[Frontiers in Bioscience 8, a167-176, September 1, 2003]

URINARY SATURATION AND RISK FACTORS FOR CALCIUM OXALATE STONE DISEASE BASED ON SPOT AND 24-HOUR URINE SPECIMENS

Yoshihide Ogawa, Hiroyuki Yonou, Sanehiro Hokama, Masami Oda, Makoto Morozumi, and Kimio Sugaya

Department of Urology, University of the Ryukyus, Okinawa, Japan 903-0215

TABLE OF CONTENTS

1. ABSTRACT

In 222 random spot urine specimens, the calcium concentration and calcium oxalate saturation {DG(CaOx) } were significantly higher among stone formers than among non-stone formers, while the citrate and creatinine-corrected citrate concentrations were lower. In 188 24-hour urine specimens, magnesium excretion was lower among stone formers than non-stone formers, while the creatinine-corrected calcium concentration and DG(CaOx) were higher. Among stone formers, there was no gender difference in the urinary concentrations of calcium, oxalate, citrate, magnesium, and DG(CaOx), but the creatinine-corrected calcium, citrate, and magnesium concentrations were higher in women, as well as 24-hour citrate excretion. The levels of calcium and oxalate have a major influence on DG(CaOx), while citrate and magnesium levels have a minor influence. DG(CaOx) was correlated with calcium and oxalate excretion, as well as with the creatinine-corrected calcium and oxalate concentrations. Approximately 5% of 24-hour urine specimens showed critical supersaturation, 80% showed metastable supersaturation, and 15% were unsaturated. Hypercalciuria or hyperoxaluria was fairly common (30% and 40%) in critically supersaturated urine, while it was less common (22.4% and 8.6%) in metastably supersaturated urine and was not detected in unsaturated urine. Hypocitraturia and/or hypomagnesiuria was more common (63.8-80%) at any saturation. The urinary calcium, oxalate, and citrate concentrations, as well as the creatinine-corrected calcium, oxalate, citrate, and magnesium concentrations and DG(CaOx), showed a significant correlation between 57 paired early morning spot urine and 24-hour urine specimens. The creatinine-corrected calcium and citrate concentrations of the early morning urine specimens were significantly correlated with the levels of calcium and citrate excretion in the paired 24-hour urine specimens. In conclusion, no parameter other than urinary saturation gives more than a vague indication of the risk of lithogenesis, so DG(CaOx) in either early morning urine or 24-hour urine specimens appears to be the best predictor of stone risk. Finally, the creatinine-corrected calcium and citrate concentrations in early morning urine can be used as a substitute for measuring 24-hour excretion.

2. INTRODUCTION

Urinary stones can be considered as fossils representing long-standing specific conditions of urinary supersaturation in the collecting system. The diurnal circadian rhythm, seasonal variation, heterogeneity of nephrons, and variations of pathological conditions all influence the saturation of urine (1-3). Urinary supersaturation is a prerequisite for stone formation and it should be documented during the period of stone formation and growth. With respect to lithogenesis, the circadian rhythm of stone parameters (calcium, oxalate, citrate, and magnesium) was investigated and discussed by Marshall, Vahlensieck, and Ogawa (4-6). However, it is not easy to measure all of these parameters in every patient during various situations in a day or year and individual parameters do not reflect the lithogenic potential or supersaturation of urine. Despite this, 24-hour urine collection to measure stone parameters remains the gold standard for finding the lithogenic etiology and potential (7-9).

Historically, urinary supersaturation has been used as scientific evidence of the potential for stone formation (10, 11), but complicated mathematical methods of calculating urinary saturation have made the practical use of such parameters difficult. Although a simplified method for estimating saturation was introduced by Marshall and Robertson (12), urinary saturation does not closely reflect clinical stone episodes, so various other parameters have been tested (13, 14). As a result, measurement of the risk of crystallization or saturation has subsequently been modified by various researchers (15-23). We previously used the simplified method of Marshall and Robertson (12) clinically and concluded that late night and early morning was the high-risk period for calcium oxalate crystallization (24), and similar findings were subsequently confirmed by Ahlstrand, Ogawa, and Robert (25-27).

The risk of calcium oxalate stone formation is generally discussed on the basis of several parameters (hypercalciuria, hyperoxaluria, hypocitraturia, and hypomagnesuria), but the lithogenic potential has been discussed in terms of urinary calcium oxalate saturation

(24, 26, 28-33). There is no clear-cut relationship between the risk indices and the rate of stone formation or between stone formers and non-stone formers (34-37), but there is a tendency for a higher risk of stone formation in supersaturated urine (38-40). We also reported that the CaOx-risk index of Tiselius (16) was higher in 9 children with calcium oxalate stones than in 92 non-stone forming children (41), followed by a similar report (42) but the lithogenic potential in children still tends to be discussed only on the basis of citrate and magnesium (43). The efficacy of drugs can be evaluated in terms of changes in saturation (32, 44-57), but it is often interpreted only on the basis of calcium, oxalate, or citrate excretion (58, 59).

Using 390 urine specimens from healthy volunteers, we previously showed that hypocitraturia, hypomagnesiuria, and a high Ca/Mg ratio were poor indicators of calcium oxalate supersaturation (DG value > 0) and confirmed that critical calcium oxalate supersaturation of urine occurs late at night and early in the morning (60). Citrate and magnesium are known as important inhibitors of crystallization (61-63), and in a larger series than we reported previously (64) we studied how the levels of these parameters contribute to supersaturation and whether isolated hypocitraturia or hypomagnesiuria is clinically important. In addition, we compared the creatinine-corrected levels of calcium, oxalate, citrate, and magnesium in early morning spot urine with their respective 24-hour urinary excretion values.

3. SUBJECTS AND METHODS

A total of 222 random spot urine specimens were obtained in the morning at the outpatient clinic from 110 non-stone formers (37 women aged 46.4±22.8 years and 73 men aged 47.6±21.4 years) and 112 idiopathic calcium oxalate stone formers (37 women aged 55.3±16.4 years and 75 men aged 47.3±17.8 years), and 188 24-hour urine specimens were obtained in the ward from 22 non-stone formers (6 women aged 53.8±8.8 years and 16 men aged 54.2±18.6 years) and 166 inpatients with idiopathic calcium oxalate stones (49 women aged 56.6±13.1 years and 117 men aged 49.2±14.6 years). All of the patients had normal renal function and no serious diseases. Both 24-hour urine and early morning spot urine specimens were obtained from 57 inpatients with idiopathic calcium oxalate stones (who were aged 53±15 years) and were used for comparison of various urinary parameters. Urinary calcium (Ca) and magnesium (Mg) were measured by ICP spectrophotometry, oxalate (Ox) and citrate (Cit) were measured by capillary electrophoresis (65, 66), and urinary creatinine (Cre) was determined by the enzymatic method. The DG(CaOx) values were calculated with Finlayson's Equil2 program. The critical level for calcium oxalate supersaturation was defined as a DG(CaOx) value > 2.8 {The DG(CaOx) values of the solubility product and formation product were reported by Ahlstrand to be -0.15 and 3.3, respectively (25) and by Robertson -0.36 and 2.8, respectively (38) }. The DG(CaOx) value and the values of [Ca], Ca/Cre (mg/mg), [Ox], Ox/Cre, [Cit], Cit/Cre, [Mg], and Mg/Cre were compared by Student's t-test, the Mann-Whitney test, and Scheffe's test. Correlations between parameters or groups (stone formers vs. non-stone formers) were determined by simple regression analysis and multiple regression analysis. Statistical significance was set at $p<0.05$ for all comparisons.

