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Problems in the metabolic evaluation of renal stone disease: audit of intra-individual variation in urine metabolites

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Abstract Preliminary metabolic assessment of patients with renal stones includes measurement of urine metabolites. This paper reports on the degree of intra-individual variation in some key urine metabolites. Over 80 medically untreated patients under initial metabolic investigation were audited from whom 24-h urine results were available as three separate urine pairs collected at intervals not less than 1 month apart. Ranking patients by intra-individual variation, above the 75th centile, the highest calcium was at least 216% of the lowest calcium, the respective figures for phosphate, urate, oxalate, citrate, creatinine and sodium were 207, 190, 271, 412, 175 and 233%. In order to estimate pre-treatment excretion within 30% of a true mean at the 95% confidence limit, for calcium and oxalate, the number of 24-h samples required were 3 and 4 respectively with 6 and 9 required to be within 20%. These observations illustrate significant practical clinical problems in assessing patients with renal stones when assessing these basic parameters. Regimens based on small numbers of urine collections are flawed, hence evidence based protocols should be devised. A minimum of three pairs of 24-h urine samples based upon predicting metabolite output within 20–30% or less of the true mean is recommended.

Keywords Intra-individual variation · Urolithiasis · Twenty-four-hour urine analysis

Introduction

Progress in the understanding of the complex physical chemistry of renal stone disease is set against a background of uncertainty in respect of medical treatment. The 1990s saw challenge to the 20-year practice of advocating low calcium diets in hypercalciuria when Curhan et al. [1] reported that the risk of male symptomatic stone disease was actually lower for those on a higher calcium diet. More recent dietary and experimental evidence also suggests that a higher calcium intake is associated with lower urinary oxalate and decreased renal stone formation [2–11]. A key component of renal stone metabolic investigation continues to be the collection of 24-h urine samples. An Internet search of urology associations/societies/foundations does not reveal any statistically explicit evidence based guidelines for the desirable number, or type, of urine samples to be collected and over what period of time. Strohmaier et al. [12], comparing spot urine with 24-h analysis, noted considerable intra-individual variation in the analytes tested on three consecutive days which included calcium, phosphate, urate, oxalate and citrate. In 2001 Pak [13] defended the suitability of a single 24-h urine sample for "simplified" investigation purposes, based on a study of two random 24-h urine samples. More recently, Parks et al. [14] showed unpredicted significant differences in stone risk factors during different parts of the year. Urine calcium was 10% lower overall in both men and women in the summer compared with winter, whilst calcium oxalate supersaturation was higher in men in the summer, but lower in women. That study, however, was based upon no more than two contemporaneous pre-treatment 24-h urine specimens and, although its validity rested upon the very high number of observations, it does not address intra-individual variation. Concern over intra-individual variation has been evident since the 1960s. Ryall and Marshall [15] have reviewed evidence drawing attention to intra-individual variation observed over periods of up to 14 consecutive days both in the context of recurrent stone formation and the absence of any known stone formation.

Most stones are predominantly calcium oxalate, with up to half of these patients said to have hypercalciuria.

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Such patients have been classified as having absorptive hypercalciuria, renal hypercalciuria, renal phosphate leak, or primary enhancement of 1, 25-dihydroxycholecalciferol [13]. We contend that, on account of intra-individual variation, definitive classification is unreliable and have challenged the basis of the classification [16].

Metabolic investigational practice varies. In an audit of 14 Welsh hospitals [17], there were two specialist metabolic clinics for stone patients and only one laboratory had written explicit investigational criteria. The majority (9 of the 14) of laboratories had no formal or informal advice to offer on how many urine samples over what period of time might indicate a patient is unlikely to have hypercalciuria or hyperoxaluria. Only half the laboratories screened for cystine and only four measured citrate, although six offered an ammonium chloride loading test for partial renal tubular acidosis.

We have experienced a steady growth in local interest in the metabolic investigation of patients with renal stone disease. In our practice, within limited competing resources, all newly referred patients have entered an enlarging pool (600) of patients undergoing active metabolic management by a single clinician, the outcome of which has been increasing delays. These create further delays as some patients default (temporarily) from the clinics. We have taken the opportunity to capitalise in retrospect on this chain reaction by auditing intra-individual variation over an extended period not covered within the existing literature. Our aim has been to provide a statistical rationale as a basis of practice in terms of the number of 24-h urine specimens required to predict 24-h urine metabolite mean values to limits which are of clinical relevance and at the same time are practical to achieve.

Methods

Data were obtained retrospectively from laboratory computer records of 82 new patients. The local research ethics committee did not require specific approval. There were 24 females and 58 males, with mean ages of 44.5 years (SD 14.5) and 50.6 years (SD 12.2), respectively. These patients collected three pairs of 24-h urine specimens, spaced at least 1 month apart. The period of accumulation of data was 5.9 years. For individuals, the mean period over which their urine collections took place was 39 weeks (SD 30 weeks).

