephrolithiasis [17–19].

After the age of 65, a mild decline in HA excretion was documented. However, this phenomenon could have little clinical significance. A recent study has confirmed that older individuals generally have a higher HA excretion than young adults, even when they follow the same diet, and demonstrated that HA excretion after an acute flavonoid load is enhanced in older compared with younger individuals [26].

We have also shown a significant increase in urinary citrate excretion in SFs from the age of 10 to the age of 55 y, which was inversely correlated with the prevalence of very low FAV intake. However, urinary citrate excretion declined after the age of 55 despite increasing FAV consumption. This apparent contradiction can be explained considering the physiological age-related subclinical decline of GFR (average GFR in participants <55 y 119 ± 32 mL/min, in participants >65 y 94 ± 27 mL/min). Even mild reductions of renal function in the absence of an established diagnosis of chronic renal insufficiency are in fact associated with reduced net acid excretion capacity [27,28]. This may result in a trend toward subclinical metabolic acidosis. Citrate handling in the kidney is influenced by the acid–base balance, with acidosis or a trend toward it, significantly reducing its excretion [14].

Significant sex-related differences in citrate excretion were also found in SFs, especially in the 17- to 55-y-old groups, supporting an influence of sexual hormones on citrate handling. Parks and Coe found significantly higher levels of urinary citrate excretion in female than in male SFs [29], whereas Curhan et al. did not confirm this difference in a large group of SFs participating in three different population-based cohort studies in the United States [30]. Similarly, the estrogen supplementation in menopausal female SFs resulted in a higher citrate excretion in one study [31], although it did not influence citrate excretion in another [32].

Dietary factors could also be involved in the age-related difference of citrate excretion. Our data showed that all the urinary parameters of lithogenic risk were higher in men than in women, with the only exceptions of citrate and pH, which were lower. After adjustment for BSA, the only parameters showing significant sex-related differences were citrate (higher in females) and creatinine, urea, sulfate, phosphorus, oxalate, and ammonium (higher in males). These differences allow hypothesizing that male SFs had a higher dietary intake of animal proteins and phosphorus [33,34], resulting in an increased dietary acid load [35,36] that could reduce citrate excretion regardless of FAV intake [14].

As such, urinary HA may be considered to be a more "direct" biomarker of FAV intake, whereas the association between FAV intake and citrate excretion may be mediated by the acid–base balance. In this scenario, the citrate-to-HA ratio can be regarded as the trend of citrate excretion capacity of the kidneys. In adolescents and young adults, the citrate-to-HA ratio is maximum, reflecting the optimal capacity of the kidneys to eliminate a dietary acid load with a very low risk for developing subclinical metabolic acidosis [27,28]. Conversely, a 6% to 7% increase in plasma concentrations of H+ ions can be detected at the age of 80 compared with the age of 20, as a consequence of physiological decline in renal function [28]. Renal net acid excretion capacity might thus be 8 mEq/d lower in healthy individuals >55 y of age than in healthy young adults [27]. Thus, a 250 g/d increase in FAV intake would be needed to counteract this loss of renal net acid excretion capacity [27]. The detected age-related decline of the urinary citrate-to-HA ratio in SFs may be a simple way of measuring these complex phenomena.

In the metabolic evaluation of patients with nephrolithiasis, citrate excretion is generally considered to be a marker of FAV intake [5,9], being associated with healthy dietary habits [37]. The present