4. RESULTS

In random spot urine specimens, the mean calcium concentration was 2.329±1.614 and 2.879±1.751 mmol/l in non-stone formers and stone formers, respectively, with the latter value being significantly higher ($p<0.01$), as shown in Table 1. The oxalate and magnesium concentrations were not significantly different between the groups, as shown in Tables 1 and 2. The citrate concentration was 2.257±2.038 and 1.498±1.279 mmol/l in non-stone formers and stone formers, respectively, with the former value being significantly higher ($p<0.01$), as shown in Table 2. The Ca/Cre (mg/mg), Ox/Cre, and Mg/Cre ratios did not differ between the groups. The Cit/Cre ratio was 0.480±0.324 and 0.342±0.267 in non-stone formers and stone formers, respectively, with the former value being significantly higher ($p<0.01$). DG(CaOx) was 1.074±1.289 and 1.393±1.132 in non-stone formers and stone formers, respectively, being significantly higher ($p<0.05$) in the latter, as shown in Table 3.

In 24-hour urine specimens, the mean calcium, oxalate, citrate, and magnesium concentrations did not differ between the groups (Tables 1 and 2). Urinary calcium, oxalate, and citrate excretion were not different between the groups, while magnesium excretion was 3.414±1.078 and 2.755±1.361 mmol/day in non-stone formers and stone formers, respectively, being significantly higher in the former group ($p<0.05$). The Ca/Cre (mg/mg) ratio was 0.116±0.051 and 0.148±0.075 in non-stone formers and stone formers, respectively, with the latter value being significantly higher ($p<0.05$), as shown in Table 3. The Ox/Cre, Cit/Cre, and Mg/Cre ratios did not differ between the groups. The mean DG(CaOx) value was 0.651±1.238 and 1.209±1.090 in non-stone formers and stone formers, respectively, with the latter value being significantly higher ($p<0.05$). Among stone formers, urinary calcium, oxalate, and magnesium excretion were not different between men and women, while 24-hour citrate excretion was significantly higher in women than in men ($p<0.05$). This gender difference was not present for the 24-hour concentrations of urinary calcium, oxalate, citrate, and magnesium or for the DG(CaOx) values, while the 24-hour calcium/creatinine, citrate/creatinine, and magnesium/creatinine ratios were significantly higher in women ($p<0.05$).

DG(CaOx) was significantly correlated with urinary calcium and oxalate concentrations ($p<0.01$), calcium and oxalate excretion ($p<0.01$), and the calcium/creatinine and oxalate/creatinine ratios ($p<0.01$), as shown in Table 4. DG(CaOx) was also positively correlated with the urinary citrate and magnesium concentrations ($p<0.01$) and magnesium excretion ($p<0.05$), but it was not correlated with urinary citrate excretion or with the citrate/creatinine and magnesium/creatinine ratios. Therefore, urinary citrate

Table 1. Urinary concentrations and excretion of Ca and Ox in stone formers and non-stone formers

		Age year	[Ca] mM	Ca mmol/day	[Ox] mM	Ox mmol/day
Spot urine	Stone (-)	47.3±22.0	2.329±1.614		0.188±0.140	
	Stone (+)	50.5±17.6	2.879±1.751[1]		0.161±0.093	
24-hour urine	Stone (-)	54.1±16.2	1.680±0.931	2.942±1.373	0.115±0.076	0.196±0.105
	Stone (+)	51.6±14.5	2.121±1.274	3.481±2.011	0.131±0.078	0.206±0.116
	M (+)	49.2±14.6	2.114±1.304	3.428±2.146	0.137±0.078	0.209±0.110
	F (+)	56.6±13.1	2.135±1.217	3.626±1.609	0.117±0.075	0.196±0.133

Urinary calcium & oxalate concentrations {[Ca] and [Ox]} and 24-hour urinary calcium & oxalate excretion {(Ca) and (Ox)} in 222 random spot urine specimens and 188 24-hour urine specimens are compared between stone formers and non-stone formers, as well as between male {M (+)} and female stone formers {F (+)}. Data are shown as mean±SD. [1]: $p< 0.01$.

Table 2. Urinary concentrations and excretion of Cit and Mg in stone formers and non-stone formers

		[Cit] mM	Cit mmol/day	[Mg] mM	Mg mmol/day
Spot urine	Stone (-)	2.257±2.038		2.509±1.841	
	Stone (+)	1.498±1.279[1]		2.168±1.257	
24-hour urine	Stone (-)	1.206±0.656	2.205±1.133	1.922±0.678	3.414±1.078
	Stone (+)	0.898±0.799	1.459±1.313	1.700±1.036	2.755±1.361[2]
	M (+)	0.821±0.781	1.335±1.314	1.774±1.085	2.859±1.421
	F (+)	1.073±0.818	1.818±1.260[3]	1.537±0.907	2.479±1.162

Urinary citrate & magnesium concentrations {[Cit] and [Mg]} and 24-hour urinary citrate & magnesium excretion {(Cit) and (Mg)} in 222 random spot urine specimens and 188 24-hour urine specimens are compared between stone formers and non-stone formers, as well as between male {M (+)} and female stone formers {F (+)}. Data are shown as mean±SD. [1]: $p< 0.01$, comparison with non-stone formers; [2]: $p< 0.05$, comparison with non-stone formers; [3]: $p<0.05$, comparison with male stone formers {M (+)}.

Table 3. Urinary Cre-corrected Ca, Ox, Cit, and Mg concentrations and DG(CaOx) in stone formers and non-stone formers

		Ca/Cre mg/mg	Ox/Cre mg/mg	Cit/Cre mg/mg	Mg/Cre mg/mg	DG(CaOx)
Spot urine	Stone (-)	0.116±0.078	0.020±0.015	0.480±0.324	0.074±0.043	1.074±1.289
	Stone (+)	0.140±0.103	0.018±0.011	0.342±0.267[1]	0.063±0.041	1.393±1.132[2]
24-hour urine	Stone (-)	0.116±0.051	0.017±0.007	0.431±0.250	0.084±0.031	0.651±1.238
	Stone (+)	0.148±0.075[2]	0.021±0.012	0.313±0.274	0.071±0.034	1.209±1.090[2]
	M (+)	0.125±0.063	0.019±0.009	0.237±0.180	0.063±0.028	1.241±1.117
	F (+)	0.200±0.076[3]	0.025±0.016	0.485±0.362[3]	0.089±0.038[4]	1.138±1.034

Urinary Ca/Cre, Ox/Cre, Cit/Cre, and Mg/Cre ratios and the DG(CaOx) value in 222 random spot urine specimens and 188 24-hour urine specimens are compared between stone formers and non-stone formers, as well as between male {M (+)} and female stone formers {F (+)}. Data are shown as mean±SD. [1]: $p< 0.01$, comparison with non-stone formers; [2]: $p< 0.05$, comparison with non-stone formers; [3]: $p<0.01$, comparison with male stone formers {M (+)}; [4]: $p<0.05$, comparison with male stone formers {M (+)}.

excretion is independent of its urinary saturation. As expected, there was a significant positive correlation between the concentrations of calcium, oxalate, citrate, magnesium, and creatinine ($p<0.01$), except in the case of calcium and oxalate ($p>0.05$). DG(CaOx) could be expressed as follows on the basis of multiple regression analysis of 408 spot and 24-hour urine specimens ($r = 0.9779$, $p <0.01$). DG(CaOx) = 3.0070 + 2.6455xlog([Ca]) + 2.6353xlog([Ox]) - 0.6292xlog([Cit]) - 0.6122xlog([Mg]). This formula combined multiple variables and showed that DG(CaOx) was positively correlated with [Ca] and [Ox], while it was negatively correlated with [Cit] and [Mg], findings that were theoretically reasonable.

