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ABSTRACTS

11th International Symposium on Urolithiasis, Nice, France, 2–5 September 2008

Tuesday 2 September		
07.30 09.30	**Registration**	
09.30 09.45	Welcome	
09.45 10.30	**Keynote Lecture - Prof FL Coe**	
10.30 11.00	**Tea & Coffee**	
	Session 1 - Pathogenesis of Stones	
11.00 11.40	Oral Presentations (1-3)	
11.40 12.10	**Guest Lecture - Prof M Fenech**	
12.10 13.00	Posters (1-11) (Pathogenesis of Stones)	
13.00 14.00	**Lunch**	
	Session 2 – Epidemiology and Nutrition	
14.00 14.30	**Review Lecture - Prof G Curhan**	
14.30 15.00	**Guest Lecture - Prof P Aronson**	
15.00 16.00	Oral Presentations (4-8)	
16.00 16.30	**Tea & Coffee**	
	Session 2 – Epidemiology and Nutrition	
16.30 17.00	Oral Presentations (9-10)	
17.00 18.00	Posters (12-22) (Epidemiology and Nutrition)	
19.00	**Welcome Reception**	19.00
Wednesday 3 September		
	Session 3A – Stones in Pets	**Session 3C - Urological Management of Stones**
08.30 09.00	**Review Lecture - Dr A Moore**	**Review Lecture - Prof G Preminger**
09.00 09.30	**Guest Lecture - Dr V Biourge**	Oral Presentations (21-27)
09.30 10.30	Oral Presentations (11-15)	
10.30 11.00	**Tea & Coffee**	**Tea & Coffee**
	Session 3B – Metabolic Factors	**Session 3C - Urological Management of Stones**
11.00 12.00	Oral Presentations (16-20)	Oral Presentations (28-32)
12.00 13.00	Posters (23-28) (Stones in Pets)	Posters (29-40) (Urological Management of Stones)
13.00 14.00	**Lunch**	
	Session 4A - Inhibitors and Promoters	
14.00 14.30	**Review Lecture - Prof RL Ryall**	
14.30 15.00	**Guest Lecture - Prof M Ketteler**	
15.00 16.00	Oral Presentations (33-37)	
16.00 16.20	**Tea & Coffee**	
	Session 4B - Medical Management of Stones	
16.20 17.00	Oral Presentations (38-40)	
17.00 18.00	Posters (41-58) (Inhibitors and Promoters and Medical Management of Stones)	
	Dinner for Chairs/Speakers	

Thursday 4 September		
	Session 5 – Crystals and Cells	
08.30	**Review Lecture - Prof S Khan**	
09.00		
09.00	**Guest Lecture - Prof M De Broe**	
09.30		
09.30	Oral Presentations (41-45)	**Live Urological Operations**
10.30		
10.30	**Tea & Coffee**	
11.00		
	Session 5 – Crystals and Cells	
11.00	Oral Presentations (46-50)	
12.00		
12.00	Posters (59-70)	
13.00	(Crystals and Cells)	
13.00	**Lunch**	
14.00		
	Session 6A - Metabolic Factors and Stones	
14.00	**Review Lecture - Prof O Moe**	
14.30		
14.30	**Guest Lecture - Prof G Capasso**	
15.00		
15.00	Oral Presentations (51-55)	
16.00		
16.00	**Tea & Coffee**	
16.30		
	Session 6B - Medical Management of Stones	
16.30	Oral Presentations (56-57)	
17.00		
17.00	Posters (71-98)	
18.00	(Metabolic Factors, Genetic Factors and Paediatric Stones)	
19.30	**Gala Dinner**	
Friday 5 September		
	Session 7 – Genetic Factors and Paediatric Stones	
08.30	**Review Lecture - Prof C Langman**	
09.00		
09.00	**Guest Lecture - Prof O Devuyst**	
09.30		
09.30	Oral Presentations (58-62)	
10.30		
10.30	**Tea & Coffee**	
11.00		
11.00	**Session 7 – Genetic Factors and Paediatric Stones**	
11.30	Oral Presentations (63-64)	
11.30	**Round-Table Discussion - Genetics of Stone Disease**	
12.00	(G Curhan, O Moe, O Devuyst and C Langman)	
12.00	**Plenary Debate -**	
13.00	(FL Coe & AP Evan versus RL Ryall & WG Robertson)	
13.00	Closing Remarks	
13.15		

Index of abstracts

FINAL NUMBER	Session	Session Day
OP-001	Pathogenesis of Stones	Tuesday 2 September
OP-002	Pathogenesis of Stones	Tuesday 2 September
OP-003	Pathogenesis of Stones	Tuesday 2 September
Guest Lecture	Pathogenesis of Stones	Tuesday 2 September
PP-001	Pathogenesis of Stones	Tuesday 2 September
PP-002	Pathogenesis of Stones	Tuesday 2 September
PP-003	Session 1: Pathogenesis of Stones	Tuesday 2 September
PP-004	Session 1: Pathogenesis of Stones	Tuesday 2 September
PP-005	Session 1: Pathogenesis of Stones	Tuesday 2 September
PP-006	Session 1: Pathogenesis of Stones	Tuesday 2 September
PP-007	Session 1: Pathogenesis of Stones	Tuesday 2 September
PP-008	Session 1: Pathogenesis of Stones	Tuesday 2 September
PP-009	Session 1: Pathogenesis of Stones	Tuesday 2 September
PP-010	Session 1: Pathogenesis of Stones	Tuesday 2 September
PP-011	Session 1: Pathogenesis of Stones	Tuesday 2 September
OP-004	Session 2: Epidemiology and Nutrition	Tuesday 2 September
OP-005	Session 2: Epidemiology and Nutrition	Tuesday 2 September
OP-006	Session 2: Epidemiology and Nutrition	Tuesday 2 September
OP-007	Session 2: Epidemiology and Nutrition	Tuesday 2 September
OP-008	Session 2: Epidemiology and Nutrition	Tuesday 2 September
OP-009	Session 2: Epidemiology and Nutrition	Tuesday 2 September
OP-010	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-012	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-013	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-014	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-015	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-016	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-017	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-018	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-019	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-020	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-021	Session 2: Epidemiology and Nutrition	Tuesday 2 September
PP-022	Session 2: Epidemiology and Nutrition	Tuesday 2 September
OP-011	Session 3a: Stones in Pets	Wednesday 3 September
OP-012	Session 3a: Stones in Pets	Wednesday 3 September
OP-013	Session 3a: Stones in Pets	Wednesday 3 September
OP-014	Session 3a: Stones in Pets	Wednesday 3 September
OP-015	Session 3a: Stones in Pets	Wednesday 3 September
PP-023	Session 3a: Stones in Pets	Wednesday 3 September
PP-024	Session 3a: Stones in Pets	Wednesday 3 September
PP-025	Session 3a: Stones in Pets	Wednesday 3 September
PP-026	Session 3a: Stones in Pets	Wednesday 3 September
PP-027	Session 3a: Stones in Pets	Wednesday 3 September
PP-028	Session 3a: Stones in Pets	Wednesday 3 September
OP-016	Session 3a: Stones in Pets	Wednesday 3 September
OP-017	Session 3a: Stones in Pets	Wednesday 3 September
OP-018	Session 3a: Stones in Pets	Wednesday 3 September
OP-019	Session 3a: Stones in Pets	Wednesday 3 September

Index of abstracts continued

FINAL NUMBER	Session	Session Day
OP-020	Session 3a: Stones in Pets	Wednesday 3 September
OP-021	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-022	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-023	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-025	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-026	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-027	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-028	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-029	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-030	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-031	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-032	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-029	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-030	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-031	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-032	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-033	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-034	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-035	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-036	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-037	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-038	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-039	Session 3c: Urological Management of Stones	Wednesday 3 September
PP-040	Session 3c: Urological Management of Stones	Wednesday 3 September
OP-033	Session 4a: Inhibitors and Promotors	Wednesday 3 September
OP-034	Session 4a: Inhibitors and Promotors	Wednesday 3 September
OP-035	Session 4a: Inhibitors and Promotors	Wednesday 3 September
OP-036	Session 4a: Inhibitors and Promotors	Wednesday 3 September
OP-037	Session 4a: Inhibitors and Promotors	Wednesday 3 September
OP-039	Session 4b: Medical Management of Stones	Wednesday 3 September
OP-040	Session 4b: Medical Management of Stones	Wednesday 3 September
PP-041	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-043	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-044	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-045	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-046	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-047	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-048	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-049	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-050	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-051	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-052	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-053	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-054	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-055	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-056	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-057	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
PP-058	Session 4b: Inhibitors and Promotors and Medical Management of Stones	Wednesday 3 September
OP-041	Session 5: Crystals and Cells	Thursday 4 September

Index of abstracts continued

FINAL NUMBER	Session	Session Day
OP-042	Session 5: Crystals and Cells	Thursday 4 September
OP-043	Session 5: Crystals and Cells	Thursday 4 September
OP-044	Session 5: Crystals and Cells	Thursday 4 September
OP-045	Session 5: Crystals and Cells	Thursday 4 September
OP-046	Session 5: Crystals and Cells	Thursday 4 September
OP-047	Session 5: Crystals and Cells	Thursday 4 September
OP-048	Session 5: Crystals and Cells	Thursday 4 September
OP-049	Session 5: Crystals and Cells	Thursday 4 September
OP-050	Session 5: Crystals and Cells	Thursday 4 September
PP-059	Session 5: Crystals and Cells	Thursday 4 September
PP-060	Session 5: Crystals and Cells	Thursday 4 September
PP-061	Session 5: Crystals and Cells	Thursday 4 September
PP-062	Session 5: Crystals and Cells	Thursday 4 September
PP-063	Session 5: Crystals and Cells	Thursday 4 September
PP-064	Session 5: Crystals and Cells	Thursday 4 September
PP-065	Session 5: Crystals and Cells	Thursday 4 September
PP-066	Session 5: Crystals and Cells	Thursday 4 September
PP-067	Session 5: Crystals and Cells	Thursday 4 September
PP-068	Session 5: Crystals and Cells	Thursday 4 September
PP-069	Session 5: Crystals and Cells	Thursday 4 September
PP-070	Session 5: Crystals and Cells	Thursday 4 September
OP-051	Session 6a: Metabolic Factors and Stones	Thursday 4 September
OP-052	Session 6a: Metabolic Factors and Stones	Thursday 4 September
OP-053	Session 6a: Metabolic Factors and Stones	Thursday 4 September
OP-054	Session 6a: Metabolic Factors and Stones	Thursday 4 September
OP-055	Session 6a: Metabolic Factors and Stones	Thursday 4 September
OP-056	Session 6a: Metabolic Factors and Stones	Thursday 4 September
OP-057	Session 6a: Metabolic Factors and Stones	Thursday 4 September
PP-071	Session 6b: Medical Management of Stones	Thursday 4 September
PP-072	Session 6b: Medical Management of Stones	Thursday 4 September
PP-073	Session 6b: Medical Management of Stones	Thursday 4 September
PP-074	Session 6b: Medical Management of Stones	Thursday 4 September
PP-075	Session 6b: Medical Management of Stones	Thursday 4 September
PP-076	Session 6b: Medical Management of Stones	Thursday 4 September
PP-077	Session 6b: Medical Management of Stones	Thursday 4 September
PP-078	Session 6b: Medical Management of Stones	Thursday 4 September
PP-079	Session 6b: Medical Management of Stones	Thursday 4 September
PP-080	Session 6b: Medical Management of Stones	Thursday 4 September
PP-081	Session 6b: Medical Management of Stones	Thursday 4 September
PP-082	Session 6b: Medical Management of Stones	Thursday 4 September
PP-083	Session 6b: Medical Management of Stones	Thursday 4 September
PP-084	Session 6b: Medical Management of Stones	Thursday 4 September
PP-085	Session 6b: Medical Management of Stones	Thursday 4 September
PP-086	Session 6b: Medical Management of Stones	Thursday 4 September
PP-087	Session 6b: Medical Management of Stones	Thursday 4 September
PP-088	Session 6b: Medical Management of Stones	Thursday 4 September
PP-089	Session 6b: Medical Management of Stones	Thursday 4 September
PP-090	Session 6b: Medical Management of Stones	Thursday 4 September
PP-091	Session 6b: Medical Management of Stones	Thursday 4 September

Index of abstracts continued

FINAL NUMBER	Session	Session Day
PP-092	Session 6b: Medical Management of Stones	Thursday 4 September
PP-093	Session 6b: Medical Management of Stones	Thursday 4 September
PP-094	Session 6b: Medical Management of Stones	Thursday 4 September
PP-095	Session 6b: Medical Management of Stones	Thursday 4 September
PP-096	Session 6b: Medical Management of Stones	Thursday 4 September
PP-097	Session 6b: Medical Management of Stones	Thursday 4 September
PP-098	Session 6b: Medical Management of Stones	Thursday 4 September
OP-058	Session 7: Genetic Factors and Paediatric Stones	Friday 5 September
OP-059	Session 7: Genetic Factors and Paediatric Stones	Friday 5 September
OP-060	Session 7: Genetic Factors and Paediatric Stones	Friday 5 September
OP-061	Session 7: Genetic Factors and Paediatric Stones	Friday 5 September
OP-062	Session 7: Genetic Factors and Paediatric Stones	Friday 5 September
OP-063	Session 7: Genetic Factors and Paediatric Stones	Friday 5 September

OP-001 Randall's plaque: an increasingly frequent and complex process in calcium stone formation

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Introduction: The prevalence of calcium stones is constantly increasing in industrialized countries. In parallel, presence of Randall's plaques (RPs) in calcium stones is found with increasing frequency. Stones exhibiting a concave region ("umbilication") with a footprint of RP suggest they had developed while attached on calcified papilla.
Objectives: To evaluate the evolution of the prevalence of "umbilicated" calcium stones over the 25 past years and the prevalence of RPs found during ureterorenoscopy in calcium stone formers. To analyze the physicochemical composition of RP in "umbilicated" calculi in order to identify the mechanisms involved in their formation.
Methods: We examined the proportion of stones exhibiting an "umbilication" among 54,700 stones referred to our laboratory between 1980 and 2006. Reno-ureteroscopic examination was performed in 289 calcium stone formers. In addition, 6 calcium oxalate stones developed on a RP were comprehensively analyzed using physical methods including infrared spectroscopy (FTIR), X-ray diffraction, and scanning electron microscopy (SEM). Heavy metals content was analyzed by X-ray fluorescence and PIXE.
Results: The proportion of "umbilicated" stones markedly increased from 8% in the early 1980 s up to 20.6% in the recent period ($P < 0.0001$), and was significantly higher in males than in females. Whewellite stones, the commonest type of stones, had the highest prevalence of umbilication, found in 43% of cases in males and 34% in females. There was a shift in the frequency peak to younger patients in the recent years. Reno-ureteroscopy disclosed one or more RPs in 57% of stone formers.
FTIR disclosed carbapatite (CA) as the main or unique component in 92.2% of RPs. Other components were less frequently found as the main component, such as sodium acid urate (4%), amorphous carbonated calcium phosphate (1.6%), whitlockite, brushite or uric acid (<0.5% each). In about 90% of cases, the morphology of the RP in the umbilication appeared as a bulky CA deposit. In other cases, RP presented either as a wide plaque with visible holes corresponding to calcified tubules, or as a cylindrical form, highly suggestive of CA deposits within the lumen of a collecting duct.

The carbonate content of CA in RP varied from less than 5% up to 30%. Most RPs contained a high proportion of carbonate ions, suggestive of a medullary origin. In about 40% of cases, the carbonate content was low, thus suggesting that CA originated, at least in part, from urine.

By SEM imaging, prints of tubule ends were observed in several RPs. Several heavy metals, namely Zn, Sr, and Pb, were found in RPs from stones and from a papillary biopsy. In addition to these metals, Cu was found in the papillary biopsy, and collocated with Ca, at variance with the other heavy metals, suggesting a close relationship between Ca and Cu.
Conclusion: Our data based on stone analysis confirm the increasing prevalence of RPs as reflected by stone morphology in calcium stone formers. Physico-chemical analysis of RPs in stones suggests a variable composition of plaques and variable pathogenic mechanisms. The finding of copper in collocation with calcium in a papillary plaque opens new perspectives as to the pathophysiology of RPs and needs further investigation.

OP-002 Does Randall's plaque represent a necessary condition in the pathogenesis of the idiopathic calcium oxalate stones?

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Introduction: According to the Randall's plaque theory of stone formation, urinary crystals may attach to focal crystalline deposits of interstitial calcium phosphate (CaP), localized in the tip of the renal papillae and exposed on the surface following loss of the normal urothelial covering. Several observations recently have supported this theory in idiopathic calcium oxalate (CaOx) stone pathogenesis.
Objectives: The aims of this study were: (1) to confirm practicability and safety of the papillary biopsy, performed during ureterorenoscopy (URS) or percutaneous nephrolithotomy (PCNL), in order to obtain in vivo appropriate specimens for the research of the interstitial CaP deposits, representing precursors of Randall's plaques; (2) to analyze whether Randall's plaques are a necessary condition for idiopathic CaOx stone pathogenesis.
Methods: Twenty-eight renal stone patients underwent URS (8 cases) or PCNL (20 cases) and renal papillae biopsy. None had a history of intestinal bypass or resection, long immobilization periods, sarcoidosis, hypervitaminosis D or Milk-alkali syndrome. Two patients had primary hyperparathyroidism; one had distal tubular acidosis. Stone analysis was obtained in each patient. Papillary specimens were obtained using three to seven forceps. The same

pathologist analyzed all histological specimens. Interstitial Ca deposits, representing Randall's plaque precursors were examined by von Kossa staining.
Results: Twenty-seven of the 28 bioptic specimens (96.4%) were adequate for an accurate pathological analysis. One sample was inadequate because of important regressive phenomena. Histological analysis showed interstitial Ca deposits in 42.9% of cases (group A: 12 patients), while no Ca deposit was observed in 15 patients (group B). Pure or mixed idiopathic CaOx calculi were found in all group A patients and in 9 out of 15 group B patients (three of whom had metabolic diseases).

During the post-operative period two patients had fever <38°C and one >38°C. Though a significant decrease in hematocrit values was registered, no patients required haemotransfusion. In a concurrent group of patients who, during URS or PCNL for renal stones, did not undergo papillary biopsy, similar clinical findings were also observed.
Conclusion: Percutaneous or transureteral renal papillae biopsy represents a safe and appropriate procedure to obtain papillary specimens in order to identify Randall's plaques. According to the low number of inadequate specimens, there is no difference between the percutaneous and retrograde transurethral pathways. No clinical significant biopsy complication was observed.
Preliminary results do not support the hypothesis that Randall's plaques play a central pathogenetic role in all idiopathic CaOx stone cases, suggesting the co-existence of different pathogenetic mechanisms.

OP-003 Speciation modeling in urines from two groups of patients: high relative supersaturation of calcium phosphate is a risk factor for multiple stone-types

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Introduction: Assessment of the probability of crystal formation in urine is a key step in the work-up of kidney stone patients. For over 20 years, the relative supersaturation of stone-forming salts has been calculated using the program EQUIL. However, recently we used a different chemical speciation program, JESS, for our modelling and for calculating the so-called saturation index (SI) of such salts in urines from healthy subjects who had been given a citrate preparation to alter their urine chemistry and concomitant speciation. The application of this powerful approach for modelling abnormal urines has not been previously reported.
Objectives: In the present study, we undertook to determine SI values and to model precipitation of stone-forming salts in the urines of hypocitraturic patients (Group A) and patients with abnormally high-calcium phosphate relative supersaturation values (Group B), with a view to predicting relative risks for stone formation by comparison with healthy controls (Group C).
Methods: Urine data for 50 patients in each of Group A and Group B as well as 50 controls in Group C were provided by the Litholink Corporation in Chicago. These data were used as input for JESS.
Results: Consideration of the log SI values shows (1) that the risk of calcium oxalate (CaOx) stone formation is the same for both patient groups, (2) that group A patients are at risk of forming uric acid stones (in addition to CaOx stones) and (3) that group B patients are at risk of forming CaOx, octacalcium phosphate, brushite, whitlockite and hydroxyapatite stones, as well as those composed of sodium urate. Our modelling shows that precipitation of CaOx has no effect on the SI values of other stone forming salts but that precipitation of any of the salts of calcium phosphate significantly decreases the SI of CaOx (Fig. 1).
Conclusion: Patients who have high urinary relative supersaturation with respect to calcium phosphate are at risk for the formation of a wide range of stone types. In the event of calcium phosphate stone formation in this group, it is unlikely that co-precipitation of calcium oxalate will occur.

Guest speaker

Nutrigenomics and nutrigenetics: a new approach to prevention of urolithiasis?

M. Fenech*[1]
[1]Nutrigenomics and Nutrigenetics, CSIRO Human Nutrition, Adelaide, Australia

Introduction: The emerging new sciences of nutrigenomics and nutrigenetics are providing the basis for improved strategies for disease prevention based on personalised nutrition. The term nutrigenomics refers to the effect of diet on gene expression and chromosomal stability. The term nutrigenetics refers to the impact of inherited traits on the response to a specific dietary pattern, functional food or supplement on a specific health outcome. Both genetic and dietary factors are involved in the aetiology of urolithiasis. In this presentation I will provide a brief introduction to the concepts of nutrigenetics and nutrigenomics and their application in prevention of a variety of pathogenic states. Furthermore, I will explore the potential application of these disciplines to determine the impact of key nutritional factors (such as intake of fluids, calcium, oxalate, citrate, vitamin C, cystine, and phytate) and their interactive effects with genetic backgrounds that predispose to kidney and bladder stone formation (e.g., cystinuria, Dent disease, PH type1, hNaDC-1 I550 V polymorphism) and associated disorders such as osteoporosis. Such approaches may lead to more efficacious personalised preventive nutritional strategies for urolithiasis.

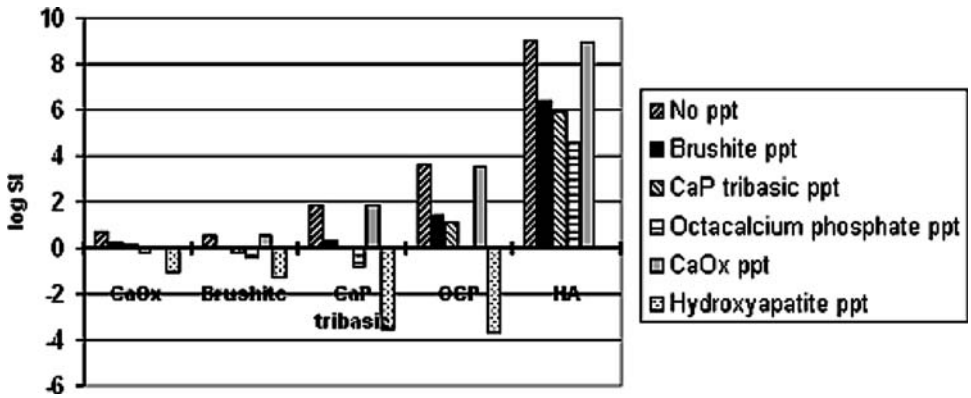

Fig. 1 Effect of precipitation on log SI

PP-001 Change of the stone composition at recurrent stone formation and in different sides of urinary tract

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Introduction: The stone analysis is an essential pre-condition for effective metaphylaxis of urolithiasis. There is lack of data on change or constancy of stone composition at recurrent stone formers. The same is valid for stone formation in different sides of urinary tract.
Objectives: The aim of this study is to provide assured data to the problems of change or constancy in cases of recurrent stone formation and in different sides of urinary tract.
Methods: For all patients whose stones were sent for stone analysis to our laboratory between 2004 and 2007 it was proven whether earlier stone analyses exist. This has been the case for 608 patients out of 9,719 analyses within 3 years.
For the purpose of evaluating of the variability or constancy of stone composition, three groups were formed:

Group A: First-degree analytical differences—occurrence of components, which determine the metaphylactic strategy essentially: Uric acid, struvite, brushite, cystine and ammonium urate.

Group B: Second-degree analytical differences—change of the main component in the system, whewellite–weddellite–apatite for example, different occurrence of components, which are not essentially for metaphylaxis.

Group C: No or only small analytical differences, small quantitative differences.

Results: A total of 65.2% of the patients with multiple stone analyses assigned to the group C showed no or only small analytical differences; 34.8% of the patients had multiple stone analyses with analytical differences (Group A and B); 15.9% of patients had very significant analytical differences (Group A with relevance for metaphylaxis) and 18.9% had analytical differences of the second degree (Group B).
A total of 146 patients (24% of 608 patients) showed multiple stone analyses on both sides of the urinary tract; 30.2% out of these patients had analytical differences (Groups A and B) and 69.8% had similar analyses (Group C).
Conclusion: A total of 34.8% of all patients with multiple stone analyses show significant differences in the stone composition. This fact shows that a change of the stone composition at recurrent stone formers is not as rare as commonly assumed. The same is valid for the change of stone composition between the both sides of urinary tract at 30% of patients.
The results of this study show that a change of the pathogenetic conditions of recurrent stone formation and at lateral different stone formation, respectively, is found in one third of recurrent stone formers. This change of pathogenetic conditions has to be taken into consideration in the prophylaxis of recurrent stone formation. This includes repeated stone analysis.

PP-002 Determination of substances in urine of stone formers to elucidate urolithiasis development

I. Yoshioka*[1], C. Momohara[1], M. Tsujihata[1], A. Okuyama[1]
[1]Urology, Osaka University Graduate School of Medicine, Osaka, Japan

Objectives: Most of urinary stone matrices consist of urinary macromolecules, which are thought to have both promotion and inhibition effect on stone crystallization, crystal aggregation and crystal–cell interactions. The composition of urinary macromolecules is assumed to differ between stone formers and healthy individuals. To evaluate the origin elucidation of urolithiasis formation, we compared substances found in urine of stone formers with those in urine from healthy subjects to determine their characteristics.
Methods: We obtained 24-h urine samples from ten male stone formers, who were without urinary stones, abnormal metabolism and microhaematuria at the time of the study, as well as from ten healthy subjects. Urine parameters in relation to urolithiasis were measured, then, the urine samples were ultra-filtered at a cut-off molecular weight of 3kD for urinary macromolecules (UMMs) extraction. Median Darby canine kidney (MDCK) cells were used to evaluate the protective activity of the UMMs from oxalate-induced cytotoxicity using a methyl-thiazolyl tetrazolium (MTT) assay. Furthermore, the concentration of glycosaminoglycans (GAGs) in the UMMs was determined using a modified DMB assay. Proteins were extracted from the UMMs and their compositions determined using the Lowry method. The protein compositions in both groups were compared using sodium dodecyl sulfate polyacrylamide gel electrophoresis, and then compared using liquid chromatography–mass spectrometry.
Results: Table shows comparison of urinary parameters between the stone formers and healthy subjects. Although the urine volume in stone formers was greater than healthy subjects, the difference was not significant, whereas the concentration of calcium was significantly greater in the stone formers. No other differences were found for the urinary parameters. The concentration of UMMs was 0.069 ± 0.03 mg/ml in the stone formers and 0.112 ± 0.03 mg/ml in the healthy subjects, which was significantly different. The result of the MTT assay showed that UMMs from the healthy subjects had a greater ability to protect the cells from oxalate-induced cytotoxicity as compared to those from the stone formers. In addition, the concentration of GAGs in the UMMs was 15.9 ± 2.22 μg/mg in the stone formers and 9.98 ± 2.57 μg/mg in the healthy subjects, which was not significantly different. Proteins in the UMMs totalled 0.66 ± 0.31 mg/ml in stone formers and 0.59 ± 0.31 mg/mg in the healthy subjects, which were not significantly different. Although we assumed the existence of a minute difference, the results obtained with sodium dodecyl sulfate polyacrylamide gel electrophoresis were not clear. Therefore, in order to clarify the difference, we tried a liquid chromatography-mass spectrometry technique.

	Stone formers	Normals	*P* value
Body weight (kg)	70.8 ± 8.4	67.1 ± 5.7	0.45
BMI	24.2 ± 2.1	22.5 ± 2.4	0.15
Urine volume (ml)	1770.0 ± 565.5	1346.3 ± 402.7	0.12
pH	5.9 ± 0.4	6.1 ± 0.6	0.57
Citrate (mg/dl)	30.7 ± 21.9	33.6 ± 18.8	0.59
Uric Acid (mg/dl)	41.7 ± 26.0	56.0 ± 14.3	0.08
Ca (mg/dl)	16.9 ± 9.5	12.3 ± 9.5	0.009
IP (mg/dl)	60.4 ± 40.9	79.0 ± 23.5	0.16
Mg (mg/dl)	5.0 ± 2.2	4.9 ± 1.5	0.18
Oxalate (mg/dl)	2.0 ± 1.6	2.4 ± 0.9	0.53

Conclusion: UMMs from the healthy subjects have a protect ability from oxalate-induced cytotoxicity. The reason for the difference in composition is not clear. It is necessary to continue further search.

PP-003 Hyperoxaluria-induced tubular ischemia : evaluation of tissue HIF-1 alpha levels in renal parenchyma in rabbit model

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Objectives: In this experimental study we aimed to evaluate possible tubular ischemia formation as demonstrated by HIF-1 alpha positivity in tubular cells following hyperoxaluria induction in a rabbit model.
Methods: A total of 24 rabbits was included in the study and divided into two groups. Group I: The experimental animals ($n = 6$ for the 7-day time period and $n = 6$ for the 28-day period) were given a hyperoxaluria-inducing diet of 0.75% ethylene glycol (EG) in distilled drinking water for a 2-week period before undergoing specific examination after 1–4 weeks respectively (depending on which time period group involved). Group II: The control animals ($n = 6$ for the 7-day time period and $n = 6$ for the 28-day period) received distilled drinking water. Animals were sacrificed after 1 or 4 weeks and the removed kidneys were sent for histopathological evaluation for the presence and degree of HIF-1 alpha positivity at different levels of the renal parenchymal tissue.
Results: Of the specimens obtained from the study group animals (hyperoxaluria induced group, $n = 6$), all demonstrated nuclear staining. While severe nuclear staining was shown in four, moderate degree staining was present in the remaining two specimens. Cell nuclei in both the cortical as well as the medullary region of kidneys were found to be stained. On the other hand, evaluation of the kidneys from the control group animals demonstrated a minimal degree of staining in one but no positivity in the remaining 5 specimens. Comparative evaluation of cortical, medullary and papillary sections of the kidney showed similar changes in all sections especially in the distal and collecting ducts.
During long-term evaluation (4 weeks) of the specimens obtained from the study group animals, while only two of them demonstrated a minimal degree of nuclear staining, no staining could be shown in the remaining four specimens. On the other hand, evaluation of the kidneys from the control group animals showed that no specimen demonstrated staining with respect to HIF-1 alpha positivity.
Conclusion: As a key mediator distributed mainly in tubular cells, HIF-1alpha plays a certain role in the cellular adaptation to hypoxia. Possible ischemia formation following hyperoxaluria induction has been found to be responsible for subsequent alterations in the renal tubules resulting in stone formation and the presence of hypoxic changes as documented by HIF-1 alpha positivity in this study also support the presence of ischemic insult after hyperoxaluria induction in this animal model.

PP-004 Ultrastructural study of laminated urinary stones

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Introduction: Several modalities of stone analysis are practised in different laboratories. However, help is not obtained for the treating clinician to assess the initiation and pathogenesis of stone formation. So progression of calculogenesis remains a myth.
Objectives: The purpose of this paper is to assess the pathological mechanisms of stone nucleation and growth by observing the ultra microscopic morphology of the different layers of laminated stones.
Methods: A total of 130 segments from 28 randomly selected laminated stones of more than 10 mm diameter were analysed. Surface morphology and cross sectional morphology were assessed by using ordinary camera images in close-up mode (9.5 mega-pixels). Optical microscopy was performed using zoom stereo microscopy up to magnification of 125 and recorded with 8 mega-pixel camera. Each segment thus analysed was used for Fourier Transmission Infra Red Analysis after powdering and mixing with potassium bromide 1 in 200.
Results: The weight of stones studied varied between 5.1 and 7.2 g. The surface was black in five, yellow in six and brown in ten. Eight had multiple patches. The cross sections showed concentric laminations in 17, radial striations in nine, frond formation in three and no designs in five. The colour of the cross section was not uniform in any stone. Black, brown, yellow and dirty white were intermixed all the stones studied. Cavities were noted in seven stones. Seventeen were friable and eleven were not. Qualitative wet chemical analysis, optical microscopic study and FTIR analysis of the whole stones were compared with similar observations of the surface and interior layers. An average of 4.64 segments was assessed for each stone. By surface morphology, Whewellite (COM) was identified by the mulberry appearance on the surface and by the concentric laminations and radial striations on section, weddellite (COD) by the spiculated surface and bi-pyramidal crystals (sugar candy appearance), uric acid (UA) by the smooth reddish appearance with fibrous strands (cotton candy appearance), phosphates by the white amorphous appearance, struvite by coffin lid appearance and newberyite by parchment appearance. By FTIR, it was seen that COM was the most common component (69%), followed by UA 42%, COD 32%, ammonium urate 11%, brushite 7%, struvite 4%, apatite 5% and newberyite 3%. A total of 77% had different components in different areas; 23% were not significantly different. Components of mixed stones were COM (87), COD (46%), UA (42%) and phosphates (26%). Mixtures were mostly COM + UA, COM + COD and Phosphate + UA. Radial striations were seen mostly along with concentric lamination in COM stones. Frond formation was characteristic in phosphate stones.
Conclusion: The surface and interior were different in those stones containing predominantly COM, UA and phosphates. The analytical findings differed in the different layer segments of the stone. Uric acid was more common in the inner layers of mixed stones than on the surface. COM was the outer component in most mixed stones.