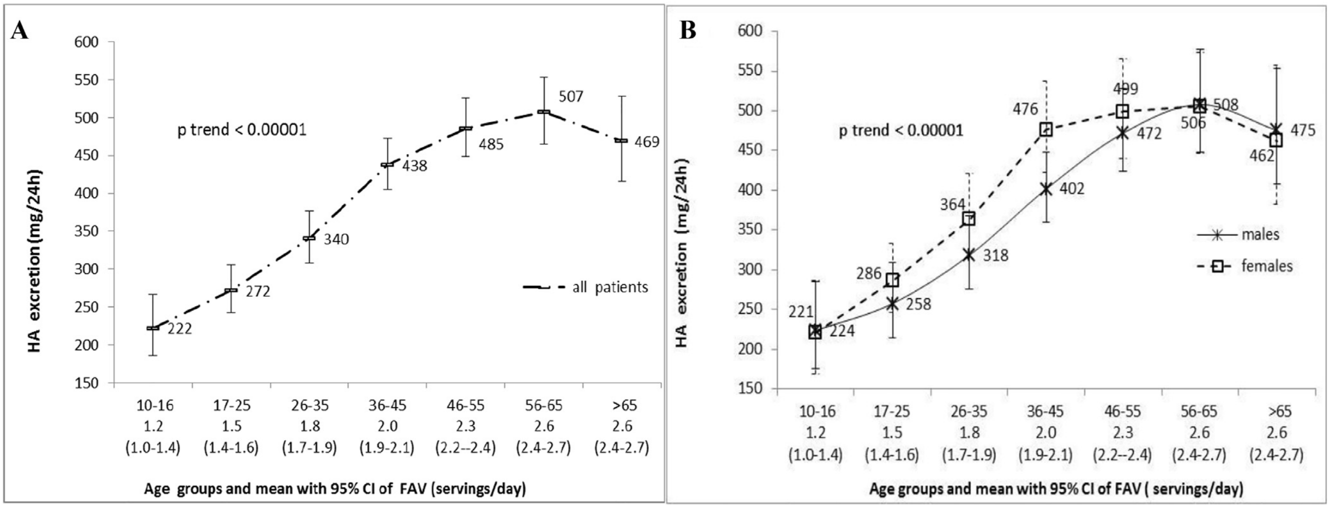

Fig. 3. Geometric means and 95% CI of 24-h urinary HA excretion (mg/24 h) adjusted for body surface area in different age groups in 1185 stone formers altogether (**A**) and stratified by sex (**B**). In the 36- to 45-y-old group, HA excretion was significantly higher in women than in men ($P < 0.05$). No differences were detected in other age groups. CI, confidence interval; FAV, fruit and vegetable; HA, hippuric acid.