For a total of 146 24-hour urine specimens, hypercalciuria (> 200 mg/day), hyperoxaluria (> 30 mg/day), hypocitraturia (< 320 mg/day), and hypomagnesiuria (< 75 mg/day) were stratified into three saturation levels {critical supersaturation (DG>2.8) 6.8%, metastable supersaturation (2.8>DG>0) 79.5%, and unsaturated (0>DG) 13.7%} according to our previous report (60), as shown in Figures 1-3. Hypercalciuria or hyperoxaluria was fairly common (30% + 40%) in critically supersaturated urine (10 specimens), less common (22.4% + 8.6%) in metastably supersaturated urine (116 specimens), and was not detected in unsaturated urine (20 specimens). In contrast, hypocitraturia and/or hypomagnesiuria, either isolated or in combination with other abnormalities, was common (63.8-80%) at any saturation level. Therefore, hypocitraturia and hypomagnesiuria were nonspecific changes that could be present irrespective of the calcium oxalate saturation. However, there was a small subset of patients with isolated hypocitraturia (approximately 10%) or isolated hypomagnesiuria (approximately 10%) that was associated with critical supersaturation.

In 402 spot and 24-hour urine specimens, the creatinine-corrected values for hypercalciuria (Ca/Cre > 0.200), hyperoxaluria (Ox/Cre > 0.300), hypocitraturia (Cit/Cre < 0.320), and hypomagnesiuria (Mg/Cre < 0.075)

Table 4. Relationship between DG(CaOx) and various urinary risk factors

Risk factor	Y: DG(CaOx) X:Risk factor Regression function	Regression Coeffcicient (r)	Probability p
log[Ca]	Y = 2.96993X + 0.36858	0.78440	0.0000
log(Ca)	Y = 2.02111X + 0.19658	0.48183	0.0000
log(Ca/Cre)	Y = 1.45260X + 2.56207	0.34707	0.0000
log[Ox]	Y = 3.06383X – 5.19166	0.81512	0.0000
log(Ox)	Y = 2.73124X – 4.97490	0.66648	0.0000
log(Ox/Cre)	Y = 1.95392X + 4.8557	0.42523	0.0000
log[Cit]	Y = 1.06793X – 1.98474	0.36557	0.0000
log(Cit)	Y = 0.08502X + 0.88565	0.02745	0.7223
log(Cit/Cre)	Y = –0.00638X + 1.19365	–0.00188	0.9691
log[Mg]	Y = 2.35477X + 0.64117	0.54092	0.0000
log(Mg)	Y = 0.95186X + 0.73471	0.18386	0.0199
log(Mg/Cre)	Y = –0.00334X + 1.17903	–0.00064	0.9898

Correlation of DG(CaOx) with the calcium concentration {[Ca] mM}, 24-h urinary calcium excretion {(Ca) mmol/day}, and calcium/creatinine ratio (Ca/Cre mg/mg); the oxalate concentration {[Ox] mM}, 24-h urinary oxalate excretion {(Ox) mmol/day}, and oxalate/creatinine ratio (Ox/Cre mg/mg); the citrate concentration {[Cit] mM}, 24-h urinary citrate excretion {(Cit) mmol/day}, and citrate/creatinine ratio (Cit/Cre mg/mg); the magnesium concentration {[Mg] mM}, 24-h urinary magnesium excretion {(Mg) mmol/day}, and magnesium/creatinine ratio (Mg/Cre mg/mg).

Table 5. Correlations of urinary parameters between early spot urine and 24-hour urine specimens

	[Ca] mM	[Ox] mM	[Cit] mM	[Mg] mM	[Cre] mg/dl
Spot urine	2.900±1.620	0.167±0.096	1.492±1.515	2.211±1.340	98.26±59.68
24-h urine	2.120±1.363[1]	0.119±0.070[2]	0.813±0.651[1]	1.627±1.100	58.24±28.17[1]
	Ca/Cre mg/mg	**Ox/Cre mg/mg**	**Cit/Cre mg/mg**	**Mg/Cre mg/mg**	**DG(CaOx)**
Spot urine	0.147±0.121	0.018±0.010	0.306±0.228	0.064±0.037	1.471±1.107
24-h urine	0.157±0.093[1]	0.020±0.013[1]	0.287±0.200[1]	0.071±0.036[1]	1.041±1.274[2]

Correlations between early spot urine specimens (Y) and 24-hour urine specimens (X) obtained from 57 inpatients with idiopathic calcium oxalate stones for urinary calcium, oxalate, citrate, magnesium, creatinine, DG(CaOx) value, calcium/creatinine, oxalate/creatinine, citrate/creatinine, and magnesium/creatinine ratios. The following correlations were obtained. [Ca]: Y = 0.492X + 1.856 (r = 0.41, p = 0.001); [Ox]: Y = 0.435X + 0.115 (r = 0.32, p = 0.016); [Cit]: Y = 1.069X + 0.623 (r = 0.46, p = 0.0003); [Mg]: No correlation; [Cre]: Y = 0.908X + 45.364 (r = 0.43, p = 0.001); Ca/Cre: Y = 0.671X + 0.042 (r = 0.52, p = 0.0000); Ox/Cre: Y = 0.259X + 0.012 (r = 0.35, p = 0.008); Cit/Cre: Y = 0.839X + 0.066 (r = 0.74, p = 0.0000); Mg/Cre: Y = 0.475X + 0.030 (r = 0.46, p = 0.0003); DG(CaOx): Y = 0.277X + 1.1831 (r = 0.32, p = 0.016). Data are shown as mean±SD. [1]: p< 0.01, comparison with spot urine; [2]: p< 0.05, comparison with spot urine.

(13) were stratified into three saturation levels (critically supersaturated 7.7%, metastably supersaturated 77.4%, and unsaturated 14.9%), as shown in Figures 4-6. Even after correction for creatinine, hypercalciuria or hyperoxaluria was fairly common (22.6 + 25.8%) in critically supersaturated urine (31 specimens), was somewhat less common (21.2 + 13.2%) in metastably supersaturated urine (311 specimens), and was even less common (11.7 + 5.0%) in unsaturated urine (60 specimens). In contrast, Cre-corrected hypocitraturia and/or Cre-corrected hypomagnesiuria, either isolated or in combination with other abnormalities, was commonly (55.6-77.4%) seen at any level of saturation. Therefore, Cre-corrected hypocitraturia and Cre-corrected hypomagnesiuria were nonspecific findings irrespective of the calcium-oxalate saturation level. However, there were small subsets of patients with isolated Cre-corrected hypocitraturia (approximately 3%) or isolated Cre-corrected hypomagnesiuria (also approximately 3%) that was associated with critical supersaturation.