PP-005 Urodynamic hypothesis of urolithiasis: the risk factors

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Introduction: Metabolic and inflammatory factors are widely believed to be the main pathogenetic factors in renal stone formation. The urodynamic changes are usually referred to structural anomalies in the upper urinary tract (UUT) and its dilation. However, little attention is paid to the functional abnormalities in the renal pelvis and the ureter (especially their contractile activity) during stone disease.
Objectives: The aim of this study was to evaluate UUT urodynamic parameters, which affect renal function and promote stone formation during urolithiasis.
Methods: A group of 60 patients with renal and ureteral stone disease with indications for urinary diversion (nephrostomy tube or catheter indwelling) were observed during 2-month follow-up. They had proximal (26) or distal (34) ureteral obstruction. During this period, 17 patients in each group were subjected to lithotripsy procedures. The UUT urodynamics was assessed with the help of electromanometry and multichannel impedance ureterography. The documented data included renal pelvic pressure (RPP) and the

number of ureteric contractility parameters such as peristalsis amplitude (PA), peristalsis rate, the ureteral wall tone (T), the characteristics of contractile wave form and direction (antegrade or retrograde). Urinary biochemistry and enzymuria were studied to characterize the lithogenic activity (Ca, oxalates, citrates) and renal tubular function according to gamma-glutamyltransferase (GGT).
Results: According to elevated calcium and oxalate levels in the urine (4.95 ± 0.25 mM and 504 ± 35 μM, respectively) and the low level of citrate (2.5 ± 0.1 mM), lithogenic activity was found in 54% patients. The basal RPP values were high (28.7 ± 2.6 cm H_2O) in the presence of an obstruction and inflammation; they dropped to 15.6 ± 1.9 cm H_2O when inflammation resumed in partially obstructed patients (with residual stone fragments) and fell down to 3.6 ± 1.4 cm H_2O in unobstructed patients. Enhanced GGT urine excretion indicated renal tubular deterioration in the above-mentioned groups, respectively: 11.5 ± 3.2; 8.1 ± 2.0, and 1.6 ± 0.5 unit/L. These data assumed that elevated RPP is harmful to the renal function. The possible risk factors leading to RPP elevation can be disorders in UUT contractile function such as a high T (13.2 ± 2.1 1/Ohm) recorded in 42% patients, a low PA (0.3 ± 0.03 Ohm), decreased breathing RPP oscillations, which decreased pronouncedly when the patients changed their position from recumbent to orthostatic (20 vs. 5% relative to the basal level), and frequent manifestations of the retrograde (84%) or chaotic (86%) peristaltic waves. Other risk factors are the retrograde peristaltic waves of high PA (2.8 ± 0.5 Ohm) and T (7.9 ± 1.8 1/Ohm) recorded in 71% patients that can provoke the urine refluxes into renal pelvis and elevated peristaltic RPP (29.0 ± 0.8, range 21–34 cm H_2O) as compared to the patients with proximal obstruction (20.3 ± 1.6 cm H_2O, range 16–27 cm H_2O). The noted RPP differences in patients with different level of the UUT obstruction were absent in the patients who received lithotripsy procedures. Probably, the origin of these urodynamic abnormalities is neurogenic.
Conclusion: UUT urodynamic changes in stone formers that manifested with basal and/or peristaltic pressure rise might be the supplementary pathogenetic factors promoting stone formation and causing deterioration of renal function, which must be controlled in the course of urolithiasis procedures. These changes can be prevented by specialised medical treatment.

PP-006 Clinical risk index in urolithiasis

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Introduction: Various risk indices have been propounded by various authors to assess the severity of stone formation in the human urinary tract. However, most of these indices are laboratory-oriented and not feasible to be performed in a hospital setting. Most of these do not take into consideration all the possible influences on stone formation.
Objectives: In this paper, the correlation of various clinically relevant risk indices has been assessed to understand the relevance of the prediction in the possibility of future stone formation.
Methods: A total of 500 stone patients were studied to determine the various possible risk factors. The total score of the index was fixed as 100. A total of 44 variables was used to calculate the index and each variable has given a score ranging from one to five. They included Age 20–40 (1), Sex M/F(2/1), FH ++/+ (3/2), Gulf + (1), Occupation Ext (1), Primary (1), Recurrent (5), Symptoms ++/+ (2/1), RBC + (1), PC ++ (1), COD + (1), COM + (2), UA + (2), Crystal aggregation + (2), Urinary infection + (1), pH below 6 (1), Bilateral / unilateral (2/1), Kidney/U/B/U (1), Passer + (2), Multiple organ (2), Multiple/Single (2/1), Incomplete Removal + (5), Serum Calcium (3/2/1), Serum Phosphorus (1), Serum Magnesium (1), Serum Creatinine (1), Serum Uric Acid (4/2/1), Urine Volume (1), Urine specific gravity (1), Urine calcium (1), Urine phosphorus (1), Urine uric acid (5), Urine magnesium (2), Urine oxalate (8/5), Urine citrate (8/5), Calcium magnesium ratio (2), TRP (4), Creatinine clearance (1), Calcium oxalate ratio (2/1), Oxalate citrate ratio(5), Oxalate uric acid ratio (2/1), Calcium uric acid ratio (2), Stone COM/COD (2/1) and Stone UA/Cystine (2/2). After calculating the index, it was correlated with the clinical severity index. The Severity Status of each patient was considered as +/++/+++/++++ (Nil/Low/Moderate/Severe) depending on the status of the disease in long term assessment. In 127 patients, the risk index was calculated after a period of one year to see the change in index score.
Results: On calculating the risk index and correlating with the severity grade of the stone disease, the correlation coefficient r value was +0.67 which was significant at $P < 0.001$ level. The risk index could be altered by dietary habit changes, drugs, life style changes and appropriate drug schedules. The second assessment after on year of the 127 patients showed that the mean risk index could be reduced from 43.08 to 36.56. This difference was statistically significant ($P < 0.01$).
Conclusion: It is concluded that by performing the various clinical and investigative studies, it is possible to arrive at a prediction regarding future stone formation in any individual. It is also possible to reduce the risk of stone formation by appropriate diet, drug and life style change schedules.

PP-007 Temporary risk identification in urolithiasis

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Introduction: We have been using a risk index calculation for Urolithiasis, which included most of the identifiable factors promoting calculogenesis. However, it was observed that the frequency of a patient forming stones was not uniform in spite of a similarity in the risk index in the permanent setting. Further, many of the risk indices could be changed by dietary or life style modifications.
Objectives: The objective of this paper was to calculate the temporary risk index of a patient at the time of each visit and correlate with stone activity during such periods, so that appropriate advice could be given on drugs, diet and life style changes.
Methods: The temporary risk index score was based on four symptoms namely pain (0—nil, 1—vague pain, 2—disturbing, 3—colic, 4—severe, 5—excruciating), haematuria (0—nil, 1—turbid, 2—cloudy, 3—reddish, 4—occasional frank blood, 5—continued frank blood), burning sensation (0—nil, 1—minimal, 2—moderate, 3—terminal severe, 4—excruciating occasional, 5—excruciating continuous), and dysuria (0—nil, 1—minimal, 2—moderate, 3—terminal severe, 4—excruciating occasional, 5—excruciating continuous), ultrasonography for back pressure (0—nil, 1—mild, 2—moderate, 3—severe kidney and ureter, 4—unilateral total, 5—bilateral total-anuria) and eight urine deposit findings (0—nil, 1—+, 2—2+ , 3—3+ , 4—4+ , 5—plenty), red blood cells, pus cells, whewellite crystals, weddellite crystals, phosphate crystals, uric acid/ammonium urate crystals, crystal clumping and crystal aggregation making a total of thirteen parameters. Each parameter was given values ranging from 0 to 5. The total score was calculated and chemotherapeutic regimes were decided based on the score, which would vary from 0 to 65. A total of 100 randomly selected patients who had been visiting the stone clinic for a minimum of five occasions were included in the study. The total scores of temporary risk were compared with the permanent clinical risk score mentioned above.
Results: The temporary risk of the 100 patients during the total of 500 visits ranged from 0 to 43 out of 65. The risk score reduced

significantly from visit 1 to 5 in all the patients. On correlating the mean index of the five visits with the permanent risk index, the correlation coefficient was +0.39 ($P < 0.01$). It is observed that the patients go through periods of hyperactivity of stone metabolism and present with symptoms, producing temporary phases of overactivity.
Conclusion: It is concluded that the temporary risk index is correlated with the permanent risk index of the patients forming urinary stones. It can be used as a method for scientific prediction regarding future stone formation in any individual. The dose of drugs and need for continuing chemotherapy for patients should be based on the temporary risk index. Blind prescription of drugs should be discouraged.

PP-008 Electrical conductivity and total dissolved solids in urine

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Introduction: Various theories have been put forward regarding the initiation and growth of urinary crystals. However, the role of electrical conductivity and total dissolved solids has not been discussed in the literature.
Objectives: The objective of this paper is to study the relevance of electrical conductivity and total dissolved solids in early morning and random samples of urine of urinary stone patients. A total of 2,000 urine samples was studied. The two parameters were correlated with the extent of various urinary concrements and biochemical status of the stone patients.
Methods: The early morning and random urine samples of the patients who attended the urinary stone clinic were analysed routinely. The pH, specific gravity, electrical conductivity, total dissolved solids, redox potential, albumin, sugar and microscopic study of the urinary sediments including the extent of crystalluria and presence of RBC and PC were correlated with each other.
Results: The values of total dissolved solids ranged from 3,028 to 18,480, the mean value being 7,012 ppm. The levels corresponded with the Electrical Conductivity of urine, There was a statistically significant correlation between the level of abnormality in the urinary deposits ($r = +0.27$ $P < 0.05$). The samples where the total dissolved solids constituted more than 12,000 contained more crystals than those samples containing total dissolved solids less than 12,000 ppm. There were, however, certain urine samples in which the TDS was over 12,000, which did not contain any urinary crystals. However, the urinary citrate values were higher than 300 mg per day in the corresponding 24-h urine samples.
Conclusion: It is concluded that the value of TDS has relevance in the process of stone formation. The extent of TDS can be controlled by increasing the urinary citrate levels of the urine.

PP-009 Morphological examination of stones in primary hyperoxaluria: a tool for early diagnosis

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Introduction: Primary hyperoxaluria type 1 (PH1) is a rare, but severe hereditary disease whose diagnosis is often missed or delayed, especially if the disease first manifests at the adult age, thus depriving patients of the benefits of timely instituted preventive measures. The great majority of PH1 patients present with nephrolithiasis. Stones are composed of pure or nearly pure calcium oxalate monohydrate (whewellite), but as whewellite is a very common component of idiopathic calcium stones, stone analysis was not considered as contributing to etiologic diagnosis. Because in PH1, hyperoxaluria is both massive and permanent, inducing a very active lithogenic process, we hypothesized that PH1 whewellite stones might exhibit some distinctive characteristics of interest to early orient etiologic diagnosis.
Objectives: To examine whether whewellite stones produced by PH1 patients have distinctive morphological characteristics both at stereomicroscopic examination and at the mesoscopic level allowing discrimination between common-type idiopathic whewellite stones.
Methods: Among 47,500 renal calculi referred to our laboratory between 1991 and 2007, 7,820 (16.4%) were made of pure or nearly pure (>95%) whewellite. Of the latter, 74 came from patients with undisputable diagnosis of PH1 based on full urine biochemistry and evidence of enzymatic defect. All calculi were examined by means of stereomicroscopy to determine the morphology of their surface and section and by infrared spectroscopy (pellet technique) to identify stone composition. In addition, 6 PH1 stones and 6 common type whewellite stones were examined by scanning electron microscopy (SEM).
Results: Morphology of stones clearly differed between PH1 and common type whewellite calculi. All PH1 calculi exhibited a whitish or pale yellow surface and a loose, unorganized section, sharply contrasting with the dark brown surface and radiating inner structure of common whewellite stones. Infrared spectra of PH1 stones exhibited a lower 1,100 cm^{-1} peak than common whewellite stones. By SEM examination, crystalline organization of PH1 stones markedly differed from that of common-type whewellite stones. Common whewellite stones exhibited a dense, radiating charcoal-like structure, whereas PH1 stones had a non-homogeneous, loose structure with crystal aggregates of various sizes and shapes, including curious, characteristic spherical structures of about 50 micrometers in diameter resembling balls of wool.
Conclusion: The peculiar morphology of pure whewellite stones consistently observed in PH1 patients appears to be pathognomonic, as it was never encountered in whewellite stones from any other origin, including enteric hyperoxaluria. Therefore, we propose morphologic examination of stones (1), in addition to component analysis by means of infrared spectroscopy or X-ray diffraction, as a simple, rapid, cheap, and reliable tool allowing to early orient the diagnosis of PH1 and thus timely institute active therapeutic strategy.

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PP-010 Clinical relevance of the carbonate content of calcium phosphate stones

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Introduction: Stones made of carbonated apatite (CA) admixed with struvite resulting from urinary tract infection by urea-splitting bacteria have a high carbonate content (1) but little is known as to the clinical significance of the carbonate content of CA in the absence of struvite in idiopathic calcium phosphate stones.
Objectives: To examine the carbonate content of calcium-phosphate stones without struvite, and the relationships between the carbonate content and presence of amorphous carbonate-containing calcium

phosphate (ACCP) or whitlockite, and the morphological characteristics of CA at the mesoscopic scale.
Methods: We analyzed 39 urinary calculi (from 20 male and 19 female patients) mainly composed of CA without presence of struvite. They were examined by stereomicroscopy for morphological typing, and by FT-IR spectroscopy for assessing stone components and determine their respective proportions. FT-IR also allowed determining the proportion of carbonate in CA and ACCP (carbonate content). The fine morphology of stones was examined by scanning electron microscopy (SEM), including search for bacterial prints (2).
Results: The total calcium phosphate content of stones (mean ± sd) was 91.3 ± 6.7%, including 60.1 ± 7.8% for CA and 31.2 ± 5.5% for ACCP. The mean proportion of carbonate in CA or ACCP was 14 ± 8%, and was similar in males and females. At SEM examination, CA essentially presented as spherules with a mean diameter of 4.5 ± 3.3 μm, significantly lower in males than in females. In 16 cases, SEM showed at the surface of the spherules imprints of short sticks, suggestive of bacterial prints. The size of the spherules did not differ between stones containing bacterial prints or not. In 14 stones containing both CA and ACCP, the carbonate content was significantly higher (21 ± 7%) than in those without bacterial prints (9 ± 5%, $P < 0.001$), and it was found to increase in parallel with the number of bacterial prints. Two stones with bacterial prints contained whitlockite (a non-carbonated phosphate), without ACCP; their carbonate content was <14%. Finally, ACCP (or whitlockite) was present in all 16 calculi with prints and in none of the 23 stones without prints ($P < 0.0001$).
Conclusion: Bacterial prints (indicative of past or current urinary tract infection) were found in phosphatic stones with a high carbonate content of CA and concomitant presence of ACCP, and in stones containing CA associated with whitlockite. Thus, a high carbonate content of CA and ACCP, or the presence of whitlockite, as determined by FT-IR appears to be indicative that an infectious process contributed in stone formation.

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PP-011 Distinctive morphologies in urinary stones as revealed by micro CT

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Introduction: Analysis of urinary stones is done by examination with binocular microscope and subsequent analysis of mineral from selected regions of the stone, usually using Fourier transform infrared spectroscopy (FT-IR). Because FT-IR analysis is often performed from selected parts of the stone, this method may miss minor stone components, and it results in destruction of the stone.
Objectives: The aim of this investigation was to assess the ability of micro CT for identifying stone minerals in 3D, non-destructively.
Methods: A total of 110 stone specimens were used for this study, of a variety of mineral types. The actual composition of these stones was taken to be that found by dissection and FT-IR of either a portion of the stone in this study or of a cohort stone collected from the same patient event. Stones were scanned by micro CT and compared by mineral type, in 151 distinctive mineral regions within the stones.
Results: Stones of apatite yielded attenuation values significantly higher than other stone types (21,340 ± 2,803, $n = 28$). The next set of stone types did not differ from one another, with calcium oxalate monohydrate (COM, 14,866 ± 713, $n = 35$), calcium oxalate dihydrate (COD, 14,046 ± 939, $n = 7$), mixed COM/COD (14,297 ± 1,411, $n = 20$), and closely admixed apatite and struvite (14,736 ± 1,994, $n = 6$) all falling within the same range. Significantly lower came cystine (7,685 ± 209, $n = 9$) and struvite stones (6,481 ± 806, $n = 20$). Stones of uric acid were significantly lower in attenuation value than other types (3,226 ± 413, $n = 26$). Significant morphological cues were also observed for several mineral types.
Conclusion: Variability of mineral attenuation values is greater than was originally reported for kidney stones, but the differences still afford considerable help in distinguishing common mineral types. Combined with morphological clues, this technique offers remarkable capability for identifying kidney stone composition, and in three dimensions.

OP-004 Determinants of 24-h urinary oxalate excretion

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Introduction: Higher levels of urinary oxalate substantially increase the risk of calcium oxalate kidney stone formation. However, the determinants of urinary oxalate excretion are unclear.
Objectives: To examine the impact of dietary factors, age, body size, diabetes, and urinary factors on 24-h urinary oxalate.
Methods: We conducted a cross-sectional study of 3426 stone forming and non-stone forming participants in the Health Professionals Follow-up Study (men), the Nurses' Health Study (older women), and the Nurses' Health Study II (younger women).
Results: Median urinary oxalate was 39 mg/day in men, 27 mg/day in older women, and 26 mg/day in younger women. Participants in the highest quartile of dietary oxalate excreted 1.7 mg/day (95% CI 0.8 to 2.6; P trend <0.001) more urinary oxalate than participants in the lowest quartile. The relation between dietary and urinary oxalate was similar in individuals with and without a history of nephrolithiasis. Dietary and supplemental calcium intakes were inversely associated with urinary oxalate. Participants consuming 1,000 mg/day or more of vitamin C excreted 6.7 mg/d (95% CI 5.2–8.3; P trend <0.001) more urinary oxalate than participants consuming <90 mg/day. Total fructose intake and 24-h urinary potassium, magnesium, and phosphorus levels also were positively associated with urinary oxalate. Age was inversely and body mass index was positively associated with urinary oxalate. After multivariate adjustment, participants with diabetes excreted 2.1 mg/day (95% CI 0.8–3.4) more urinary oxalate than those without diabetes.
Conclusion: The impact of dietary oxalate on urinary oxalate appears to be small. Further investigation of factors influencing urinary oxalate may lead to new approaches to prevent calcium oxalate kidney stones.

OP-005 Risk factors for renal calcium stone formation in South African and European young adults

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Introduction: The different susceptibility to renal stone disease of white and black populations has been explained in terms of intrinsic factors (genetic background), but also in terms of extrinsic factors such as diet and lifestyle. However, in South Africa, the absence of stone disease in the black population has not yet been fully explained by either intrinsic or extrinsic factors [1].

Objectives: The aim of this study was to identify any differences between black and white subjects in South Africa and Europe in the dietary and urinary risk factors, which predispose them to renal stone formation.
Methods: A total of 72 healthy subjects (45 males and 27 females, age range 21–30 years) with no previous history of renal stone disease or specific diseases predisposing to renal stone formation were recruited in South Africa (SA) and in Italy (IT). They were divided in three groups: South African blacks (SA-B), South African whites (SA-W) and Italian whites (IT-W).
Each participant was subjected to a investigation including a 24-h dietary record and 24-h urine sample taken over the same period. Nutrients and calories were calculated by means of food composition tables using a computerised procedure. Urinary concentrations of potassium, sodium, calcium, phosphate, oxalate, urate, citrate, magnesium, and creatinine, together with the pH and urinary volumes, were measured.
Results: The mean carbohydrate intake was significantly higher in SA-B (293 + 90 g/day) than in both SA-W (194 + 74, P = 0.002) and IT-W (212 ± 81; $P = 0.000$). Daily magnesium intake was higher in SA-B (290 + 124 mg/day) than in IT-W (176 + 73 mg/day, $P = 0.002$).
The mean daily urinary excretion of calcium was significantly ($P = 0.029$) lower in SA-B (3.07 ± 1.68 mmol/day) with respect to SA-W (4.65 ± 2.44 mmol/day) and IT-W (4.51 ± 1.89 mmol/day) whereas mean daily urinary excretion of citrate was significantly ($P = 0.012$) higher in SA-B (3.36 ± 1.4 mmol/day) than in SA-W (3.09 ± 1.45 mmol/day) and IT-W (2.36 ± 0.98 mmol/day).
Conclusion: Although the carbohydrate intake and the percent of energy from carbohydrate of black subjects in this study were higher with respect to white controls, we were not able to show any other relevant difference of the known dietary stone risk patterns between black and white subjects.
On the other hand the urinary patterns of black controls seem to be only slightly more favorable in term of risk for stone formation than those of white controls showing a lower calcium excretion and an higher citrate excretion in the urine.

Our result of higher carbohydrate intake in black subjects is counter-intuitive as it suggests a higher risk of stone formation in this group. This puzzling result may have arisen because our subjects were recruited from the urban population rather than from rural areas, suggesting that western diets and lifestyles may ultimately change the stone incidence profile in the black population [2,3].

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OP-006 Could the intervention of an Okinawan vegetable-based diet change urinary stone-risk parameters in healthy subjects in Okinawa?

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Objectives: A cross-over prospective study was conducted to compare the effects of dietary intervention with Okinawan vegetable-based diet versus Western meat-based diet on urinary parameters related to the risk of stone formation in healthy subjects in Okinawa.
Methods: A total of 126 [48 men (age: 49.26–6.95 years) and 78 women (age: 49.15–7.40 years)] volunteers in Okinawa were randomly assigned to Group A and Group B and 24-h baseline urine samples were collected from all subjects. Those in Group A received Okinawan diet by home-parcel delivery for 1 month followed by Western diet for another month; while those in Group B received Western diet for 1 month followed by Okinawan diet for another month. Twenty-4-h urine samples were also collected at every month after each dietary regimen, respectively. Urine volume, pH, urinary excretions of oxalate, glycolate, citrate, uric acid, sulfate, phosphate, calcium, magnesium, sodium, and potassium were determined. Relative supersaturation of calcium oxalate [SS(CaOx)] was calculated using the EQUIL 2 computer program.
Results: Twenty-four-hour urinary excretions of calcium, oxalate, glycolate, and uric acid as well as urinary SS(CaOx) indices were lower than baseline, while urinary excretion of potassium was higher in both groups after the dietary intervention with Okinawan vegetables. The obese subjects (BMI > 25 kg/m^2) had lower pH, higher oxalate, higher calcium, higher uric acid, and higher SS(CaOx) than the non-obese subjects (BMI < 25 kg/m^2).
Conclusion: The dietary intervention for one month with Okinawan vegetables caused minimal changes in urine parameters and relative supersaturation of calcium oxalate. The obese subjects showed lower pH, higher oxalate level, higher calcium level, and higher uric acid level, suggesting some tendency for stone formation. In addition, urinary oxalate level was associated with citrate, 4-pyridoxic acid, glycolate levels as well as BMI but not with ascorbate level. Urinary citrate level was associated with oxalate and glycolate levels, but not with 4-pyridoxic acid level and pH.

OP-007 The effect of magnesium on the calcium binding capacity of different brans under simulated gastrointestinal conditions. In vitro study with ^{45}Ca

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Introduction: Bran as a source of dietary fibre can bind calcium during gastrointestinal transit and thus reduce calcium absorption, urinary excretion and the risk of calcium oxalate stone formation.
Objectives: The present study was performed to investigate calcium binding characteristics of different brans under simulated gastrointestinal pH conditions and to explore the influence of magnesium on calcium binding capacity.
Methods: Portions (1 g) of different brans (rice, rye, soy, fine wheat, coarse wheat, oat) and 50 ml of varying $CaCl_2/MgCl_2$ solutions containing ^{45}Ca were incubated at 37°C at gastric pH (2.2) followed by buffering steps with Tris buffer to pH 6 and pH 8. Calcium binding was calculated on unbound ^{45}Ca, determined with a liquid scintillation counter, referred to a control and the initial calcium concentration. The Ca/Mg ratio of the $CaCl_2/MgCl_2$ solutions varied between 1:1 (12.5 mmol/l Ca/12.5 mmol/l Mg), 1:2, 1:5 and 1:8 (12.5 mmol/l Ca/100.0 mmol/l Mg).
Results: Calcium binding capacity of brans showed a clear pH-dependence. At gastric pH, calcium binding was low. Calcium binding increased in all brans with increasing pH values leading to different capacities at duodenal/jejunal pH 6 or ileal pH 8, respectively. Increasing magnesium proportion of solutions resulted in a

decrease in calcium binding capacity after adjustment to pH 6.0 or 8.0, respectively.
Conclusion: At isomolar concentrations of calcium and magnesium the decrease in calcium binding is about 7–16%. The decrease in calcium binding was highest (45–60%) at a Ca/Mg ratio of 1:8. The results suggest that magnesium has an effect on calcium binding capacities of brans under conditions of isomolar concentration.

OP-008 Randall's plaques, family history of stones and stone event number: are they associated?

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Introduction: A number of observations have recently suggested that Randall's plaque plays a central role in idiopathic calcium oxalate (CaOx) stone pathogenesis.
Objectives: The aim of this study was to evaluate if family history of stones and personal stone history are associated with the presence of interstitial crystalline deposits of calcium-phosphate (CaP) in the papilla, which are precursors of the Randall's plaques.
Methods: Twenty-seven patients affected by renal stones underwent percutaneous nephrolithotomy (20 cases) or ureteroscopy (7 cases), followed by forceps biopsy of renal papillae, aiming to research interstitial Ca deposits. According to finding of these crystalline deposits, patients were stratified into two groups: group A (12 cases) with Randall's plaques; group B (15 cases) with no interstitial Ca deposits. Presence of family history of stones and number of relatives affected by nephrolithiasis as far as number of personal stone events were registered in each patient. Statistical analysis was carried out using Mann–Whitney and Chi-square tests.

Results: The median number of stones treatment procedures was 2 [Interquartile range (IQR) 1–3] in group A: only one treatment was performed in four cases; two in four patients; three in two patients and four in the remaining two cases. Only one previous spontaneous passage of renal stone occurred in one case, while two patients had three stone expulsions and one patient six; the remaining 66.7% patients had no spontaneous stone passage. No family history of stones was reported in 58.3% of cases, while three patients had one relative affected by nephrolithiasis; one patient 2 and one 4 relatives.

The median number of stone treatment procedures was 2 (IQR 1–3) in group B too: one treatment was performed in five cases; two in six patients; four in two patients and six in the remaining two cases. Only one previous spontaneous passage of a renal stone occurred in three cases, while one patient reported two events, one patient 3 and two patients 5 stone expulsions; the remaining 53.3% patients had no spontaneous stone passage. 66.7% of group B patients had no family history of stone disease; two patients had 1 relative affected by renal stone; two patients 2 and the remaining one 3.

Chi square and Mann–Whitney tests did not show any statistically significant association between evidence for the presence of Randall's plaques and family history or number of parents affected by renal stones as far as personal stone experience was concerned.
Conclusion: Our data do not prove significant associations between presence of the Randall's plaques and personal history or family history of stone disease, although patients characterized by interstitial crystalline Ca deposits show a number of affected relatives greater than in group B (41.7 vs. 33.3%).

OP-009 Colonization with *Oxalobacter formigenes* and urinary oxalate excretion

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Introduction: The impact of the colonization of the gut with *Oxalobacter formigenes* (OxF) on urinary oxalate excretion and calcium oxalate stone disease is unclear.
Objectives: To determine the effects of OxF colonization on fecal oxalate, fecal OxF numbers and urinary oxalate, in colonized and non-colonized normal subjects.
Methods: Subjects consumed diets controlled in their contents of oxalate, calcium and other key nutrients in a crossover design. Oxalate in samples was measured by IC and OxF numbers determined by real-time PCR.
Results: In both groups, stool and urinary oxalate content increased as dietary oxalate increased and dietary calcium decreased. Numbers of OxF increased over tenfold as the oxalate content of the diet increased from 50 to 750 mg mg/day and the calcium content decreased from 2,000 to 400 mg/day. Although all individuals showed a dietary response there was over a 100-fold difference between individuals in the levels of OxF sustained by similar diets. Comparisons between colonized and non-colonized individuals indicated that stool oxalate content was 2–120 times higher in non-colonized individuals and urinary oxalate 20–40% higher.
Conclusion: These results suggest that colonization with OxF may reduce the intestinal absorption of oxalate and lower urinary oxalate excretion. This is compatible with a lack of colonization of the gut with OxF being a risk factor for calcium oxalate stone disease.

OP-010 A diet to reduce mild hyperoxaluria in idiopathic calcium oxalate stone formers: a pilot study

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Introduction: Hyperoxaluria is a critical factor for the crystallization of calcium oxalate and the formation of kidney stones. Clinicians routinely recommend a low-oxalate diet to patients with calcium oxalate nephrolithiasis, but none of the diets proposed up to now has been proved efficacious in reducing oxalate excretion.
Objectives: To assess whether a normal-calcium, low-protein, low-salt diet is effective to reduce hyperoxaluria in idiopathic calcium oxalate nephrolithiasis in comparison with traditional low-oxalate diet, routinely recommended by clinicians.
Methods: We treated 56 idiopathic calcium oxalate stone formers presenting with mild hyperoxaluria (>40 mg/day) while on free diet, with a normal-calcium, low-protein, low-salt diet for a period of three months. We compared the results obtained on this diet with a historical control group formed by 20 hyperoxaluric patients treated in the traditional way with a low-oxalate diet.
Results: After 3 months of therapy, mean oxaluria decreased from 50.2 to 35.5 mg/day during the normal-calcium, low-protein, low-salt diet, and from 45.9 to 40.2 mg/day during the traditional diet (adjusted difference between post-treatment means: −7.3 mg/day [95 percent confidence interval: −12.3 to −2.2, $P = 0.005$]).
Conclusion: The results suggest that a normal-calcium, low-protein, low-salt diet can reduce oxalate excretion in hyperoxaluric patients. It encourages the undertaking of a randomized-control study to confer more solid evidence in support of our findings.

PP-012 Anthropometric and urinary risk factors for calcium oxalate kidney stone formation in black and white South African male subjects

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Introduction: Calcium oxalate stones are commonly thought of as a multifactorial disease, involving environmental risk factors as well as diet, gender and race. Several studies have demonstrated that body size is another risk factor and that it is independent of dietary intake [1]. Interestingly, body composition [fat mass and fat free mass (FFM)] has been shown to differ between different racial groups [2]. As is well known, the incidence of urolithiasis in the black population in South Africa is extremely rare, while in the white population it occurs to the same extent as in other western societies [3].
Objectives: The present study was undertaken to investigate the relationship, between some anthropometric, dietary and urinary risk factors for calcium oxalate (CaOx) urolithiasis in black and white South African male subjects.
Methods: Urinary, anthropometric and dietary variables were measured in healthy black ($n = 10$) and white ($n = 12$) males. Diets were assessed using a semi-quantitative food questionnaire and five day food record. Twenty-four urine samples were collected on day 5 and analysed using routine modern laboratory techniques.
Results: Blacks consumed significantly less calcium and protein compared to whites ($P < 0.05$). Urinary calcium and oxalate did not differ between the two groups ($P > 0.05$). FFM and body fat percentages did not differ between blacks and whites ($P > 0.05$). In blacks, body mass index (BMI) and waist to hip ratios showed a significant positive correlation with urinary calcium ($r = 0.79$ and 0.60, respectively, $P < 0.05$). In whites, BMI and urinary calcium had a negative association which was not statistically significant ($P > 0.05$), whereas body fat percentage and waist to hip ratio showed a significant negative relationship with urinary calcium ($r = -0.62$ and 0.68, respectively, $P < 0.05$). Contrary to whites, FFM percentage increased in blacks, as urinary calcium decreased ($P < 0.05$).
Conclusion: Body composition may be an independent risk factor for calcium oxalate urolithiasis through varying calcium excretion levels.

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PP-013 Determinants of 24-h urinary calcium excretion

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Introduction: Urinary calcium losses can lead to reduced bone mass, and higher urinary calcium levels are a major risk factor for kidney stones.
Objectives: To identify determinants of 24-h urinary calcium excretion.
Methods: We conducted a cross-sectional study of 3,193 stone formers (SF) and non-stone formers (NSF) in the Health Professionals Follow-up Study (men), the Nurses' Health Study (older women), and the Nurses' Health Study II (younger women).
Results: Mean urinary calcium was higher in SF than NSF and was 197 mg/day in men, 194 mg/day in older women, and 207 mg/day in younger women. Urinary calcium as a fraction of dietary calcium in SF was 25% in men, 24% in older women, and 24% in younger women, and in NSF was 18% in men, 21% in older women, and 20% in younger women (P values comparing SF and NSF <0.01). After adjustment for 24-h urinary creatinine and other factors, participants in the highest quartiles of urinary magnesium, sodium, sulfate, citrate, phosphorus, and volume excreted 71, 37, 43, 61, 37, and 23 mg/day more urinary calcium, respectively, than participants in the lowest quartiles (P values trend <0.01). Participants in the highest quartile of dietary calcium excreted 10 mg/day more urinary calcium than those in the lowest quartile (P trend <0.01), and participants taking more than 500 mg/d of supplemental calcium had 15 mg/d more urinary calcium than those taking no supplements (P trend <0.01). Caffeine intake and family history of stone disease were positively associated and urinary potassium, thiazide use, history of gout, and age was inversely associated with urinary calcium.
Conclusion: Intestinal absorption of dietary calcium and/or negative calcium balance is greater in SF than NSF. Many factors have greater impact on urinary calcium excretion than calcium intake. Further investigation of factors influencing urinary calcium may lead to new approaches to prevent osteoporosis and calcium kidney stones.

PP-014 Calcium oxalate stone and gout

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Introduction: Gout is well known to be produced by increased uric acid level in blood. The role of uric acid metabolism in calcium oxalate urinary stone disease has been debated in recent times. Even though uric acid stones and gout are known metabolic combinations, there is not much known about the relationship between gout and calcium oxalate stone disease.
Objectives: The objective of this paper is to assess the relationship between gout and calcium oxalate stone formation in humans.
Methods: A total of 48 patients with the combination of gout and calcium oxalate stones were included in the study. The biochemical values of this group were compared with 38 randomly selected uric acid stone patients with gout and 43 patients with gout alone, 100 calcium oxalate stone patients without gout and 30 controls, making a total of 259 patients. Serum was collected from these patients and 24-h urine samples were analysed for various biochemical parameters such as calcium, phosphorus, uric acid, oxalate, citrate and magnesium. ANOVA and Duncan's multiple range test were performed to assess statistical significance of the differences.
Results: The promoters of stone formation namely urine calcium ($P < 0.05$), uric acid ($P < 0.05$), oxalate ($P < 0.05$), serum phosphorus ($P < 0.05$) and uric acid ($P < 0.05$) were significantly different in the various groups. The promoters, namely urine uric acid ($Pp < 0.05$) and serum calcium ($P < 0.05$) were in a significantly higher range in the gouty patients, gouty uric acid stone patients and gouty calcium oxalate stone patients compared to the other groups. Urine uric acid ($P < 0.0001$), oxalate ($P < 0.0001$) and serum phosphorus ($P < 0.05$) were significantly higher in the gouty stone formers compared to the non-gouty patients and controls. The inhibitor, urinary citrate ($P < 0.001$) was significantly lower in the gouty, gouty uric acid and gouty calcium oxalate patients. Serum uric

acid was highest in the stone formers with gout, followed by the calcium oxalate stone patients and controls.
Conclusion: The high values of promoters, namely uric acid and calcium, in gouty stone patients indicate the tendency for lithiasis in these patients. There is probably a correlation between gout and calcium oxalate urinary stone. It is presumed that this mechanism is achieved through uric acid metabolism. The findings point to the summation effect of metabolic changes in development of stone disease.