Calcium, phosphate, urate, sodium and creatinine were measured using a Synchron LX 20 analyser (Beckman Coulter United Kingdom Ltd, High Wycombe, UK) using the manufacturer's recommended protocols. Oxalate was measured using an oxalate oxidase method (Trinity Biotech, Bray Co., Wicklow, Ireland). Citrate was measured using a citrate lyase method (R-Biopharm, Glasgow, UK) adapted for use on the Synchron LX 20 analyser. Urine sample preparation followed that of Ng et al. [18]. Quality control of analyses was assessed daily using a ''normal'' and ''elevated'' control. The analytical variance of the assays was selected from the long-term between-batch variance of the control value closest to the mean patient values for that particular analyte. Patients were instructed by both medical staff and nursing staff, supplemented by written instructions, on the urine collection protocol each time they undertook a pair of urine collections. Patients were also provided with a direct line telephone contact to discuss any issues relating to their urine collections or investigations. Collections were rejected if the second of the pair was more than 3 days old and if the urine volume was less than 800 ml. No inter-current illness known to influence urine results was observed in any of the patients during the time period of the audit.

The biological variation of all analytes was calculated as inter- and intra-individual components of variance by analysis of variance with correction for unequal sample sizes [19] and subsequent subtraction of analytical variance [20]. The variances were expressed as coefficients of variation (CV%). These components of variance were used to calculate the number of urine samples that would need to be analysed to determine an individual's true long-term mean values for urine calcium, phosphate, oxalate urate, citrate and sodium excretion within ± 10, ± 20 and $\pm 30\%$ for $P < 0.05$ [21].

Results

The 82 patients collected a mean of six (SD 0.7) urine samples during the course of the study. Mean values for all analytes, stratified by gender, are shown in Table 1.

The inter-, intra- and analytical variance, expressed as coefficients of variation, are shown in Table 2 for all analytes measured during the present study. In addition, the number of samples required to estimate the patients'

Table 1 Mean and standard deviation for all analytes, stratified by gender

	Female		Male		*t* Test *P* value
	Mean	SD	Mean	SD	
Calcium (mmol/day)	5.6	2.7	8.4	3.5	0.001
Phosphate (mmol/day)	24.6	6.1	36.2	12.3	< 0.001
Urate (mmol/day)	3.3	0.6	4.2	1.2	0.0005
Oxalate (μmol/day)	360.8	153.0	392.4	166.6	0.4
Citrate (mmol/day)	3.4	2.2	3.4	2.2	0.9
Sodium (mmol/day)	133.1	46.9	199.0	68.7	< 0.001
Creatinine (mmol/day)	10.6	2.3	16.6	4.7	< 0.001

Differences between males and females were assessed using the Student's *t* test

true mean values ±10, ±20 and ±30% are shown. Expressing the urine analytes as creatinine ratios did not greatly affect the intra-individual variances: CV_i% for calcium/creatinine, phosphate/creatinine, urate/creatinine, oxalate/creatinine, citrate/creatinine and sodium/creatinine were 22.0, 16.5, 15.3, 36.1, 33.8 and 27.6, respectively.

To assess the effect of age and gender upon intra-individual variation, the patients were stratified by age tertile and by gender and the intra-individual variation re-calculated (Table 3)

To emphasise the impact of intra-individual variation on the interpretation of results, patients' highest value for each analyte obtained during the study was divided by their lowest value. These ratios were sorted numerically, lowest to highest, and expressed as percentiles (Figs. 1, 2). At the 25th centile, the ratio of highest to lowest calcium excretion was 1.5, suggesting that in 75% of the population, over a period of months, their highest calcium excretion is at least 50% greater than their lowest. Similar figures for phosphate, urate, oxalate, citrate, creatinine and sodium were 43, 41, 56, 61, 31 and 62%. At the 75th centile, in 25% of the population, the highest calcium, phosphate, urate, oxalate, citrate, creatinine and sodium excretions were at least 216, 207, 190, 271, 412, 175 and 233% higher than the lowest.

Discussion

Every test result is subject to a number of sources of variation, of which analytical imprecision and intra-individual biological variation are particularly important [22]. The effect of both of these on the dispersion of a single test result and on the number of samples required to make clinical decisions can easily be calculated. Biological information on a large number of common laboratory analytes has been published [23], mainly on healthy individuals. However, for certain analytes, biological variation in pathological states may be higher than in the healthy state. In addition, Ricos et al. [24] have discussed the discrepancies between various studies on the biological variation of common urine analytes in normal individuals. They suggest that the reasons for these discrepancies include the number of subjects studied, differences in analytical variation and the ratios of men and women studied. This study, unlike others, examined biological variation in a reasonably sized population of patients with a documented history of a well-defined clinical condition, namely urolithiasis. In addition, having been accumulated over a period of years, the study represents a typical age/sex mix of patients being investigated for this disease.