Regarding comparison between 57 paired early morning spot urine specimens and 57 24-hour urine specimens, the urinary calcium, oxalate, citrate, and creatinine concentrations were found to be significantly correlated between the groups ($p<0.05$), except for magnesium ($p>0.05$). The mean values of these parameters were higher in spot urine specimens than in 24-hour urine specimens ($p<0.05$). DG(CaOx) showed a significant correlation between early spot urine and 24-hour urine specimens ($p<0.05$). The mean values were 1.471±1.107 and 1.041±1.274, respectively, and the former value was significantly higher than the latter ($p>0.05$), as shown in Table 5. The mean 24-hour urinary calcium excretion was 137.6±90.8 mg/day and the calcium/creatinine ratio (mg/mg) was 0.1471±0.1203 (mean±SD) in early morning spot urine specimens, and there was a significant correlation between the two parameters ($p<0.01$), as shown in Table 6. The mean 24-hour urinary oxalate excretion was 17.3±12.1 mg/day and the oxalate/creatinine ratio (mg/mg) was 0.0175±0.0096 in spot urine specimens, with no significant correlation between the two parameters ($p>0.10$). The mean 24-hour urinary citrate excretion was 240.7±165.9 mg/day and the citrate/creatinine ratio (mg/mg) was 0.3061±0.2283 in spot urine specimens, with a significant correlation between the two parameters

Table 6. Correlations between 24-hour excretion and Cre-corrected concentrations of urinary parameters

Risk factor (f) f/Cre	Mean±SD	Y: log(f) X: log(f/Cre) Regression function	Regression Coefficient (r)	Probability p
Ca (mg/day) Ca/Cre (mg/mg)	137.6±90.8 0.1474±0.1206	Y = 0.3732X + 2.3942	0.3835	0.0032
Ox (mg/day) Ox/Cre (mg/mg)	17.3±12.1 0.0175±0.0096	Y = 0.0313X + 1.1931	0.0246	0.8558
Cit (mg/day) Cit/Cre (mg/mg)	240.7±165.9 0.3061±0.2283	Y = 0.7074X + 2.7104	0.6757	0.0000
Mg (mg/day) Mg/Cre (mg/mg)	59.9±30.1 0.0636±0.0368	Y = 0.0394X + 1.7729	0.0439	0.7455

Comparison of the 24-hour urinary calcium (Ca mg/day), oxalate (Ox mg/day), citrate (Cit mg/day), and magnesium (Mg mg/day) excretion with the Ca/Cre, Ox/Cre, Cit/Cre, and Mg/Cre ratio (mg/mg), respectively, of early spot urine specimens obtained from 57 inpatients with idiopathic calcium oxalate stones. Data are shown as mean±SD.

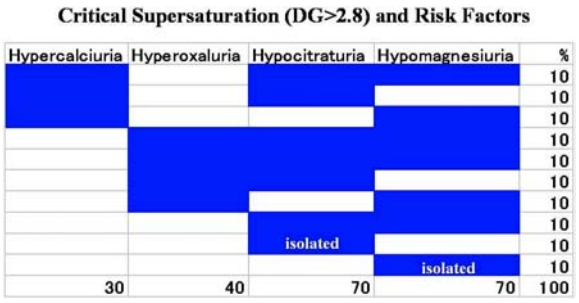

Figure 1. The prevalence of hypercalciuria (> 200 mg/day), hyperoxaluria (> 30 mg/day), hypocitraturia (< 320 mg/day), and hypomagnesiuria (< 75 mg/day) in 10 critically supersaturated 24-hour urine specimens.

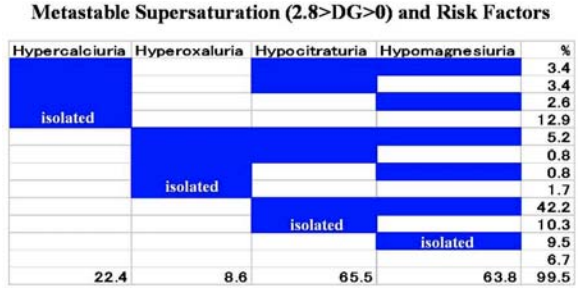

Figure 2. The prevalence of hypercalciuria (> 200 mg/day), hyperoxaluria (> 30 mg/day), hypocitraturia (< 320 mg/day), and hypomagnesiuria (< 75 mg/day) in 116 metastably supersaturated specimens.

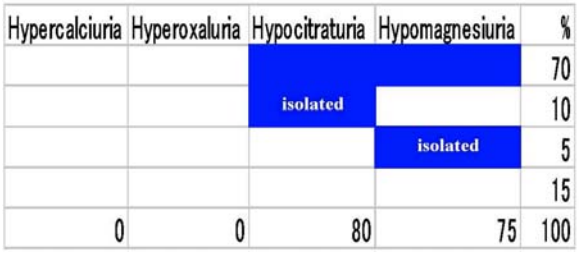

Figure 3. The prevalence of hypercalciuria (> 200 mg/day), hyperoxaluria (> 30 mg/day), hypocitraturia (< 320 mg/day), and hypomagnesiuria (< 75 mg/day) in 20 unsaturated specimens.

($p<0.01$). The mean 24-hour urinary magnesium excretion was 59.9±30.1 mg/day and the magnesium/creatinine ratio (mg/mg) was 0.0636±0.0368 in spot urine specimens, again with no significant correlation between the two parameters ($p>0.10$), as shown in Table 6. The calcium/creatinine, oxalate/creatinine, citrate/creatinine, and magnesium/creatinine ratios were significantly correlated between early morning spot urine and 24-hour urine specimens, with the regression coefficients (r values) being 0.5162, 0.3489, 0.7360, and 0.4646 ($p<0.01$), respectively. The mean±SD was 0.1474±0.1206 and 0.1565±0.0927, 0.0175±0.0096 and 0.0199±0.0129, 0.3061±0.2283 and 0.2866±0.2002, and 0.0636±0.0368 and 0.0709±0.0360, respectively. The former set of mean ratios showed slightly lower values than the latter set except for the mean citrate/creatinine ratio.

5. DISCUSSION

In this study, the measurement of 24-hour urinary calcium, oxalate, and citrate excretion could not discriminate stone formers from non-stone formers, while the mean 24-hour urinary magnesium excretion was significantly higher in non-stone formers than in stone formers. The creatinine-corrected urinary calcium, oxalate, and magnesium levels in spot urine were also unable to discriminate stone formers from non-stone formers, but the mean creatinine-corrected citrate level was higher in non-stone formers than in stone formers. Because nephrolithiasis is a heterogenous group of disorders, stones develop due to a wide variety of metabolic or environmental disturbances (1-7). Therefore, determination of calcium oxalate saturation is necessary to gain an understanding the physicochemical events involved in renal stone formation (38, 67). We found that the DG(CaOx) value for either 24-hour urine or spot urine could discriminate stone formers from non-stone formers despite the heterogenous composition of these groups of patients. Finlayson's Equil program seems to be useful for discriminating stone formers from non-stone formers. The differential Gibbs' free energy (DG) value of calcium oxalate represents the chemical potential of the calcium oxalate system in states from a solution to a precipitate, thus reflecting the extent of calcium oxalate saturation (17). We have reported previously that all of the methods for estimating the ion-activity product of calcium oxalate,

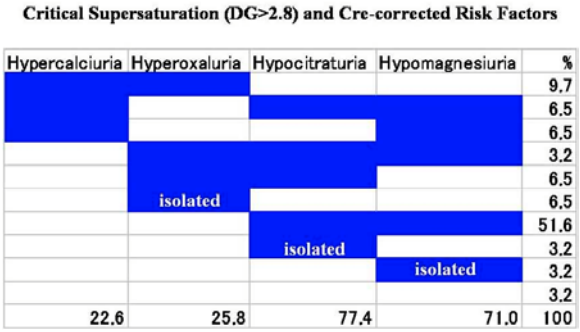

Figure 4. The prevalence of creatinine-corrected hypercalciuria (Ca/Cre > 0.200), hyperoxaluria (Ox/Cre > 0.300), hypocitraturia (Cit/Cre < 0.320), and hypomagnesiuria (Mg/Cre < 0.075) in 31 critically supersaturated urine specimens.