PP-015 Investigation of the effect of vitamin E (DL-α-tocopheryl acetate) ingestion on plasma antioxidant status in black and white South Africans

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Introduction: Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress [1]. A previous study has shown that supplementation with vitamin E (200 mg/day) in urogenital tuberculosis patients for 60 days ameliorates oxidative stress, a predisposing factor for renal stone formation [2]. We have previously reported different renal handling mechanisms of this nutrient on kidney stone risk factors in South Africa's stone-prone (white) and relatively stone-free (black) population groups [3].
Objectives: The present study was undertaken to investigate the effects of vitamin E ingestion on plasma antioxidant status in the two race groups.
Methods: Healthy black ($n = 5$) and white ($n = 5$) South African males (age 18–30 years) participated in this study. Each subject ingested one vitamin E capsule (400 IU) every day immediately after supper for 60 days, while on a free and unrestricted diet. 24-h food diaries and blood samples were collected on day 0 (baseline) and day 60. Plasma vitamin E levels were measured by HPLC. Data were statistically analysed using repeated measures ANOVA.
Results: At baseline, there were no significant differences in mean plasma vitamin E levels between black and white males ($P > 0.05$). Following supplementation, a favourable increase (from 9.48 ± 1.09 to 15.14 ± 1.83 mg/L) was observed for black males, which approached significance ($P = 0.069$). However, there was a significant and favourable increase (from 10.22 ± 0.77 to 20.72 ± 4.13 mg/L) in white subjects ($P < 0.005$).
Conclusion: Supplementation with vitamin E for 60 days improved the antioxidant status of plasma in both groups but to different degrees. Surprisingly, the effect was greater in the white group. Investigation of lipid peroxidation products in both groups is necessary before any firm conclusions can be drawn regarding the potential role of this mechanism in providing protection against urolithiasis in the black population.

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PP-016 Chemical components of urinary stones according to age and sex of adult patients in northeastern Thailand

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Introduction: Studies in the West have demonstrated an association between stone composition and both age and sex [1,2].
Objectives: Since urinary disease is endemic in the north-eastern region of Thailand [3], we were interested to examined the relationship between stone composition, age and sex, which has never been reported in Thailand.
Methods: A series of 426 urinary stones, 33 from the lower (LUT) and 393 from the upper urinary tract (UUT) surgically removed from adult patients aged over 20 years were used in this study. After washing with distilled water, the stones were cut in half with a fine saw and the resultant "sawdust" analyzed for chemical composition using infrared spectroscopy
Results: Calcium oxalate (CaOx) and uric acid and urate (UA-UR) were the main constituents in LUT stones of males and UA-UR and magnesium ammonium phosphate (MAP) of females. While UA-UR was distributed in all age classes of males, it was only detected in elderly females. In cases of UUT stones, the peak finding for both sexes was for the 50 to 59-year-olds age class. The MAP component was found more commonly in UUT stones of females, particularly in the younger age groups. CaOx and calcium phosphate (CaP) were the main components of UUT stones in both sexes (CaP was slightly more common in females) with the highest proportion in the 30 to 49-year-olds age class, thereafter they declined and were replaced with UA-UR. Although the proportion of LUT stones in our study was small, our findings agree with previous studies on the role of both age and sex in the aetiopathogenesis of urinary stones.
Conclusion: Our results support previous reports on the role of age and sex on the aetiopathogenesis of both LUT and UUT stones, which should be taken into consideration in stone management and intervention.

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PP-017 Pediatric urolithiasis in Armenia, a country in transition

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Introduction: Socio-economic factors play an important role in formation and composition of urinary stones in children [1]. Armenia has undergone considerable economical progress during the last 8 years which could influence the spectrum of pediatric urolithiasis.
Objectives: To analyze in a prospective study current trends in pediatric stone formation and stone composition in Armenia.

Methods: We compared all patients aged 4 months to 15 years with proven urolithiasis admitted in period A (1991–1999) with those seen in period B (2000–2007). Stones were mainly obtained by open surgery (64% in A vs 42% in B) and by ESWL (7% vs 28%). The rest passed spontaneously, and a minority was extracted. Calculi were analyzed by infrared spectroscopy in Bonn (until 1999) and in Yerevan (since 2000). The Arabkir hospital is the only place in Armenia treating pediatric urolithiasis.
Results: We observed 198 patients (69% males) in period A [2] and 94 (66% males) in period B. The incidence of primary (endemic) bladder stones fell considerably from 2.0/year in A ($n = 18$) to 1.1/year. in B ($n = 9$), but the proportion of bladder stones to all urinary stones remained unchanged (9–10%). The incidence of kidney stones fell similarly from 20/year. in A ($n = 180$) to 10.6/year. in B ($n = 85$). The predominant constituent of kidney stones remained calcium oxalate (62% in A vs 66% in B). Infection stones (struvite, calcium carbonate and partly NH_4 urate) decreased to the same extent from 40 in A to 19 in B, but the proportion remained unchanged (20 vs. 22%).
Conclusion: The incidence both of primary bladder stones and of kidney stones, including the infection stones, has decreased by almost one-half. Pediatric urolithiasis in Armenia tends to become closer to the situation in industrialized countries.

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PP-018 Female stone disease: the changing trend

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Introduction: The prevalence of stone disease has been relentlessly increasing in parallel with profound changes in living standards and dietary habits. It has also been reported that there is an appreciable decrease in the male predominance of nephrolithiasis. Studies on biochemical changes relating to the changing gender prevalence of stone disease are scarce.
Objectives: In this paper, the changing trends in the biochemical parameters over the years in males and females have been assessed in the period 1971 to 2008.
Methods: A descriptive clinical prospective study was performed on 8,590 stone patients belonging to both sexes treated at the urinary stone clinic, Trivandrum from 1971 to March 2008. The various metabolic parameters including 24-h urine volume, urine calcium, phosphorus, uric acid, oxalate, magnesium, creatinine and citrate, serum creatinine, calcium, phosphorus, uric acid and magnesium and other calculated parameters like creatinine clearance, tubular reabsorption of phosphate, calcium: magnesium ratio and calcium:oxalate ratio were studied. The incidence of stone disease between the two sexes was plotted. The biochemical values were compared between the males and females.
The recorded data were transformed into nonparametric form and frequencies and cross tabs were obtained. To elucidate the associations and comparisons between different parameters, Chi square (χ^2) test was used as nonparametric test. Multivariate logistic regression analysis was performed to assess the risk factors (Odds ratio) of different factors for each group. The possible causes for the change in incidence of stone disease in the two sexes were elucidated.
Results: Of the 8590 stone patients studied, 12.7% (1,091) were females. There was a definite increase in the incidence of female urolithiasis over the past 37 years ($P < 0.001$). There were significant variations in urine biochemical parameters. There is a definite increase in the excretion of urinary calcium over the years ($P < 0.001$). The excretion rate of oxalate in urine of females also increased steadily over the years ($P < 0.001$). The magnesium in urine of females has fallen over the years ($P < 0.001$). Urinary citric acid has however shown an increase over the years ($P < 0.001$). Urinary excretion of phosphorus ($P < 0.001$) and urinary uric acid ($P < 0.001$) showed a decreasing trend. There was a definite decrease in female patients with hypercalcemia over the years. There was a considerable increase in the percentage of females with a high calcium:magnesium ratio over the years ($P < 0.001$).
Conclusion: The decrease in the excretion rate of magnesium which is inhibitory to stone genesis, together with the increased excretion of calcium and oxalate may have contributed to the increasing incidence of stone disease in females. This might be due to changes in living standards and dietary habits.

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PP-019 Pattern of family history in stone patients

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Introduction: Genetic predisposition to urolithiasis is a much talked about subject. However, in-depth knowledge of the types of family members involved is not forthcoming in most studies. In order to understand the possible pattern of inheritance, a detailed survey is needed.
Objectives: The objective of this paper is to identify the types of family members of proved urinary stone patients who have predisposition to have familial incidence of stone disease.
Methods: The study population consisted of 2,157 patients with proved urinary stone disease interviewed in 2003 2007 in the urinary stone clinic. Patients who reported to have a positive family history were interviewed in further detail to identify the details of familial occurrence. The positive family history was classified as Group 1—First order single (one person in the immediate family—father, mother, siblings or children), Group 2—First order multiple (more than one member in the above group), Group 3—Second order single (one person in the blood relatives in family grandparents, grand children, uncles, aunts, cousins etc) and Group 4—Second order multiple (more than one member in the above group).
Results: Of the 2,157 patients studied, 349 patients gave a positive history of stone disease constituting 16.18%. Of these 321 were males and 28 were females. Subdivision of the family members showed that 282 patients (80.80%) had a single family member with stones and the rest 67 (19.20%) had multiple family members with history of stone disease. Group 1 which constituted one family member in the immediate family had 255 involvements (father, 88; mother, 16; brother, 135; sister, 2; daughter, 4; and son, 10); Group 2 with multiple members in the immediate family constituted 51 relatives; Of these Father and brother combination was the most common with 35

occurrences. Group 3 with one person in the distant relatives in family namely grandparents, grand children, uncles, aunts, cousins etc. constituted 27 occurrences and Group 4 with more than one member in the distant family constituted 16 occurrences.
Conclusion: Single family member involvement was more common than multiple family member involvement. Males predominated. Stone occurrence was more in the immediate family members of the stone patients than in distant relatives. Males predominated over the females in the family. Brothers formed the most common group to be involved with stone disease. Study of stone risk in the family members should be centred on brothers and sons of stone patients.

PP-020 Effects of vitamin B_1 and B_6 depletion on urinary stone-risk parameters in rats

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Objectives: It has previously been reported that vitamin B6 deficiency causes hyperoxaluria, hypocitraturia, and increases the risk of urinary calcium oxalate stone formation. In the present study, we investigated the effects of vitamin B1 depletion with or without vitamin B6 depletion on urinary stone-risk parameters, and relative supersaturation indices of calcium oxalate and uric acid.
Methods: Twenty-four male Wistar rats were divided into four groups of 6 animals each. All animals were acclimatized at the University Animal Center for several days and then they were fed a standard diet (Control group), or a vitamin B1-deficient diet (Vit-B1-def group), or a vitamin B6-deficient diet (Vit-B6-def group), or a combination of vitamin B1- and B6-deficient diet (Vit-B1&B6-def group) for 3 weeks, respectively. Twenty-four-hour urine samples were collected before and after feeding respective diets. Urine volume, pH, creatinine, urinary excretions of oxalate, citrate, uric acid, magnesium, potassium, calcium, and phosphate were determined. Relative supersaturation indices of calcium oxalate and uric acid were calculated by EQUIL 2 computer program.
Results: Urinary citrate, pH, magnesium, and potassium levels were lower than baseline, while urinary uric acid excretion as well as relative supersaturation indices of calcium oxalate and uric acid was higher than baseline after feeding respective diets for 3 weeks in Vit-B1-def, Vit-B6-def, and Vit-B1&B6-def groups.
Conclusion: The results of this study demonstrated that depletion of vitamin B1 and/or vitamin B6 increases the relative supersaturation indices of calcium oxalate and uric acid, and thereby, may increase the risk of urinary calcium oxalate and uric acid stone formation.

PP-021 Oxalate content of green tea

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Introduction: A high dietary oxalate intake may decrease the bioavailability of minerals and trace elements essential for human health and lead to calcium oxalate stone formation in the urinary tract. Due to its aroma and stimulation effect, green tea (*Camellia sinensis*) is one of the most widely consumed beverages worldwide. Many investigations deal with the health benefits of green tea consumption.
Objectives: The purpose of the present study was to analyze the oxalate content of green tea depending on origin, quality, time of harvest and preparation.
Methods: The oxalate content of 48 samples was measured using a validated HPLC-enzyme-reactor method. The samples were collected, for the most part, in 4 regions of China: Zhe Jiang, Guang Xi, Yun Nan and Jiang Su. The samples were prepared by steeping 3.5 g leaves in 200 ml 90°C water for 10 min.
Results: The oxalate content of green tea ranged from 8.3 to 139.8 mg/l. In samples from known provenances, the highest oxalate concentration was found in green tea from Zhe Jiang. Specified quality had only a slight influence on oxalate concentration. The low grade quality showed a tendency to lower oxalate concentration. Leaves reaped in the autumn when grown to full size yielded more oxalate than small and young leaves reaped in the spring. Modifications in steeping duration of tea leaves had no significant influence on the oxalate content of the beverage.
Conclusion: Patients at risk for recurrent stone formation should take into account the oxalate content of green tea.

PP-022 Recurrent urolithiasis: an innovative approach using participative instruction to prevent stone recurrence. Evaluation by the patients

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Introduction: Theoretically, patients suffering from recurrent urolithiasis have been told the importance of dietetics to prevent continued stone recurrence, which may lead potentially to chronic renal failure. However, most of them form recurrent stones. We developed an innovative care program for these patients based on a multidisciplinary approach using participative instruction to make them sensitive to what they can do practically to prevent stones recurrence, whatever the etiology of the urolithiasis. The aim of this study was to evaluate the perceived impact of this approach by the patients.
Objectives: Since June 2006, 189 have been seen in this multidisciplinary program divided in two parts: the first in the morning with the groups of 5 patients attending the presentations of the nurse, the nephrologist, the biochemist and the dietician about the global objectives, written in a special personal file. After the meal taken together with care-givers, the second part consisted of individual consultations with the same health workers plus the urologist and the psychosomatic therapist. A questionnaire of 10 semi-quantitative questions evaluating the usefulness and the satisfaction of the patients was provided at the end of the day.
Methods: From this cohort of patients, 92% answered the questionnaire: 91% found the concept of this approach very interesting or interesting; 89% found that the day was very useful or useful to reduce the recurrence of urolithiasis. This was due to a better understanding of the factors that lead to stone formation and to logical objectives to minimize stone recurrence. The received information about the important objectives to prevent recurrence was very good or good in 97% of patients. The personal approach was found to help them reach their objectives in 80% of cases.
Results: A majority of patients with recurrent stones expressed satisfaction with this new multidisciplinary approach using participative instruction. They reported that prior to this session, the importance of this information had not been included in the therapeutic approach to reduce urolithiasis recurrence. Most of the patients needed to write additional information in addition to the written questions and semi-quantitative answers. They expressed their thanks for this multidisciplinary approach, informing us that they had learned a lot about urolithiasis. Many of them explained the positive effect of the group to understand their pathology. A large percentage underlined the positive contribution made by the associated psychosomatic therapist.

Conclusion: In conclusion, this new multidisciplinary participative approach could be interesting to reduce urolithiasis recurrence. A study of the impact of this new approach is still going on.

OP-011 Urate urolithiasis in cats

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Introduction: Urate uroliths have been reported to account for 5.6–9% of uroliths from cats. An association between Siamese cats and urate uroliths has been previously reported, yet there has been only superficial evaluation of breed associations and other risk factors for urate urolithiasis in cats.
Objectives: The objective of this study was to describe the prevalence of urate urolithiasis in cats and evaluate possible breed associations.
Methods: Data from feline uroliths submitted to the Canadian Veterinary Urolith Centre between February 2, 1998 and July 7, 2007 were evaluated. The prevalence of urate urolithiasis was described. Categorical comparisons were performed using chi-square test. Odds ratios and 95% confidence intervals were calculated. Wilcoxon test was used to evaluate the association between age and urate urolithiasis.
Results: 12378 uroliths were examined during the study period. Urate uroliths were identified in 473 (3.8%) cases, including 436 ammonium urate, 23 mixed struvite/urate and 14 uric acid. Among the urate stone formers, 279 (59%) were male and 194 (41%) were female ($P = 0.96$). The prevalence of urate urolithiasis was highest in Egyptian Maus at 80% (16 urate/20 total urolith submissions). This breed had a significantly higher prevalence of urate urolithiasis compared to all other breeds combined ($P < 0.0001$, OR 104, 95% CI 34.7–313). There was also a significantly higher prevalence of urate urolithiasis in Birman cats, where this stone type accounted for 3/13 (23.1%) submitted uroliths ($P = 0.004$, OR 7.6, 95% CI 2.1 – 27.7). Siamese were similarly overrepresented, with urate uroliths accounting for 30/209 (12.6%) submissions ($P < 0.0001$, OR 3.8, 95% CI 2.6 – 5.6). There was not a significant association between urate urolithiasis and any other breed. There was an association between urate urolithiasis and age ($P = 0.0013$). The mean age of cats with urate urolithiasis was 6.2 + 0.2 years (mean + SEM) compared to 6.7 + 0.04 years for non-urate stone formers. Egyptian Maus were significantly younger compared to other breeds combined among all stone formers (5.0 + 0.6 vs. 6.7 + 0.3 y, $P = 0.04$) and among urate stone formers (4.3 + 0.9 vs. 6.2 + 0.2y, $P = 0.034$). Siamese cats were not significantly younger than other cats when all urolith types were included ($P = 0.77$) but were significantly younger than non-Siamese urate stone formers (4.8 + 0.6 vs. 6.3 + 0.2 y, $P = 0.018$).
References: The association between Egyptian Mau cats and urate urolithiasis was astounding. The association of urate urolithiasis and Siamese cats is consistent with a previous report and confirms the predisposition in this breed, while the association with Birman cats was unexpected and requires further study. The significant younger age of urate stone formers overall, and Egyptian Maus in particular, is consistent with a genetic predisposition yet such data must be interpreted with caution. In dogs, male Dalmatians are at particularly high risk for urate urolithiasis because of a change in purine metabolism that leads to excretion of urate. Such a defect has not reported in cats, nor are cats with urate uroliths typically identified with potentially predisposing co-morbidities such as portosystemic shunt and hepatopathies. Therefore, the pathophysiology for urate stone formation in cats is unclear and requires further study. Studies of high risk breeds, particularly the Egyptian Mau, are indicated to determine the reason for the association between breed and urate urolithiasis.

OP-012 Struvite relative supersaturation: a good predictor of struvite stones dissolution in vitro

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Introduction: Relative supersaturation (RSS) is a method that allows to measure the potential for a urine to dissolve or form crystals and that has been validated in cats [1].
Objectives: The aim of this study was to assess if struvite RSS is a good predictor of in vitro struvite dissolution kinetic in cat urine.
Methods: Two different commercial complete dry expanded diets with 2 levels of Na content, designed to dissolve struvite uroliths were selected: diet A [2] (1.3% Na as fed) and diet B [3] (0.4% Na). Those diets were fed successively to seven Chartreux cats for 2 weeks. Urinary volume, pH, specific gravity and concentrations of 10 solutes (Ca, Mg, Na, K, NH_4^+, phosphate, citrate, sulfate, oxalate, uric acid) were measured on the pooled urine collected during the last 7 days of each study period. Based on those data, the urinary relative supersaturation (RSS) for struvite (MAP) was calculated using SUPERSAT™ [1]. Each pooled urine was divided in aliquots based on mean urinary volume produced daily per cats. The aliquots were placed in bottles and stored at −20°C pending the test. Two groups of 2 feline struvite stones (218 ± 0 mg) homogeneous in source, shape and weight were selected. On day 0, a bottle of each urine was defrosted, struvite stones were added. The bottles were then placed in a water bath at 38°C during 24 h. After that, the urines were filtered to collect the stones. The stones were lightly dried on an absorbent paper and weighed, and the process restarted until complete dissolution of the stones.
Results: During the study, cats remained healthy, maintained their body weight and consumed their diet adequately. The Table summarizes the results.

	Diet A	Diet B
Mean urine volume (mL/cat/day)	97.2	55.7
Urinary pH	6.1	6.1
Specific gravity	1.047	1.068
MAP RSS	0.2	0.4
Number of days for complete dissolution	23	34
Dissolution kinetic (mg/day)	10.0	6.8

Conclusion: When RSS is below 1 (undersaturation zone), urine dissolves struvite stones efficiently and the lower the RSS, the quicker the dissolution kinetic. This observation shows that RSS is thus a good predictor of urine potential to dissolve struvite stones. Diet B has also been shown to induce struvite dissolution in vivo. This in vitro model might thus be a good way to assess efficacy in vivo.

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OP-013 Evaluation of trends in canine urolithiasis: 1985–2006

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Introduction: Urolithiasis is a common and recurrent problem in dogs, and literature supports trend changes for struvite and calcium oxalate [1] but does not address other minerals and risk factors that occur.
Objectives: To characterize the types of calculi, age, breed, gender distribution, and other risk factors for dogs with urolithiasis. The Cochran-Armitage test was used to evaluate significance in trends. $P < 0.05$ was considered significant.
Methods: A retrospective review of 25,481 canine uroliths submitted to the Stone Lab between January 1985–December 2006 were included.
Results: Documented mineral types were: struvite, CaOx, urate, apatite, brushite, cystine, silica, potassium magnesium pyrophosphate, sulfa drug, xanthine, and newberyite. Calculi were located throughout the urinary tract, but struvite was the stone type most commonly voided in the urine. A strong inverse linear relationship between CaOx and struvite stones was noted ($R = 0.94$, $P < 0.0001$). Significant decreases in silica, cystine and urate were noted ($P < 0.007$) but these trends were not linear. No significant trend changes were noted for brushite or apatite. Struvite occurred significantly more often in younger dogs ($P < 0.0001$); 56% of the struvite stones were from dogs <6 whereas only 25% of CaOx stones were submitted from this age group. Urate and Cystine calculi occurred in dogs <6 years of age >50% of the time.
Conclusion: As was previously published, CaOx calculi continue to increase in dogs which is in contrast to a recent report in cats [2]. Monitoring changes in trends and risk factors for canine urolithiasis can be important in developing better management strategies.

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OP-014 Is the lack of oxalate degrading bacteria a risk factor for calcium oxalate urolith formation in dogs?

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Introduction: Calcium oxalate (CaOx) is the most common urinary stone in dogs and its prevalence has significantly increased over the last two decades. Supersaturation of calcium and oxalate in urine promotes formation of uroliths. Therefore, reducing urine concentrations of these stone components are essential to prevent CaOx precipitation. Enteric colonization of oxalate degrading bacteria (ODB) is correlated with the absence of hyperoxaluria and/or CaOx formation in humans and rats [2, 6, 8, 10]. Several ODB have been reported in mammals [1–5, 11] but only *Oxalobacter formigenes* depends on oxalate for its sole energy needs. Therefore, *Oxalobacter formigenes* is an efficient oxalate degrader in the GI tract. Oxalobacter formigenes has two enzymes, formyl CoA transferase and oxalyl CoA decarboxylase that metabolize oxalic acid into formate and CO_2 [7, 9]. The role of *Oxalobacter formigenes* in dogs with CaOx urolithiasis is unknown. We hypothesize that decreased colonization of the intestine with ODB is a risk factor for canine CaOx urolith formation.
Methods: We evaluated the presence of *Oxalobacter formigenes* in canine feces by PCR to detect a fragment of the oxalyl CoA decarboxylase (oxc) gene of *O. formigenes*. Feces from healthy ($n = 8$) and CaOx ($n = 10$) dogs were screened by PCR.
Results: Seven of nine healthy dog samples, but only two of ten diseased dog samples, were positive for oxc gene of *O. formigenes*. These findings indicate that the O. formigenes gene relevant to oxalate degradation is present more frequently in healthy dogs compared to the stone-forming dogs. Future studies will focus on determining the sources of oxalate degrading activity in feces from healthy and stone-forming dogs.

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OP-015 A mutation in a novel urate transporter is responsible for hyperuricosuria and the predisposition to urate urolithiasis in dogs

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Introduction: Dalmatian dogs are predisposed to urate urolithiasis due to a change in purine metabolism which results in the excess production and excretion of uric acid in their urine [1]. They also have relative hyperuricaemia from the same etiology. It has long been postulated that the defect in Dalmatians is in the transport of urate into the liver and kidney [2, 3]. Dalmatian hyperuricosuria and hyperuricemia (huu) is inherited as a simple autosomal recessive trait; however it is fixed within the Dalmatian breed [4, 5].

Results: In order to identify the gene responsible for this change in purine metabolism, we used a Dalmatian X Pointer backcross that segregates the high and low uric acid phenotype [6]. The locus was successfully mapped to a position on CFA03 with a LOD score of 17.45. Using a combination of fine structure mapping techniques based on informative backcross recombinants and homozygosity within the Dalmatian breed, the critical interval was narrowed to ~500 kb containing four candidate genes. These four genes were sequenced and their expression levels were determined in both liver and kidney from affected and unaffected dogs. No expression differences were identified; however, a missense mutation was identified in one of the candidate genes. The mutation segregates with the phenotype not only in the backcross dogs but also in other breeds with hyperuricosuria. The gene responsible has been associated with uric acid levels in multiple independent cohorts in people [7–9]. It has recently been identified as a urate transporter although causative mutations have not been identified in people [10].

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OP-016 The role of "Metabolic Syndrome" in the formation of uric acid-containing stones

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Objectives: The aim of the study was to investigate the role of "Metabolic Syndrome" in the formation of idiopathic uric acid-containing stones.

Methods: A group of 759 stone-formers attending Stone Clinics at UCH and RFH over the past 11 years were studied using a comprehensive stone screening procedure. The study involved analysis of a fasting blood and spot urine sample, a 7-day Diet Diary recorded on the patient's free, home diet, two 24-h urines collected on the final two days of the Diary, a detailed patient history and quantitative stone analysis. The Potential Renal Acid Load (PRAL) for each patient was calculated from the composition of his/her diet [1] and from this the estimated net acid excretion (NAE) calculated [1]. The predicted urinary pH was then interpolated from the relationship between urinary pH and NAE [1] and compared with the value measured in the fresh 24-h urine sample from the same patient.

Results: From the Stone Screen, the patients were classified into four groups: (a) idiopathic Ca stones ($n = 631$), (b) uric acid-containing stones ($n = 103$), (c) infected stones (n = 16), (d) stones due to distal renal tubular acidosis (dRTA) ($n = 5$) and (e) stones as a result of an ileostomy (4).

Within the idiopathic Ca stone-formers, the predicted urine pH was almost identical to the measured pH between pH 6.3 and 7.5. Below pH 6.3, the patients passed urines that were increasingly more acidic than those anticipated from the composition of their diets. Furthermore, below pH 6.3, the patients became increasingly obese and more hypertensive than the idiopathic patients whose urine pH values

Table 1 Comparison of subjects with type 2 diabetes versus controls (mean ± SD)

Parameter (units)	Type 2 diabetes (N = 10)	Non-diabetic controls (N = 16)	*P*-value type 2 diabetes vs. controls
Age (years)	54 ± 10	52 ± 10	0.49
Body Mass Index (kg/m^2)	31 ± 3	30 ± 3	0.29
24-h Urine Potassium (mEq/day)	36 ± 10	32 ± 11	0.28
24-h Urine Sulfate (mEq/day)	40 ± 10	38 ± 6	0.38
24-h Urine Phosphorus (mg/day)	569 ± 243	526 ± 156	0.44
24-h Urine NAE (mEq/day)	67 ± 14	48 ± 14	$P < 0.001$
24-h Urine Uric Acid (mg/day)	498 ± 179	519 ± 132	0.64
24-h Urine pH	5.41 ± 0.28	5.90 ± 0.42	$P < 0.001$
24-h Urine Undissociated Uric Acid (mg/day)	229 ± 86	126 ± 76	$P < 0.001$
Urine Ammonium/NAE	0.67 ± 0.19	0.90 ± 0.23	$P = 0.003$

were >6.3, and this trend was accentuated the greater the difference between the predicted and measured urinary pH. Below pH 5.6, uric acid progressively became the predominant constituent of the patients' stones. Overall, the uric acid stone-formers were more obese (87% with a BMI > 25) and had more type 2 diabetes (21%) and hypertension (34%) [ie so-called "Metabolic Syndrome"] than the idiopathic Ca stone-formers (56% with a BMI > 25, 4% with type 2 diabetes and 15% with hypertension).

At the other end of the pH scale, patients with infection stones or with dRTA, excreted urines that were more alkaline than that expected from the composition of their diets, as would be anticipated. A further study on 138 patients showed that the patients with "Metabolic Syndrome" had a defect in the renal production of ammonia, which caused their urine to be more acidic than would be expected under conditions of normal ammoniagenesis.

Conclusion: Comparison of predicted 24-h urinary pH with measured 24-h urinary pH is helpful in the identification of patients with dRTA, urinary tract infection and so-called "Metabolic Syndrome" involving obesity, hypertension and insulin-resistance amongst the stone-forming population. This study also supports the reported association between uric acid stone-formation and type 2 diabetes [2], possibly arising from a defect in the renal production of ammonia.

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OP-017 Type 2 diabetes and the risk of uric acid nephrolithiasis

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Introduction: Individuals with type 2 diabetes mellitus (T2DM) are at increased propensity for uric acid nephrolithiasis due to an unduly acidic urine. It is not clear if the overly acidic urine in T2DM is due to dietary factors, obesity, or the older age of diabetic stone formers.

Objectives: The objective of this study was to assess the metabolic features that place individuals with T2DM at increased risk for uric acid nephrolithiasis while controlling for dietary factors, age and body mass index (BMI).

Methods: A total of 10 non-stone forming patients with T2DM and 16 age- and BMI-matched non-stone forming volunteers without T2DM (controls) were recruited for this study. None of the subjects was taking medications known to alter urinary stone risk parameters (such as diuretics, allopurinol, or alkali therapy). Subjects were placed on the same constant metabolic diet with fixed acid ash content for 6 days, and 24-h urine was collected on the last 2 days. Net acid excretion (NAE) was calculated as urine [(ammonium + titratable acidity) — (bicarbonate + citrate), all in mEq]. Urinary parameters were compared between the two groups, with data shown as mean ± standard deviation.

Results: The 24-h urine sodium, potassium (a marker of dietary alkali), phosphorus, and sulfate (marker of dietary acid) were similar between the two groups of subjects, indicating good dietary compliance (Table 1). Urinary uric acid was not different between the two groups (519 ± 132 mg/day for controls vs. 498 ± 179 mg/day in T2DM, $P = 0.64$); 24-hr urine pH was significantly lower in T2DM (5.41 ± 0.28 vs. 5.90 ± 0.42 in controls, $P < 0.001$), leading to higher undissociated urinary uric acid content (229 ± 86 mg/day vs. 126 ± 76 mg/day in controls). Urine NAE was significantly higher in T2DM than controls (67 ± 14 vs. 48 ± 14 mEq/day, $P < 0.001$). Urine ammonium/NAE, a marker of renal ammonium excretion, was significantly lower in T2DM suggesting impaired urinary buffering in this group of subjects.

Conclusion: Patients with T2DM are at increased risk for uric acid nephrolithiasis because of an unduly acidic urine and impaired urinary buffering that are independent of dietary factors, obesity and age.

OP-018 Impact of body weight on metabolic diagnosis in stone formers

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Introduction: Some of the metabolic abnormalities that favour stone formation have a strong dependence of environmental and nutritional factors. Overweight and obesity are associated with an increased risk of nephrolithiasis. The increased prevalence of kidney stones around the world is also parallel to the growing rate of obesity.

Objectives: The aim of the study was to assess if patients with overweight /obesity differed in their metabolic abnormalities from patients with normal BMI.

Methods: We evaluated 817 renal stone formers (459 men and 358 Woman) in an outpatient setting with a standard protocol (two 24 h urine collections and serum parameters). Patients were classified according to their BMI in normal (BMI 18.5–24.9) overweight (BMI 25–29.9) and obese (BMI > 30). Cystinuria and primary hyperparathyroidism diagnoses were excluded from the study.

Results: Women < 50 years, 67.6% had normal BMI, 18.4% were overweight and 14% obese, while women >50 years 37% had normal weight, 36.2% were overweight and 26.8% obese In all of them hypercalciuria was the most prevalent diagnosis. Hyperuricosuria increased significantly its prevalence only in overweight and obese women < 50 years old, from 4.7% (normal BMI) to 17 and 22%, respectively ($P < 0.01$). Men < 50 years, 35.7% had normal BMI, 48.1% were overweight and 16.2% obese, whereas men > 50 years 11.9% had normal BMI, 58.9% were overweight and 29.2% obese. Hypercalciuria was the most prevalent diagnosis in normal weight men < 50 years of age, whereas Gouty Diathesis was the most frequent one in the oldest normal weight men. Hyperuricosuria was the most frequent abnormality in overweight and obese men and increased significantly its prevalence from normal to obese men in both groups of ages while Hypercalciuria was second in frequency in overweight and obese men also in both groups of ages. The association of hypercalciuria with hyperuricosuria was the only combination that increased in frequency in both sexes with greater BMI. Logistic regression analysis also showed that Hyperoxaluria and Gouty Diathesis were significantly more prevalent in males.

Conclusion: The only abnormality that increased in prevalence with increasing BMI in both sexes was hyperuricosuria. In men, this increase was independent of age, whereas in women, it occurred only in those <50 years old. This was the most prevalent abnormality seen in overweight and obese men. Hypercalciuria with hyperuricosuria was the only combination that increased significantly in both sexes with increasing BMI. Idiopathic hypercalciuria was the most frequent abnormality in women independently of their weight and age.

OP-019 Parameters of metabolic syndrome in uric acid and calcium oxalate stone formers

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Introduction: Previous investigations showed that the metabolic syndrome (MS) as an expression of insulin resistance plays a major role in the pathogenesis of uric acid stones. This is in correspondence to the characteristics found in uric acid stone formers (UASF). Obesity, for example, is seen, however, also in many calcium oxalate stone formers (CaOxSF) and has been described as a risk factor.
Objectives: Therefore we compared parameters of MS and metabolic risk factors in UASF and CaOxSF to see whether MS influences the risk for stone formation in a special way.
Methods: We studied $n = 100$ consecutive CaOxSF and $n = 50$ UASF treated in our hospital. The following parameters were examined: Age, sex, stone frequency, stone analysis, arterial blood pressure, diabetes mellitus, serum levels of creatinine, calcium and uric acid and 24 h-urine parameters (volume, pH, calcium, uric acid, citrate, urea, and ammonia). For statistical analysis Gaussian distribution and equal variance were checked. Significance was analyzed by Student's *t* test or Mann–Whitney test, respectively.
Results: BMI (27.7 vs. 25.3) and urinary calcium (7.3 vs. 4.2 mmol/day) were significantly higher in CaOxSF, systolic blood pressure (161 vs. 148 mmHg) and serum uric acid (6.3 vs. 5.3 mg/dl) in UASF.

All the other parameters examined were not significantly different.
Conclusion: We could demonstrate that parameters of MS are equally prevalent in CaOxSF and UASF. MS is not a characteristic finding only in UASF. There was no clear relation of these parameters to the special pathogenesis of uric acid lithiasis. Especially urinary pH was not significantly different between CaOxSF and UASF. MS seems to be a general finding in stone patients and cannot be related to a special stone composition.

OP-020 Obesity in children: is there any risk for increased urinary stone forming risk factors?

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Objectives: To evaluate the possible effect of overweight on the urinary stone forming risk factors in children.
Methods: A total of 64 children were included into the study programme. Following a detailed stone disease history, systolic and diastolic blood pressures were precisely measured and recorded in all patients BMI, 24-h urine, and serum stone forming risk parameters were also evaluated in overweight (n:40; 16 boy, 24 girls: Group I) and normal cases (n:24; 10 boy, 14 girls, Group II) (38 girls and 26 boys, M:F 1:1.4) without any medical or dietetic pretreatment.
Results: Evaluation of the stone forming risk factors in both groups has revealed the obesity problem to increase the excretion of these substances in Group I. The majority of the children in this group demonstrated hypocitraturia 18/40 (45.0%) when compared with the ones in Group II ($P < 0.001$). While mean urinary citrate level was 343.4 ± 124.8 mg/24 h in Group I, this value was noted as 486.6 ± 143.59 mg/24 h in Group II ($P < 0.001$). Similarly children in the first group did have elevated mean urinary oxalate and calcium excretion together with lower magnesium excretion when compared with the other ones.
Conclusion: Our results clearly show that, overweight status may be associated with an elevated risk of stone formation in both genders of children possibly due to the alterations in urine composition. Children suffering from overweight could be more prone to stone formation and they should be carefully evaluated and followed from this point of view.