The collection of complete 24-h urines is a perennial problem. The use of both creatinine excretion [25] and recovery of *p*-aminobenzoic acid [26] as markers of completeness of collection have been criticised. In this present study, those urine samples with a volume of less than 800 ml were rejected for analysis on the grounds of possible incompleteness. The remainder were analysed as being representative of samples normally presented to the laboratory for routine analysis. Any errors in collection therefore have contributed to the intra-individual variation for all analytes.

The data presented in Table 1 show gender differences in all analytes apart from urine oxalate and citrate. It is therefore reasonable to investigate whether stratification by gender had any influence on the intra-individual variation reported in Table 2. Additionally, as intestinal absorption of calcium decreases with age [27] it is also of interest to investigate the effect of age upon this variation. Stratification by age tertile and gender (Table 3), however, showed no consistent pattern of difference between age and gender groups, apart, perhaps, for a tendency for intra-individual variation in some analytes to be lower in the 44- to 55-year age group, compared to older or younger patients.

Of 600 patients we have investigated in our clinics, there are only two in whom urine chemistry has not revealed any evidence of abnormal values over a 3-year period of clinical follow-up. In a report [28] on 2,000 patients with upper urinary tract stones, a metabolic abnormality was found at initial screening in roughly half of the patients with idiopathic calcium stones and struvite stones. In those with uric acid stones and those with mixed uric acid/calcium oxalate a metabolic abnormality was evident in less then 10%. In a group of 49 patients [29], 41% were initially classified as metabolically inactive, but on follow-up abnormal findings were found in 40% of this group. We observe that

Table 2 Intra- and inter-individual variation

Test	CV_i%	CV_g%	CV_a%	Mean	*n* for mean ± 10%	*n* for mean ± 20%	*n* for mean ± 30%
Calcium (mmol/day)	24.6	36.4	3.58	7.8	24	6	3
Phosphate (mmol/day)	19.3	21.9	5.32	33.9	15	4	2
Urate (mmol/day)	24.0	19.1	4.81	4.2	23	6	3
Oxalate (μmol/day)	29.7	26.1	4.39	426.4	35	9	3
Citrate (mmol/day)	32.2	38.4	7.81	3.5	42	11	5
Creatinine (mmol/day)	17.4	23.6	2.56	15.4	12	3	1
Sodium (mmol/day)	26.9	28.7	0.9	184	28	7	3

Intra-, inter- and analytical variation expressed as coefficients of variance (CV_i%, CV_g% and CV_a%, respectively). The number (*n*) of samples required to determine the patients' "true" mean values ±10, 20 and 30% are shown for $P < 0.05$

Table 3 Intra-individual variation (CV_i%) stratified by age tertile and gender

	n	Calcium (mmol/day)	Phosphate (mmol/day)	Urate (mmol/day)	Oxalate (μmol/day)	Citrate (mmol/day)	Sodium (mmol/day)	Creatinine (mmol/day)
Males < 44 years	17	27.4	21.3	14.1	27.3	25.9	28.0	16.0
Males 44–55 years	21	27.9	9.2	13.9	16.1	38.7	21.2	11.6
Males > 55 years	20	37.3	24.2	19.6	32.8	45.6	26.0	19.7
Females < 44 years	12	38.9	10.9	7.8	25.4	37.8	12.1	6.1
Females 44–55 years	6	23.6	7.8	8.0	10.6	32.8	14.0	12.7
Females > 55 years	6	29.1	5.3	15.7	17.4	40.3	21.4	9.2

practically all patients presenting with renal stones and who have been studied for long enough will manifest at least one urinary abnormality, and that it is common for their metabolic patterns to be inconsistent. The whole basis of what is normal and abnormal has been questioned in a key work [30]. Among subjects from the Nurses' Health Study and the Health Professionals Study with and without a history of renal stones (cases and controls, respectively), up to 27% of controls had hypercalciuria. Comparing the two groups, the risk of renal stones increased with urine calcium levels, but not with oxalate or citrate. Male cases actually had lower uric acid levels than controls. The authors [30] noted the importance of urine chemistries for predicting stone formation, but observed that the significance and the magnitudes of the associations appeared to differ by age and gender.

Although the American convention is to undertake extensive metabolic evaluation of renal stone disease, within a commercial medical setting there is a tension between the desirability of extended investigation and the need to progress medical treatment: the "seeming desire for austerity" in this context has been questioned [31]. Our patients were on a waiting list for metabolic treatment and whilst waiting they collected urine samples. The protracted length of the waiting list has yielded information that questions the conventional approach to investigation. In a recent key paper, Parks et al. [31] have promulgated the inadequacy of a single 24-h urine specimen for the medical evaluation of nephrolithiasis. In that paper, the group collecting the maximum number of urine samples collected three 24-h urine specimens usually sequentially on a 3-day basis. Our data are directly in keeping with those findings but reflect greater intra-individual variation in concert with a much longer period of pre-medical treatment observation. From our data, the relatively high number of collections required to estimate the excretion of some metabolites to even 30% from an actual mean should be noted.