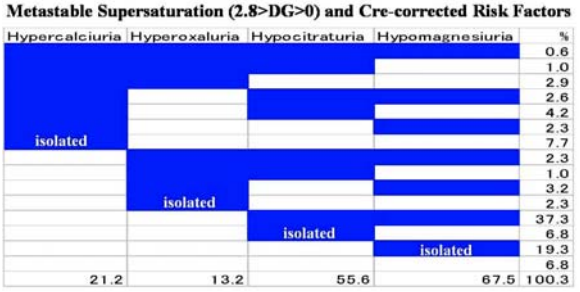

Figure 5. The prevalence of creatinine-corrected hypercalciuria (Ca/Cre > 0.200), hyperoxaluria (Ox/Cre > 0.300), hypocitraturia (Cit/Cre < 0.320), and hypomagnesiuria (Mg/Cre < 0.075) in 311 metastably supersaturated urine specimens.

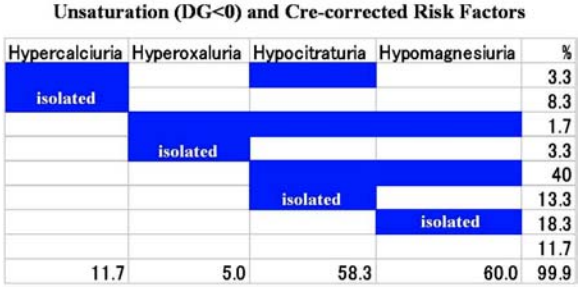

Figure 6. The prevalence of creatinine-corrected hypercalciuria (Ca/Cre > 0.200), hyperoxaluria (Ox/Cre > 0.300), hypocitraturia (Cit/Cre < 0.320), and hypomagnesiuria (Mg/Cre < 0.075) in 60 unsaturated urine specimens.

which also reflects the saturation, were correlated well with each other (68), so that any indices accounting for urinary calcium oxalate saturation could discriminate the two groups better than any other single parameters. Similarly several risk formulas {AP(CaOx) index, calcium/magnesium, calcium/citrate, calciumxoxalate/magnesium/citrate, and Parks and Coe score} were confirmed by Tiselius to be equally useful in terms of the predictive power of stone risk and in discriminating between stone formers and non-stone formers (23). The risk indices are governed by the strong contribution of the calcium and oxalate concentrations as well as the weak inhibitory effects of citrate and magnesium. However, the calcium, oxalate, citrate, and magnesium concentrations were positively associated with DG(CaOx) if compared individually. Citrate inhibits urinary stone formation by making a complex with calcium, while magnesium exerts a solubilizing effect because magnesium oxalate has a slightly greater stability constant than calcium oxalate (69). These two inhibitors apparently have an important influence on stone formation and their inhibitory effect can be easily calculated and expressed as the urinary saturation (e.g., the DG(CaOx) value). Although most researchers believed that urinary citrate and magnesium levels should be lower in the supersaturated urine of stone formers because these are inhibitory factors, it was actually the other way around because the DG(CaOx) value increased along with increasing citrate and magnesium levels (68). This paradoxical and puzzling phenomenon may be accounted for by the fact that the urinary levels of all these substances are closely associated with each other depending on the extent of concentration or dilution of the urine, and that their relative ratios influenced by urinary concentration and dilution may play an important role in determining the DG(CaOx) value or the extent of saturation. Therefore, the Ca/Mg and Ca/Cit ratios have been used as better indices of stone risk by some researchers (23, 70-72). According to the formula for calculation of the DG(CaOx) value { DG(CaOx) = 3.0070 + 2.6455xlog([Ca]) + 2.6353xlog([Ox]) - 0.6292xlog([Cit]) - 0.6122xlog([Mg])}, DG(CaOx) is negatively associated with the citrate and magnesium concentrations. Interpreting this formula suggests that the calcium and oxalate concentrations make an almost equally important contribution to the DG(CaOx) value, while citrate and magnesium make an almost equal minor contribution. The former vs. the latter shows a ratio of approximately 2.6:0.6, so calcium and oxalate concentrations exert 4-fold more influence on the saturation than citrate and magnesium concentrations. Finlayson reported that urinary oxalate is about 15 times more important than urinary calcium for stone formation (73). However, our findings do not support Finlayson because the DG(CaOx) formula shows that calcium and oxalate have an equal influence on stone formation.

In this study, the 24-hour urinary calcium, oxalate, and magnesium excretion were shown to be positively correlated with the DG(CaOx) value, but 24-hour urinary citrate excretion was not. This may suggest that 24-hour urinary citrate excretion is not an important risk factor for stone formation. Hypercalciuria and hyperoxaluria were mostly observed in CaOx-supersaturated urine and their prevalence was almost equal. Mild hyperoxaluria was emphasized to be the main clinical cause of idiopathic calcium stone disease by Robertson and Hughes (74), who claimed that urinary oxalate is the most important risk factor for calcium oxalate stone formation. We do not deny the concept of mild hyperoxaluria, but the clinical prevalence of hypercalciuria and hyperoxaluria appears to be equal, while hypocitraturia and hypomagnesiuria were even more common, but were not associated with any particular level of urinary saturation. Because hypocitraturia tended to be a non-specific finding, both

hypocitraturia and hypomagnesiuria are not good predictors of urinary calcium oxalate saturation, but may be associated with the underlying etiologic conditions. However, there was a small group of patients who had isolated hypocitraturia with critical calcium oxalate supersaturation, where both the calcium and oxalate concentrations were borderline. When we introduced "isolated hypocitraturia" as a concept, we intended it to mean CaOx-supersaturated urine that showed hypocitraturia without obvious hypercalcuria, hyperoxaluria, or hypomagnesiuria, excluding unsaturated urine, while "isolated hypomagnesiuria" meant CaOx-supersaturated urine that showed hypomagnesiuria without hypercalcuria, hyperoxaluria, or hypocitraturia (again excluding unsaturated urine) (60). Hypomagnesiuric hypocitraturia was introduced as a new entity by Preminger (62), but we would like to define this as calcium oxalate supersaturation associated with hypomagnesiuria and hypocitraturia. In the present study, 10% of critically supersaturated urine filled this definition. Although citrate and magnesium are important inhibitors of stone formation, isolated hypocitraturia or hypomagnesiuria is often an inadequate explanation for stone formation/non formation. Assessment of the urinary saturation of stone components should be included in metabolic screening and is mandatory for clinical evaluation of drug efficacy, however, the magnitude of the drug effect was minor in terms of saturation (75). Hypocitraturia or hypomagnesiuria is a poor indicator of urinary saturation as such, but to compare the frequency of these risk factors may be a good idea (76).

Risk factors for stone formation, including hypercalciuria and hyperoxaluria, are often observed in association with high urinary saturation. Supersaturation, as expressed by a high DG(CaOx) value caused by one or more of these risk factors, can undoubtedly lead to stone formation irrespective of the underlying etiologic condition if it persists for a long time. More than 90% of critical calcium oxalate supersaturation occurs late at night or in the early morning, so examination of early morning spot urine may generally be sufficient to predict the risk of critical supersaturation (60). In the present study, the urinary calcium, oxalate, citrate, and magnesium concentrations, as well as the DG(CaOx) value, were well correlated between early morning spot urine and 24-hour urine, although generally higher in early morning urine, so the DG(CaOx) value of early morning spot urine specimens may be a better indicator of stone risk because it could represent the highest saturation throughout the day. Therefore, the target of therapy for stone formers should be to reduce the saturation of early morning spot urine. The urinary calcium/creatinine, oxalate/creatinine, magnesium/creatinine, and citrate/creatinine ratios were well correlated between early morning and 24-hour urine, with the former three ratios being lower in spot urine. Therefore, calculation of the urinary calcium/creatinine, oxalate/creatinine, and magnesium/creatinine ratios from early morning spot urine specimens may slightly underestimate urinary excretion relative to the values obtained from 24-hour urine specimens.