PP-023 Extracorporeal shock-wave lithotripsy for the treatment of urocystoliths in dogs

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Introduction: We have recently reported cystoscopic guided laser lithotripsy for fragmentation and removal of uroliths as an alternative to surgery for removal of lower urinary tract stones in dogs. Cystoscopic guided laser lithotripsy has limited success in smaller male dogs because of the difficulty in transurethral passage of flexible ureteroscopes in male dogs weighing less than 5 kg.
Objectives: The objectives of this study were to describe fragmentation of urocystoliths by use of extracorporeal shockwave lithotripsy (ESWL), to determine success rates for stone fragmentation and removal, and to describe procedural complications in a series of dogs.
Methods: Dogs treated for urocystoliths by use of ESWL at the Purdue University Veterinary Teaching Hospital from September 1, 2005 to March 31, 2007 were included in this study. ESWL was performed with a first generation Dornier HM-3 Lithotripter. A median of 2,000 shockwaves generated at 18 kV (range 1,000–2,200 shockwaves generated at 17–19 kV) was delivered to the urinary bladder in order to fragment the uroliths. Biplanar fluoroscopy was used to target the uroliths within the urinary bladder. The uroliths were considered effectively fragmented when they were no longer visible on fluoroscopy. Small urolith fragments remaining in the bladder after ESWL were removed by use of basket extraction or voiding urohydropropulsion.
ESWL fragmentation of cystic uroliths was performed in 16 dogs (ten males and six females). The median body weight for all dogs was 4.1 kg and seven of ten male dogs weighed 5 kg or less. The majority of dogs (13/16; 81%) had nephroliths and/or ureteroliths in addition to their urocystoliths. Upper and lower tract uroliths were treated by use of ESWL during a single anesthetic episode. The remaining three dogs only had urocystoliths. These three male dogs weighed less than 5 kg, prohibiting transurethral cystoscopy.
Results: ESWL resulted in partial fragmentation of urocystoliths in all cases. Effective urolith fragmentation with successful removal of all urocystolith fragments by basket extraction and/or voiding urohydropropulsion was achieved in 5/6 female dogs (83%) and 9/10 male dogs (90%). In one male dog and one female dog, the stone fragments after ESWL were too large to remove through the urethra by basket extraction. In these two dogs, the remaining stone fragments were fragmented by laser lithotripsy and successfully retrieved via basket extraction and voiding urohydropropulsion.
Short-term complications related to ESWL occurred in one male and one female dog. Urethral obstruction secondary to intravesicular hemorrhage and clot formation occurred in one female dog, necessitating placement of a urinary catheter for 48 h. In one male dog, a urinary tract infection resulted from liberation of bacteria during urolith fragmentation. There were no long-term complications.
Conclusion: Extracorporeal shockwave lithotripsy successfully fragmented urocystoliths into fragments small enough to remove through the urethra in 14 of 16 dogs. This treatment modality is an appropriate non-invasive alternative to surgery in small dogs that are too small for transurethral cystoscopy. Additionally, dogs with nephroliths or ureteroliths and concurrent urocystoliths may be effectively treated by ESWL of their upper and lower tract uroliths in the same anesthetic episode.

PP-024 Factors associated with suture-nidus urolith submissions from 163 dogs and 13 cats submitted to the Canadian Veterinary Urolith Centre from 1999 to 2006

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Objectives: To characterize suture-associated uroliths and compare historical data accompanying these uroliths to non-suture-associated uroliths.
Methods: Design—Retrospective case control study.
Animals—163 dogs and 13 cats with uroliths associated, or apparently associated, with suture material and submitted to the Canadian Veterinary Urolith Centre (CVUC) were included. Controls consisted of 326 canines and 26 felines from the population of recurrent non-suture associated uroliths submitted closest in time to the sample case.

Procedures—Submission records from November 12, 1999 to October 5, 2006 were reviewed. Associations between urolith composition, recurrence times, and gender, age and breed predisposition were evaluated.
Results: The cases consisted of 92 dogs and 7 cats in the suture visible nidus group and 71 dogs and 6 cats in the suture dissolved nidus group. Suture-associated uroliths represented 0.6% of canine urolith submissions, and 9.4% of recurrent canine uroliths; 0.17% of feline submissions and 4% of recurrent feline uroliths. Males were significantly more likely to form suture-associated uroliths than controls. Shih Tzus, Lhasa Apsos and Pomeranians were significantly predisposed to forming suture associated uroliths. Compound suture-associated uroliths were significantly more likely compared to other urolith types (OR, 8.6). Dogs with suture-associated uroliths had significantly faster recurrence times ($P = 0.011$) than control dogs.
Conclusion: Suture remnants in the bladder play a significant role in recurrent urolithiasis in dogs. Identification of risk factors is important to avoiding iatrogenic urolith recurrence.

PP-025 Atorvastatin inhibits calcium oxalate urolithiasis formation

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Introduction: The interactions between crystals and renal tubular cells are important factors in urolithiasis formation. Moreover, some reports have suggested the involvement of renal tubular cell injury in crystal-cell interaction processes. Atorvastatin, which is a competitive inhibitor of statins 3-hydroxy-3-methylglutaryl coenzyme A, is prescribed to reduce high cholesterol levels and has anti-inflammation and anti-oxidization activities. Atorvastatin is also reported to control the expression of transforming growth factor-fÀ1.
Objectives: We investigated whether atorvastatin can prevent renal tubular cell injury by oxalate and inhibit calcium oxalate urolithiasis formation.
Methods: Ten-week-old, specific pathogen-free, male Sprague–Dawley rats were used.
Atorvastatin (2 mg/kg.) in 0.5% carboxymethyl cellulose was administered orally daily for 2 weeks. The rats were separated into four experimental groups (A, B, C, D), group A, given water and 0.5% carboxymethyl cellulose every day, group B, given water and atorvastatin in 0.5% carboxymethyl cellulose every day, group C, given 1% ethylene glycol dissolved in water and 0.5% carboxymethyl cellulose every day, and group D, given 1% ethylene glycol dissolved in water and atorvastatin in 0.5% carboxymethyl cellulose every day. The ethylene glycol model of hyperoxaluria and the effect of atorvastatin treatment (groups A, B, C, and D) were analyzed. Urine samples were collected every 24-h in metabolic cages, and were analyzed immediately or stored at −70°C until analysis. The rats were sacrificed after 2 weeks, and the kidneys were removed for further examination. We measured urinary *N*-acetyl glycosaminidase (NAG) levels as a biomarker of renal tubular cell injury and urinary 8-hydroxy-2-f-deoxyguanosine (8-OHdG) as a biomarker of oxidative stress in the 24-h urine samples. The removed kidneys were used for quantitative analysis of superoxide dismutase (SOD) activity and detection of apoptosis. And we measured the amounts of calcium oxalate crystal deposits in renal tubular cells. Finally, nox 1 mRNA, the subunit of NADPH oxidase system, was measured by quantitative RT-PCR.
Results: Urinary NAG and 8-OHdG levels were reduced significantly by atorvastatin treatment in this stone-forming rat model. Atorvastatin treatment increased SOD activity and inhibited the degree of apoptosis of renal tubular cells compared with the stone-forming control group (group C). A decrease in calcium oxalate crystal formation was recognized in the evaluation of excised kidneys following atorvastatin treatment. And atorvastatin treatment decreased the expression of nox 1 mRNA.
Conclusion: Atorvastatin was found to have inhibitory effects on renal tubular cell injury and oxidative stress caused by oxalate and calcium oxalate crystals. Atorvastatin inhibited calcium oxalate urolithiasis formation. It was speculated that the mechanism was to inhibit NADPH oxidase. We believe that atorvastatin could helpful in the prevention and treatment of calcium oxalate urolithiasis.

PP-026 Stone composition of urinary stones of domestic and zoo animals

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Introduction: The nature of urinary stone components is roughly the same in humans and animals. The stone components which occur in animals are also found in the analysis of human stones.
However, the frequency of the different components is not in accordance with animal and human stones. Compared to each other, dogs and cats have different distribution pattern of stone components. Compared to human urinary stones also significantly different abundance pattern occur.
Objectives: The purpose of this study is to conduct a survey of the stone composition of dogs, cats and other pets and as well of zoo animals. The frequency of stone minerals in the several groups is discussed.
Methods: The stones sent for analysis to our laboratory were investigated by a combination of polarization microscopy, X-ray diffraction and infrared spectroscopy. A total of 145 urinary stones of animals were analysed. 69 stones were from dogs, 51 stones from cats, 6 stones from other pets and 19 stones from zoo animals.
Results: The table represents the abundances of the several stone components (in%). The evaluation was performed separately for the four animal groups. Struvite is the most frequent component in canine stones. The high frequency of ammonium hydrogen urate and cystine is also remarkable. In cats, whewellite, weddellite and struvite were the most frequent stone minerals. In the group "other pets" stone material of rabbits, hares and guinea-pigs were analysed; calcite and apatite have exclusively been found. The group "zoo animals" included the following animals: ass, giraffe, deer, goat, camel, tiger, panther, wolf, wool-pig, cow, turtle, reptile and otter. In this group, apatite was the most frequent component. The majority of animal

Table 1 The frequency of occurrence of stone minerals in animals

Stone mineral	Dogs $n = 69$	Cats $n = 51$	Other pets $n = 9$	Zoo animals $n = 19$
Struvite	47.8	45.0		15.8
Whewellite	27.5	54.9		21.1
Weddellite	27.5	31.4		36.6
Calcium phosphate (Apatite etc.)	18.8	14.7	66.7	52.7
Urate (Ammonium-, Sodium-)	18.8	2.0		15.8
Uric acid	1.4			
Xanthine		2.0		5.3
Cystine	4.3			5.3
Calcite			83.3	
Silicon dioxide	1.4			5.3

stones contained only one mineral (58.6%); 35.2% consisted of two components and only 6.2% consisted of three components (Table 1).
Conclusion: We found the same stone minerals in the urinary stones of animals as in human stones with the exception of calcite and silicon dioxide. However, the frequency patterns of the animal stones differed significantly from that of human stones. In canine stones, struvite was the most frequent mineral (47.8%). In feline stones, the abundance of struvite (45.0%) was also significantly higher than the 5.9% found in 111,196 human stones. The frequencies of ammonium hydrogen urate and of cystine (15.9 and 4.3%, respectively) found in the stones from dogs were considerably higher than those in stones from humans (0.9 and 0.3%, respectively). The investigated stones of zoo animals showed a very different frequency pattern of stone minerals compared to the frequency pattern in human stones although the number of investigated stones is not so high. Remarkable is the very high abundance of ammonium hydrogen urate in stones from zoo animals (15.8%). With reference to the number of constituents, stones from animals are more simply composed than stones from . In animal urinary calculi, single mineral stones predominate (58.6%), whereas in human calculi stones with two minerals predominate.

PP-027 Composition of canine low urinary tract stones in Mexico City

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Introduction: Effective long-term management of urolithiasis depends on identification and manipulation of factors contributing to the initial stone formation that, in turn, depends on accurate identification of the mineral composition of the urolith involved.
Objectives: The purpose of the study was to know the chemical composition of the uroliths obtained of the low urinary tract in dogs of Mexico City.
Methods: One hundred and five cases of stones obtained surgically of the urinary bladder or urethra in dogs (67 males and 38 females) attended in different clinics of Mexico City were studied. The chemical composition of the uroliths was determined for quantitative and qualitative analysis by stereoscopic microscopy, IR-spectroscopy, scanning electron microscopy and X-ray microanalysis. Uroliths were classified as simple or pure (one layer containing more of 70% of the mineral), mixed (one layer with less than 70% of single mineral) and compound (uroliths with nidus and others layers of different predominant mineral composition). In this study the terms calcium oxalate (CaOx) include CaOx monohydrate, dihydrate or both, and urates include ammonium urate and sodium urate.

Table 1 Composition and distribution of the uroliths

	% (n) total	% Female/ Male	<5	% (years) 6–10	>10
Struvite	38.1 (40)	60/40	70	23	7
CaOx	26.6 (28)	21.5/78.5	36	53	11
Silica	13.3 (14)	0/100	86	14	0
Urate	7.6 (8)	37.5/62.5	62	25	13
Cystine	0.9 (1)	0/100	0	100	0
Mixed	11.4 (12)	41.6/58.4	58	42	0
Compounds	1.9 (2)	0/100	50	50	0

Results: Age of animals ranged from 4 months to 14 years old, with a median of 5 years old. Dogs less than 1 year old only formed struvite stones, representing a 15% of the all cases of this mineral. Composition and distribution of the uroliths is show in the table.
Most uroliths were found in pure breed (77%); twenty-three different breeds they were identified, but more than half of submissions were from 6 breeds: Schnauzer (22%), Poodle (14%), Labrador retriever (7%), Dalmatian (4%), Cocker spaniel (4%), and German shepherd (3%).

The mixed-uroliths were formed by combination of CaOx with ammonium urate (58%), struvite with CaOx (25%) and struvite with calcium phosphate (17%). The two compounds uroliths had a nucleus of silica with a stone of CaOx (Table 1).
Conclusion: In our study the frequency of struvite, CaOx, cystine, urates, mixed and compounds stones are in agreement with other papers that reports dog populations of America and Europe, but a higher frequency of silica uroliths was observed.
Acknowledgement: This project has been support partially by the "Waltham Foundation".

PP-028 Evaluation of urinary calculi from guinea pigs (*Cavia porcellus*): 127 cases

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Introduction: Guinea pigs with urolithiasis are frequently presented to veterinarians, but the aetiopathogenesis is unknown.
Objectives: An dual study to determine the composition of 127 urinary calculi in guinea pigs and evaluate urinalyses and lower urinary tract cultures.
Methods: Analysis records of guinea pig urinary calculi submitted to the UCD Stone Laboratory between1985 and 2003 were reviewed. Furthermore, a 2004–2007 prospective study was performed which requested submissions of guinea pig urinary calculi. These submissions were accompanied by a urine sample for urinalysis and culture and a questionnaire. All stones were analyzed using polarized light microscopy and infrared spectroscopy (IR). X-ray diffractometry (XRD) was performed on a subset of stones.
Results: Of the 52 stones from the Stone Lab database and the 75 that we solicited, 83% and 93% respectively were composed of 100% calcium carbonate. The others were primarily calcium carbonate mixed with various other minerals, mostly struvite. It was determined by XRD that 5/6 of the stone subset were composed of 100% calcite (Fig. 1). The stones were located throughout the urinary tract. From the prospective group of 75 stones, no significant

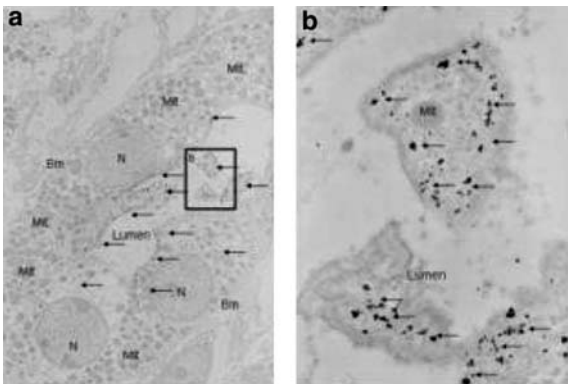

Fig. 1 In the lumen of distal tubular cell osteoporen existed on the epithelial cells of the lumen side and on the cell debris in the lumen (mouse kidney of 24 h after glyoxylate administration). **a** 3,000×, **b** 25,000×, *N* nuclear, *Bm* basement membranre, *Mt* mitochondria, *arrows* osteoporin

differences were noted among gender, reproductive status, or age. Although many guinea pigs were given antibiotics before cultures were performed, Corynebacterium renale was cultured from 5 samples.
Conclusion: We have determined that most often urinary calculi from guinea pigs are composed of 100% calcium carbonate, and methodologies, such as IR or XRD are necessary to differentiate this mineral from others. Treatment modalities, including diet and husbandry, should be developed to help prevent calcium carbonate stones in guinea pigs.

OP-021 SWL tolerance with non-opioid analgesia

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Introduction: Success rates of extracorporeal shock wave lithotripsy (SWL) depend amongst other factors on the pain tolerance of the patient. With a lower pain threshold, SWL is usually applied with a lower energy which may affect clinical outcomes. We present our experience with a non-opioid analgesia protocol for SWL of kidney stones on an outpatient basis.
Methods: We analysed retrospectively 179 patients who received their first SWL for a solitary kidney stone during 2006. Mean age was 48 years (22–80). There were 105 male and 74 female patients. All patients were treated on a Siemens Lithostar Multiline® lithotripter. The target was to deliver 4000 shock waves at an energy level of 4 which corresponds to manufacturers recommendations for renal stones. As per protocol, all patients received pre-SWL 100 mg Diclofenac per rectum only. Patients who could tolerate the targeted shock wave number and energy under this analgesia were allocated in group A. those who required a reduction in either energy levels or shock wave numbers were allocated in group B. We recorded patient parameters (gender, age, body mass index BMI), stone parameters (side, size, location), and treatment parameters (applied energy, applied number of shock waves, operator). For univariate statistical analysis we used the chi-square test and a *t* test, while for multivariate statistical analysis the logistic regression test was used.
Results: 95 patients (53%) could tolerate the full treatment and were in group A. 84 patients (47%) could not tolerate the full treatment and were in group B. Group A contained the majority of males (64%), whereas the majority of females were in group B (62%) ($P < 0.001$). Also, patients in group A were significantly older ($P < 0.05$). The majority of patients with a higher BMI were in group A (68%). Patients with a lower BMI tended to be less pain tolerant (group B) ($P < 0.05$). For renal pelvic stones the majority of patients could tolerate the full treatment (70% group A), whereas for calyceal stones the numbers were equal (51% group A). Operator, stone size, and stone side were not found statistically different. Multivariate analysis confirmed that gender and BMI were the most important independent predictors for tolerating full treatment.
Conclusion: Under simple non-opioid analgesia, being male, older, and of a higher BMI are independent predictors for a better pain tolerance, and, in consequence, more efficient treatment.

OP-022 Minimal invasive PCNL in the treatment of nephrolithiasis: analysis of efficiency and complications in 335 consecutive patients

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Introduction: Extracorporeal shock wave lithotripsy (SWL) or percutaneous nephrolithotomy (PCNL) are the treatment modalities of choice in nephrolithiasis. SWL however is associated with a relatively high probability of residual fragments, whereas PCNL demonstrates better results, but is more prone to complications. To reduce the invasiveness and consequentially the complication rate a miniaturized 18F instrument for PCNL (MPCNL) has been developed. A group of 335 consecutive patients were prospectively evaluated to determine the outcome of the method.
Methods: A group of 335 patients (mean age 53.8 ± 16.5) were treated. Data on the stone size and location, stone-free rate, blood transfusions, operating time and complications were recorded. A subgroup with a stone mass larger than 5 cm^2 on the plain X-ray film ($n = 103$) was analyzed separately to determine the applicability to larger stone loads.
Results: In 333 patients access was possible. On average, the re-treatment rate was 0.30 (subgroup: 0.42). The mean stone size was 4.3 cm^2 (subgroup: 8.9 cm^2) The average operating time was 67 ± 30 min (subgroup: 76 ± 30 min). Overall, the stone-free rate was 90.8% (subgroup: 91.3%). Blood transfusion was needed in six cases (1.9%, subgroup: 1%). Febrile pyelonephritis was observed in 22 patients (6.5%, subgroup: 7.7%). Except for one arterio-venous fistula, which had to be treated by interventional radiology, no major complications were observed.
Conclusion: MPCNL was a reliable alternative to SWL for renal calculi with a size from 1 to 2 cm located in the renal pelvis and calices, especially the lower calyx. The advantages are shorter treatment time and a higher stone-free rate. The complication rate is similar to that of SWL and significantly lower when compared to conventional PCNL. Despite the reduced diameter of the instruments, treatment of larger stone burdens is possible only a slightly increased operating time. A high stone-free rate and low level of complications are maintained. MPCNL is worth consideration as a alternatively to conventional PCNL in suitable cases.

OP-023 Minimal invasive PCNL in older patients: analysis of outcome and morbidity in 57 consecutive patients aged 70 and above

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Introduction: Minimal invasive PCNL (MPCNL) is used in the treatment of nephrolithiasis as an alternative treatment to shock wave

lithotripsy or conventional PCNL in suitable cases. Previously published data have demonstrated a high efficiency and low morbidity of the method.
This study analyses the data of 57 consecutive patients aged 70 years (y) or above compared with 278 younger patients to evaluate the applicability of the method in aged patients.
Methods: A group of 57 patients (age range 70–92 y, mean age 75.0 ± 4.2 y) were treated by a miniaturized PCNL (MPCNL) equipment, including a 12 F nephroscope and an 15 F Amplatz sheath. Data on the stone size and location, stone-free rate, blood transfusions, operating time and complications were recorded prospectively. Results were compared with the data of 278 consecutive younger patients. Student's *t* test or Chi-square test were used for statistical analysis.
Results: The average stone size was 4.7 cm^2 on the plain X-ray film and did not differ from that of controls (4.2 cm^2). Furthermore, no statistically significant differences could be detected for mean operating time (62 ± 28 vs. 68 ± 30 min), stone-free rate (90 vs. 89%) or transfusion rate (1.8 vs. 1.8%). The necessity for re-treatment to achieve endoscopically and radiographically assured absence of residual fragments tended to be lower (21 vs. 32%) but did not reach statistical significance. A significant difference ($P < 0.05$) however was revealed for postoperative febrile episodes. There was no relevant fever episode in older patients (0%) whereas fever was observed in 7.9% of controls.
Conclusion: MPCNL was demonstrated to be applicable for use in older patients. The results are independent of age in terms of stone-free rates and treatable stone burden. There was no higher morbidity in older persons compared with younger individuals. Therefore, in older patients MPCNL can be regarded as a safe and reliable alternative treatment modality to SWL and PCNL in suitable cases.

OP-024 A comparative evaluation of the efficacy of the use of Tamsulosin and/or Tolterodine for the medical treatment of distal ureteral stones

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Objectives: The goal of this study was to evaluate the activity of the therapeutics (Tamsulosin or/and Tolterodine) used to accelerate the expulsion of stone and to reduce probable complications during the observation of the medical treatment of distal ureteral stones allowing spontaneous passage.
Methods: A total of 120 patients possessing distal ureteral stones were included to the study. Stones of <10 mm allowing urinary flow were included to the study. The patients were randomly divided into four groups. Group 1 patients received Tamsulosin 0.4 mg/day, Group 2 patients received Tamsulosin 0.4 mg/day plus Tolterodine 2 mg 2 ×1, Group 3 patients received Tolterodine 2 mg 2 × 1; whereas, the patients in Group 4 did not receive any medical treatment and were accepted as the control group patients.
Results: No statistical significance concerning age and stone dimensions between the groups was determined ($P > 0.05$). Stone expulsion rates were higher ($P < 0.05$) in Groups 1 and 2 when compared with those of Groups 3 and 4. Significant variation ($P < 0.05$) regarding the period of time required for stone expulsion was observed in Groups 1 and 2.
Conclusion: In our study, the use of Tamsulosin for the expulsion of distal ureteral stones was demonstrated to be effective, whereas the use of Tolterodine provided no additional advantages.

OP-025 Percutaneous nephrolithotomy (PCNL) in patients with previous open stone surgery

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Objectives: To investigate the effects of previous open nephrolithotomy on technical features, outcomes and morbidities of subsequent percutaneous nephrolithotomy (PCNL).
Methods: One hundred and sixty patients underwent PCNL between December 2004 and September 2006. The patients were divided into those who had previous open nephrolithotomy on the same kidney (group-1; 55 patients) and those who had no previous open surgery (Group 2; 105 patients). Technical features encountered during operation and the outcomes of each of the groups were compared.
Results: There were no significant differences between groups with respect to mean age (group-1:42.6 ± 10 vs. Group 2:45.5 ± 9.6 years), body mass index (24.8 ± 2.11 vs. 24.6 ± 2.14) and stone burden (385.6 ± 140.,6 mm^2 vs. 401.05 ± 142 mm^2). In Group 1, 28 and 27 stones were located in the right and left kidney, while the location was 51 and 54 for the right and left kidney in Group 2. When the groups were compared, the mean operative time was significantly longer (155 ± 30 vs. 137 ± 30 min) in Group 1.But there was no significant difference with respect to requirement for secondary intervention (11 vs. 10%). Sepsis developed in 2 patients and 1 patient in Group 1 and Group 2, respectively. One patient in Group 1 died as a result of septic shock. Ten (18.2%) patients in Group 1 and 13 (12.4%) patients in Group 2 required blood transfusion.
Conclusion: When PCNL is performed after previous open nephrolithotomy, the operative time increases. There is no difference with respect to success rate and morbidity.

OP-026 Cost-effectiveness of different management strategies for ureteral stones

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Introduction: Ureteroscopy (URS) and extracorporeal shock wave lithotripsy (SWL) are alternative options for the management of ureteral calculi that are eventually offered as equivalent by the guidelines of the most important urological associations. The modality of treatment has to be chosen in consideration of several factors such as characteristics of the stone and of the urinary tract, association with obstructive uropathy and deterioration in renal function, urinary tract infection or other complicating factors, symptoms of the patient, availability of instrumentation and experience of the operator. Finally, cost is an important factor that should be considered when determining an appropriate treatment strategy.
Objectives: The aim of this study was to compare the cost of alternative treatment strategies for ureteral calculi.
Methods: The total cost for each treatment procedure was estimated considering direct costs (personnel, pharmaceuticals and consumables, depreciation and maintenance), indirect costs (related to use of the operating theatre) and general costs of the hospital. Finally the cost of each management option was computed in relation to the stone free rate, and the number of patients with re-treatment or auxiliary procedures [1].
Results: The cost was 634 Euros for a single SWL treatment and 1285 Euros for URS. These figures increased to respectively 872 and 1,368 Euros when the costs of re-treatment and auxiliary procedures were taken into account.
Conclusion: In Europe, the cost of URS has generally been computed higher than those for SWL whereas in the United States URS has been evaluated less costly than SWL for stones at all ureteral locations [2, 3]. We confirmed that in our health organisation the cost of SWL

is lower than the cost of URS. The cost of purchasing and maintaining a lithotripter is a main determinant of the treatment cost associated with SWL, but its relative contribution to the total cost varies with the annual volume of procedures. On the other hand, the cost of URS is conditioned by the need for general anesthesia. The use of flexible scopes could reduce the need for general anesthesia, but involves higher cost for instrumentation in consideration of the fragility of the instruments and the cost of disposables.

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OP-027 Improved training model for percutaneous nephrolithotomy

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Introduction: Percutaneous nephrolithotomy (PCNL) and similar endourological procedures require an advanced level of skills. To facilitate the training of the proper technique, simulators are helpful. Non-biological models, useful for learning the basic steps, do not represent the clinical situation in an ideal way. Several years ago, we developed a porcine urinary tract model for PCNL using silicone and gelatine, respectively.
Objectives: When dilating the percutaneous tract, silicone and gelatine quite frequently were damaged thus inhibiting proper working with the endoscopes. Therefore, we improved our training model and made it closer to the clinical situation in humans.
Methods: The kidney along with the ureter was dissected from the retroperitoneal organ package of freshly slaughtered pigs. The kidneys were put into bags cut into parts of the thoracic/abdominal wall of these pigs. The renal pelvis can be filled with saline to simulate hydronephrosis; stones can be implanted for PCNL.
Results: Our new model allows for even better training of all percutaneous endourological procedures (e.g. percutaneous nephrostomy, PCNL, endopyelotomy). In particular, puncturing is extremely close to the situation in humans as the porcine thoracic/abdominal wall in principle has the same anatomy as that of the human.
Conclusion: Our new training model has already been used with great success in hands-on courses. Concerning "tissue feeling", the anatomical relationships and the great variety of procedures that can be used for the purposes of training, the model is superior to that of non-biological models. Furthermore, it is easily available and inexpensive.

OP-028 Can flexible ureterorenoscopy (FURS) be recommended in the treatment of stone masses greater than 100 mm^2 in the upper urinary tract?

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Introduction: Flexible ureterorenoscopy is widely used for the treatment of calyceal stones in combination or as an alternative to Extracorporeal Shockwave lithotripsy (SWL). For a reasonable procedure, the diameter of the ureteral access seems to limit the maximal stone size to 100 mm^2. With enhanced surgical ability, the treatment of greater stone masses is possible.
Methods: A prospective study of 400 consecutive flexible URS-procedures for stone treatment, diagnostic purpose or laser surgery of calyceal stenosis was conducted. We compared a subgroup A (75 procedures on 51 patients with stone masses > 100 mm^2) with those of all studied patients (group B: 400 procedures on 350 patients). Endoscopic procedures were performed by means of 270°-deflectable or double-bending ureterorenoscopes using an ureteral access sheath of 14 or 16 F. Lithotripsy was done by means of a Holmium-Laser. Data for stone-free rate, operating time, hospital stay length, complication rate, stone analysis, stone localisation and auxiliary procedures was collected.
Results: In 344 cases urinary tract stones were detected and treated. The stone-free rates were 90.2% (A) and 88.2% (B). Mean operating time was 70 min (A) and 53 min (B). The re-operating rate was 47% in group A and 12% in group B. The complication rate was 8% (A) versus 4.2% (B) for pyelonephritis and 2.6% (A) versus 1.8% (B) for ureteral perforation.
Conclusion: Flexible ureterorenoscopy is a reliable and effective diagnostic and treatment modality for stones in the upper urinary tract. With acceptance of a multi-time approach and a slightly higher complication rate, stones of more than 100 mm^2 can be treated successfully. Until recently SWL has been the guideline recommended therapy for stones between 1 and 2 cm diameter. Randomized studies are needed to determine the significance of flexible ureterorenoscopy in the treatment of stones >100 mm^2 in the upper urinary tract.

OP-029 Prospective study of 400 consecutive flexible ureterorenoscopies (FURS) of the upper urinary tract

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Introduction: Since the advent of new flexible ureterorenoscopes, flexible endoscopy of the upper urinary tract has become easier but its importance has to be determined.
Pathologies of the upper urinary tract, such as calyceal and renal pelvic stones, calyceal diverticulum stones, morphological obstructions and tumors, can be treated under direct endoscopic vision. Especially in cases with lower pole stones, flexible ureterorenoscopy shows more favourable results than Extracorporeal Shockwave lithotripsy (SWL).
Methods: A prospective study of 400 consecutive flexible URS-procedures for stone treatment, diagnosis or laser surgery of calyceal stenosis was carried out. Endoscopic procedures were performed by means of 270°-deflectable or double-bending ureterorenoscopes using an ureteral access sheath of 14 or 16 F. Lithotripsy or incision was performed by means of a Holmium-Laser. Data for stone-free rate, operating time, hospital stay length, complication rate, stone analysis, localisation of the stone and auxiliary procedures was collected in 400 procedures (350 subjects).
Results: Mean operating time was 53 min. In 344 cases urinary tract stones were detected and treated. Mean stone size was 49.6 mm^2. In 43.7% Holmium-Laser Lithotripsy was performed. Thirty percent of the patients had been pre-treated by SWL and flexible ureterorenoscopy was used to remove residual fragments. The complication rate was 4.2% due to pyelonephritis and 1.8% due to ureteral perforation. The overall stone-free rate was 88%. We successfully performed incision of calyceal stenosis in nine cases.
Conclusions: Flexible ureterorenoscopy is a reliable and effective diagnostic and treatment modality for different pathologies of the upper urinary tract. Stones up to 100 mm^2 can be treated successfully. The main advantage in comparison to SWL is an increased stone-free rate

and a reduced treatment time. Anatomical abnormalities, e.g., calyceal stenosis, can be treated simultaneously with stone therapy.

Currently flexible ureterorenoscopy of the upper urinary tract is not recommended as a first line therapy by international guidelines. Based on the present results randomized studies are needed to determine the significance of flexible ureterorenoscopy in the upper urinary tract.

OP-030 Aid to percutaneous renal access by virtual projection of the ultrasound puncture on to fluoroscopic images

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Introduction: Percutaneous renal access in the context of PCNL is a challenging technique. We present a computerized system designed to improve percutaneous renal access by virtually projecting the ultrasound nephrostomy tract on to fluoroscopic images.
Objectives: To evaluate the feasibility and safety of the navigation system (Koelis) in a clinical trial, comparing residents and experts on the same patients.
Methods: The software developed runs on a computer using a stereo camera [1].

- The first step consists in acquiring fluoroscopic images in various orientations which are particularly useful to visualize all of the calices and to determine the axis of calyceal stalks. As the kidney moves with breathing, the anaesthetist is asked to stop ventilation for a 10-s period at the end of expiration to take images, so that the acquired images are coherent. The fluoroscope is then removed from the operative field to leave more space for the operator.
- From this point on the computer system, that has integrated the spatial position of the fluoroscopic images, is able to display the nephrostomy tract selected on the ultrasound transducer without any human intervention. The ultrasound transducer is the only device in motion. The operator visualizes, in real time, both the puncture tract on the ultrasound image and its virtual representation on all fluoroscopic views. The operator is therefore able to determine the optimal puncture position and to visualize the progression of the needle on the ultrasound monitor.

Results: We validated this technique in the operating room during 20 PCNL.

- For 16 patients, a single puncture was sufficient to reach the target in an optimal way.
- For 4 other patients, 3 puncture trials were necessary. The distance between the computerized needle tract and the visualized tract by fluoroscopic images were on average of 1 cm. This error was only due to the deformation of the needle during the puncture.

No intra-operative complication was observed for all cases.
Conclusion: Those two imaging systems provide a real synergy, while leaving the surgeon completely free to perform the operative procedure. Navigation reduces radiation exposure, is also an excellent tool for training and seems to be useful for experts too.

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OP-031 Investigations for recognising urinary stone

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Introduction: Identification of stones in the urinary tract in patients presenting with various symptoms has been a problem for clinicians from time immemorial. With the advancement of modern investigative modalities, the clinicians have been benefited in identifying presence of stones in the patients. The Ultra Sounds Scan (USS) and Computerised Tomogram (CT) scan are newer additions to the investigative modalities already available. Digitalization of the X-ray has improved the outlook of identifying Radio Opaque Shadows (ROS)in the plane X-ray of Kidney, Ureter and Bladder (KUB) region.
Objectives: This study was done to identify the value of the commonly performed investigative modalities available namely X-ray KUB regions and USS to recognize stones in patients suspected to have the disease.
Methods: A total of 200 patients who attended the stone clinic with symptoms of suggestive of urinary stone disease and had either their stone removed or had been followed up for minimum of six months, were interviewed. The final opinion on the presence or absence of stones was made after follow-up to assess the efficacy of the initial opinion based on the plain X ray KUB or USS. The patients were classified as proven stone patients only after retrieval of stones. The efficacy of the initial screening investigation was assessed 1 to calculate the specificity and sensitivity of the two modalities of investigation.
Results: Of the 200 patients studied, all had plain X-ray KUB. Only 166 patients had Ultrasonogram for recognizing stones in the urinary tract. 74 patients showed positive evidence of stones either by X ray or USS. The findings of the two modalities of investigation are given below:

Number of X-rays done 200
Number positive 24
Proved positive 24 (Stone retrieved)
Proved negative 0
Number negative 176
Proved positive 32 (Stone retrieved)
Proved negative 144
Number of USS done 166
Number positive 120
Proved positive 50 (Stone retrieved)
Proved negative 70
Number negative 46
Proved positive 14 (Stone retrieved)
Proved negative 32

USS showed back presence effects in 62 patients. Of these, 12% showed stones in the ureter, whereas the rest did not show evidence of stones. Those selected as positive stones finally had either passed stones or had PCNL, URS, Cystolithotripsy, open surgery or were put on high dose chemotherapy. 46 patients who had no ROS in KUB and no stones in USS passed stones subsequently.
Conclusion: It is concluded that it is necessary to perform both plain X-ray KUB and USS on suspected cases of stones to verify whether or not they have stones and also to exclude other pathology, which may produce similar urinary symptoms.