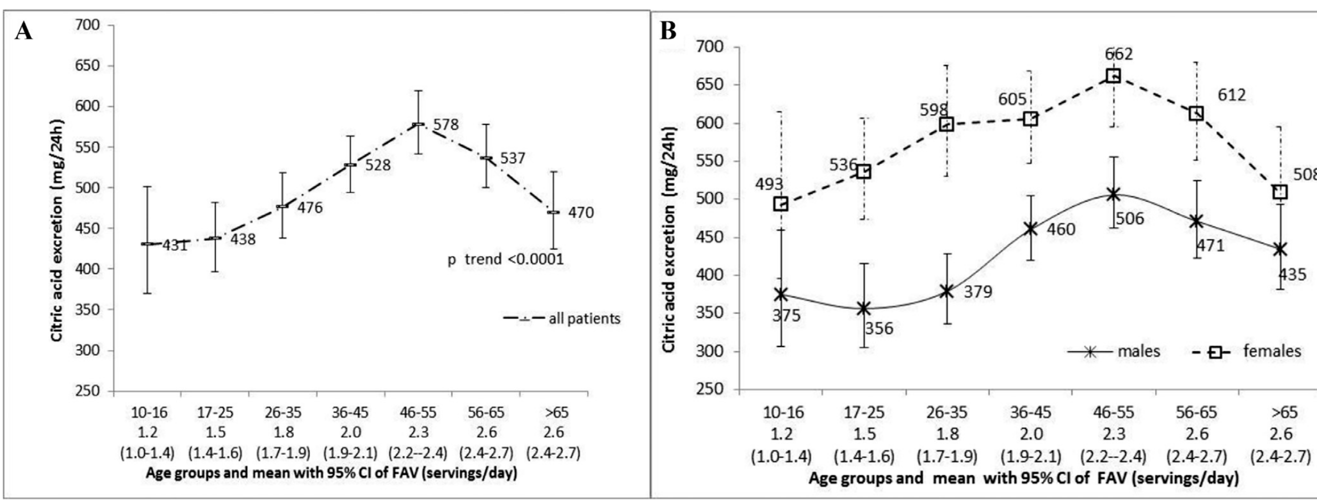

Fig. 4. Geometric means and 95% CI of 24-h urinary citrate excretion (mg/24 h) adjusted for body surface area in different age groups in 1185 stone formers altogether (**A**) and stratified by sex (**B**). Citrate excretion was significantly higher in women than in men ($P < 0.05$) in all the considered age groups, except for the youngest (10–16 y) and the oldest (>65 y) groups. CI, confidence interval; FAV, fruit and vegetable.

results highlighted that this biomarker may overestimate FAV intake in young individuals, particularly in young women, and underestimate it in older individuals. HA excretion may provide a more reliable biomarker of FAV intake, but is not involved in inhibition of lithogenesis. As such, the combined use of these biomarkers may be very useful in guiding the clinical management of hypocitraturia. In young SFs with hypocitraturia and low HA excretion, dietary advice including the increase of FAV intake may be sufficient to reduce the lithogenic risk [38]. Conversely, in older individuals with hypocitraturia and sufficient FAV intake documented by HA levels, the prescription of citrate salts should be preferred [39], considering also that this treatment may be beneficial on the kidney function [40].

Some limitations of our study should be considered. The absence of a comprehensive nutritional evaluation is the main one. The circumstance that flavonoids, the HA precursors, cannot be found only in FAV, but also in other foods, such as chocolate, red wine, tea, and coffee, should be considered [41]. This may have partly biased our results because the frequency of habitual intake of these foods was not assessed in the present study.

However, recent studies have highlighted that, in free diets and particularly in younger individuals, the contribution of cocoa, tea, and coffee to total dietary flavonoids and HA excretion is much lower than that of FAV [25]. Thus, HA can be considered to be a marker of FAV intake regardless of the intake of these foods [19]. In fact, studies associating the intake of red wine, coffee, tea, and chocolate with increased HA excretion have been mainly performed after the administration of an acute load of these foods and have not considered habitual intakes [26]. For example, coffee consumption significantly affects HA excretion only when it is more than two cups daily, which is actually more than the average consumption in the Italian population [42].

Another important limitation is the absence of data on chronic drug treatments taken at the moment of 24-h urine sample collection. Even if drugs with known influence on urinary lithogenic risk factors, including thiazide diuretics [37], were suspended the week before urine collection, citrate excretion can be influenced by