Regarding the comparison of urinary data between males and females, urinary calcium and oxalate concentrations were not significantly different between men and women as well as calcium supersaturation (77,78), while urinary calcium, oxalate, and magnesium excretion were all reported to be significantly higher in stone-forming men than women (79). Our data obtained from stone-forming inpatients on a hospital diet are consistent with the former report except that urinary citrate excretion was higher in stone-forming women than in men and the creatinine-corrected urinary substances (Ca, Ox, Cit, and Mg) tended to be higher in women. Urinary excretion of various substances is influenced by the diet (80), so a prospective study is warranted on groups of stone formers with well-defined diets.

Although 24-hour urine collection remains the gold standard for metabolic evaluation and can detect a metabolic abnormality in approximately 70% of stone formers if performed properly (81), it is influenced by various factors, including the season (1, 2, 82), day-to-day variations, exercise, dietary changes (80), and activity levels. Incorrect sampling can cause significant errors in the excretion data (83), and a single 24-hour sample is not sufficient for evaluating patients before metabolic treatment (84). Supersaturation values are, however, reasonably stable in most patients during months to years required for stones to form, and urine samples collected in standard practice and sent to a central laboratory may accurately reflect the supersaturation values (85). Use of spot urine specimens has been recommended by some authors because of simplicity and the good correlation with 24-hour urine specimens for various parameters, but spot urine values have not been verified as a surrogate measure for 24-hour urinary excretion of all the substances of interest (86, 87). In the present study, urinary oxalate and magnesium excretion were not correlated with the oxalate/creatinine ratio or the magnesium/creatinine ratio, respectively. There are some substances (e.g., uric acid) for which the creatinine ratio does not accurately predict the 24-hour excretion (88). Therefore, measurement of early morning spot urine may be useful for identifying some risk factors, including hypercalciuria and hypocitraturia as well as calcium oxalate saturation, but hyperoxaluria and hypomagnesiuria in early morning spot urine do not have the same implications as these findings in 24-hour urine. However, the currently accepted definitions of normal values are not firmly supported, so the traditional definitions of normal 24-hour urine values need to be reassessed (89).

We have not mentioned macromolecular promoters and inhibitors or the concept of inhibitory macromolecule deficiency, but routine determination of such substances is impractical (90-94). In the future, macromolecular factors may eventually be incorporated in the Equil2 program.

6. ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research of the Japanese Ministry of Education, Culture, Sports, Science and Technology (B2 13470338). We are grateful for the technical assistance of Ms. Tomoko Maeda.

7. REFERENCES

1. Robertson WG, M. Peacock, R.W. Marshall, R. Speed, & B.E.C. Nordin. Seasonal variations in the composition of urine in relation to calcium stone-formation. *Clin Sci Mol Med* 49, 597-602 (1975)
2. Hallson PC & G. Alan Rose. Seasonal variations in urinary crystals. *Br J Urol* 49, 277-284 (1977)
3. Elomaa I, S-L. Karonen, A-L. Kairento & R. Pelkonen. Seasonal variation of urinary calcium and oxalate excretion, serum 25(OH)D3 and albumin level in relation to renal stone formation. *Scan J Urol Nephrol* 16, 155-161 (1982)
4. Marshall RW, M. Cochran, W.G. Robertson, A. Hodgkinson & B.E.C. Nordin. The relation between the concentration of calcium salts in the urine and reanl stone composition in patients with calcium-containing renal stones. *Clin Sci* 43, 433-441, (1972)
5. Vahlensieck EW, D. Back & A. Hesse. Circadian rhythm of lithogenic substances in the urine. *Urol Res* 10, 195-203 (1982)
6. Ogawa Y, R. Kitagawa & T. Umeyama. Diurnal variation of calcium, phosphorus, and magnesium in normal and calcium oxalate stone-forming urine. *Jpn J Nephrol* 25, 1127-1130 (1983)
7. Pak CYC. Etiology and treatment of urolithiasis. *Am J Kidney Dis* 18, 624-637 (1991)
8. Levy FL, B. Adams-Huet & C.Y.C. Pak. Ambulatory evaluation of nephrolithiasis: An update of a 1980 Protocol. Am J Med 98, 50-59 (1995)
9. Curhan GC, W.C. Willett, F.E. Speizer & M.J. Stampfer. Twenty-four-hour urine chemistries and the risk of kidney stones among women and men. *Kidney Int* 59, 2290-2298 (2001)
10. Finlayson B & G.H. Miller Jr. Urine ion equilibria: a numerical approach demonstrated by application to antistone therapy. *Invest Urol* 6, 428-440 (1969)
11. Finlayson B. Calcium stones:some physical and clinical aspects. In: Calcium metabolism in renal failure and nephrolithiasis. New York: John Willey & Sons, 337-382 (1977)
12. Marshall RW & W.G. Robertson. Nomograms for the estimation of the saturation of urine with calcium oxalate, calcium phosphate, uric acid, sodium acid urate, ammonium acid urate and cystine. *Clin Chim Acta* 72, 253-260 (1976)
13. Tiselius H-G, L.E. Almgard, L. Larsson & B. Sorbo. A biochemical basis for grouping of patients with urolithiasis. *Eur Urol* 4, 241-249 (1978)
14. Robertson WG, M. Peacock, P.J. Heyburn, D.H. Marshall & P.B. Clark. Risk factors in calcium stone disease of the urinary tract. *Br J Urol* 50, 449-454 (1978)
15. Pak CYC, Y. Hayashi, B. Finlayson & S. Chu. Estimation of the state of saturation of brushite and calcium oxalate in urine: a comparison of three methods. J Lab Clin Med 89, 891-901 (1977)
16. Tiselius H-G. An improved method for the routine biochemical evaluation of patients with recurrent calcium oxalate stone disease. *Clin Chim Acta* 122, 409-418 (1982)
17. Werness PG, C.M. Brown, L.H. Smith & B. Finlayson. Equil2: a basic computer program for the calculation of urinary saturation. *J Urol* 17, 1242-1244 (1985)
18. Berg C, L. Larsson & H-G. Tiselius. The composition of four-hour urine samples from patients with calcium oxalate stone disease. *J Urol* 60, 301-306 (1987)
19. Tiselius H-G. Standardized estimation of the ion activity product of calcium oxalate in urine from renal stone formers. *Eur Urol* 16, 48-50 (1989)
20. Tiselius H-G. Aspects on estimation of the risk of calcium oxalate crystallization in urine. *Urol Int* 47, 255-259 (1991)
21. Ogawa Y. Modification of estimation of the urinary ion-activity products of calcium oxalate and calcium phosphate. *Acta Urol Jpn* 39, 407-411 (1993)
22. Brown CM, D.K. Ackerman & D.L. Purich. Equil93: a tool for experimental and clinical urolithiasis. *Urol Res* 22, 119-26 (1994)
23. Tiselius H-G: Risk formulas in calcium oxalate urolithiasis. *World J Urol* 15, 176-185 (1997)
24. Ogawa Y, S. Takahashi, R. Kitagawa, T. Umeyama & K. Aoyagi. Diurnal variation in calcium-oxalate supersaturation level in normal and stone-forming urine. *Jpn J Nephrol* 25, 1131-1134 (1983)
25. Ahlstrand C, L. Larsson & H-G. Tiselius. Variations in urine composition during the day in patients with calcium oxalate stone disease. *J Urol* 131, 77-81 (1984)
26. Ogawa Y. Circadian rhythms of urinary saturation levels of calcium oxalate and calcium phosphate in normal male individuals. *Acta Urol Jpn* 39, 785-789 (1993)
27. Robert M, J.O. Roux, F. Bourelly, A.M. Boularan, J. Guiter & L. Monnier. Circadian variations in the risk of urinary calcium oxalate stone formation. *Br J Urol* 74, 294-297 (1994)
28. Tiselius H-G. Measurement of the risk of calcium oxalate crystallization in urine. *Urol Res* 13, 297-300 (1985)
29. Schwille PO, J.H. Weippert, W. Baushc & G. Rumenapf. Acute oral alkaline citrate load in healthy humans - response of blood and urinary citrate, mineral metabolism, and factors related to stone formation. *Urol Res* 13, 161-168 (1985)
30. Sriboonlue P, V. Prasongwattana, K. Tungsanga & V. Sitprija. Measurements of urinary state of saturation with respect to calcium oxalate and brushite in renal stone formers. *J Med Assoc Thai* 73, 684-689 (1990)
31. Berg C, L. Larsson & H-G. Tiselius. Effects of different doses of alkaline citrate on urine composition and crystalization of calcium oxalate. *Urol Res* 18, 13-16 (1990)
32. Ogawa Y. Impact of sodium-potassium citrate on the diurnal variations in urinary calcium oxalate and calcium phosphate saturation levels in normal individuals. *Br J Urol* 73, 136-141 (1994)
33. Parks JH, M. Coward & F.L. Coe. Correspondence between stone composition and urine supersaturation in nephrolithiasis. *Kidney Int* 51, 894-900 (1997)
34. Ryall RL, J.N. Darroch & V.R. Marshall. The evaluation of risk factors in male stone-formers attending a General Hospital out-patient clinic. *Br J Urol* 56, 116-121 (1984)
35. Marangella M. Urine saturation with calcium salts in normal subjects and idiopathic stone formers estimated by an improved computer model system. *Urol Res* 13, 189-93 (1985)