OP-032 A correlation between CT renal stone characteristics and lithotripsy treatment outcome: is smaller and softer better?

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Introduction: Renal calculi occur in approximately six percent of the population. They are associated with episodes of acute pain, urinary obstruction and sepsis and account for a significant number of lost work man-hours.
Prophylactic management of stone disease has been shown to be worthwhile in reducing complications and hospital inpatient stay.

Lithotripsy is a safe and universally available option for the treatment of stones. It has a reported overall success rate of 65–70% in fragmenting individual calculi.

Stone size is currently the predominant factor in selection of patients for lithotripsy and lithotripsy is the treatment of choice for stones of less than one centimetre. Stone density may also play a part in determining fragmentation success, but its contribution remains to be adequately evaluated.

Accurately predicting the likelihood of success of lithotripsy treatment would be extremely useful in patient management.

Improvements in multi-slice CT have resulted in the use of low dose CT-KUB in the pre-treatment evaluation of stone disease. These hi-resolution studies allow a reliable assessment of stone density, size and location to be made.

Objectives: To determine the relationship between stone parameters as assessed by axial and coronal multi-slice CT and lithotripsy success.

Methods: A single observer, without prior knowledge of treatment outcome, assessed 25 pre-treatment CT-KUB studies (64 slice CT, coronal slice thickness 1.0 mm, axial slice thickness 3.0 mm). Measurements were taken of a single calculus from axial and coronal images demonstrating the maximum stone diameter. Maximum stone diameter, average Hounsfield units from a region of interest (ROI) encompassing but not extending outside the stone (HUav), and maximum Hounsfield units of the stone (HUmax) were recorded.

Patient outcome was classified as stone unchanged (UC), fragmented (F) or stone free (SF).

Correlation between CT determined stone parameters and patient outcome was assessed.

Results: Only three of the 25 stones assessed were greater than 1 cm in diameter.

There was no significant difference in stone size, HUav or HUmax for axial measurements between the treatment outcomes ($P = 0.22$, $P = 0.28$ and $P = 0.12$, respectively).

Average coronal HUmax was 809 (SD ± 370) for the fragmented and stone free groups combined, and 1205 (SD ± 456) for the unchanged group. This difference neared statistical significance ($p = 0.07$). Average coronal HUav was 482.9 (SD ± 213) for the fragmented and stone free groups combined, and 709 (SD ± 272) for the unchanged group, and a similar level of statistical significance for a difference between these values was present ($p = 0.08$). Differences in coronal size were not statistically significant ($P = 0.25$).

Conclusion: The lack of correlation between stone size and treatment outcome is contradictory to previously published results and is likely to have arisen from the a priori selection of patients on basis of size for lithotripsy treatment itself.

In this small study, there was a trend towards higher peak and average Hounsfield unit values in stones that failed to be treated by lithotripsy. Interestingly, these trends were found when evaluation was made on the coronal 1 mm CT images and not on the 3 mm axial slices. It is likely that partial voluming on 3 mm slices significantly effects Hounsfield unit measurement, particularly with small calculi.

Assessment of a larger patient cohort would be useful to confirm the trends found in this initial work.

PP-029 Influence of extracorporeal shock wave lithotripsy (ESWL) on erythropoietin production in the kidney

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Introduction: Erythropoietin (Epo) is produced by interstitial cells near the proximal tubules in the kidney. Its production is stimulated by blood loss and disturbances of the intrarenal blood circulation; renal insufficiency impairs the synthesis of Epo.

Objectives: We were interested to learn whether or not extracorporeal shock wave lithotripsy (ESWL), which is able to induce morphological and functional damages in the kidney as well as intrarenal bleeding, can influence the production of Epo in the clinical setting.

Methods: A group of 48 patients (mean age 48 ± 15 years) was treated for renal stones by ESWL. ESWL was performed using an MFL 5000 lithotripter (Dornier/Philips). Blood samples were taken at admission (−1), immediately before and after ESWL (−0 and +0) and days 1, 3 and 5 after ESWL. Hemoglobin (Hb) and Epo were measured in these samples. For the determination of Hb, an automatic blood cell counter, for Epo the EPO-ELISA (Medac, Hamburg, Germany) was used. The *U*-test was used for statistical analysis of significance.

Results: At day 1 and 3 after ESWL there was a transient significant increase in Epo levels (−1: 6.64 ± 4.24 U/l; −0: 5.80 ± 4.49 U/l; +1: 7.38 ± 3.91 U/l; +3: 7.81 ± 5.34 U/l; $P < 0.001$). At day 5, the pre-treatment levels were found. There was a significant inverse correlation between the pre-treatment levels of Epo and Hb ($r = 0.382$; $P = 0.012$), however, there was no correlation between the transient increase in Epo and Hb.

References

1. ESWL induces a transient increase in Epo, which is independent of Hb. This demonstrates that the ESWL induced increase in Epo is caused disturbances of the intrarenal microcirculation resulting in tissue hypoxia. Since the increase of Epo after ESWL is within the physiological range and only transient, the influence of ESWL on Epo production in the kidney is not harmful.

PP-030 Is it worth upgrading your flexible ureterorenoscope? A clinical analysis

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Introduction: The new generation semi-flexible ureterorenoscopes (URS) offer an improved deflection of 270° in both directions and a stiffer sheath. It is postulated that stone access and removal are facilitated compared to the conventional devices.

Objectives: In our study, we aimed to determine the difference in efficacy of stone treatment comparing the new and the conventional flexible URS.

Methods: A total of 90 patients with upper urinary tract stones was included in a retrospective study. Twenty-nine cases were treated with the last generation of flexible URS (11274 AA, Karl Storz Endoscopy, Germany) between 01/2000 and 07/2003 and 61 cases were treated with the new flexible URS (Flex-X, Karl Storz Endoscopy, Germany) between 12/2005 and 05/2007. The patients underwent standard clinical and radiological workup before the procedure. The stone characteristics, stone free rate and complications were compared between the two groups. Treatment success was defined as complete stone removal or insignificant residual fragments smaller than 4 mm requiring no further intervention.

Results: The mean patient age for conventional and new flexible URS was 58.6 and 52.2 years, respectively, the mean stone burden was 8.8 mm and 8.5 mm. Stone access was possible in 96.6 and 100% for the conventional and new URS group ($P = 0.4$). Immediate treatment success was 32% for the conventional versus 70% for the new flexible scope ($P = 0.0032$). For the subgroup of lower pole stones, mean stone size was 6.6 mm (conventional scope, 16 patients) versus 5.3 mm (new scope, 51 patients). Stone access was possible in 93.75% versus 100% ($P = 0.433$) and treatment success was 31.2% versus 68.6% ($P = 0.0004$) for the old compared to the new scope. No major complications were observed. Minor complications,

including perforation (3.44 vs. 1.63%), abortion because of bleeding or impaired visibility (7 vs. 6.5%) and fever (6.89 vs. 4.91%) were comparable in both groups.
Conclusion: Our study suggests an advantage of the new flexible ureterorenoscopes compared to their predecessors. They offer an increased stone free rate especially in the treatment of lower pole stones. Despite the high initial acquisition cost, it is therefore advisable to switch to the latest generation flexible devices.

PP-031 MINI-PCNL in children: indications and results

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Introduction: Although Extracorporeal Shock Wave Lithotripsy (SWL) is the first line therapy to treat urolithiasis of upper urinary tract in children; a percutaneous procedure is indicated in case of ESWL failure, simultaneous treatment of obstruction or large stone mass.
Mini-percutaneous nephrolithotomy (MPCNL) is a miniaturised, minimally invasive procedure for percutaneous treatment of urolithiasis. This prospective study evaluates the significance of MPCNL in children.
Methods: A total of eight percutaneous nephrolithotomies were carried out in children (four male and four female, mean age 8.4 year) and were reviewed in relation to stone size, stone localisation, operative duration, stone-free rate, complications and adjuvant procedures. A 12 Ch miniature nephroscope or 5 Ch flexible nephroscope was used. The puncture of collecting system was ultrasound guided. Ultrasound disintegration, ballistic or Holmium laser lithotripsy was used.
Stone-free rate was verified endoscopically, radiological and by means of ultrasound examination.
Results: In total, 2 staghorn calculi, 2 renal pelvic calculi, 3 lower pole stones and 1 proximal ureteral stone were treated. The average stone burden was 1.7 cm^2. Ultrasound guided puncture was successful in all cases. The mean operation time was 95 min including retrograde endoscopic procedures. Stone-free rate was 100%. In 50% of cases at least one second-look PCNL was necessary. No complications like febrile pyelonephritis, perforation or bleeding were observed.
Conclusion: Percutaneous treatment of upper urinary calculi using miniaturised instruments can reach a high success and stone free rate, with minimal risk. Indications for percutaneous stone treatment in children are SWL failure, simultaneous treatment of obstruction and staghorn calculi. The results of this study justify the use of MPCNL in individual cases in children.

PP-032 Results of flexible retrograde ureterorenoscopy by means of ureteral access sheaths

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Introduction: The use of ureteral access sheaths (UAS) in flexible retrograde ureterorenoscopy is still discussed controversial. Main advantage of UAS is to make ureteral access easier, to facilitate the procedure and to reduce the intra-pelvic pressure. A potential disadvantage is the risk of developing an ureteral stricture.
Methods: A prospective study consisting of 99 patients (64 male, 35 female) has been performed. Flexible ureteroscopy was performed by means of UAS (12/14F or 14/16F) and a 8.5F flexible ureteroscope (R. Wolf, Knittlingen, Germany, ACMI, USA). The mean age was 50.9y (16–86 year). The follow-up was at least 3 months. During follow-up the upper urinary tract was examined by ultrasound and intravenous urography.
Results: In 95% ($n = 94$) no ureteral stricture was detected. Only 5% ($n = 5$) had dilatation of the upper urinary tract due to uretero-pelvic junction obstruction. The stone-free rate was 89.1%. The stone recurrence rate was 7.1%. Reports in the literature show a low complication rate, an easier approach to the upper urinary tract, a decreased intrapelvic pressure, and an enhanced stone-free rate. Results of the meta-analysis were similar to the results reported in the present study.
Conclusion: The use of UAS in flexible retrograde ureterorenoscopy enhances feasibility of the procedure, makes it possible to re-enter the upper urinary tract more easily, decreases treatment time, reduces risk of febrile pyelonephritis and provides a clear endoscopic view due to enhanced irrigation. Post-operative stricture formation as a result of ureteric dilatation is rare. In summary, the use of UAS can be recommended as the technique of choice in retrograde flexible ureterorenoscopy.

PP-033 Minimally invasive percutaneous nephrolithotomy (MINI-PERC) in transplanted kidneys

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Introduction: Due to the anatomic conditions there is a difference in the treatment of urolithiasis in transplanted kidneys and orthotopic kidneys. Location of the transplanted kidney, morphology of the ureter and fragility of the neo-ostium of the ureter lead to poor results with shockwave lithotripsy and endourological procedures. Therefore, the treatment modality should be chosen accurately. Due to the particularities of transplanted kidneys, the evidence of a primary percutaneous approach has to be determined.
Methods: In a 62-year-old male suffering from a 10 mm mid-calyceal stone in a transplanted kidney located in the right hypogastric region, a minimally percutaneous nephrolithotomy (Mini-Perc) was performed. A 12F miniaturized nephroscope was used. Under ultrasound guidance, including B-mode and Doppler ultrasound and fluoroscopy, puncture of the kidney was performed. A 15F Amplatz sheath was placed. Holmium laser lithotripsy was used. In order to determine the significance of percutaneous procedures in transplanted kidneys a meta-analysis consisting of 25 patients had been performed.
Results: The patient was rendered stone-free without any complications. After a 17 months follow-up he was still stone-free and the renal function was unchanged. The meta-analysis showed similar results: no major complications and a stone-free rate ranging from 70 to 100% in percutaneous stone removal in transplanted kidneys.
Conclusion: A primary percutaneous stone removal of calculi in transplanted kidneys provides favourable results, as this procedure is reliable and the complication rate is rather low. The percutaneous access can be established easily by means of ultrasound. Iliac vessels can be identified by Doppler ultrasound. As the stone fragments are removed via an Amplatz sheath, any damage to the ureter is prevented. Additional use of flexible scopes allow the achievement of a high stone-free rate, as almost all calyces can be examined. If miniaturized instruments are used, percutaneous stone removal is a feasible procedure in transplanted kidneys.

PP-034 The impact of alfa-1 adrenergic blockers on stone free rates for the management of ureteral stones in patients who underwent concomitant ESWL

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Introduction: ESWL is one of the best options for the management of ureteral stones because of its low morbidity rate and lack of requirement for general anesthesia. According to several randomized

clinical trials, alfa-1 adrenergic blockers improve the spontaneous stone passage particularly for distal ureteral stones. Alfa-1 adrenergic blockers can be combined with ESWL.
Objectives: The objective of this study was to investigate whether alfa-1 blockers have any effect on stone clearance in the patients who underwent concomitant ESWL for ureteral calculi.
Methods: A total of 146 patients who had ureteral calculi (94 proximal-mid ureter and 52 distal ureter) were divided into two groups as upper and lower ureteral stones. Each group was randomized into two subgroups as the first group received alfa-1 blockers and the other group did not. Regarding the upper ureteral stone cases, the first group with alfa-1 blockers had a mean stone size of 10 mm, and in the second group the mean stone size was 11 mm. The mean stone size was 9 mm in both groups of distal ureteral calculi. All patients were followed-up with weekly plain abdominal x-rays and a urinary ultrasonography at the end of 6 weeks.
Results: In the first group ($n = 47$) of upper ureteral stones who received alfa-1 blockers, 38 out of 47 patients (80.8%) became stone free. In the second group ($n = 47$) of upper ureteral stones without alfa-1 blockers 33 out of 47 patients (70.2%) became stone free. On the other hand, in the first group ($n = 26$) of lower ureteral stones with alfa-1 blockers, 23 out of 26 patients (84.6%) became stone free, while a stone free rate of 70.1% (18 of 26 patients) was observed in the second group ($n = 26$) of lower ureteral stones.
In both first groups of upper and lower ureteral stones higher success rates have been documented when compared with second groups without alfa-1 blockers. The preliminary results of this ongoing trial demonstrated a higher rate of stone clearance with alfa-1 blockers.
Conclusion: It is suggested that the combination of alfa-1 blockers with ESWL would enhance the stone free rates in the management of ureteral stones.

PP-035 Feasibility study to measure the thermal effect of laser on a ureteric wire stent

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Introduction: Lasers are nowadays widely used for the treatment of urinary tract stones. Often the laser is deployed in the immediate vicinity of intra-ureteric wire devices such as safety guide wires and baskets. Sometimes it is even used directly on to those devices, i.e. for encrusted wire stents or entrapped baskets. It is known that lasers generate temperatures of up to 2,000°C, and damage to the outer coating of the wire devices or—worse—breakage of the metal core has been observed.
Objectives: We developed an in vitro model allowing us to measure the heat affecting the devices in order to ultimately determine a safe distance of the laser from the wire for a given applied energy.
Methods: In our model, we used a Holmium:YAG laser, which is to date the most often used laser for stone fragmentation, and as a wire device a 0.35'' ZebrastentTM, which is a new-concept lumen-less Teflon-coated double-J wire stent designed to facilitate stone passage. A Perspex® tank was filled with 0.9% saline solution and the temperature was maintained at a physiological 37°C by using a thermoregulator (TE-10D, Techne). The Ho:YAG laser fibre (Slimline 200, Lumenis) and the wire stent were clamped into position via spring loaded mechanisms. The fibre was initially positioned in direct contact with the stent. For this feasibility study, the maximum exposure of 2.0 J (20 Hertz; 40 W) was applied until a total energy of 1 and 1.5 kJ, respectively, had been deposited. The fibre was moved in 1 mm increments away from the stent.
Results: This incremental increase of distance between fibre and wire enabled us to construct a 1-dimensional thermal distribution.
Conclusion: This model is able accurately to reflect the applied laser energy and resulting heat on to the wire device depending on the distance between them. Further experiments are underway to determine the nature and extent of damage to various wire devices at given laser settings, and—ultimately—to determine a safe distance of the laser from various wire devices at a given energy setting.

PP-036 Endourological treatment of calyceal diverticulum stones: indications and results

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Introduction: Due to the anatomical conditions associated with stones located in the calyceal diverticulum, the outcome of extracorporeal shockwave lithotripsy (SWL) is very poor. Even after successful disintegration, the stone-free rate is only about 50% because of diverticular neck obstruction. Since the introduction of new miniaturized endourological instruments, the technical feasibility of these procedures had been significantly improved. The main advantage is to be able to combine treatment of the morphological obstruction and the removal of the stone.
Methods: A prospective study consisting of 32 patients suffering from diverticulum stones had been performed. The stone distribution was 9 upper calyx, 9 mid calyx and 10 lower calyx. Six patients had been treated by URS (group A), 17 had been treated by minimal invasive PCNL (MPCNL, group B) and 9 had received a combined treatment (group C).
Results: The overall stone-free rate was 78.8%. The stone-free rate in group A was 66.7%, in group B 94.1% and in group C 77.8%, respectively. In group B, the stone-free rate of lower calyceal stones was 87.5%, of mid calyceal stones 100% and upper calyceal stones 100%. In group A, the stone-free rate of lower calyceal stones was 50% and upper calyceal stones 80%. In group C, the stone-free rate of lower calyceal stones was 100%, of mid calyceal stones 100% and upper calyceal stones 50%. All morphological obstructions of the diverticulum neck could be treated successfully. No severe complications were observed. No transfusion was necessary.
Conclusion: In comparison to the results using SWL, the stone-free rate can be increased by means of an endourological approach. Although flexible ureteroscopy allows almost all parts of the calyceal system to be reached, the data show that a percutaneous approach is more successful. The main advantage of endourological procedures is the possibility of a simultaneous incision of the morphological obstruction. In summary, a primary endourological approach to diverticulum stones is superior to SWL. According to the stone location, a decision should be made as to whether flexible URS or MPCNL is the more suitable option.

PP-037 Tensile, flexural and compressive strength studies on natural and artificial phosphate urinary stones

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Introduction: Mechanical properties of renal calculi dictate how a stone interacts and disintegrates with mechanical forces produced by shock wave or intracorporeal lithotripsy techniques [1]. Renal stones of different types with different compositions have a large variation in their mechanical strength and susceptibility to shock waves [2,3]. Phosphate stones account for 12–20% of all urinary stones and rank

Table 1

Material	Flexural Strength MNm-2	Flexural Modulus GNm-2	Tensile Strength MNm-2	Compressive Strength MNm-2
Natural Urinary Stone-1 (031)	9.18	5.79	0.812	2.04
Natural Urinary Stone-2 (0902)	4.21	1.68	0.749	1.44
Brushite Stone	–	–	0.436	10.42
Whitlockite Stone	–	–	1.20	32.85

first in the list of recurrent calculi [4]. The ability to predict how easily a renal stone will break in vivo by lithotripsy techniques would be advantageous [5] and hence the in vitro mechanical strengths of natural and artificial phosphate urinary stones were studied.
Objectives: Operated urinary stones and artificially developed urinary stones composed of phosphates were subjected to tensile, flexural and compressive strength studies in order to understand their mechanism of disintegration by in vivo fragmentation using non-invasive as well as invasive ultrasonic and laser techniques.
Methods: Operated urinary stones and artificial stones prepared using the standard pharmaceutical operations namely granulation, tableting and coating [1,6] were cut into rectangular slabs of maximum possible sizes. Tensile, flexural and compressive strength tests were performed for natural urinary stones and tensile and compression tests were performed for artificial stones using universal testing machine (Tinius Oslen, Model H5KN). The FTIR and X-ray diffraction techniques were used to analyze the samples.
Results: Tensile and compressive strengths for all the stones and the flexural strength and flexural modulus for the natural stone samples were determined and their average values are tabulated (Table 1).
Conclusion: The FTIR spectrum confirmed the presence of hydroxyapatite in both the natural stones and magnesium ammonium phosphate with calcium oxalate trihydrate in one stone (031). The X-ray diffraction analysis of the stones confirmed their crystalline nature. The tensile and flexural strength measurements and hence their flexural modulus, the in vitro mechanical strength of the urinary stones were studied directly using universal testing machine. It has been observed that the flexural properties depend on the size of the sample even for the samples cut from a single stone. The compressive strengths were almost double the tensile strengths of the respective natural stones while these were around twenty five times larger for the artificial stones prepared.

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PP-038 Retrieval methods for urinary stones

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Introduction: Several newer modalities for the treatment of stones have been developed in recent years. The advent of shock-wave treatment and endoscopic instrumentation has given a fillip to the range of minimally-invasive treatments for stone disease.
Objectives: This paper attempts to assess the current status of the various modalities of available treatment for urinary stone disease in the Kerala situation.
Methods: A total of 300 patients who attended the stone clinic with urinary stone disease and who had stones removed by different modalities were selected for the study. Their clinical symptoms, demographic profile, size, number and position of stones, metabolic profiles, retrieval modalities and outcome of treatment in terms of stone clearance were assessed. Instances of failure, incomplete clearance and complication events were noted. Based on these experiences, a flow chart was created for appropriate decision making in urinary stone management.
Results: The modalities of retrieval included Nephrectomy, Nephrolithotomy, Pyelo-nephrolithotomy, Extended Pyelolithotomy, Pyelolithotomy, Ureterolithotomy, Cystolithotomy, Urethrolithotomy, ESWL, PCNL, URS, Cystolithotripsy, Urethrolithotripsy and Spontaneous passage. The clearance rate of stone was maximum in open surgery. The extent of stone clearance by ESWL depended upon various factors. PCNL was mostly limited by the difficulties in achieving puncture to the stone site. Availability of variety of flexible nephroscopes also altered the success rate of the procedure. Pushing stones from ureter up to the pelvis followed by PCNL was having good success rates. In patients who had successful PCNL, post-operative morbidity was significantly reduced in terms of the number of days of hospitalization, time taken for return to work, absence of urinary leak, site infection, urinoma formation and urinary tract infection. URS was performed in many patients and stones removed. However, the indication for the procedure remains doubtful, as the size of the stones thus retrieved has been less than 6 mm in size. These would have passed out spontaneously or with chemotherapeutic support. URS, lithotripsy and basketing were confronted by upward migration of stones to the kidney, requiring further procedures for retrieval. Introduction of double J stents helped in relieving urinary obstruction particularly in patients presenting with anuria, but retained stents, forgotten stents and failed stone retrieval were common following the procedure. The procedure of URS was simplified by the presence of dilated ureter in patients who spontaneously passed their stones or those with distal obstruction and proximal dilatation.
Conclusion: It is concluded from the study that open surgery remains the sheet anchor of treatment of urinary stones in many patients in Kerala. Newer less invasive procedures should be ethically selected. Decisions should be patient-based taking into consideration.

PP-039 The initial experience of 3-month long use of the Sonolith i-sys electroconductive shockwave lithotripter in an outpatient setting

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Introduction: The era of extracorporeal shock wave lithotripsy was started in 1980 in Grosshadern Clinical Hospital in Munich, Germany

with a HM3 machine. Many types of shockwave lithotriptors have been constructed since that time offering treatment to patients worldwide. ESWL remains the first choice recommendation for treatment of renal stones up to 20 mm size. Larger stones and ureteric stones, especially in the lumbar part of the ureter, may also be successfully treated by means of ESWL. Electro-conductive technology was introduced first in 1994 and is still being developed in modern generation lithotripters. In that device, the electrode is encapsulated in a highly conductive solution in order to make all electrical discharges identical. As a the result, all waves were generated from the same geometric point F1 and the full power of the shock wave is transferred to the focal point F2, thus enabling sufficient lithotripsy with a penetration depth of 170 mm. The Sonolith i-sys machine (EDAP TMS, Vaulx-en-Velin, France) has a dual positioning system containing X-ray and ultrasound imaging, both of them displayed on one 20'' touch-screen monitor.
Objectives: To perform an initial evaluation of the efficacy of a new Sonolith i-sys electro-conductive shockwave lithotriptor for treating renal and ureteral calculi in an outpatient setting.
Methods: A total of 289 patients—135 female (47%) and 154 male (53%)—were treated during the first three months of our experience with a Sonolith i-sys machine, from January till the end of March 2008. A sub-group of 144 cases had right side stones and 145 the left side stones. A total of 253(86%) were the renal stones, while 36(13%) were located in the ureter. All patients were treated as outpatients without sedation or analgesia either prior to or after treatment. The assessment of potential immediate stone disintegration was performed each time immediately after the treatment by an X-ray image stored in a device's memory.
Results: In 177 cases (61%) the treatment was described by the patients as completely painless, 104 (36%) patients described the pain as moderate and only 8 of them as a severe (3%). Visible stone fragmentation at the time of the treatment was observed in 151 cases (52%). The highest rate of visible fragmentation was achieved in upper calyceal stones (61%), dropping to 51% and 55% for the middle and lower calyceal stones, respectively; immediate fragmentation of renal pelvis stones was described as 51%. A 48% fragmentation rate was reported for ureteric calculi. No immediate fragmentation was observed for staghorn calculi and those in the distal ureter.
Conclusion: Sonolith i-sys is a sufficient, easy to operate and reliable machine enabling the treatment of renal and ureteral calculi in a completely outpatient situation, without any sedation or analgesia, with complete safety and with only moderate complaint of pain from the patients. Immediate stone fragmentation at the time of the procedure was observed in 52% of cases. Our conclusions, based on a short period of time, are provisional and obviously require a longer period of observation and analysis for a'final and accurate assessment to be made.

PP-040 Initial experience with extended lithotomy position for percutaneous nephrolithotomy (PCNL)

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Introduction: Percutaneous nephrolithotomy is conventionally performed in the prone position requiring the repositioning of the patient from a lithotomy position. We describe our initial experience with the extended lithotomy position in which the patient's procedure is performed in the supine position.
Methods: Under general anaesthetic, the patient is placed in the supine position, with a 3 litre irrigation bag full of air underneath the ipsilateral operating flank. With the legs in the stirrups, the ipsilateral leg is extended and the contralateral one abducted to allow for retrograde access into the pelvi-calcyceal system. The percutaneous puncture of the kidney is performed under ultrasound and fluoroscopic control. The tract is dilated using Alken dilators with a 26F Amplatz sheath that allows us to work with low intrarenal pressures. The stones are fragmented and cleared with the Swiss Lithoclast master.
Results: Ten cases performed with a mean age of 56(45–72). Sex distribution was seven males to three females. Stone burden ranged from staghorn calculi to 2 cm renal pelvic stones. Mean operating time 75mins (45–128 min). All were fit for discharge 48 h post-operatively. No sepsis, blood transfusion or intestinal injuries were reported. Stone analysis revealed calcium oxalate in six cases and struvite stones in the other four cases.
Conclusion: The extended lithotomy position is a safe and effective position, which allows simultaneous percutaneous and transurethral access to the renal system. It offers the patient a more comfortable intra-operative position and allows for safer anaesthetic management. The stone clearance rate is similar to that of the prone position. A prospective randomised study is needed to compare the extended lithotomy and the prone position when performing percutaneous nephrolithotomy.

OP-033 Proteomic analysis of stone former urine

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Introduction: Combinations of anionic and cationic proteins forming protein aggregates can cause calcium oxalate monohydrate (COM) crystals to aggregate [1]. Thus, knowledge of the composition of proteins presented in stone former urine may improve understanding of the mechanism of stone formation. While many proteins have been linked in some way to stone formation, it is possible that some are more strongly associated than others [2]. The development of proteomics analysis by mass spectrum has the potential to identify proteins that could be involved in protein-protein interactions leading to stone formation.
Objectives: Our present study employs proteomic analysis of stone former urine macromolecules (SFU) compared to normal urine samples (NU) to identify anionic and cationic proteins that could form protein aggregates.
Methods: Urine samples were collected from both normal ($n = 9$) and idiopathic calcium oxalate stone formers ($n = 9$), and urine macromolecules were isolated by ultradiafiltration (10 kDa MiniKros, Spectrum Laboratories, CA, USA) against 100 mM NaCl. Prior to mass spectral analysis, protease inhibitors were removed by one dimensional polyacrylamide gel electrophoresis (15 μg/lane). Urine protein bands were in-gel digested using proteomic grade trypsin and further analyzed by a Voyager-DE PRO MALDI-TOF mass spectrometer. The analysis generated a list of proteins present, along with the relative abundance of the protein based on total ionic current (TIC). Their characteristics (isoelectric point [pI], hydropathicity, and amino acid composition) were obtained from the Swiss protein database (http://www.expasy.org). Using TIC data, weighted average values for pI and amino acid composition were generated for each urine sample.
Results: Both pI and Lys content were significantly increased in SFU relative to NU, while the content of Arg, Glu, Asp, and hydropathicity were not significantly different. The total of all anionic proteins ($pI < 6.5$) was significantly decreased in relative abundance in SFU, but only Tamm-Horsfall Protein and apolipoprotein D were significantly decreased individually. Conversely, the total of all cationic

proteins (pI > 6.5) was significantly increased in SFU, with only alpha-1-antitrypsin, hemoglobin alpha and beta, transferrin, IgG gamma and kappa light chains decreased individually. Osteopontin (OPN) and human serum albumin (HSA) were not significantly different between SFU and NU. While this analysis excludes information on post-translational modifications, the increased abundance of cationic proteins and decreased abundance of anionic proteins in SFU relative to NU was consistent with the reduced net negative charge per unit mass of protein observed empirically in SFU samples.
Conclusion: The observed shift in protein distribution in SFU samples compared to NU samples is in the direction expected to favor crystal aggregation and stone formation, based on our prior observations showing that mixtures of polyanions and polycations with nearly equal proportions promote calcium oxalate crystal aggregation [1].

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OP-034 Selective binding of prothrombin fragment 1 and albumin to specific faces of inorganic and urinary com crystals

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Introduction: Although intracrystalline proteins may prevent calcium oxalate (CaOx) stones by facilitating the degradation and dissolution of crystals retained in the kidney, the mechanisms by which they bind to and become incarcerated within the mineral phase of individual crystals are largely unexplored. Prothrombin fragment 1 (PTF1) and human serum albumin (HSA), both of which have been implicated in stone formation, are intracrystalline components of CaOx.
Objectives: (1) To compare the intracrystalline distributions of PTF1 and HSA within inorganic and urinary CaOx monohydrate (COM) crystals and (2) To determine whether binding of PTF1 can be explained by interactions between particular/gamma-carboxyglutamic (Gla) residues and atomic arrays on the top (100), side (010) or apical (021) faces of the COM crystal.
Methods: PTF1 and HSA were labelled with fluorescein isothiocyanate (FITC) and/or Alexa Fluor 647. COM crystals were generated in: (1) inorganic solutions at PTF1 concentrations of 0–0.5 mg/L; (2) ultrafiltered (UF: 10 kDa) urine at PTF1 concentrations of 0–2.0 mg/L; (3) inorganic solutions and (4) UF urine containing HSA or PTF1 alone, equal amounts of both, or relative urinary concentrations of both. Crystals were examined by fluorescence microscopy. The Accelrys Materials Studio and Discovery Studio programs were used to model coordination of Gla residues 26, 30 and 33 of bovine PTF1 to Ca atoms on each face of COM. Coordination of Gla groups to surface oxalates via Ca bridging was also modelled.
Results: INORGANIC CRYSTALS: Fluorescence was not seen in COM precipitated in the absence of proteins. Crystals containing HSA alone fluoresced with a bow-tie distribution consistent with binding to the two side faces, while those containing only PTF1 showed attachment to the two apical surfaces. The same distribution was seen when both were present at equal concentrations, but at urinary concentrations, the HSA bound to the side and apical faces.
URINARY CRYSTALS: Bow-tie patterns were never seen in crystals precipitated without protein. PTF1 showed the same pattern as in iCOM crystals, with intense fluorescence at the crystal centre. When both proteins were present at urinary concentrations, HSA was visible only at the apical extremities, demonstrating that PTF1 and HSA compete for the apical faces and that PTF1 attachment dominates over that of HSA.

Irrespective of whether modelling was based on coordination of Gla to surface calcium, or protein-bound calcium ions to surface oxalate, PTF1 showed equal bonding potential for all 3 COM surfaces.
Conclusion:

1. HSA and PTF1 are incorporated into COM crystals from the point of nucleation, which may involve templating.
2. The inclusion of HSA and PTF1 into urinary and inorganic COM crystals is anisotropic.
3. The anisotropy results from preferential binding of HSA and PTF1 to specific COM faces.
4. The binding affinity of HSA for individual COM surfaces differs in urine and inorganic solutions.
5. Preferential binding of PTF1 to the apical faces of COM cannot be explained by interactions between Gla groups and surface atomic arrays: other factors such as step structures and heights must play a role.
6. Studies of interactions between proteins and crystal surfaces should be performed in urine.

OP-035 Mechanism of formation of concentrically laminated spherules

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Introduction: Randall's plaque has been implicated as playing an important role in idiopathic calcium oxalate stone (CaOx) formation. As originally described by Randall [1], and further examined in more detail by Evan and co-workers [2], this pathological mineralization consists of calcium phosphate (CaP) particles that appear to form first at the basement membrane of the thin loops of Henle in the form of multi-laminated spheres. Ryall [3] has presented an interesting treatise on the striking similarity between these multi-laminated spherules found as the nidus of stone formation, to the storage granules found in a wide variety of biomineralizing organism, and has suggested that valuable insight might be gained by studying the mechanisms that organisms use to control such mineral deposits. This in turn may provide information on the lack of control that seemingly occurs in urolithiasis, and hopefully lead to new diagnostic and treatment options.
Objectives: Rather than searching for animal models that form calcifying granules, we have developed an in vitro model system that can provide mechanistic information on the formation of such multi-laminated mineral morphologies. The objective of these studies is to provide further insight into the materials chemistry involved in stone formation.
Methods: Our work has focused on a non-classical crystallization process in which acidic peptides mimicking the acidic proteins associated with biominerals (both pathological and controlled) are used to induce an amorphous precursor to the mineral phase. When the amorphous phase is highly hydrated, it can have fluidic character, leading to nanoscopic droplets that agglomerate and coalesce into spherulitic morphologies [4]. We have examined calcium phosphate, oxalate, and carbonate, using this in vitro model system, and have found that this polymer-induced liquid-precursor (PILP) process is operative for all of these calcifying systems.
Results: Using fluorescently labeled polymer, we find that the concentric laminations in our biomimetic mineral spherules are the result of diffusion-limited exclusion of the polymeric impurity as the precursor undergoes the amorphous-to-crystalline transformation. A nanogranular texture is also observed in crystals formed by the PILP

process, emulating the nanoscale features seen on urinary CaOx crystals [3].
Conclusion: These results suggest that the similarities observed in the multi-laminated spherule morphologies seen in a variety of organisms lies not with specific biological control mechanisms, but rather arises from non-specific processes associated with the basic materials chemistry involved in mineralization via an amorphous precursor, and particularly in the presence of acidic proteins and/or organic matrix, which very often induce a liquid or gel-like amorphous precursor phase.