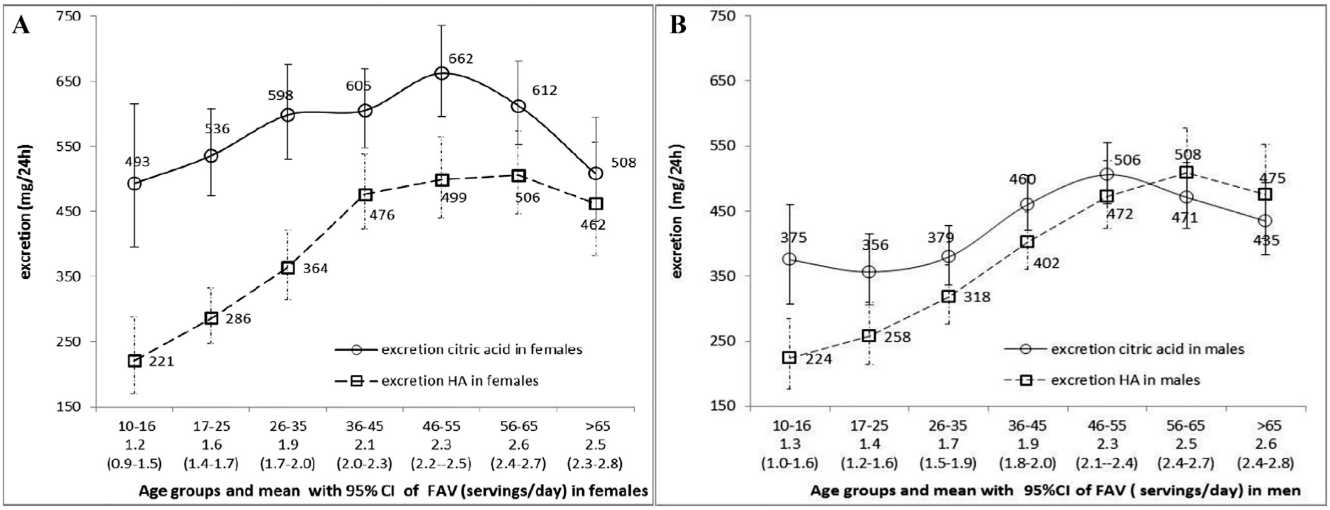

Fig. 5. Geometric means and 95% confidence intervals of 24-h citrate and HA excretion adjusted for body surface in different age groups of female (n = 560; **A**) and male (n = 625; **B**) stone formers. CI, confidence interval; FAV, fruit and vegetable; HA, hippuric acid.

Table 4
Geometric means and 95% CI of 24-h HA excretion (mg/d) and of 24-h citric acid excretion (mg/d) adjusted for body surface area in 1185 stone formers stratified by FAV (servings/d).

			HA excretion		Citric acid excretion	
Group	FAV servings per day, n	Stone formers, n	Geometric means (95% CI)	$P < 0.05$ (Bonferroni test)	Geometric means (95% CI)	$P < 0.05$ (Bonferroni test)
1	≤1	225	270 (250–293)	(1) vs (2) vs (3) vs (4)	404 (376–434)	(1) vs (2) vs (3) vs (4)
2	2	607	375 (357–394)	(2) vs (3) vs (4)	532 (509–556)	
3	3	291	622 (580–667)		536 (503–570)	
4	≥4	62	716 (613–839)		605 (528–694)	

CI, confidence interval; FAV, fruit and vegetable; HA, hippuric acid

polypharmacy in a complex way [14]. Thus, this circumstance also should be considered when interpreting the present findings.

Despite these limitations, this study provided important insights about the real-world clinical significance of urinary citrate and HA determination as possible biomarkers of FAV intake and could have relevance also outside the specific field of nephrolithiasis.

Conclusions

Both urinary citrate and HA excretion were confirmed as significant biomarkers of FAV intake in a large group of Italian SFs. Although urinary HA can be considered to be a direct biomarker of FAV intake, being influenced only by renal function, urinary citrate also may be modulated by other variables, such as age, sex, dietary acid load, and drugs. Citrate excretion may in fact overestimate FAV intake in young individuals, in whom renal function is optimal, and conversely underestimate FAV intake in those >55 y of age. These concepts, suggested by our data, may guide the clinical interpretation of hypocitraturia and the choice of the best treatment in SFs.

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The authors dedicate this article to the memory of their master, Professor Loris Borghi, who pioneered clinical research in the field of urolithiasis and recently passed away at the age of 69.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nut.2018.07.112.