36. Kok DJ & S.E. Papapoulos. Physicochemical considerations in the development and prevention of calcium oxalate urolithiasis. *Bone Miner* 20, 1-15 (1993)
37. Robert M, A.M. Boularan, C. Colette, M. Averous & L. Monnier. Urinary calcium oxalate saturation in 'stone formers' and normal subjects: an application of the EQUIL2 program. *Br J Urol* 73, 358-361 (1994)
38. Robertson WG, M. Peacock & B.E. Nordin. Activity products in stone-forming and non-stone-forming urine. *Clin Sci* 34, 579-594 (1968)
39. Robertson WG, M. Peacock, R.W. Marshall, D.H. Marshall & C. Nordin. Saturation-inhibition index as a measure of the risk of calcium oxalate stone formation in the urinary tract. *N E J Med* 294, 249-252 (1976)
40. Bek-Jensen H & H-G. Tiselius. Stone formation and urine composition in calcium stone formers without medical treatment. *Eur Urol* 16, 144-150 (1989)
41. Ogawa Y, T. Umeyama, A. Hasegawa & T. Kawamura. Calcium-oxalate crystallization levels among pediatric patients. *Jpn J Ped Surg* 25, 231-235 (1989)
42. Hoppe B, A. Hesse, T. Neuhaus, S. Fanconi, I. Forster, N. Blau & E. Leumann. Urinary saturation and nephrocalcinosis in preterm infants: effect of parenteral nutrition. *Arch Dis Child* 69, 299-303 (1993)
43. Miyake O, K. Yoshimura, T. Yoshioka, T. Koide & A. Okuyama. High urinary excretion level of citrate and magnesium in children: potential etiology for the reduced incidence of pediatric urolithiasis. *Urol Res* 26, 209-213 (1998)
44. Pak CYC, J.W. Cox, E. Powell & F.C. Bartter. Effect of the oral administration of ammonium chloride, sodium phosphate, cellulose phosphate and parathyroid extract on the activity product of brushite in urine. Am J Med 50, 67-76 (1971)
45. Pak CYC. Effects of cellulose phosphate and sodium phosphate on formation product and activity product of brushite in urine. *Metabolism* 21, 447-455 (1972)
46. Pak CYC, D.E. Barilla, K. Holt, L. Brinkley, R. Tolentino & J. Zerwekh. Effect of oral purine load and allopurinol on the crystallization of calcium salts in urine of patients with hyperuricosuric calcium urolithiasis. *Am J Med* 65, 593-599 (1978)
47. Zerwekh JE, O. Lawoyin & C.Y.C. Pak. Quantitation of response to therapy in calcium urolithiasis. *Urol Res* 7, 209-213 (1979)
48. Weber DV, F.L. Coe, J.H. Parks, M.S.L. Dunn & V. Tembe. Urinary saturation measurements in calcium nephrolithiasis. *Ann Int Med* 90, 180-184 (1979)
49. Pak CYC, K. Sakhaee, C. Crowther & L. Brinkley. Evidence justifying a high fluid intake in treatment of nephrolithiasis. *Ann Int Med* 93, 36-39 (1980)
50. Preminger GM, K. Sakhaee, C. Skurla & C.Y.C. Pak. Prevention of recurrent calcium stone formation with potassium citrate therapy in patients with distal renal tubular acidosis. *J Urol* 134, 20-23 (1985)
51. Pak CYC & C. Fuller. Idiopathic hypocitraturic calcium-oxalate nephrolithiasis successfully treated with potassium citrate. *Ann Int Med* 104, 33-37 (1986)
52. Pak CYC & R. Peterson. Successful treatment of hyperuricosuric calcium oxalate nephrolithiasis with potassium citrate. *Arch Intern Med* 146, 863-867 (1986)
53. Tiselius H-G, C. Alhstrand, H. Bek-Jensen, C. Berg, L. Larsson & E. Palmqvist. To what extent is urine composition related to stone formation, and how efficient are we in affecting urinary risk factors? *Fortschr Urol Nephrol* 26, 26-29 (1987)
54. Preminger GM, K. Sakhaee & C.Y.C. Pak. Alkali action on the urinary crystallization of calcium salts: contrasting responses to sodium citrate and potassium citrate. *J Urol* 139, 240-242 (1988)
55. Pak CYC, K. Koenig, R. Khan, S. Haynes & P. Padalino. Physicochemical action of potassium-magnesium citrate in nephrolithiasis. *J Bone Mener Res* 7, 281-285 (1992)
56. Levine BS, J.S. Rodman, S. Wienerman, R.S. Bockman, J.M. Lane & D.S. Chapman. Effect of calcium citrate supplementation on urinary calcium oxalate saturation in female stone formers: implications for prevention of osteoporosis. *Am J Clin Nutr* 60, 592-6 (1994)
57. Preminger GM. Is there a need for medical evaluation and treatment of nephrolithiasis in the "Age of Lithotripsy"? *Semin Urol* 12, 51-64 (1994)
58. Hobarth KH, J. Hofbauer & N. Szabo. Value of repeated analyses of 24-hour urine in recurrent calcium urolithiasis. *Urology* 44, 20-25 (1994)
59. Schwartz BF, J. Bruce, S. Leslie & M.L. Stoller. Rethinking the role of urinary magnesium in calcium urolithiasis. *J Endourol* 15, 233-235 (2001)
60. Ogawa Y & T. Hatano. Risk factors in urinary calcium oxalate stone formation and their relation to urinary calcium oxalate supersaturation. *Int J Urol* 3, 356-360 (1996)
61. Hodgkinson A. Citric acid excretion in normal adults and in patients with renal calculus. *Clin Sci* 23, 203-12 (1962)
62. Preminger GM, S. Baker, R. Peterson, J. Poindexter & C.Y.C. Pak. Hypomagnesiuric hypocitraturia: an apparent new entity for calcium nephrolithiasis. *J Lithotripsy & Stone Dis* 1, 22-25 (1989)
63. Ogawa Y, K. Yamaguchi & M. Morozumi. Effects of magnesium salts in preventing experimental oxalate urolithiasis in rats. *J Urol* 144, 385-389 (1990)
64. Ogawa Y, S. Nishijima, T. Miyazato & T. Hatano: Meaning of hypocitraturia in clinical stone practice. In: Urolithiasis 2000. Eds: Rodgers AL, Hibbert BE, Hess B, Khan SR & Preminger GM. the Univ. of Cape Town, Cape Town, South Africa, 84-87 (2000)
65. Holmes RP: Measurement of urinary oxalate and citrate by capillary electrophoresis and indirect ultraviolet absorbance. *Clin Chem* 41, 1297-1301 (1995)
66. Ogawa Y & T. Hatano. Urinary oxalate and citrate determination. *Kidney and Dialysis* Suppl 43, 151-157 (1997)
67. Pak CYC. Physicochemical basis for formation of calcium phospahte origin: Calculation of the degree of saturation of urine with respect to brushite. *J Clin Invest* 48, 1914-1922 (1969)
68. Ogawa Y & T. Hatano. Comparison of the Equil2 program and other methods for estimating the ion-activity product of urinary calcium oxalate: a new simplified method is proposed. *Int J Urol* 3, 383-385 (1996)