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OP-036 A comparison of the complete intracrystalline protein profiles of urinary calcium oxalate monohydrate and dihydrate crystals

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Introduction: The prevalence of calcium oxalate (CaOx) monohydrate (COM) crystals in stones, and CaOx dihydrate (COD) crystals in urine, has prompted the hypothesis that preferential formation of COD protects against urolithiasis. Both polymorphs contain intracrystalline proteins, which in other biomineralization systems are well known to influence the texture and physical properties of their mineral hosts. Intracrystalline proteins in COM and COD crystals could thus determine the role that urinary proteins play in the development or prevention of kidney stones.
Objectives: To establish a comprehensive 2-D profile of proteins incorporated into urinary COM and COD crystals.
Methods: Crystals were precipitated from the same pooled healthy urine sample by addition of sodium oxalate, after adjusting the Ca concentration to 2 and 8 mM, respectively. After washing exhaustively with water, they were demineralised in 0.25 M EDTA and dialysed against 150 mM NaCl for 3 days with twice daily changes of the saline solution to remove residual EDTA. The extract was further dialysed against sequentially weaker saline solutions for 2 days, and finally, distilled water. Proteins were isoelectrically focused onto 24 cm, pH 3–11 strips overnight and separated on 12.5% SDS-PAGE gels. Protein spots were visualised with Eriochrome black-T silver stain and compared using ImageMasterTM.
Results: More than 120 spots were visible in the COM extracts, many of which were charge variants of the same protein. Proteins were distributed evenly throughout the entire Mr ($\sim$20–100 kDa) and pI (3–11) range. The predominant proteins were prothrombin fragment 1 (seen as 6 major charge variants at $\sim$30 kDa), serum albumin, and an unidentified protein migrating at $\sim$25 kDa and pI-4, none of which were detected in the COD extract.
Approximately 80 spots were seen in the COD extract, migrating from $<$ 10 to 75 kDa, with pI values between 3 and 6. The majority had Mr values $<$25 kDa and several heavily staining spots were seen at Mr $\sim$ 10 kDa. OPN, which was not visible in the COM crystal extract, was observed as a range of Mr and charge variants at pI $\sim$ 4–6. No proteins with pI $>$ $\sim$9.8 were observed.

The identities of the dominant spots are currently being confirmed using mass spectrometry.
Conclusion:

1. The 2-dimensional patterns of the intracrystalline urinary proteins in COM and COD crystals differed markedly.
2. Proteins associated with COD crystals tended to be fewer, smaller and more acidic than those from COM.
3. No single protein was associated with both crystal habits.
4. Differences in the intracrystalline protein content of COM and COD crystals may explain the disparate predominance of the two polymorphs in urine and stones, and thus, the likelihood of stone formation.

OP-037 Calcium oxalate monohydrate aggregation; a polymer aggregation problem?

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Introduction: Kidney stones most frequently form as aggregates of calcium oxalate monohydrate (COM) crystals with organic layers between them, and the organic layers contain principally proteins. The pathway leading to the formation of these crystal aggregates in affected people has not been identified, but it has long been suspected that stone forming patients have a defect in the structure or distribution of their urinary proteins, which circumvents the normally protective affects of typical urine proteins.
Objectives: To test the hypothesis that COM crystal aggregation is induced through interactions with urinary proteins, two possible protein models were studied. First, mixtures of polyanionic and polycationic proteins, which were shown to induce COM aggregation [1], were studied in greater detail. Second, a protein model with reduced charge was tested for protein aggregation and COM aggregation.
Methods: Polyelectrolyte (protein) aggregation and COM crystal aggregation were both determined by particle size distribution (PSD) measurements with an Accusizer 780 (Particle Sizing Systems, USA). Crystal aggregation was characterized by the ratio (RD) of resultant weight averaged diameter (DW) to the initial DW for the COM seed PSD, after stirring for 1 h in a buffer solution with the protein or mixture to be tested. The typical buffer contained 150 mM NaCl, 10 mM HEPES (at pH = 7.5), and 0.25 mM CaCl2 and 0.25 mM Na2C2O4; corresponding to a supersaturation, s = 1.1. Tests were also performed at low salt (no added NaCl) and/or low pH (pH = 5.5; sodium acetate buffer). Native Tamm-Horsfall Protein (n-THP) was isolated by salt precipitation from normal urine. A reduced charge version of THP was obtained by treatment with neuraminidase to remove sialic acid residues (ds-THP).
Results: With no protein added, the COM aggregation experiment yielded RD = 1.06 $\pm$ 0.10; statistically no change in size. Polyanions (pD and pE) as individual components caused a 10–15% decrease in RD, and polycations (pR and pK) demonstrated a 5–10% reduction in RD. However, mixtures of polycations and polyanions at charge ratios close to 1:1 promoted COM crystal aggregation in a concentration dependent manner, with RD $>$ 2 above the cloud point (lowest concentration with visible protein aggregate formation), except for mixtures containing pE as the polyanion. Non-stoichiometric

mixtures of polycations and polyanions were much less effective at increasing RD. The cloud point concentration was roughly equal for different combinations of polycations and polyanions, but about tenfold higher polymer concentrations were required in low salt buffer. In the second model system, n-THP showed no polymer aggregate formation and reduced RD in the COM aggregation experiment, while ds-THP showed polymer aggregation at concentrations >5nM, which promoted COM aggregation. Maximal COM aggregation with was observed at a specific ratio of COM seed to polymer aggregate. Both polymer and crystal aggregation were substantially reduced for ds-THP at low salt concentrations.
Conclusion: Both models demonstrated polymer aggregation at solution conditions where COM aggregation was observed. Correlation with data from other bulk crystallization measurements suggest that the anionic side chains form critical binding interactions with COM surfaces that are necessary, in conjunction with the polymer aggregation, to induce COM aggregation.

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OP-038 Evaluation of urinary oxalate levels in patients receiving gastrointestinal lipase inhibitors

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Objectives: This study aimed to examine the possible effects of a gastrointestinal lipase inhibitor (Orlistat; Xenical) on the intestinal absorption of oxalate, and therefore on the urinary levels of oxalate excretion in obese patients.
Methods: Long-term follow-up data of 95 patients (57 males, 38 females; M/F:1/5) were documented. Patients were randomly assigned into two groups. While the patients in Group I ($n = 55$) were treated with Orlistat (Xenical), the patients in Group II ($n = 40$) received no specific medication. Urinary calcium, oxalate, and citrate levels were detected for 24 h in the collected urine by using the enzymatic spectrophotometric method. To evaluate the significance in each group, as well as the differences between the two groups, the ANOVA test was used, and the results were given as mean ± SD.

Results: Comparative evaluation of urinary oxalate levels during the 3-month follow-up clearly showed that urinary oxalate excretion significantly increased in 34/55 patients (61.8%) in the first group ($P < 0.05$). Of these patients, 30/34 (88.2%) continued to have increased urinary oxalate excretion during the 6-month follow-up ($P = 0.001$). However, the data generated by the authors did not show any significant effect of this medication on urinary citrate and calcium levels during the 3- and 6-month follow-up evaluation ($P = 0.05$).
Conclusion: The results suggest that increased intestinal absorption of dietary oxalate due to this type of medication in obese patients could make a substantial contribution to urinary oxalate excretion, and may increase the risk of stone formation.

OP-039 Oral therapy with ALTU-242 reduces hyperuricemia and hyperuricosuria in mice lacking urate oxidase (Uox−/−)

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Introduction: Elevated plasma uric acid, hyperuricemia, has been increasing in Western countries over the last decade and correlates well with an increase in prevalence of renal disease, gout, hypertension and metabolic syndrome. It occurs either as a result of excessive urate production or decrease in renal excretion of uric acid or both. Standard urate lowering therapies have limited effectiveness and are not always well tolerated.
Objectives: We posited a new approach for treatment of hyperuricemia, and tested it with the oral use of a modified urate-specific enzyme (ALTU-242), that is stable and active in the pH- and protease-challenging environment of the intestine. We tested its efficacy on reduction of plasma and urinary urate in uricase deficient mice (Uox−/−), a model with severe hyperuricemia and urate nephropathy. We hypothesized that a stable and active urate-specific enzyme will reduce the body pool of urate by degrading intestinal urate and promoting a blood to lumen transepithelial gradient that will enhance enteric excretion and thereby reduce plasma urate levels.
Methods: In the first experiment, three different doses of ALTU-242 (5, 50, and 200 mg/day, $n = 7$) or placebo were mixed with the food and administered to Uox−/− mice for three weeks. All mice were kept on allopurinol (10 mg/dL) during the breeding and post-weaning periods. To increase the severity of disease, allopurinol was removed one week before the study. In the second experiment, the efficacy of ALTU-242 or placebo was monitored for 15 days in mice that had been treated with allopurinol continuously until being switched to ALTU-242 (200 mg/day, $n = 7$).
Results: Hyperuricemia and uricosuria were reduced considerably, upon daily oral administration of ALTU-242, when compared to untreated controls in both experiments.
In the first study, mice fed 200 mg of ALTU-242 for 3 weeks had a mean overall reduction in urinary urate of 66% (2.45 ± 0.77 mg/18 h vs. 7.1 ± 0.49 mg/18 h, $P < 0.05$) and plasma urate was reduced 26% (6.19 ± 0.68 mg/dL vs. 8.35 ± 0.68 mg/dL, $p < 0.05$). Using the same model we tested two doses of ALTU-242 (50 and 5 mg/day) for 3 weeks. Compared with the untreated controls, we again demonstrated a constant reduction in uricosuria of 46% with the 50 mg dose (3.59 ± 0.74 mg/24 h vs. 6.63 ± 1.15 mg/24 h, $P < 0.05$) while the lower dose had minimal or no effect, implying specificity of the drug action. In the second experiment, when the mice were switched from allopurinol treatment immediately to oral treatment with a 200 mg dose of ALTU-242 for 15 days, reduction in hyperuricemia was even more significant compared to placebo than in the first study. Plasma urate was reduced 52% (4.86 ± 0.55 vs. 10.06 ± 1.23 mg/dL), compared to control mice. Similarly, the mean overall reduction in urinary urate was 76% compared to untreated mice ($P < 0.05$). Further studies will be necessary to better understand the mechanism of ALTU-242 action on the metabolism of uric acid.
Conclusion: Based on these initial studies demonstrating efficacy, ALTU-242 has promising potential as a new oral agent for treatment of hyperuricemia, hyperuricosuria, and related diseases.

OP-040 Hyperoxaluria regardless of cause is reduced and nephrocalcinosis prevented with crystalline oxalate degrading enzyme in animal models

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Introduction: Hyperoxaluria is a major risk factor for recurrent urolithiasis and progressive nephrocalcinosis. Present therapies to reduce hyperoxaluria in patients with primary (PH) and enteric hyperoxaluria (EH) are limited and may not forestall disease progression.
Objectives: We tested an oral therapy with a crystalline, cross-linked formulation of oxalate decarboxylase (ALTU-237) on the reduction of urinary oxalate and decrease in the severity of kidney injury in four animal models. We used two animal models for EH; diet induced hyperoxaluria rats (EH rats) (diet supplemented with 1.1% potassium oxalate and 0.5% calcium) and SCL26a null mice (PAT1 mice), where ileal oxalate absorption is increased due to deficiency of Cl–oxalate exchanger (diet supplemented with 0.5% potassium oxalate and 0.8% calcium) and in two mouse models for PH: AGT1KO mice, where hyperoxaluria is a result of altered glyoxylate metabolism due to absence of the hepatic AGT1 gene and in AGT1KO mice challenged with ethylene glycol (EG-AGT1KO).
Methods: Four different doses of ALTU-237 (5, 25, 80 and 200 mg/day, $n = 5$–7) or placebo mixed with the food were tested in 4 different models: EH rats, PAT1 mice and AGT1KO and EG-AGT1KO mice, for 16–90 days.
Results: Daily oral treatment with ALTU-237 continually and significantly reduced urinary oxalate in all four models compared to corresponding controls. Initially, we demonstrated in the EH model, during a 90-day trial, a mean overall reduction in urinary oxalate of 40–60% (from 11.43 ± 1.01 mmol/24 h to 6.9 ± 0.67 and $6.146.9 \pm 0.56$ mmol/24 h, respectively, $n = 6$, $P < 0.05$) when rats were fed with 80 mg and 200 mg dose. In the same model, we tested three doses of ALTU-237 (5, 25, 80 mg/day, $n = 5$) and again demonstrated a constant and dose-dependent reduction in urinary oxalate of 15–40% over a one month period. In addition, we showed that 200 mg daily dose of ALTU-237 prevented hyperabsorption of oxalate and minimized nephrocalcinosis in PAT1 mice when fed diet with 0.5% oxalate and 0.8% calcium (2.80 ± 0.7 mmol/24 h vs. 1.17 ± 0.35 mmol/24 h, $P < 0.05$) during 23 days. Thus, daily oral treatment with ALTU-237 significantly lowered urinary oxalate in both models where hyperoxaluria was solely resulting from increased absorption of oxalate from dietary source.
Furthermore, ALTU-237 treatment in EG-AGT1KO mice (PH1 model), where hyperoxaluria is result of endogenously increased oxalate, was coincident with reduction in urinary oxalate of 40% or more. When mice were fed with the dose of 80 mg/day, this resulted in complete prevention of nephrocalcinosis and urolithiasis, and also resulted in 100% animal survival. Also, 200 mg of ALTU-237 in AGT1KO mice reduced both urinary (44%) and fecal oxalate (72%) when compared to controls implying that stable crystals of oxalate decarboxylase both degraded intraluminal oxalate and promoted enteric excretion and further elimination of oxalate from circulation. Histopathology analysis of the gut confirmed that oral therapy did not produce any structural or morphological changes.
Conclusion: These data demonstrate in four animal models of hyperoxaluria resembling human disease, that oral therapy with crystalline oxalate decarboxylase reduces hyperoxaluria in a sustained and dose dependent manner and prevent calcium oxalate nephrocalcinosis and urolithiasis. Thus, ALTU-237, which is presently being studied in humans, can present a realistic option for the treatment of human hyperoxaluria independent of cause.

PP-041 Investigation of the effects of a Chinese herbal preparation on calcium oxalate crystallization in synthetic urine

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Introduction: Herbal remedies have been found to be useful in the treatment of urinary stone disease in animal models, with fewer side effects than contemporary Western medicine [1]. These may therefore be suitable for long-term use in humans.
Objectives: This study was undertaken to investigate the effect of two Chinese herbs, *Shi Wei* (*Folium Pyrrosiae*) and *Jin Qian Cao* (*Desmodium Styracifolium Merr.*) on calcium oxalate (CaOx) crystallization in synthetic urine (SU).
Methods: Separate stock solutions of Shi Wei and Jin Qian Cao were prepared by dissolving these herbs in distilled water (0.15 g/25 mL). The solutions were refluxed for 15 min and micro-filtered. SU was made according to Walton et al. [2]. Crystallization experiments were profound in synthetic urines alone and then dosed with each of the two stock solutions (5%(v/v)). The CaOx metastable limits (MSL) were measured. The 3 samples were dosed with sodium oxalate 30 μM above their respective MSL and particle size distributions were determined using a Coulter Counter [3]. Crystallization kinetics were measured by determining nucleation [4], aggregation [4] and growth rates [5] of CaOx crystals. All experiments were performed in triplicate.
Results: The metastable limit of SU (105 μM) was increased on addition of *Shi Wei* and *Jin Qian Cao* (both were 120 μM). *Shi Wei* and *Jin Qian Cao* induced a decrease in the average particle size of CaOx crystals. The results of crystallization kinetics experiments are summarized in Table 1.

Table 1 Inhibitory activity data for Shi Wei and Jin Qian Cao

	Nucleation inhibition (%)	Aggregation inhibition (%)	Growth inhibition (%)
SU	31.8	−376	35.1
SU + *Shi Wei*	40.8	−373	31.8
SU + *Jin Qian Cao*	38.9	−237	56.1

The rates of crystal nucleation, aggregation and growth decreased in all experiments. Overall there was an increase in the inhibition of nucleation and growth but a promotion of aggregation. Both herb additives caused a greater inhibition of crystal nucleation than SU alone. *Jin Qian Cao* showed a significantly lesser effect on promoting aggregation than *Shi Wei*. *Jin Qian Cao* also demonstrated a greater inhibitory effect on crystal growth relative to the other two.
Conclusion: Both Chinese herbs demonstrated inhibition of CaOx crystallization, but *Jin Qian Cao* was more efficacious than *Shi Wei*. Thus, these herbs might have the capacity for therapeutic action in the management of CaOx urolithiasis. However, studies of their effects in real urine are needed prior to embarking on a clinical trial.

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PP-042 MALDI-TOF mass spectrometry and MicroBCA protein assay: a new combined approach for urinary analysis of medium size peptides in calcium oxalate lithiasis

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Introduction: Urinary lithiasis is one of the most frequent benign urological diseases. The risk of developing urinary stones throughout the life is 15%. The recurrence is 50% in the first 5–10 years, 75% in 20 years. The exact series of events that lead from urinary supersaturation to the crystals until the stone is not well defined. There are not evidences on how the proteins and the peptides work in physiopathologic pathways, however it is likely that urinary proteins and peptides are critical mediators of crystallization process. We have not a marker of disease: it is not possible to identify the subjects predisposed to the development of lithiasis, nor to its recurrence after treatment.
Objectives: Our experience proposes to analyse the urinary proteic composition of patients affected by urinary calcium oxalate lithiasis, in order to isolate, ultimately, a biomarker or a proteic predisposing factor.
Methods: We have collected urine samples of 17 patients (11 male, 6 female; mean age (years) = 45 ± 14 SD) affected by urinary calcium oxalate lithiasis; 17 controls have also been enrolled, matched close to age and sex with patients. The samples were analysed by a proteomic approach using a MALDI-TOF mass spectrometer, in a range between 1000 and 5000 Da (medium size peptides). By this procedure, we try to find significant qualitative differences in urinary proteome of the two populations analysed. We have also performed a subsequent numerical analysis (quantitative) on the urinary samples, always in the same mass spectrum, using specific filters and MicroBCA (Bicinchoninic Acid) Protein Assay.
Results: Overlapping the medium mass spectrum of the patients and that of controls, there are no differences, since in both graphs all the peaks correspond perfectly. The mass spectrum of the controls are also characterized by comparable values of peaks intensity than those of patients. Subsequent numerical analysis (quantitative), using filters limiting weight to 5000 Da and MicroBCA protein assay, suggests that could exist higher concentrations of proteic species in control samples in respect to patients suffering from urinary stones. The MALDI–TOF is not able to evidence protein concentration differences but on the basis of BCA analysis we can say that patients are characterized by a lower concentration of urinary peptides in the range mentioned above, compared to the controls. This difference is statistically significant (Mann-Withney Test; alpha = 0.05; 95% CI; $P < 0.005$).
Conclusion: The results of the study in the range of medium size peptides, have not detected a urinary biomarker or a predisposing protein factor in "stone formers" patients compared to the controls. The evaluation of the results obtained, as regards the quantitative differences, lead to further research.

PP-043 Investigation of the effect of a solution of lime powder on calcium oxalate crystallization in synthetic urine: preliminary results

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Introduction: Citrus fruits and juices are a known natural source of dietary citrate which is one of the major inhibitors of stone forming calcium salts [1]. Lime contains high amounts of citrate, potassium and other beneficial antioxidants [2]. It might be of great interest in the context of urolithiasis research.
Objectives: In this study we investigated the effect of a solution of the citrus fruit lime on calcium oxalate crystallization in synthetic urine.
Methods: Lime powder (LP) prepared from concentrated lime juice, was used to prepare solutions with four different concentrations (0.125–1.00 mg/ml) in distilled water. The effect of LP on CaOx crystallization was tested in synthetic urine. Crystallization experiments included measuring the CaOx metastable limit, particle volume-size distribution, nucleation, aggregation and growth assays. All the experiments were performed in duplicate.
Results: The CaOx metastable limit was found to be the same after the addition of different concentrations of lime. Scanning electron microscopy showed that there were more crystals with increasing concentration of lime solutions but that they were proportionally smaller. CaOx crystal aggregation was inhibited whereas nucleation was promoted in the presence of lime powder.
Conclusion: Lime promoted the nucleation of calcium oxalate crystals, increasing their number but decreasing their size. Furthermore, it inhibited calcium oxalate aggregation. These results are promising as they suggest that lime might be an effective therapeutic agent for the treatment of calcium oxalate urolithiasis. However, additional studies using real urine in both in vitro and in vivo investigations should be conducted to confirm this preliminary conclusion.

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PP-044 Osteopontin has a crucial role in the ultrastructural conversion of calcium oxalate crystals into matrix-involving kidney stones

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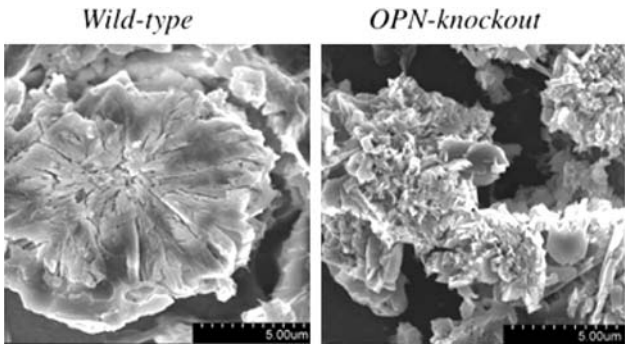
Introduction: The starting point of kidney stone formation is the conversion of retained intratubular crystals into concrete stones.
We previously reported a strong expression of OPN on renal tubular cells in the stone-forming kidney, suggesting that OPN plays important roles in crystal–cell interaction and stone formation.
Objectives: In the present study, we analyzed pathological and ultrastructural differences of generated kidney crystal deposition, and determined the role of OPN in the morphological changes of calcium oxalate crystals using OPN knockout mice.
Methods: Saline and glyoxylate (80 or 100 mg/kg) were intraabdominally injected into wild-type mice (WT) and OPN knockout mice (KO) for a week, and kidneys and 24 h urine samples were taken on day 7 after the initiation of glyoxylate administration. OPN expression was detected by quantitative RT-PCR, in situ hybridization and immunohistochemical staining. Urine biochemistry was determined from the 24 h urine samples. Kidney crystal formation was estimated by light microscopy and morphological observation was performed by polarized light optical microphotography and scanning electron microphotography. Crystal components were detected by X-ray diffraction.

Results: Urine oxalate excretion showed no significant difference between WT and KO. Kidney crystal depositions were detected in WT administered with 80 and 100 mg/kg glyoxylate and KO administered 100 mg/kg glyoxylate, but there were no crystals in KO with 80 mg/kg administration. The number of kidney crystals generated in the kidney of KO administered 100 mg/kg glyoxylate was significantly fewer than that of WT administered 100 mg/kg glyoxylate. OPN expression was detected in WT mouse kidney, but not KO kidneys, and OPN protein was contained in WT crystals by observation of immunohistochemical staining. Polarized light optical microphotography and SEM showed large rosette-shaped crystals growing in renal tubules in WT, whereas small and disorderly scattered crystals in KO. The crystal component of both genotypes was calcium oxalate monohydrate.



Conclusion: OPN plays a crucial role in morphological conversion of CaOx crystals into stones in mouse kidneys. This study provides important information that the control of OPN expression may be a target for inhibition of stone formation in the future.

PP-045 The mechanism of calcium oxalate urolithiasis formation

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Introduction: The epidemiologic prevalence of pediatric urolithiasis is very low compared with that of adult urolithiasis in the industrialized countries such as North America and Europe as well as Japan. Therefore, we have thought that there are some findings concerning the mechanism of calcium oxalate urolithiasis formation in the pediatric urine.
Objectives: Previously we have demonstrated that pediatric urinary macromolecules (UMMs) are stronger inhibitors than those of adults of calcium oxalate (CaOx) aggregation and adhesion to renal tubular cell of CaOx crystal. To investigate the CaOx urolithiasis formation mechanism, we evaluated the difference in the inhibitory activity against the renal tubular epithelial cell injury by oxidative stress of oxalate between pediatric and adult UMMs. Then the difference of the component in UMMs, especially proteins and glycosaminoglycans (GAGs), was analyzed between each lineage.
Methods: Urine samples were collected from boys and their fathers during a 24-h period, and the parameters related to urolithiasis were measured. Urinary components with a molecular weight of 3 kDa or greater were extracted as UMMs. The protective effect of UMMs for Madin–Darby canine kidney (MDCK) cell injury under exposure to oxalate was examined by measuring lactate dehydrogenase (LDH). Furthermore, the immuno-histochemical staining for assessment of apoptosis and oxidative stress was performed. The GAGs content was measured using the modified dimethylmethylene-blue (DMB) assay. Using Western blotting, we studied variations in the electrophoretic mobility patterns and relative abundances of proteins concerning stone formation such as Tamm–Horsfall glycoprotein, albumin, urinary prothrombin fragment 1 and osteopontin.
Results: Although the dose of uric acid, oxalate, magnesium and the amount of protein in the urine of children were significantly higher than in adults, there was no significant difference between children and adults in both NAG (*N*-acetyl-beta-D-glucosaminidase) and 8-OHdG (8-hydroxy-2-fdeoxyguanosine). The dose of UMMs was significantly higher in children. UMMs inhibited the cell injury by oxalate in concentration dependence, which has stronger action in children than in adults. TUNEL staining revealed that the level of apoptosis was suppressed using UMMs of children than of adults. Moreover, 8-OHdG immuno-histochemical staining revealed that the inhibition of oxidative stress was stronger using UMMs of children. Although there was no significant difference between children and adults in protein concentrations of UMMs, the dose of GAGs retarding CaOx crystallization was significantly higher in children. The expression of osteopontin was stronger in adults between each lineage by the result of Western blotting.
Conclusion: We speculated that the low incidence of pediatric urolithiasis was additionally led with a current result that pediatric UMMs potentially inhibited both apoptosis and oxidative stress which induced renal injury. Pediatric UMMs had stronger inhibition in all phases of CaOx stone formation. In the difference of both, pediatric UMMs had significant higher concentration of GAGs and adult UMMs had stronger expression of osteopontin as a matrix protein in the formation of calcium-containing stones.

PP-046 Inhibition of crystallisation by extract of *Tamarix gallica* L.

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Introduction: Medicinal plants are traditionally used for treating renal problems, particularly *Tamarix gallica* L. which is found in the Sahara region of Algeria.
Objective: In our study, we investigated the inhibitory effect of the extract of the *Tamarix gallica* L. on the crystallisation of calcium oxalate in vitro.
Methods: The extract of *Tamarix gallica*L. is obtained using a Soxhlet apparatus for 48 h. Our study of the crystallisation of calcium oxalate is based on the model turbidimetric (Wavelength 620 at 37°C) by means of a spectrophotometer (Beckman Ultra violet visible UV/Vis).The formation of calcium oxalate is induced by the addition of the oxalate and calcium solutions. The addition of inhibitor at different concentrations enabled us to obtain the percentage of inhibition caused by the extract.
Results: It was found that the percentage of inhibition augment is maximal at high concentrations of extract. The variation of the absorbance decreases gradually with increase in the concentration of the inhibitor; the variation reaches a minimum at the highest concentration of inhibitor.
Conclusion: We conclude that the extract of *Tamarix gallica* L. inhibits the formation of calcium oxalate crystals and therefore may inhibit calcium oxalate dihydrate (COD) urinary lithiasis. The inhibition is due to the presence of acidic compounds. The calcium ions ($Ca^{+\ 2}$) present in the calcium oxalate solution can form a complex with these acids and so reduce the risk of forming calcium oxalate crystals.

PP-047 Why some plant extracts prevent stone formation?

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Introduction: Since ancient times a variety of single and combined herbal preparations have been used with supposed success in renal lithiasis therapy. Conclusive scientific data on the exact clinical role and efficacy of these herbs remains to be determined. Recently, some antioxidant capacity has been potentially attributed to *Trigonella foenum-graecum* L. seeds, supporting folk information regarding antilithiasic activity of the plant.
Objectives: The aim of the present communication is to evaluate the antilithiasic capacity of a traditional Mallorcan herbal preparation and to compare the obtained results with those corresponding to two typical antioxidant flavonoids such as catechin and epicatechin.
Methods: Thirty six male Wistar rats were used. The animals were assigned to four groups (n = 9). Rats of the CTR-group were not treated and were used as controls, CAT-group was treated with drinking water supplemented with 100 mg/L catechin, EPI- group with drinking water supplemented with 100 mg/L epicatechin, and FHE- group with drinking water supplemented with 7 ml/L of a folk herbal extract with assigned antilithiasic activity very popular in the Balearic Islands. The composition of such herbal extract is: fluid extract of *Arctotaphylos uva-ursi* L. (2.16%), Fluid extract of *Zea mays* L. (2.16%) tincture of *Sabal serrulata* L. (21.5%), tincture mother of *Agathosma betulina* L. (17.5%), fluid extract of *Ricinus zanzibariensis* L. (46.48%), glycerine (10%), anise essence (0.2%) (Farmacia Salva Trobat, Palma de Mallorca, Spain). After 16 days of treatment period, calcium oxalate lithiasis was induced during 8 days, by adding 0.8% V/V ethylene glycol (EG) plus 1% W/V NH_4Cl to drinking water of each group. On the last day of the experiment, 24 h rat urine was collected by metabolic cages. Finally, the animals were sacrificed and their kidneys removed for histological and mineral evaluation.
Results: The calcium concentration in the kidney tissue showed a significant decrease in the CAT-group (2.35 ± 0.34 mg/g), EPI-group (1.76 ± 0.29 mg/g) and FHE- group (2.04 ± 0.27 mg/g) versus CTR-group (5.38 ± 1.39 mg/g); nevertheless no significant differences between the three treated groups (CAT-group, EPI- group and FHE- group) were detected. Examination of kidney paraffin sections showed that CTR-group rats had the greatest amount of calcification. Main urinary biochemical data (Ca, Mg, P, oxalate and citrate) presented no significant differences between treated and untreated groups.
Conclusion: Studies on experimental EG renal lithiasis appeared in the 1960s but the importance of the oxidative damage caused by hyperoxaluria was not proposed until the end of the century. The present study clearly recognized preventive action of antioxidant flavonoids and the results were compared with those obtained using a folk herbal extract that also contained antioxidants. As in previous studies, the medicinal plants used had little effect on urinary chemistry of urolithiasis. In fact the consumption of catechin or epicatechin did not cause any effect on urinary chemistry. Nevertheless, when EG rats were treated with the folk herbal extract, the calcium deposits in the kidney were significantly reduced, being the obtained results similar to those obtained when EG rats were treated with antioxidants as catechin and epicatechin. There results clearly demonstrate the ability of antioxidants to prevent the development of papillary and renal parenchymal calcifications on the kidney, consequently preventing the development of papillary and parenchymal calculi.

PP-048 Significance of the Bonn Risk Index (BRI) for metabolic monitoring of patients with calcium oxalate urolithiasis: a clinical use study of the UROLIZER

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Introduction: To date, clinical-chemical parameters to determine the individual metabolic risk of calcium oxalate (CaOx) urolithiasis have often been connected with high analysis costs and insufficient diagnostic selectivity.
Objectives: The aim of the present study was to investigate the diagnostic relevance of the Bonn Risk Index (BRI), a new method to determine the urinary CaOx crystallisation risk.
Methods: A group of 39 recurrent CaOx stone patients was studied. Measurement was made of significant lithogenic parameters (volume, pH, density, calcium, oxalic acid, citrate, magnesium, uric acid and phosphate) and com pared with UROLIZER^{TM} readings (BRI, $[Ca^{2+}]$, pH) from urine samples collected over 24 h in patients with and without metaphylaxis measures to prevent stone formation. For precision, repeat tests (each $n = 6$) were carried out on 34 urine samples of BRI and $[Ca^{2+}]$. Calculation of relative supersaturation (RSS [EQUIL 2]) and activity product (AP according to TISELIUS) were made on 24 h urine samples. Two treatment groups were studied: alkaline citrates (Alk) $n = 20$ with Ca 5–8 mmol/day, hydrochlorothiazides (HCT) n = 19 with Ca > 8 mmol/d; they were studied after three months. Median comparison with Mann-Whitney U Test; Spearman correlation.
Results: The study showed a high precision for the UROLIZER^{TM} measurements: Variation coefficients for BRI in the range of 0.5–5/l was 10%, for $[Ca^{2+}]$ in the range of 0.2–1.5 mmol/l, 5%. The mean BRI fell by 10%, respectively, 4% after 24 h storage (24°C/4°C). There was a high correlation: BRI to AP $r = 0.720$ ($P < 0.01$); BRI to RSS r = 0.730 (p < 0.01). Clear reduction of BRI (1/l) during metaphylaxis after 3 months—$Alk_{without}$ 1.4 vs. Alk_{with} 0.8 ($P < 0.05$) (Table 1); $HCT_{without}$ 3.2 vs. HCT_{with} 1.5 ($P < 0.01$).
Conclusion: Our test results justify the assertion that in a urological outpatient practice, the UROLIZER^{TM} shows high analytical reliability in diagnosing the metabolic risk of CaOx stone formation in the individual patient's urine. It reflects the effect of metaphylaxis and improves patient compliance. The innovative analysis device is especially suited for metabolically oriented "urolithiasis centres", which work closely with urological practices in their respective areas.
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Table 1 Values of the UROLIZER and citrate during metaphylaxis with alkaline citrates (Alk)

urine parameter (24-h urine)	median without *Alk*	median with *Alk*
BRI (1/l)	1.4	0.8
$[Ca^{2+}]$ (mmol/l)	0.60	0.47
Citrate (mmol/d)	1.95	3.85
pH	5.9	6.7

PP-049 Effects of diuretic herbs on urinary stone risk factors

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Introduction: Phytotherapy is an alternative management for urinary stone disease. According to the Thai traditional medicine, *Hibiscus sabdariffa* (HS) and *Phyllanthus amarus* (PA) are known as diuretic herbs and claimed to be effective in the stone treatment [1].
Objectives: The objective of this study was to evaluate the effects of these two herbs on serum and urinary parameters related with urinary stone risk factors.
Methods: Two groups of male subjects, i.e., 9 non-stone (49.3 ± 10.5 years old) and 9 stone (54.8 ± 8.7 years old) formers participated in the study protocol. The subjects were assigned to take a cup of tea (250 ml made from 1.5 g dried herb) twice daily, for a period of two weeks for each herb (with one week washout period between the taking of each herb). Heparinized blood and 24-h urine samples were collected from each subject both before and after the intake of each herbal tea and analyzed for serum electrolytes and urinary pH and excretions of creatinine, calcium, potassium, sodium, magnesium, phosphate, uric acid, citrate and oxalate.
Results: While the intake of HS tea did not affect on any serum parameters, the PA tea caused the increase of creatinine in both groups ($P < 0.05$). For urinary parameters, the intake of HS tea caused the increase in both uric acid excretion and uric acid clearance but significant values were detected only in the stone forming group ($P < 0.02$ and $P < 0.018$, respectively). While the intake of PA tea caused only slightly increase in urinary output, it had only little effect on most urinary parameters.
Conclusion: In conclusion, the intake of the two diuretic herbs did not reveal any prominent prophylactic effect with regard to the changes in urinary stone risk factors, Moreover, the increase in urinary excretion of uric aid after the intake of HS tea should be considered as a risk factor.