References

[1] Romero V, Akpinar H, Assimos DG. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. Rev Urol 2010;12:e86–96.
[2] Gambaro G, Croppi E, Bushinsky D, Jaeger P, Cupisti A, Ticinesi A, et al. The risk of chronic kidney disease associated with urolithiasis and its urological treatments: a review. J Urol 2017;198:268–73.
[3] Daudon M. Epidemiology of nephrolithiasis in France. Ann Urol 2005;39:209–31.
[4] Rendina D, De Filippo G, D'Elia L, Strazzullo P. Metabolic syndrome and nephrolithiasis: a systematic review and meta-analysis of the scientific evidence. J Nephrol 2014;27:371–6.
[5] Gambaro G, Croppi E, Coe F, Lingeman J, Moe O, Worcester E, et al. Metabolic diagnosis and medical prevention of calcium nephrolithiasis and its systemic manifestations: a consensus statement. J Nephrol 2016;29:715–34.
[6] Ticinesi A, Nouvenne A, Maalouf NM, Borghi L, Meschi T. Salt and nephrolithiasis. Nephrol Dial Transplant 2016;31:39–45.
[7] Borghi L, Schianchi T, Meschi T, Guerra A, Allegri F, Maggiore U, et al. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. N Engl J Med 2002;346:77–84.
[8] Taylor EN, Curhan GC. Diet and fluid prescription in stone disease. Kidney Int 2006;70:835–9.
[9] Prezioso D, Strazzullo P, Lotti T, Bianchi G, Borghi L, Caione P, et al. Dietary treatment of urinary risk factors for renal stone formation. A review of CLU Working Group. Arch Ital Urol Androl 2015;87:105–20.
[10] Fink HA, Akornor JW, Garimella PS, MacDonald R, Cutting A, Rutks IR, et al. Diet, fluid, or supplements for secondary prevention of nephrolithiasis: a