69. Boyce WH. Editorial. Basic mechanisms in urolithiasis. Abstractor's comments. *Urol Survey* 5, 194 (1955)
70. Takasaki E. The magnesium:calcium ratio in the concentrated urines of patients with calcium oxalate calculi. *Invest Urol* 10(2), 147-50 (1972)
71. Parks JH & F.L. Coe. A urinary calcium-citrate index for the evaluation of nephrolithiasis. *Kidney Int* 30, 85-90 (1986)
72. Robert M, Roux J-O, Bpularan A-M, Bpurelly F, Monnier L & Grasset D. Idiopathic calcium oxalate urinary lithiasis: usefulness of Parks' and Tiselius' indices in the evaluation of the risk of stone formation. *Urol Int* 55, 88-92 (1995)
73. Finlayson B. Renal lithiasis in review. *Urol Clin North Am* 1, 181-212 (1974)
74. Robertson WG & Hughes H. Importance of mild hyperoxaluria in the pathogenesis of urolithiasis: New evidence from studies in the Arabian Peninsula. *Scanning Microsc* 7, 391-402 (1993)
75. Coe FL, H. Wise, J.H. Parks & J.R. Asplin. Proportional reduction of urine supersaturation during nephrolithiasis treatment. *J Urol* 166(4), 1247-51 (2001)
76. Yagisawa T, P.S. Chandhoke & J. Fan. Metabolic risk factors in patients with first-time and recurrent stone formations as determined by comprehensive metabolic evaluation. *Urology* 52(5), 750-5 (1998)
77. Buchholz NP, D.S. Kim, P.K. Grover & R.L. Ryall. Calcium oxalate crystallization in urine of healthy men and women: a comparative study. *Scanning Microsc* 10(2), 435-42 (1996)
78. Bergsland KJ, J.M. Kinder, J.R. Asplin, B.J. Coe & F.L. Coe. Influence of gender and age on calcium oxalate crystal growth inhibition by urine from relatives of stone forming patients. *J Urol* 167(6), 2372-6 (2002)
79. Heller HJ, K. Sakhaee, O.W. Moe & C.Y.C. Pak. Etiological role of estrogen status in renal stone formation. *J Urol* 168(5), 1923-7 (2002)
80. Siener R, D. Ebert, C. Nicolay & A. Hesse. Dietary risk factors for hyperoxaluria in calcium oxalate stone formers. *Kidney Int* 63(3), 1037-43 (2003)
81. Yagisawa T, P.S. Chandhoke & J. Fan. Comparison of comprehensive and limited metabolic evaluations in the treatment of patients with recurrent calcium urolithiasis. *J Urol* 161(5), 1449-52 (1999)
82. Stuart RO, K. Hill, J. Poindexter & C.Y. C. Pak. Seasonal variations in urinary risk factors among patients with nephrolithiasis. *J Lithotr Stone Dis* 3(1), 18-27 (1991)
83. Strohmaier WL, K-J. Hoelz & K-H. Bichler. Spot urine samples for the metabolic evaluation of urolithiasis patients. *Eur Urol* 32, 294-300 (1997)
84. Parks JH, E. Goldfisher, J.R. Asplin & F.L. Coe. A single 24-hour urine collection is inadequate for the medical evaluation of nephrolithiasis. *J Urol* 167(4),1607-12 (2002)
85. Asplin J, J. Parks, J. Lingeman, R. Kahnoski, H. Mardis, S. Lacey, D. Goldfarb, M. Grasso & F. Coe. Supersaturation and stone composition in a network of dispersed treatment sites. *J Urol* 159(6), 1821-5 (1998)
86. Matsushita K & K. Tanikawa. Significance of the calcium to creatinine concentration ratio of a single-voided urine specimen in patients with hypercalciuric urolithiasis. *Tokai J Exp Clin Med* 12, 167-171 (1987)
87. Gokce C, O. Gokce, C. Baydinc, N. Ilhan, E. Alasehirli, F. Ozkucuk, M. Tasci, M.K. Atikelr, H. Celebi & N. Arslan. Use of random urine samples to estimate total urinary calcium and phosphate excretion. *Arch Intern Med* 151, 1587-1588 (1991)
88. Wortmann RL & Fox IH. Limited value of uric acid to creatinine ratios in estimating uric acid excretion. *Ann Int Med* 93, 822-825 (1980)
89. Curhan GC, W.C. Willett, F.E. Speizer & M.J. Stampfer. Twenty-four-hour urine chemistries and the risk of kidney stones among women and men. *Kidney Int* 59(6), 2290-8 (2001)
90. Robertson WG & M. Peacock. Calcium oxalate crystalluria and inhibitors of crystallization in recurrent renal stone-formers. *Clin Sci* 43, 499-506 (1972)
91. Fleisch H. Inhibitors and promoters of stone formation. Kidney Int 13, 361-371 (1978)
92. Michelacci YM, R.Q. Glashan & N. Schor. Urinary excretion of glycosaminoglycans in normal and stone forming subjects. *Kidney Int* 36(6), 1022-8 (1989)
93. Akinci M, T. Esen, T. Kocak, C. Ozsoy & S. Tellaloglu. Role of inhibitor deficiency in urolithiasis. Eur Urol 19, 240-243 (1991)
94. Ryall RL. Urinary inhibitors of calcium oxalate crystallization and their potential role in stone formation. *World J Urol* 15, 155-164 (1997)

Key Words: Calcium Oxalate, Risk Factors, Urinary Saturation, Citrate, Magnesium

Send correspondence to: Yoshihide Ogawa, M.D., Ph.D., Department of Urology, Faculty of Medicine, University of the Ryukyus, Uehara 207, Nishihara-cho, Okinawa, Japan 903-0215, Tel: 81-098-895-1184, Fax: 81-098-895-1429, E-mail: ogawa@med.u-ryukyu.ac.jp