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PP-050 Drug dosage protocol for treatment of calcium oxalate stones

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Introduction: Several drugs are now available in the market for the medical management of urinary stone disease. However, most of these have not stood the test of time. Patient compliance and therapeutic efficacy are the hallmarks of effective medical management. In earlier studies, we have confirmed that in most patients with calcium oxalate stone formation, a combination of allopurinol and pyridoxine is best suited for the treatment and prevention of the stone forming process.
Objectives: The objective of this study is to identify the most effective patient-friendly and least toxic dosage schedule for the medical management of urinary stones. The process of initiating and sustaining dosage schedules for short-term chemotherapy and long-term prophylaxis in patients with urolithiasis was based on clinical, radiological, biochemical and microscopic parameters.
Methods: A total of 444 patients with proven calcium oxalate stone disease who had been receiving a combination of allopurinol and pyridoxine for a minimum period of three years were enrolled in this prospective study. The dosage schedule of these patients was recorded for three years. Dosage adjustment was made depending upon the various clinical, biochemical, microscopic and radiological changes during the study period. The dosage schedules were divided into six categories, namely Very High Dose Chemotherapy (VHDC), i.e., Allopurinol 600 mg/day and Pyridoxine 240 mg/day, High Dose Chemotherapy (HDC), i.e., Allopurinol 300 mg/day and Pyridoxine 120 mg/day, Moderate Dose Prophylaxis (MDP), i.e., Allopurinol 200 mg/day and Pyridoxine 80 mg/day, Low dose Prophylaxis (LDP). Allopurinol 100 mg/day and Pyridoxine 40 mg/day and Very Low dose Prophylaxis (VLDP), i.e., Allopurinol 50 mg/day and Pyridoxine 20 mg/day and intermittent VLDP, wherein the VLDP was given on alternate months and still later at longerr intervals. The temporary risk was assessed at each visit and dosage adjustment made. The effect of the intervention was assessed during the next visit.
Results: All the patients involved in the study required adjustment of their dosages. The following schedules were initiated—VHDC—(12) 3.5%, HDC—(103) 23.2%, MDP—(78) 17.57% or LDP—(251) 56.53%. Patients who defaulted for more than a month were excluded from the study. During each visit for follow up, all patients were advised change over of dose depending upon the clinical situation at the time of review. Patients on VHDC were advised reduction to lower doses systematically. On passage of stones, the dose was immediately reduced to LDP in all situations unless prevented by the presence of significant crystalluria or severe pain. All patients on MDP had reduction of dose to LDP subsequently. Patients started on LDP needed elevation in dose in 63 (16.8%) to HDC and 23 patients (12.87%) to MDP. Dose of 247 patients could be reduced to VLDP (55.63%) and later on to intermittent VLDP –85 (19.14%). 111 (25%) patients continued to be on LDP though out the period of study.
Conclusion: It is concluded that in managing the stone patient, the clinical, radiological, microscopic and biochemical parameters should be taken into consideration in deciding the reduction/increase in the dose of drugs. The principle of giving chemotherapy/chemoprophylaxis should be to administer the least number of drugs in the least dosage depending upon the requirement of the disease.

PP-051 Role of scanning electron microscopy in identifying drugs used in medical practice

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Introduction: Several plant preparations are available with potential benefits for medical treatment. They are, however, often given without any scientific basis. Usefulness of any drugs will be assessed on the basis of clinical improvement and metabolic correction in blood, urine, body fluids and tissues. Clinical experiments cannot be carried out without doing in-vitro and animal experimental work. It is here that the role of scanning electron microscopy (SEM) becomes relevant.
Objectives: This paper presents the results of in-vitro and animal experimental studies using SEM in the identification of the therapeutic properties of trial drugs in medicine.
Methods: Urinary crystals, namely calcium oxalate monohydrate and calcium oxalate dehydrate, were grown in six sets of Hane's tubes in silica gel medium. Trial drugs namely scopria dulcis lynn, rotula aquatica lour, musa sapiens and dolicos biflorus were incorporated in the gel medium to identify the inhibitory effect of the trial drugs on the size and extent of crystal column growth. The changes in

morphology of crystals were studied using SEM. In the second set of studies, six male Wistar rats each were made to form stones by administering sodium oxalate and ethylene glycol and made diabetic using alloxan and streptozotocin. The SEM changes of calculogenesis were studied. The rats were administered trial drugs before, during and after being made to form stones and being made diabetic. The tissues of rats namely kidney, bladder, liver and spleen were collected and studied under the scanning electron microscope at different magnifications. Parametric data were compared between trial drugs and controls and Duncan's multiple range test used for comparing ranges of values. Microscopic changes and extent of crystallization were scored appropriately.

Results: The changes in crystal morphology produced by calculogenesis by addition of inhibitors in the form of trial drugs in the in vitro studies were pronounced in the SEM studies. Elemental distribution analysis showed that the crystal purity was not altered by the trial drugs. The trial drugs produced significant changes in the pattern of crystal growth and in the crystal morphology of both calcium oxalate monohydrate and calcium oxalate dihydrate. Scoparia dulcis linn was found to be the most effective anti-calculogenic agent. Musa sapiens and dolicos biflorus were found to have no significant effect in inhibiting crystal growth. The kidneys showed maximum changes of calculogenesis and hence the protective effects of trial drugs could be studied well in this experiment. Major components of calculogenesis included the presence of different grades of crystals in the glomerulus and interstitial tissues, extrusion of the crystals into the tubular lumen and tissue inflammatory cell infiltration. Scoparia dulsic linn was found to exhibit maximum protective effect against the changes of calculogenesis followed by rotula aquatica lour. Musa sapiens and dolicos biflorus were found to have only minimal effect in preventing crystal deposition, inflammatory cell infiltration and other changes of calculogenesis. SEM was found to be effective in assessing the effect of drugs on crystal growth morphology and tissue histology.

Conclusion: The effect of various trial drugs can be scientifically evaluated by the use of scanning electron microscopic studies of the kidney tissues in animal experiments. Scopia dulcis lynn was identified to have significant anti-calculogenic effect.

PP-052 Prospective study of the Wu-Lin-San formula for prophylaxis for recurrent calcium oxalate nephrolithiasis

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Objectives: The Wu-Ling-San formula has been proved to have a preventive effect on the treatment of calcium oxalate nephrolithiasis both in vitro and in vivo. In this study, we examined the efficacy of the Wu-Ling-San formula prophylaxis for preventing recurrent calcium oxalate nephrolithiasis.

Methods: This was a prospective clinical study that evaluated the 24-h urine parameters of 20 patients with kidney stones. All the patients had a history of recurrent calcium oxalate nephrolithiasis and were aged 30–75 years. The patients were asked to drink enough fluid to urinate at least 2 L daily during the study period. A 24-h urine collection was performed to establish the baseline levels of multiple urinary parameters before taking the medicine. The patients were randomly divided into two groups. The medication group took 2 g Wu-Ling-San formula three times daily for 1 month. The control group took 2 g placebo formula three times daily for 1 month. All the 24-h urine collections were analysed for multiple urinary parameters after completion of the study. The serum liver, renal function and electrolyte was also determined.

Results: A total of 20 patients were enrolled, of whom 14 (70%) were men and 6 (30%) were women. All the patients completed the study. The mean patient age was 54.8 years (range 30–75). The patients reported a lifetime total of 1–5 (average 2.7) stone episodes. The patients had undergone 2–6 (average 2.5) stone procedures. The baseline urine output level was 1,800 ml/day. After treatment with Wu-Ling-San formula group, the mean urine output level was 2,400 ml/day ($P < 0.05$). The baseline mean urine volume of the placebo group was 1840 ml/day.placebo therapy and this increased slightly during the placebo period to 1,900 ml/day ($P > 0.05$). No patient complained of side effects such as fatigue, dizziness, impotence, musculoskeletal symptoms or gastrointestinal disturbance. The serum liver function, renal function, electrolyte and urinary parameters were not significant changed in either group.

Conclusion: Chinese herb medicine (Wu-Ling-San Formula) is a promising adjunct to surgical and medical management of kidney stones. Compared with potassium citrate, they might be better accepted by patients than medications taken three times daily because of the lower gastrointestinal problems associated with potassium citrate. However, in the case of the Wu-Ling-San Formula versus hydration group, the urinary parameters did not improve with the Wu-Ling-San Formula. Active therapy with Wu-Ling-San Formula did have a positive effect on the urine volume without electrolyte imbalance. Patients need on-going education and encouragement to meet the goals of urinary output, and treatment with the Wu-Ling-San Formula might help patients reach these goals.

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PP-053 Prevention of nephrolithiasis by an extract of khella (*Ammi visnaga* L.) in an animal model

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Introduction: Hyperoxaluria is a major risk factor for the formation of renal stones. Although effective treatments have been developed to remove kidney stones with minimum renal damage, the treatment of chronic stone occurrence remains frustrating.

Objectives: The aim of this study was to evaluate whether or not oral administration of an aqueous extract prepared from the seeds of *Ammi visnaga* L. (Khella; Apiaceae) could prevent nephrolithiasis in an animal model.

Methods: Hyperoxaluria was induced in male Sprague-Dawley rats by giving 0.75% ethylene glycol (EG) and 1% NH4Cl via the drinking water. The Khella extract (KE; 125, 250 or 500 mg/kg) was orally administered for 14 days. Rats were sacrificed after 2 weeks and kidneys were harvested for the histopathological assay of crystal formation. 24 h urine samples were collected before the animals were sacrificed.

Results: The histopathological examination of the kidneys revealed that KE significantly reduced the incidence of calcium oxalate crystal

deposition (Control (Untreated): 0 + 0, EG: 3.13 + 1.23, EG + KE 125 mg/kg: 1.63 + 1.19, EG + KE 250 mg/kg: 1.86 + 0.83, and EG + KE 500 mg/kg: 1.25 + 0.71). In addition, KE significantly increased urinary excretion of citrate (Control: 1.38 + 0.27 mg/day, EG: 1.10 + 0.21 mg/day, EG + KE 125 mg/kg: 4.0 + 0.67 mg/day, EG + KE 250 mg/kg: 4.02 + 0.34 mg/day, and EG + KE 500 mg/kg: 5.85 + 2.45 mg/day) along with a decrease of oxalate excretion (Control: 0.45 + 0.05 mg/day, EG: 1.01 + 0.34 mg/day, EG + KE 125 mg/kg: 0.80 + 0.34 mg/day, EG + KE 250 mg/kg: 0.58 + 0.22 mg/day, and EG + KE 500 mg/kg: 0.57 + 0.15 mg/day). In addition, KE increased the urine pH (Control: 6.03 + 0.11, EG: 5.64 + 0.1, EG + KE 125 mg/kg: 5.84 + 0.17, EG + KE 250 mg/kg: 5.91 + 0.18, and EG + KE 500 mg/kg: 6.05 + 0.15) in a dose dependent manner. In summary, a reasonably good correlation was obtained between the incidence of crystal deposition and the increase in urine pH.

Conclusion: Our data suggest that KE may be used as a potential therapeutic agent for the prevention of kidney stone recurrence associated with hyperoxaluria.

PP-054 Effects of an extract of *Quercus salicina blume / Q. stenophylla makino* on urinary stone formation in a rat model of calcium oxalate urolithiasis

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Introduction: Kidney stones occur in approximately 10% of the population during their lifetime [1]. Pharmacological or other treatments are therefore needed to prevent stone formation and recurrence. Urocalun®, an extract of *Quercus salicina Blume/Q. stenophylla* Makino (QS extract) that is clinically used for the treatment of urolithiasis in Japan, has recently been shown to have anti-oxidative activity in cell-free systems. We have previously shown that QS extract suppresses superoxide anion (O_2^-) levels in a cell-free xanthine/xanthine oxidase system [2] and suppresses oxalate-induced cell injury and inhibits NADPH oxidase activity in NRK-52E cells (rat renal tubular epithelial cells) [3].

We hypothesized that QS extract suppresses stone formation by decreasing renal tubular epithelial injury through its anti-oxidative activity.

Objectives: To investigate the involvement of QS extract in the prevention of stone formation and recurrence, the effects of the extract were examined in a rat calcium oxalate urolithiasis model induced by oral administration of ethylene glycol (EG) and the vitamin D3 analogue alfacalcidiol for 14 days.

Methods: QS extract was produced by Nippon Shinyaku Co. (Kyoto, Japan). Six-week-old male Sprague-Dawley rats were divided randomly into four groups (control, EG, EG + QS extract 250, and EG + QS extract 500).

After the last administration, urine and plasma biochemistries and urinary calcium were measured and histological examination of the kidney was performed. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) were assessed as oxidative stress markers.

Results: No statistically significant differences were found in plasma creatinine, urine creatinine or UUN between the EG and control groups. BUN and plasma magnesium levels in the EG group were significantly lower than in the control group. Marked increases in the plasma calcium levels and urinary calcium and magnesium levels were found in the EG group compared with the control group.

Urinary MDA and 8-OHdG were significantly elevated in the EG group compared to the control group. The increase in MDA levels in the EG group was completely suppressed by treatment with EG + QS extract 500, while the increase in 8-OHdG levels was not significantly suppressed in either QS extract group.

Urinary calcium levels in the EG group were higher than in the control group .The increase in calcium levels in the EG group was significantly suppressed in both QS extract administration groups .

Conclusion: In conclusion, we found that QS extract suppressed renal calcium accumulation and urinary MDA excretion in a rat model of calcium oxalate urolithiasis, most likely by reducing oxidative stress. Furthermore, urinary citrate excretion was increased by QS extract. These results suggest that QS extract might be effective in preventing stone formation and recurrence in urolithiasis patients.

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PP-055 Effect of blind treatment on stone disease

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Introduction: Several medicines are being prescribed blindly for the prophylaxis of urinary stones throughout the world. Most of the drugs appear to be out of place and doing more harm than good to the patient. This leads to non-compliance in taking medication, occurrence of side-effects and continuation of the process of stone formation. There needs to be a scientific basis for deciding on the usage of chemotherapy and prophylactic drugs and of dosage for the treatment of stone disease.

Objectives: The objective of this paper is to identify the prevalence of blind chemotherapy among the patients attending the stone clinic for the first time and finding out the real indication for the drugs administered.

Methods: Patients who attended our stone clinic for the first time were interviewed to find out what drugs they had been taking before their attendance at the clinic. A total of 350 patients who had consumed specific drugs relevant to stone formation for at least a period of 15 days were selected for detailed assessment. The type of drug consumed, the dose, the duration, the side-effects, compliance rate and effect on stone disease were assessed. The biochemical profile of the patients was assessed to identify the effect of the therapeutic modalities utilised. Conclusions regarding the utility were made. The values were compared with those of patients not on medication in comparison with the laboratory normal ranges.

Results: Of the 350 patients studied, 96 patients were consuming potassium citrate in different dosages, 50 were consuming Allopurinol, 44 Cystone, 27 potassium citrate + magnesium, 25 Calcury, 24 Rowatinex, 21 ayurvedic drugs, 17 Dystone, 17 homeopathic medicines and 17 other drugs. The longest duration of compliance was for Cystone (2.5 years). All other drugs were stopped by the patients themselves because of further symptoms. However, they had restarted the drugs 15 days prior to attendance. A total of 93% of the patients did not feel that there was any significant relief of symptoms. Seven percent of the patients who had no significant symptoms had been taking Cystone. The side effects which prompted the patients to stop

their treatment were gastrointestinal upset, particularly with potassium citrate, Rowatinex and the potassium citrate + magnesium combination. The relevant biochemical changes noted were increased urinary citrate levels in patients consuming potassium citrate alone or in combination with magnesium. Serum uric acid was within normal limits in patients consuming Allopurinol. Urinary uric acid levels were also lower in patients on Allopurinol.
Conclusion: It is concluded that most of the drugs administered blindly were neither indicated nor beneficial for the patients. Such unscientific medications will be detrimental to patient care. Metabolic correction has to be based on proper metabolic assessment.

PP-056 Factors influencing renal function in cystinuric patients

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Introduction: Cystinuria is an autosomal recessive disease inducing a highly recurrent form of stone formation which requires a high rate of urological intervention.
Objectives: To determine factors which contribute to altering renal function in cystinuric patients.
Methods: We recorded clinical and laboratory data from 400 cystinuric patients (205 males aged 40.3 ± 16.3 years and 185 females aged 36.4 ± 15.5). Body mass index (BMI) was calculated from the height and weight of the patient. Clinical parameters were number of calculi, number and type of stone removing procedures. Laboratory parameters were urinary cystine excretion and glomerular filtration rate estimated according to the MDRD simplified formula (eGFR) [1]. Correlations between data were analysed by multiple regression procedures.
Results: The mean age (±SD) at onset of stones was 20.0 ± 11.0 years. The mean number of calculi per patient was 15.7 ± 31.7. It was increasing with the duration of the prevention period from 3.3 for a follow-up <10 years to 19 for 60 years of follow-up. It was also influenced by the body mass index (BMI), increasing for 8 for BMI < 20–40 for BMI ≥ 30 kg/m^2. The mean (±SD) number of urological procedures was 4 ± 3.8 /patient. An unilateral atrophy was observed in 99 patients (25%) and 53 patients underwent unilateral nephrectomy. eGFR was < 60 ml/mn/1.73 m^2 in 35.5% of patients and <30 ml/min/1.73 m^2 in 5.1% of patients. Six patients (1.5%) reached end-stage renal failure. The decrease in eGFR was correlated with the increasing number of open surgery procedures, but was not influenced by the number of PNL, ESWL or ureteroscopic treatments. It was also negatively correlated with BMI in males (not in females), the age at onset of stone disease, and the age at the beginning of medical treatment. No correlation was noted between eGFR and urinary cystine excretion or the number of calculi.
Conclusion: Our data suggest that stone forming cystinuric patients are exposed to impaired renal function due to the delay of diagnosis or of medical care, the BMI of patients, the number of open surgery procedures and the degree of atrophy of the kidney. Therefore, therapeutic measures, which are recognized to be effective for preventing stone activity and the damage caused by recurrent stones, must be early initiated in patients with cystinuria.

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PP-057 Timing of metabolic evaluation in renal stone formers

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Introduction: The timing of metabolic evaluation and the collection of 24 h urine (24hU) specimens is still a matter of debate. Several guidelines recommend metabolic evaluation on outpatient conditions after active stone therapy. The main reasons are a fear of interference with methods of stone therapy influencing results and the different environment of home versus hospital. On the other hand, metabolic evaluation under outpatient conditions is often not performed at all. There is a lack of acceptance by many practising urologists and also by family doctors of the need for metabolic evaluation as it is inconvenient to collect 24hU on an outpatient basis.
Objectives: Faced with these problems, we wondered whether or not in-patient evaluation is really different from out-patient metabolic evaluation, especially when regarding the fact that in-patient metabolic evaluation is much easier to carry out and probably more reliable.
Methods: A total of 100 consecutive stone formers who where hospitalised in our department were investigated. We collected 24 hU on an in-patient basis during the hospital stay (24hUIP) and we collected another specimen 2 weeks after the hospital stay on an out-patient basis (24hUOP). The following parameters were measured: 24 h volume, calcium, uric acid, citrate, urea and ammonia.
Statistics: Means and standard deviations, normality and variance tests; in case of normal distribution and equal variance, significance was calculated by Student′s t test. Significant difference: $P < 0.05$. Correlations were calculated for all the parameters tested.
Results: A total of 54 of our 100 patients were compliant and collected the second 24 h urine specimen on an outpatient basis about two weeks after the hospital stay.
There was no statistically significant difference between the in-patient and the out-patient collected for all the parameters. Apart from ammonia, there was a highly significant correlation for all parameters between in- and out-patient collection.
Conclusion: Our results show, that metabolic evaluation of stone formers is feasible on in-patient conditions during active stone treatment. This facilitates 24 h urine sample collection and probably also increases its accuracy. Metabolic evaluation when performed under in-patient conditions hopefully will increase the acceptance both by practising urologist and family doctors and also the patients. In our opinion, this is the first and probably most important step to reduce the number of recurrences of stone formation.

PP-058 Effect of potassium citrate treatment on bone mineral density in patients with idiopathic recurrent calcium oxalate urolithiasis

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Objectives: Bone loss is a well-known consequence of calcium oxalate (CaOx) urolithiasis. Potassium citrate (PC) is being used to prevent bone loss in patients with urolithiasis. But there are few studies investigating the effect of PC on bone mineral density (BMD). We aimed to investigate the effects of PC on BMD in patients with idiopathic recurrent CaOx urolithiasis.
Methods: Thirty patients (16 women, 14 men) with idiopathic recurrent CaOx urolithiasis were enrolled in the study. Demographic data (age, height, weight and BMI) were collected. Fasting serum calcium, uric acid, creatinine, alkaline phosphatase and PTH levels and 24-h urine volume and creatinine, calcium, citrate, oxalate and

urate levels were measured before treatment. BMDs and z-scores for total femur (TF) and L2-4 vertebrae of the patients were measured by Dual Energy X-ray Absorptiometry (DEXA) method (QDR Elite W 4-500, Hologic, Waltham, Massachusetts) before treatment. The patients were treated with PC (60 mEq/day) (Urocit-K, potassium citrate in wax matrix, Mission Pharmacal Company, San Antonio, Texas) for a period of 12 months. The fasting blood biochemical and 24-h urine biochemical parameters and BMDs for TF and L2-4 vertebrae were re-evaluated after treatment period. The results were compared using one-sample t-test for men and women separately.
Results: The mean age was 34.5 ± 8 and 32.18 ± 6 years for men and women, respectively. The mean BMI was 24.8 ± 1.9 and 22.6 ± 1.5 kg/m^2 for men and women, respectively. When compared with pre-treatment levels, there was no statistically significant change in post-treatment blood biochemical parameters ($P > 0.05$). There was also no significant change in respect to urine calcium level both in men and women ($P > 0.05$). After treatment, a significant increase in urine citrate level occurred for both genders ($P < 0.05$). On the other hand, post-treatment urine urate and oxalate levels were significantly increased according to pre-treatment levels for both genders ($P < 0.05$). The pre-treatment BMD values for TF and L2-4 vertebrae in men were 0.9040 ± 0.109 g/cm2 and 0.853 ± 0.155 g/cm^2, respectively. These values were 0.8904 ± 0.164 g/cm2 and 0.8957 ± 0.211 g/cm2 for women. The pre-treatment z-scores of TF and L2-4 vertebrae were 0.65 ± 0.15 and −0.47 ± 0.13 for men, respectively. The corresponding values for women were found to be −1.59 ± 0.30 and −0.54 ± 0.13. The post-treatment BMD values for TF and L2-4 vertebrae in men were 0.9117 ± 0.156 g/cm2 and 0.9165 ± 0.285 g/cm^2, respectively. The corresponding values in women were 0.9366 ± 0.150 g/cm2 and 0.9023 ± 0.139 g/cm^2. The post-treatment z-scores for TF and L2-4 vertebrae were found to be 0.68 ± 0.29 and −0.42 ± 0.20 in men and −0.43 ± 0.15 and −0.152 ± 0.27 in women, respectively. The BMD values for TF and L2–4 vertebrae both in men and women were significantly increased after treatment ($P < 0.05$). The z-scores for corresponding locations were also significantly elevated in both genders after treatment ($P < 0.05$).
Conclusion: It can be concluded that PC treatment improved bone mineral balance, prevented bone loss and increased low BMD in patients with idiopathic recurrent CaOx urolithiasis. We may conclude that PC can be used, as a long-term treatment, in patients with idiopathic CaOx urolithiasis who have an increased risk for osteoporosis and bone fracture due to severe bone mineral loss.

OP-041 The role of cyclooxygenase 2 and prostaglandin E2 in the attachment of calcium oxalate crystals to renal epithelial cells

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Introduction: The interaction between the renal tubular cells and calcium oxalate crystals is an important step in the early stage of calcium oxalate nephrolithiasis. We previously analyzed the gene expression profile using cDNA micro-arrays and found over-expression of genes related to the inflammatory process in nephrolithiasis [1]. Oxalate has been reported to promote the expression of cyclooxygenase2 (COX2) and prostaglandin E2 (PGE2), the product of COX2 to inhibit the attachment of oxalate calcium crystals to the renal tubular cells [2].
Objectives: The activity of a substance endogenously may not be the same as that applied exogenously. We examined the role of endogenous COX2 and PGE2 in the crystal-cell interaction using wild-type MDCK cells and COX2-overexpressing MDCK cells to examine the mechanism of crystal attachment.
Methods: A COX2-expressing plasmid was produced and introduced into MDCK cells by the lipofection method3), and the COX2-overexpressing MDCK cells were incubated at 37 C for 120 min in a CO2 incubator after addition of 14C-calcium oxalate monohydrate (COM) crystals to the culture solution to a final concentration of 0.5 mM. Then, the cell surface was washed twice, and 14C was measured with a liquid scintillation counter. Cells pre-incubated with a COX2 inhibitor were also analyzed.
Results: Analysis by thin layer chromatography revealed that COX2-overexpressing MDCK cells produced about ten times more PGE2 than the wild-type and mock-treated MDCK cells. The attachment of COM crystals was significantly reduced in the COX2-overexpressing MDCK cells than in the wild-type and mock-treated MDCK cells. The selective COX2 inhibitor dose-dependently increased the attachment of calcium oxalate. These results suggest that the expression of COX2 and the resulting production of PGE2 play a preventive role in the occurrence of lithiasis by inhibiting the attachment of COM crystals to the surface of the renal epithelial cells.
Conclusion: The production of PGE2 by introducing a COX-expressing plasmid into the MDCK cells inhibited the attachment of COM crystals. This inhibitory activity was suppressed by a selective COX2 inhibitor.

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OP-042 Binding of calcium oxalate crystals to cultured renal cells in South Africa's stone-prone and relatively stone-free population groups

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Introduction: Historically, the attachment of crystals to cells within the nephron of the kidney has been regarded as a prerequisite to kidney stone formation. However, recent studies have shown that adherent crystals are internalized and destroyed, and thus crystal attachment and subsequent intracellular destruction may be an unexpected means by which to avert stone formation. The incidence of urolithiasis in South Africa's white population is similar to that of the Western world. However, in the black population it is extremely rare. Thus, the present study aimed to investigate whether crystal-cell adhesion is a contributory factor to the rarity of stones amongst South Africa's black population.
Methods: Inorganic calcium oxalate (CaOx) monohydrate and dihydrate crystals were prepared and used for method validation studies. CaOx crystals were precipitated in 24-h urines from healthy black ($n = 8$) and white ($n = 10$) males by addition of a fixed amount of sodium oxalate in excess of the urinary metastable limits. The protein content of the crystals was analysed by SDS-PAGE and Western blotting. Crystal slurries were prepared using sterile water and stirred at a fast speed overnight. Confluent monolayers of Madin–Darby canine kidney cells were cultured [1] and aliquots of the slurries were added to the monolayers to allow for binding. The amount of adherent crystals was determined according to Ebisuno et al. [2] and expressed per

milligram of protein in the cells. Each individual's crystals were tested in triplicate.
Results: A linear increase in crystal binding was detected with increasing CaOx monohydrate and dihydrate concentration in the range 0.2–0.6 mg/ml. The binding affinity of CaOx dihydrate crystals for MDCK cells was significantly less than that of the monohydrate ones. Urinary CaOx crystals also demonstrated a linear and concentration dependent increase in binding but in the range 1–2 mg/ml. The range of binding affinities determined for the two population groups was similar and both groups had a relatively large within-group spread. However, a trend towards lower binding amongst white controls was observed compared with black controls.
Conclusion: If crystal binding in the nephron leads to stone formation, then the relatively lower binding affinity of the white population's crystals is surprising. This trend would be inconsistent with their observed higher risk for stone formation compared to the black population. Therefore, we hypothesise that amongst the black population, bound crystals do not readily allow for further crystal deposition or they are more readily disintegrated after phagocytosis. Both of these mechanisms suggest that crystal attachment may serve a protective function amongst the black population.

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OP-043 Mechanism of oxalate-induced NADPH oxidase mediated free radical injury in renal epithelial cells: role of small GTP binding protein Rac1

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Introduction: Hyperoxaluria is a major risk factor in calcium oxalate nephrolithiasis. Oxalate-induced cell injury has been demonstrated to be one of the major mechanisms implicated in calcium oxalate nucleation, aggregation and growth of kidney stone. We have previously demonstrated that oxalate-induced NADPH oxidase derived reactive oxygen species (ROS) play a significant role in cell injury in renal epithelial cells. Since NADPH oxidase activation requires several regulatory proteins, the primary goal of this study was to characterize the role of Rac GTPase in oxalate-induced NADPH oxidase-mediated oxidative injury in renal epithelial cells.
Methods: Confluent monolayers of LLC-PK1 cells pre-treated with or without inhibitors of NADPH oxidase (DPI, 20 μM) or Rac1 (NSC23766, a cell permeable pyrimidine compound; 50 μM) were exposed to 0.5–1 mM oxalate for different time periods. ROS (superoxide and hydrogen peroxide) production and cell injury (LDH release in media) were determined. NADPH oxidase activity was determined by quantifying superoxide-induced lucigenin photoemission. Rac1 activation was determined in subcellular fractions by western analysis.
Results: Oxalate time and dose dependently increased superoxide, hydrogen peroxide production, and LDH release in LLC PK1 cells. Oxalate time dependently increased NADPH oxidase activity and Rac1 membrane translocation in LLC-PK1 cells. Oxalate-induced time dependent activation of Rac1 directly correlated with stimulated NADPH oxidase activity. Pre-treatment of LLC-PK1 cells with Rac1 inhibitor significantly attenuated oxalate-induced ROS production (Ox: 2.12 ± 0.13 fold; Ox +Rac1 inhibitor: 1.38 ± 0.11), LDH release. (Ox: 3.35 ± 0.21 fold; Ox + Rac inhibitor:2.14 ± 0.15) and completely prevented Rac1 activation. In addition, inhibition of Rac1 activation completely prevented oxalate-induced NADPH oxidase suggest that oxalate-induced NADPH oxidase mediated oxidative injury is regulated by Rac1 in LLC-PK1 cells.
Conclusion: This is the first study demonstrate that an oxalate mediated cell injury involve a Rac1-dependent signaling mechanism. Our data demonstrate that Rac1 dependent activation of NADPH oxidase might be a crucial mechanism responsible for increased ROS generation in renal epithelial cells during oxalate toxicity and, therefore these pathways may represent a novel therapeutic target for patients with recurrent kidney stones.

OP-044 Tamm-Horsfall protein glycosylation and crystal aggregation

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Introduction: One defense against nephrolithiasis is provided by urine macromolecules that modulate the nucleation, growth, aggregation and retention of crystals in the kidneys. Tamm-Horsfall protein (THP) as the second most abundant protein in normal urine is likely to be a key protein. It is normally heavily glycosylated, with roughly 30% carbohydrate content [1], but sialic acid, an anionic glycosyl group, has been reported to be less abundant in THP obtained from stone-formers [2]. Moreover, others have reported that THP from stone formers self-aggregates[3].
Objectives: The aim of this study was to investigate the effects of the terminal sialic acid residues by comparing native THP (n-THP) and desialylated THP (ds-THP) with respect to calcium oxalate monohydrate (COM) crystal aggregation and protein aggregation, both with itself and other urinary macromolecules.
Methods: THP was purified from normal human urine by salt precipitation, and a portion was desialylated using neuraminidase (24 h, 37°C, 90 mmol/L sodium acetate buffer, pH = 5.5). Native and ds-THP were subjected to 2-dimensional gel electrophoresis to ascertain their isoelectric points. Particle sizing (Accusizer 780, Particle Sizing Systems, USA) was used to assess protein self association and protein effects on COM crystal aggregation. Far Western blots were used to detect THP association with other urine macromolecules. Normal urine macromolecules were separated by PAGE, blotted to nitrocellulose, incubated with n-THP and ds-THP overnight, washed extensively, and finally probed using a polyclonal THP antibody to screen the urinary macromolecules that have affinity for n-THP and ds-THP.
Results: The neuraminidase treatment removed approximately 50% of the sialic acid residues from n-THP. In 2D gel electrophoresis, n-THP demonstrated an isoelectric point range of 3.2–4.3, while ds-THP spanned the range of 3.9–5.3, consistent with the reduced quantity of carboxylate groups. In a 150 mM NaCl buffer at pH = 7.5, ds-THP showed evidence of self-aggregation above 5nM protein, increasing as the protein concentration increased, while n-THP showed no aggregation at concentrations < 50nM. In the COM crystal aggregation experiment, ds-THP induced COM aggregation at concentrations between 5 and 50 nM; whereas n-THP disaggregated COM crystals to a small extent at matching concentrations. In addition to THP self association, the loss of negatively charged terminal sialic acid in ds-THP resulted in strong binding to several bands in the Far Western blots (three major bands and three minor bands).
Conclusion: The loss of negatively charged terminal sialic acid in THP enhanced the self association, enhanced COM aggregation, and promoted association with other urinary proteins. Consequently, reduced glycosylation of THP is likely a risk factor for stone formation.

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OP-045 Prothrombin fragment 1 inhibits attachment of urinary calcium oxalate monohydrate crystals to MDCKII cells and facilitates their intracellular degradation and dissolution

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Introduction: In vivo, calcium oxalate monohydrate (COM) crystals are associated with proteins bound to their surfaces and contained within their mineral phase. Prothrombin fragment 1 (PTF1) is the predominant intracrystalline protein in urinary COM crystals, is synthesized in the human kidney and potently inhibits COM crystallization in urine. Although these properties suggest that PTF1 may fulfil a regulatory role in urolithiasis, its effects on COM adhesion to renal cells and on intracellular crystal degradation and dissolution have not been examined.
Objectives: (1) To compare the effects of intracrystalline (IC), surface-bound (SB) and intracrystalline + surface-bound (IC + SB) PTF1 on the binding of urinary COM crystals to Madin-Darby canine kidney (MDCKII) cells, and (2) To determine the effect of increasing concentrations of IC PTF1 on the degradation and dissolution of urinary COM crystal in MDCKII cells.
Methods: 14C-oxalate-COM crystals containing IC PTF1 were generated from ultrafiltered (UF: 10 kDa) urine containing 0–1.0 mg/L PTF1; SB crystals deposited from UF urine were incubated in the same urine containing 0–1.0 mg/L PTF1; IC + SB crystals were prepared by combining both procedures. Crystals were bound for 15 min in UF urine and binding was expressed as retained 14C-oxalate after removal of unbound crystals. Dissolution of crystals containing IC PTF1 was performed in culture medium for periods up to 48 h and quantified as radioactivity released into the medium. Crystal degradation and surface area were assessed by field emission scanning electron microscopy (FESEM).
Results: Crystal surface area did not alter significantly with IC PTF1 concentration. The table shows the effect of increasing IC, SB and IC + SB concentrations of PTF1 on crystal dissolution and inhibition of crystal binding. Dissolution increased proportionally with IC PTF1 concentration. FESEM showed that crystal degradation was profound and increased relative to IC PTF1 concentration. IC PTF1 had no effect on crystal binding up to 1.0 mg/L, while SB PTF1 increased inhibition relative to PTF1 concentration. However, IC + SB PTF1 significantly inhibited crystal binding in proportion to the protein's concentration, indicating that IC and SB PTF1 act synergistically.