systematic review and meta-analysis of randomized trials. Eur Urol 2009;56:72–80.
[11] Meschi T, Maggiore U, Fiaccadori E, Schianchi T, Bosi S, Adorni G, et al. The effect of fruits and vegetables on urinary stone risk factors. Kidney Int 2004;66:2402–10.
[12] Meschi T, Nouvenne A, Ticinesi A, Prati B, Guerra A, Allegri F, et al. Dietary habits in women with recurrent idiopathic calcium nephrolithiasis. J Transl Med 2012;10:63.
[13] Taylor EN, Stampfer MJ, Mount DB, Curhan GC. DASH-style diet and 24-hour urine composition. Clin J Am Soc Nephrol 2010;5:2315–22.
[14] Zuckerman JM, Assimos DG. Hypocitraturia: pathophysiology and medical management. Rev Urol 2009;11:134–44.
[15] Robertson WG. Do “inhibitors of crystallisation” play any role in the prevention of kidney stones? A critique. Urolithiasis 2017;45:43–56.
[16] Crozier A, Del Rio D, Clifford MN. Bioavailability of dietary flavonoids and phenolic compounds. Mol Aspects Med 2010;31:446–67.
[17] Guerra A, Folesani G, Mena P, Ticinesi A, Allegri F, Nouvenne A, et al. Hippuric acid in 24 h urine collections as a biomarker of fruits and vegetables intake in kidney stone formers. Int J Food Sci Nutr 2014;65:1033–8.
[18] Krupp D, Doberstein N, Shi L, Remer T. Hippuric acid in 24-hour urine collections is a potential biomarker for fruit and vegetable consumption in healthy children and adolescents. J Nutr 2012;142:1314–20.
[19] Penczynski KJ, Krupp D, Bring A, Bolzenius K, Remer T, Buyken AE. Relative validation of 24-h urinary hippuric acid excretion as a biomarker for dietary flavonoid intake from fruit and vegetables in healthy adolescents. Eur J Nutr 2017;56:757–66.
[20] Nouvenne A, Ticinesi A, Allegri F, Guerra A, Guida L, Morelli I, et al. Twenty-five years of idiopathic calcium nephrolithiasis: has anything changed? Clin Chem Lab Med 2014;52:337–44.
[21] Hsi RS, Sanford T, Goldfarb DS, Stoller ML. The role of the 24-hour urine collection in the prevention of kidney stone recurrence. J Urol 2017;197:1084–9.
[22] McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects. J Clin Epidemiol 2017;67:267–77.
[23] Berkemeyer S, Remer T. Anthropometrics provide a better estimate of urinary organic acid anion excretion than a dietary mineral intake-based estimate in children, adolescents, and young adults. J Nutr 2006;136:1203–8.
[24] Zamora-Ros R, Knaze V, Rothwell JA, Hémon B, Moskal A, Overvad K, et al. Dietary polyphenol intake in Europe: the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Eur J Nutr 2016;55:1359–75.
[25] Penczynski KJ, Herder C, Krupp D, Rienks J, Egert S, Wudy SA, et al. Flavonoid intake from fruit and vegetables during adolescence is prospectively associated with a favourable risk factor profile for type 2 diabetes in early adulthood [Epub ahead of print]. Eur J Nutr 2018.
[26] Alkhaldy A, Edwards CA, Combet E. The urinary phenolic acid profile varies between younger and older adults after a polyphenol-rich meal despite limited differences in in vitro colonic catabolism [Epub ahead of print]. Eur J Nutr 2018.
[27] Berkemeyer S, Vormann J, Gunther AL, Rylander R, Frassetto LA, Remer T. Renal net acid excretion capacity is comparable in prepubescence, adolescence, and young adulthood but falls with aging. J Am Geriatr Soc 2008;56:1442–8.
[28] Frassetto L, Sebastian A. Age and systemic acid-base equilibrium: analysis of published data. J Gerontol A Biol Sci Med Sci 1996;51:B91–9.
[29] Parks JH, Coe FL. A urinary calcium-citrate index for the evaluation of nephrolithiasis. Kidney Int 1986;30:85–90.
[30] Curhan GC, Willett WC, Speizer FE, Stampfer MJ. Twenty-four-hour urine chemistries and the risk of kidney stones among women and men. Kidney Int 2001;59:2290–8.
[31] Dey J, Creighton A, Lindberg JS, Fuselier HA, Kok DJ, et al. Estrogen replacement increased the citrate and calcium excretion rates in postmenopausal women with recurrent urolithiasis. J Urol 2002;167:169–71.
[32] Heller HJ, Sakhaee K, Moe OW, Pak CY. Etiological role of estrogen status in renal stone formation. J Urol 2002;168:1923–7.
[33] Bingham SA. Urine nitrogen as a biomarker for the validation of dietary protein intake. J Nutr 2003;133(suppl 3):921S–4S.
[34] Dragsted LO. Biomarkers of meat intake and the application of nutrigenomics. Meat Sci 2010;84:301–7.
[35] Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. J Am Diet Assoc 1995;95:791–7.
[36] Remer T. Influence of nutrition on acid-base balance—metabolic aspects. Eur J Nutr 2001;40:214–20.
[37] Mandel EI, Taylor EN, Curhan GC. Dietary and lifestyle factors and medical conditions associated with urinary citrate excretion. Clin J Am Soc Nephrol 2013;8:901–8.
[38] Pachaly MA, Baena CP, Buiar AC, de Fraga FS, Carvalho M. Effects of non-pharmacological interventions on urinary citrate levels: a systematic review and meta-analysis. Nephrol Dial Transplant 2016;31:1203–11.
[39] Phillips R, Hanchanale VS, Myatt A, Somani B, Nabi G, Biyani CS. Citrate salts for preventing and treating calcium containing kidney stones in adults. Cochrane Database Syst Rev 2015;10:CD010057.
[40] Gadola L, Noboa O, Marquez MN, Rodriguez MJ, Nin N, Boggia J, et al. Calcium citrate ameliorates the progression of chronic renal injury. Kidney Int 2004;65:1224–30.
[41] Mulder TP, Rietveld AG, van Amelsvoort JM. Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine. Am J Clin Nutr 2005;81(suppl):256S–60S.
[42] Ogawa M, Suzuki Y, Endo Y, Kawamoto T, Kayama F. Influence of coffee intake on urinary hippuric acid concentration. Ind Health 2011;49:195–202.