The question arises as to what is the value of a mean value? Ryall and Marshall [15] have reported day-to-day variation in calcium output in an individual with hypercalciuria and recurrent renal stones and in an individual with no history of renal stone disease. Over a 14-week period, the former showed a normal calcium excretion on two consecutive days, and the latter showed an increased calcium excretion on three consecutive days. In our experience nearly half our patients, with intermittent subnormal citrate values, have partial renal tubular acidosis, even though their mean values may be normal. Are the shapes, frequency and timing of urine metabolite peaks (or troughs, in the case of citrate) more

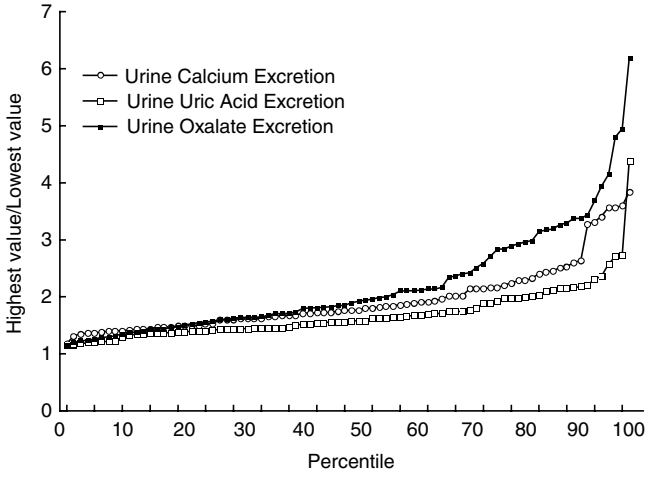

Fig. 1 Patients by rank order: ratio of minimum to maximum 24-h urine excretions of calcium (*open circles*), urate (*open boxes*) and oxalate (*filled boxes*). *X*-axis values are presented as percentiles

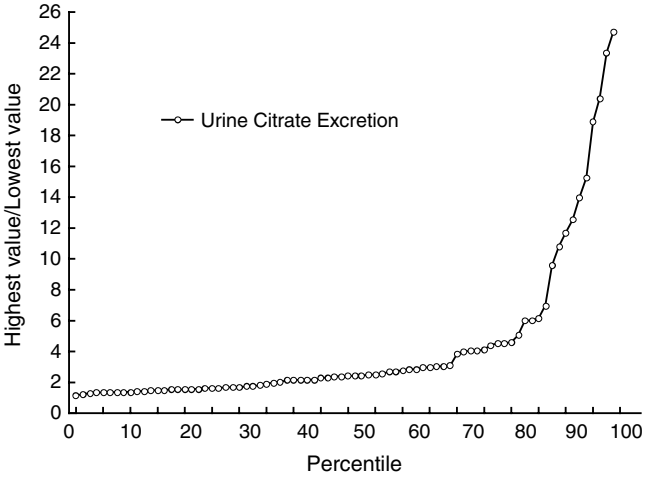

Fig. 2 Patients by rank order: ratio of minimum to maximum 24-h urine excretion of citrate. *X*-axis values are presented as percentiles

important than mean values in stone risk terms and what patterns should we focus upon? We have found patients unwilling to collect 24-h urine samples for the purpose of research, but willing to collect spot urine samples twice daily for extended periods. Extended periods of spot urine observation may possibly significantly add to the limited predictive values of 24-h urine collection subject to the parallel construction of reference ranges. Urine samples (24 h) should be collected to clinical protocols which quantitate their strengths and weaknesses. Small numbers of collections are problematical when it comes to interpretation and are therefore flawed.

Guided by Table 1, our clinical practice is now to advocate the collection of three pairs of 24-h urine samples at intervals of 1 month or more on the grounds that the mean value of the individual tests undertaken will be within 20–30% or less of the real mean. Of course this is a compromise with the realities of getting patients to complete collections and does not mean that a shorter period would be satisfactory, but it does a have a statistical basis. In practice, patients are requested to collect two pairs of 24-h urine as part of their initial metabolic assessment. They are seen in the clinic to discuss the initial findings and then asked to collect two further pairs of 24-h urine at 1-monthly intervals for their second metabolic review and initiation of interventions. From the outset they have received advice directing them to a 2.5-l diuresis (if medically appropriate) and are given a 250-ml measuring cylinder for this purpose, but are otherwise asked to eat and take medication and self-medicate ''normally.''

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