	PTF1 (mg/L)			
	0.00	0.10	0.50	1.0
IC:% dissolution	11.8	12.4	14.0*	17.0*
IC:% binding inhibition	15.0	15.0	15.0	20.5*
SB:% binding inhibition	15.0	19.0*	25.0*	28.0*
IC + SB:% binding inhibition	16.0	34.0*	52.0*	57.0*

* $P < 0.05$

Conclusion:

1. PTF1 located both within, and upon the surfaces of COM crystals, significantly inhibits the attachment of urinary COM crystals to MDCKII cells
2. Intracrystalline PTF1 slightly, but significantly, enhances the dissolution and massively increases the physical degradation of CaOx crystals attached to MDCKII cells.
3. Therefore urinary PTF1 may provide a natural defence against urinary stone pathogenesis.

OP-046 Effect of calcium on calcium oxalate crystal induced renal epithelial injury

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Introduction: Calcium oxalate (CaOx) crystals are injurious to cells and crystal-induced injury is suggested to play a significant role in stone formation. Hypercalciuria is a common feature of CaOx stone patients. Thus, crystals in the kidneys of stone patients form in the presence of high urinary calcium and renal epithelial cells are exposed to various crystals in the presence of high calcium.
Objectives: To understand the role of calcium in CaOx nephrolithiasis, we decided to determine the effect of high calcium levels on CaOx crystal induced injury.
Methods: We exposed human renal epithelial cell line, HK2 in vitro to CaOx monohydrate crystals at a concentration of 133 μg/cm2 for 1, 3, 6 or 12 h in the presence or absence of 5 or 10 mM/L calcium Ca^{2+}. We determined the release of lactate dehydrogenase (LDH) as marker of injury and hydrogen peroxide (H_2O_2) and 8-isoprostane (8-IP) as sign of oxidative stress. Cells were also examined after trypan blue and nuclear DNA staining with 4,6-diamidino-2-phenylindole (DAPI) to determine their membrane integrity and apoptosis, respectively.
Results: Exposure of cells to 5 or 10 mM/L of Ca^{2+}, for up-to 6 h, resulted in increased trypan blue and DAPI staining and production of H_2O_2. Similarly an exposure to CaOx crystals also resulted in increased trypan blue and DAPI staining and H_2O_2 production. An exposure to 5 mM/L Ca or CaOx crystals also resulted in increased production of 8-IP. A combination of the two treatments, Ca and CaOx crystals, did not show anymore changes than exposure to high Ca or CaOx crystals alone, except in the case of a longer exposure of 12 h. Longer exposures of 12 h resulted in cells sloughing from the substrate. Results of LDH release into the medium were not consistent.
Conclusion: Our results indicate that exposure to high levels of Ca or CaOx crystals is injurious to renal epithelial cells but the two do not appear to work synergistically. On the other hand, results of our earlier studies suggest that oxalate and CaOx crystals work in synergy, i.e., CaOx crystals are more injurious in the presence of high oxalate. Perhaps Ox and CaOx crystals activate different biochemical pathways while Ca and CaOx crystals affect the identical pathways.

OP-047 Immune system, crystallisation inhibitors and tissue calcification

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Introduction: Soft tissue calcification is an undesirable disorder that implies the pre-existence of an injury which induce calcium phosphate (hydroxyapatite) formation through heterogeneous nucleation. At present it is admitted that this type of calcification plays an important role in calcium oxalate monohydrate papillary calculi formation, induced by Randall's plaque. Crystallization inhibitors (such as phytate, pyrophosphate or bisphosphonates) and several proteins (such as osteopontin or osteoporotegerin) has also demonstrated to have an important role in soft tissue calcification.
Objectives: The present study examined the role of phytate and osteopontin during the development of soft tissue calcification in an animal model.
Methods: Male Wistar rats were assigned in two groups ($n = 16$). Non-phytate-treated rats were fed with AIN-76A diet (a purified diet in which phytate is undetectable) and phytate-treated rats were fed with AIN-76A enriched with a high phytin diet. After a period of 21 days consuming corresponding diets, all rats were subjected to calcinosis induction by subcutaneous injection of $KMnO_4$ in two positions on either side of the interscapular region. At 2, 5, 8 and 10 days after calcinosis induction, four rats of each group were sacrificed and the injured tissues (hydroxyapatite and surrounding tissue) were removed for histological analysis (calcium deposits, macrophages and osteopontin detection) and calcium etermination.
Results: Development of tissue calcification of phytate-treated rats was notably and significantly reduced in comparison with non-phytate-treated rats. Calcified deposits appeared as soon as 2 days after calcinosis induction, macrophages weren't present until the 5th-day and osteopontin was not detected until the 8th day and from this day, it was clearly detected and associated with calcified areas.
Conclusion: The results suggest the important role of phytate as a crystallization inhibitor in the first steps of calcification formation, avoiding the development of an important amount of hydroxyapatite crystals. Hence, the inhibition of crystal development would facilitate the reabsorption of injured tissue by the immune system. Histological analysis indicated that osteopontin is not involved in the first steps of soft tissue calcification. It appears to be related with the control of calcification rather than its genesis, regulating the activity of macrophage and macrophage-derived cells (i.e., osteoclasts), thus facilitating phagocytosis and enhancing hydroxyapatite deposits destruction. Therefore, extracellular protein matrix could have an important role as a signaling agent in the control of the cellular processes associated with tissue calcification, rather than as a crystallization inhibitor.

OP-048 Expression of annexin 2, nucleolin related protein, hyaluronan, osteopontin and CD44 during the onset of intratubular nephrocalcinosis

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Introduction: The initial phase of intratubular nephrocalcinosis (and potentially nephrolithiasis) is the retention of crystals to regenerating/dedifferentiated tubular epithelial cells. There is increasing evidence that the tubular epithelium, under pathological conditions, expresses crystal binding molecules on its luminal surface and that these phenotypical changes occur prior to firm crystal adhesion. Research on the molecular nature of the crystal binding phenotype has identified a growing list of membrane-associated molecules with affinity for calcium crystals, including hyaluronan (HA), osteopontin (OPN), nucleolin related protein (NRP) and annexin 2 (Ax2).
Objectives: To get insight in the initial phase of crystal retention.
Methods: In the present study we investigated the temporal changes of tubular epithelial phenotype and luminal expression of HA, OPN, CD44 (mutual receptor of HA and OPN), Ax2 and NRP during the onset of nephrocalcinosis in male Wistar rats, i.e., at day 1, 2, 3 and 4 during a 4-day ethylene glycol (EG) administration period and at day 2 after arrest thereof. Crystalluria and urinary crystal formation were assessed by measuring the calcium content in the pellet and supernatant of centrifuged urine, respectively. In the presence of unchanged serum calcium levels it is assumed that a decreased supernatant calcium indicates crystal formation. Total oxaluria was assessed by measuring oxalate in acidified urine. The amount of crystal containing tubules was quantified on Von-Kossa stained renal sections. Tubular injury/regeneration, HA-, OPN-, CD44-, Ax2- and Nucl-expression were microscopically evaluated by scoring ca. 1,200 tubules (out of 6 animals) per time point on PAS/PCNA and the respective immunohistochemical stained renal sections.
Results: Administration of EG resulted in an immediate and persistent increased crystalluria, as indicated by an increased pellet calcium content and decreased supernatant calcium content. During EG administration, epithelial crystal adhesion gradually increased as were injury/regeneration and luminal HA-, OPN-, CD44-, Ax2- and Nucl-expression. Two days after arrest of EG-administration supernatant calcium was still low and hyperoxaluria (although lowered) was still present, indicating calcium oxalate crystal formation. At that time however, crystalluria had decreased to control values while the amount of renal crystals as well as the amount of regenerating cells and the luminal expression of HA, CD44 and Ax2 had clearly increased. In general, luminal expression of HA, Ax2, OPN and CD44 correlated with the amount of intratubular crystals.
Conclusion: This study suggests that the extent of non-obstructive crystal retention depends on the presence of the appropriate crystal binding tubular epithelial phenotype, characterized by regeneration and luminal expression of mainly HA-, Ax2-, and CD44, and not per se on the amount of crystals formed.

OP-049 Establishment of continuous human papillary epithelial cell (chPEC) line

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Introduction: We have recently demonstrated successful primary culture and characterization of renal papillary epithelial cells from human kidney (hIMCD epithelial cells). The utility of these cells is limited by their short life span of four to five passages, after which these cells fail to divide. The goal of the present study was to establish continuous cultures of human renal papillary epithelial cells. In this study we describe the successful immortalization of human Inner Medullary collecting Duct (hIMCD) cells by over-expressing human TERT in these cells.
Objectives: Primary cultures of Human Inner Medullary Collecting Duct Cells (hIMCD) from the renal papillary region were used to establish immortalized cell line by infecting the cells with retroviral vectors. Phoenix packaging cells were transfected with plasmid pBABE expressing hTERT (human Telomerase Reverse Transcriptase). Viral preparations obtained were used to infect primary hIMCD cells and selected for puromycin resistance. Telomere maintenance was determined by Telomere Repeat Amplification Protocol (TRAP) assay. Immortalized cells were checked for the expression of epithelial and urothelial specific markers by western blotting.
Methods: hIMCD cells were successfully immortalized by expressing the hTERT enzyme in these cells. These cells were successfully cultured for many generations beyond the primary cells in culture. The telomere length was maintained in the immortalized cells through successive generations as determined by TRAP assay. Expression of Cytokeratin, an epithelial cell marker and Uroplakin Ia, Ib markers for urothelial differentiation was confirmed by Western blots.

Results: The generation of continuous cell line from human renal papillary epithelium (chPEC) line will greatly aid in our understanding of the interactions between oxalate, calcium, phosphate and other ions involved in nephrolithiasis, in the renal papillary regions.
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OP-050 Human kidney cells having "autocrine" functions during crystal-mediated injury

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Introduction and *Objectives*: The production of HA in injured cells precedes the initiation of inflammatory response. But whether or not this will promote further cell damaging or trigger cell repairing mechanism is still unclear. The cell growth effects of HA-secreting Human Kidney-2 (HK-2) cells were studied in the presence of IL-1β.
Methods: Monolayer confluent cultures of HK-2 cells were exposed to different concentrations of calcium oxalate (CaOx) crystals, in the presence or absence of pro-inflammatory IL-1β. Cytotoxic effects of CaOx were assessed by staining the cells with Trypan blue viability dye and/or by measuring the amount of lactate dehydrogenase (LDH) released into the culture media. GAGs in the conditioned media were recovered by cetylpyridinium chloride (CPC) precipitation and papain digestion, measured by carbazole reaction and dimethylmethylene blue (DMMB) assay. Hyaluronidase digestion and cellulose acetate electrophoresis (CEAE) were done to confirm the HA identity and quantified. Furthermore, CD44 expression on cell surface was analyzed with flow cytometric immunophenotyping technique. The cell viabilities were then studied in CaOx-injured cells after incubation with IL-1β.
Results: CaOx was bound on to the cell surfaces causing cytotoxic effects and increases in total GAGs and HA, in a dose-dependent manner. This is similar to an initiating inflammatory stage, subsequent to CaOx-induced injury of the tubular epithelium. The pro-inflammatory IL-1β induced the secretion of HA and expression of CD44, in dose-dependent manner. Co-incubation of CaOx and IL-1β in cell culture demonstrated further increase of HA, when compared with CaOx alone. HA may play an important roles in stone formation, and published data have indicated that relatively higher concentration of HA is able to promote nucleation but inhibit the growth of crystallization. Other evidences suggests that HA interacts with CD44 for signaling the process for wound healing. This is further confirmed by the increase of cell viability after incubating with IL-1β for 24 h in CaOx-injured HK-2 cells. Therefore, data collected herein supports not only the establishment of renal cell micro-environment to mimic inflammatory conditions during stone formation, but also indicate HK-2 cells have autocrine mechanism for repairing upon crystal-induced injury.
Conclusions: Kidney tubular epithelial cells have been demonstrated to have "autocrine" effects during a stone episode, which is a valuable tool to understand the pathophysiology of urolithiasis, and to investigate further (1) the "foe and friend" roles of inflammation in stone formation and (2) the molecular mechanism of cell adhesion of crystals.

PP-059 Relation between ionic concentrations and the growth of oxalic, uric and phosphatic urinary calculi

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Introduction: Urinary calculi are concretions consisting of ions and compounds such as oxalate, phosphate and uric acid which saturate urine and precipitate in the urinary tract of stone patients, resulting in renal colic and obstruction as they move down the urinary tract.
Objectives: The goal of this research was to evaluate the relation between the size of the calculus and the ionic concentrations measured in urinary calculi found in stone patients in the city of Cumaná, state of Sucre, Venezuela.
Methods: For that purpose, 45 urinary concretions taken from the patients characterized previously (11 of uric acid, 25 of oxalate, and 9 of phosphatic salts) were analyzed and their crystals identified by X-ray diffraction. Each calculus was washed with distilled water, dried for 24 h, crushed, and identified for the subsequent determinations of the following ions: calcium, magnesium, manganese, zinc, copper (atomic absorption), sodium, potassium (atomic emission), and phosphorus (Murphy and Riley's method).
Results: The linear regression analysis showed a significant relation between the calcium ion and length and between the phosphorus ion and width in oxalic calculi. Furthermore, a relation was observed between the phosphorus ion and length and width in phosphatic calculi.
Conclusion: These facts indicate the importance of the calcium and phosphorus ions in the growth of oxalic and phosphatic urinary concretions.

PP-060 Analysis of facilitators and inhibitors compounds of the crystalline precipitation in urines of urolithic patients

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Introduction: Urolithiasis is caused by the supersaturation of urine and precipitation of crystalline salts of uric acid, calcium oxalate and calcium phosphate, among others. This precipitation depends on the presence of facilitators and inhibitors compounds of this process.
Objectives: The present research has as goal to evaluate the urinary concentrations of facilitators and inhibitors compounds of the crystalline precipitation in stone patients.
Methods: To achieve this purpose urinary samples from 40 patients with clinical histories of urolithiasis from the University Hospital "Antonio Patricio de Alcalá" of the Cumaná city, Sucre state, Venezuela, and 40 individuals controls were analyzed. Each person was instructed on how to collect the sample of 24 h urine and proceeded to subsequent determinations of the uric acid, oxalate, calcium, magnesium and phosphorus (by spectrophotometric methodology), proteins (turbidimetric procedure) and citrate (enzymatic method).
Results: The obtained results show increased values in the parameters proteins, oxalate, uric acid, calcium, phosphorus and decreased values in the citrate and magnesium determinations in the stone patients in relation to controls.
Conclusion: We conclude that stone patients excrete significantly more of the compounds that facilitate crystalline precipitation in the urinary tract.

PP-061 Effects of microgravity enhancement of oxidative stress in human renal tubular cells

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Introduction: The increased risk of renal stone formation during space flight and during 90 days bed rest [1] has been linked primarily to increased calcium excretion from bone demineralization. Moreover, in space the human body is subjected to high levels of oxidative stress, which is associated with the pathogenesis of urolithiasis.
Objectives: With the eventual aim of preventing urolithiasis in space flights, we examined the effects of oxidative stress in human renal tubular cells using microgravity enhancement model, named a clinostat.
Methods: Cell culture
Renal proximal epithelial cells of a normal human kidney line (HK-2: human kidney cell) were grown in Dulbecco's modified Eagles medium with 5% fetal calf serum. Clinostat experiments.

The clinostat is a rotating system that converts gravity from a vector quantity into a scalar quantity. The specimen is rotated horizontally at a constant angular rate, which simulates a microgravity environment. The flask on a slide with the specimen positioned in the direction of the rotating axis was fixed in the clinostat and rotated at 50 and 100 rpm for 24 h.

Statistical analysis

The Mann–Whitney U test was used to compare data between groups, with probability values of $P < 0.05$ considered to be statistically significant.
Results: LDH, LPO, 8-OHdG, and SOD levels
The LDH level in the supernatant of the cell culture was significantly higher in the 50 and 100 rpm clinostat groups than in the control group after 24 h ($P < 0.05$ and $P < 0.01$, respectively). The LPO level increased in the 100 rpm clinostat group, but the change was not significant. The 8-OHdG level was significantly higher in the 100 rpm clinostat group than in the control group after 24 h ($P < 0.05$). The SOD level decreased in the 100 rpm clinostat group after 24 h, but the change was not significant.

Immunohistochemical staining

The Nox4 staining was weak in the control group but strong in the 100 rpm clinostat group.

Immunofluorescence

The 8-OHdG staining was weak in the control group but strong in the 100 rpm clinostat group.

Cell morphology

The use of Hoechest 33258 to investigate changes in cell nuclei revealed abundant apoptotic bodies containing nuclear fragments in the 100 rpm clinostat group but none in the control group.
Conclusion: The main cause of osteopenia in a microgravity environment is mechanical unloading. Moreover, oxidative-stress-induced activation of nuclear factor-kappa B was observed in all regions of the brain in a microgravity environment . The effects of microgravity last for several weeks after returning to a normal-gravity environment, and humans exhibit increased lipid peroxidation in erythrocyte membranes and reductions in some blood antioxidants.
The results of this study demonstrate that microgravity subjects human renal tubular cells to high levels of oxidative stress, and suggest that space-based experiments could provide data that would be useful to the clinical prevention of urolithiasis.

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PP-062 A comparison of the binding of urinary calcium oxalate monohydrate and dihydrate crystals to human kidney cells

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Introduction: The prevalence of calcium oxalate monohydrate (COM) crystals in kidney stones and dihydrate (COD) crystals in urine has led to the proposal that preferential formation of COD, rather than COM crystals, protects against urolithiasis because they are less likely to adhere to renal tubular cells. Although some studies have reported that COM crystals bind more avidly to cells, they were performed under aqueous conditions using inorganic crystals. However, in vivo, crystals form within the kidney in the presence of urinary proteins that cover their exteriors and are interred within the mineral bulk, which must affect their binding affinity for the cell surface.
Objectives:

1. To examine the binding kinetics of urinary COM and COD crystals to human kidney (HK-2) cells in ultrafiltered (UF), and centrifuged and filtered (CF) human urine.
2. To quantify the binding to HK-2 cells of COM and COD crystals in UF and CF human urine samples collected from different individuals.

Methods: Urine was collected from healthy individuals, pooled, centrifuged and filtered. 14C-oxalate-labelled COM and COD crystals were precipitated from the urine by addition of oxalate following adjustment of two aliquots of the urine to 2 and 8 mM Ca, respectively. For the kinetic study, the crystals were incubated with HK-2 cells for up to 120 min in pooled CF and UF (10 kDa) urines adjusted to 2 and 8 mM Ca. Binding was expressed as 14C-oxalate retained after removal of unbound crystals. For the quantitative study, 14C-oxalate-labelled COM and COD crystals were incubated with HK-2 cells for 50 min in separate CF and UF urines collected from 8 healthy individuals at the native Ca concentrations of the urines. Field emission electron microscopy (FESEM) was used to confirm crystal morphology and to calculate surface area.
Results: The COD crystals were considerably larger than the COM crystals.
Kinetic study

1. Binding of both COM and COD crystals was greater in UF urine than in CF urine and both generally bound more strongly at 8 mM than at 2 mM Ca.
2. The kinetic binding curves of COM were smooth, while those of COD were consistently biphasic, suggesting that the two crystal types induce different cellular metabolic responses.
3. In UF urine COM binding was greater than that of COD at 2 mM Ca, but at 8 mM Ca the binding of COD was greater at early and late time points. COD also bound more strongly at early time points in CF urine at both 2 and 8 mM Ca.
4. When data were corrected for surface area, COM always bound more strongly than COD in UF and CF urine and at 2 and 8 mM Ca.

Quantitative study

1. In both CF and UF urine, there was no difference between the binding affinity of urinary COM and COD crystals.
2. When data were corrected for surface area, however, COM crystals invariably bound significantly more strongly than COD crystals.

Conclusion:

1. Binding of both COM and COD crystals to cultured human renal epithelial cells is influenced by the ambient calcium concentration and the urinary macromolecular profile.

2. Under physiological conditions, COM crystals would be more likely than COD crystals to adhere to renal epithelial cells and be retained within the kidney.

PP-063 Photomicrography of urinary deposits in a stone clinic

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Introduction: Urinary deposits are closely related to the formation of urinary stones. However, the study of these deposits is not routinely done in the stone clinics. Patients are often unaware of the importance of this study.
Objectives: The objective of this paper was to analyse the findings of urine microscopy of urinary stone patients who attended the stone clinic.
Methods: A total of 800 patients who attended the Urinary Stone Clinic during the years 2005–2007 were selected for the study. Each patient had two samples of urine studied; Early Morning Urine and Random Urine. They were classified into different groups as (a) proven stone patients (304 patients), (b) colic patients (289 patients) and (c) crystalluria patients (207 patients). They were further classified as pre-treatment group and post-treatment group. The patients had preventative treatment depending on their biochemical abnormalities. The urine samples were centrifuged and the deposits examined under the low power and high power magnification using a binocular microscope. The appropriate fields were photographed using a micro-photographic camera.
Results: A total of 23% of the urinary samples studied contained deposits (36% of the EMU and 16% of the random samples). The most common deposits were RBC (17%), PC (13%), COM crystals (7%), COD crystals (11%), uric acid crystals (2%), amorphous phosphates (1%), epithelial cells (13%) and sperm (7%). The unusual deposits included ammonium urates and cystine. The deposits were more in the male patients (25%) compared to the females (19%). 83% of the patients with significant deposits had symptoms at the time of collection of sample, while 17% were not symptomatic. Among the patients with crystals, 53% had RBC associated and 49% had pc. RBC were seen most in the COD crystal group, compared to the mixed crystals and COM or uric acid alone group. Pus cells alone were found in 2% of patients, all of whom were female. The percentage of urinary deposits was higher in the pre-treatment group (32%) than in the post-treatment group (17%). The extent of crystalluria was higher in the colic group (38%) compared to the crystalluria (22%) and stone (13%) groups.
We did a comparative study of the results of urinary deposits of our stone lab with those of 200 randomly selected urine deposits from other laboratories. The percentage of reported deposits was much lower than in the outside laboratories. None of the samples was recorded as EMU or random and centrifuged or un-centrifuged. No report from outside labs mentioned the presence of COM crystals.
Conclusion: It is concluded from the study that accurate assessment of the urinary stone patient lies in a proper microscopic evaluation of their urine. It is mandatory that EMU should be examined as there is greater chance of identifying crystals and other deposits. Centrifuged deposits showed a greater number of deposits and these should be standard in urine examination. Regular urine deposit examination should be performed in all patients coming for regular follow up.

PP-064 Stone composition and metabolic status

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Introduction: Approximately 85% of renal stones are composed of calcium compounds, of which calcium oxalate stone predominates. There are mainly two types of calcium oxalate crystals—Calcium Oxalate Monohydrate (COM) and Calcium Oxalate Dihydrate (COD). COM stones are more common than COD stones in our population.
Objectives: The objective of this paper is to study whether there is any correlation between the biochemical risk factors of the stone former and the type of calcium oxalate stone formed. We have tried to derive this relation with biochemical values of those patients in order to get an insight into the etiology of stone formation.
Methods: A study of 487 patients who had been attending the urinary stone clinic in Trivandrum during the study period of 1998–2007 was conducted. The stones from these patients were subjected to chemical analysis and FTIR Spectrographic analysis. They were categorized into COM, COD, Mixed COM + COD and others. A total of 142 urinary stone patients of whom 87 were predominantly COM stone formers and 55 COD stone formers were included in the study. Their blood samples and urine samples were biochemically analyzed. The 24-h urine values of calcium (U.Ca), Phosphorus (U.Pho), Uric acid (U.UA), Magnesium (U.Mg), Creatinine (U.Cr), Oxalate (U.Ox), Citric acid (U. CA), Sodium (U.Na) and Potassium (U.K) and serum values of Calcium (S.Ca), Phosphorus (S.Pho), Uric acid (S.UA), Magnesium (S.Mg) and Creatinine (S.Cr) and calculated values of Creatinine Clearance (Cr.Cl), Tubular Reabsorption of Phosphate (TRP), Calcium:Magnesium ratio (Ca:Mg ratio) and Calcium:Oxalate ratio (Ca:Ox ratio) were recorded. This data was analyzed using statistics programme SPSS 10.5. Comparison was made using small sample student t-test.
Results: Patients with COM stones had significantly higher mean values for urine oxalate levels compared to COD patients ($P < 0.01$). COD patients had significantly low mean values for urine magnesium compared to COM patients ($p < 0.05$). COD patients had significantly higher mean values for urine Calcium-oxalate ratio ($P < 0.01$) compared to COM. Also COD patients had significantly higher mean values for Calcium: Magnesium ratio compared to COM patients ($P < 0.01$). All other values failed to show significant difference. In those patients who had comparatively higher values of urinary oxalate, there is more chance of forming a COM type of stone than COD. While in those who have lower values of Mg, there is more chance of forming a COD type of stone than COM. Urine calcium was high in both groups without showing significant variation from the mean. In patients with high Calcium:Oxalate and Calcium:Magnesium ratios there is more chance of forming a COD stone than COM.
Conclusion: If urine oxalate is high, there is more chance of forming a COM stone and if urine Mg value is low there is more chance of forming a COD stone. Knowledge about stone composition will help to predict the most probable cause of the calculogenesis and to decide medical treatment.

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PP-065 Elemental distribution analysis of urinary crystals

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Introduction: Various crystals are seen in human urine. Some of them, particularly calcium oxalate dihydrate, are seen normally. Pathological crystals indicate crystal formation initiating urinary stones. Unfortunately many of the relevant crystals are not recognized in light microscopic analysis of the urinary deposit performed in most of the clinical laboratories. Many crystals are not clearly identifiable under the ordinary light microscopy.
Objectives: The objective of the present study was to perform scanning electron microscopic (SEM) assessment of various urinary deposits and confirm the identity by Elemental Distribution Analysis (EDAX).
Methods: 50 samples of urinary deposits were collected from urinary stone clinic. Deposits containing significant crystalluria (more than 10 per HPF) were collected under liquid paraffin in special containers and taken up for SEM studies. The deposited crystals were retrieved with appropriate Pasteur pipettes, and placed on micro pore filter paper discs. The fluid was absorbed by thicker layers of filter paper underneath and discs were fixed to brass studs. They were then gold sputtered to 100 Å and examined under SEM (Jeol JSM 35C microscope). When crystals were seen, their morphology was recorded by taking photographs at different angles. At appropriate magnification, EDAX probe was pointed to the crystals under study and the wave patterns analyzed. Components of the crystals were recognized by utilizing the data.
Results: All the samples analyzed contained significant number of crystals. All samples contained more than one type of crystal. The commonest crystals encountered included calcium oxalate monohydrate (whewellite 22%), calcium oxalate dihydrate (weddellite 32%), uric acid (10%) and calcium phosphate in the form of apatite [4], brushite (6%), struvite (6%) and octocalcium phosphate (2%). The morphological appearance of urinary crystals described could be correlated with the wavelengths obtained through elementary distribution analysis.
Conclusion: Various urinary crystals that are not reported under light microscopy could be recognized by SEM-EDAX combination. EDAX is a significant tool for recognizing unknown crystals not identified by ordinary light microscopy or SEM alone.

PP-066 Optical microscopy versus scanning electron microscopy in stone analysis

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Introduction: Stone analysis is incompletely carried out in many clinical centres. Identification of the stone component is essential for deciding future prophylaxis. The reason for the lack of interest in performing stone analysis among clinicians is the lack of proper feasible investigative set up in most hospitals. X-ray diffraction, Fourier Transmission infrared spectroscopy and scanning electron microscopy still remain a distant dream for routine hospital work. It is in this context that optical microscopy is suggested as an alternative procedure.
Objectives: The objective of this paper is to assess the utility of an optical microscope which provides a magnification of up to 40 times and gives a clear picture of the surface and cross section of the stones. In order to authenticate the morphological analysis of urinary stones, scanning electron microscopy and elemental distribution analysis were performed.
Methods: A total of 250 urinary stones of different composition were collected from our stone clinic and observed under an optical microscopy and photographs were taken at different angles of the surface and cross sections. Twenty five representative samples among these were gold-sputtered to make them conductive and were fed into an SEM machine. Photographs of the samples were taken at different angles at magnifications up to 50,000. Elemental distribution analysis was performed to confirm the composition. The observations of the two studies were compared.
Results: The different appearances of the stones under optical illuminated microscopy were mostly standardized appearances, namely pure whewellite with bosselations, weddellite with speculations, bright yellow coloured appearance of uric acid and dirty white amorphous appearance of phosphates. SEM and EDAX gave clearer pictures, but gave added confirmation of the stone composition with clear-cut recognition of different types of phosphates namely brushite, struvite, hydroxyapatite, octocalcium phosphate and various mixtures of these. From the reference standards, it was possible to confirm the optical microscopic pictures.
Conclusion: The higher magnification capacities of the scanning electron microscope and the EDAX patterns are useful for providing support for performing optical microscopy. After standardization, routine analysis can be performed with optical microscopy. The advantage of the optical microscope is that it is easy to use and samples can be analyzed in natural colour.

PP-067 EDAX versus FTIR in mixed stone

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Introduction: Mixed stones form a significant number of all urinary stones. Accurate analysis of individual areas of stones is fraught with uncertainties. Scanning Electron Microscopy with Elementary Distribution Analysis (SEM-EDAX) is a very important tool in assessing stone composition. The morphological appearance and ultra microscopic appearance must be accurately assessed to understand the genesis of stone. However, it is not feasible to perform this in routine clinical practice. Fourier Transform Infra Red Spectroscopy (FTIR) is more popular, but fails to provide the detailed morphology of individual areas of stones. A combination of these will give insight into the actual morphology of urinary stones.
Objectives: The objective of this paper is to project the role of the combination of FTIR spectroscopy and SEM-EDAX combination is achieving a total understanding of mixed stone determined by analysis of FTIR spectra and comparing these with the spectra of pure components. Spectra for different layers were obtained. The stone samples were further studied by SEM-EDAX analysis. The findings of FTIR were correlated with SEM-EDAX and detailed data generated. Using SEM-EDAX, the spatial distribution of major and trace elements were studied to understand their initiation and formation. IR radiation was passed through the samples. Some of the infrared radiation was absorbed by the sample and some of it passed through (transmitted). The resulting spectrum representing the molecular absorption and transmission, created a molecular fingerprint of the sample. The rest of the stone fragment was taken up for SEM-EDAX. The fragments less than 1 cm size were gold sputtered to make them conductive.
Results: Eighty percent of stones studied were mixtures of calcium oxalate monohydrate (whewellite) and calcium phosphate (hydroxyapatite) in various proportions. Quantitative evaluation of components was achieved through FTIR and SEM-EDAX analysis. It was possible to get an idea about the spatial distribution of molecules using SEM analysis. The composition of different areas was identified using EDAX. Analyzing with EDAX, it was possible to obtain the percentage of different elements present in a single sample.
Conclusion: It is concluded that the most common mixed stone encountered in the study is mixture of calcium oxalate monohydrate and calcium phosphate in a definite proportion. The combination identified not only the molecular species present in the calculus, but

also the crystalline forms within chemical constituents. Using EDAX, the amount of calcium, phosphorus, oxygen and carbon present in the stone sample could be determined.

PP-068 Injured renal tubular cells and osteopontin form the nucleus of kidney stone

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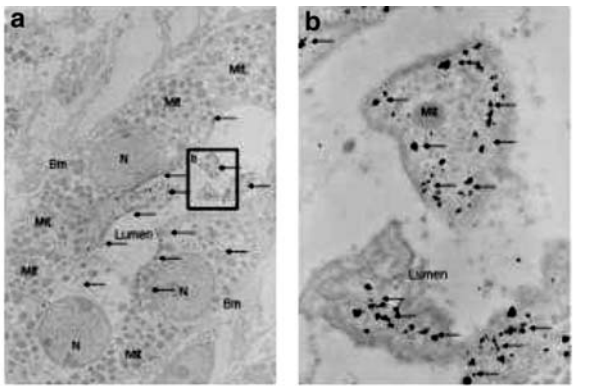
Objectives: Previously, we suggested the involvement of renal tubular epithelial cell injury in the pathogenesis of kidney stone. In this study, we evaluated the relationship between cell injury at kidney stone formation and kidney stone matrix protein, osteopontin (OPN), at the microstructural level.
Methods: Glyoxylate was administered intraperitoneally to 8-week-old male C57BL/6 mice. Kidney tissue was extracted before and 6, 12, and 24 h after administration. We examined the microstructure using a transmission electron microscope, and performed immunohistochemical staining of OPN using light, and transmission electron microscopes as well as quantitative RT-PCR of OPN.
Results: Six hours after glyoxylate administration, the internal structure of mitochondria was disordered, and the double membrane of mitochondria became indistinct. In the renal tubular epithelial cells, mitochondria and micro villi collapsed 12 h after administration. These materials were aggregated in the tubular lumen 24 h after administration, comprising the core of calcium oxalate crystal. Around the core, mitochondria and micro villi were observed. OPN began to appear on the luminal side of the renal tubular cells, and gradually appeared in the tubular lumen with the decay of microvilli or mitochondria. OPN was present in the core of the calcium oxalate crystal in the tubular lumen.



In the lumen of distal tubular cell osteopontin existed on the epithelial cells of the lumen side and on the cell debris in the lumen (mouse kidney of 24 hours after glyoxylate administration) (a ×3000 b ×25000 N:nuclear Bm:basement membrane Mt:mitochondria ←:osteopontin)

Conclusion: This study suggested the importance of tubular cell injury in the pathogenesis of kidney stone. Organelles such as mitochondria and microvilli in the tubular cell may appear in the tubular lumen, forming the core of the crystal, and leading to kidney stone. Simultaneously, OPN may also appear in the tubular lumen, and promote the aggregation of mitochondria and microvilli, playing an important role in kidney stone formation. This study clarified the pathogenesis based on the microstructure of renal tubular epithelial cells and OPN expression during the initial phase of kidney stone formation.

PP-069 A mathematical model for calculating the risk of crystallisation of calcium salts in the renal tubule

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Objectives: To devise a mathematical model to approximate tubular fluid composition in order to calculate the risk of precipitation of Ca salts in the renal tubule between glomeruli and ducts of Bellini in the human kidney.
Methods: A dynamic model of the human kidney was devised to generate tubular fluid composition continuously along the renal tubule. The model included data on pH and the concentrations of Ca, Mg, Na, K, NH_4^+, P, oxalate (Ox), citrate (Cit), SO42- and uric acid (UA) within tubular fluid. The relative supersaturation (RSS) of Ca salts was calculated at all points along the renal tubule using SUPERSAT [1]. The effect of varying the tubular reabsorption of water and of H^+, Ca, P and Ox on RSS was evaluated. In a pilot study, plasma and renal conditions were constructed to yield average urine compositions produced by normal subjects (N) (volume 1.5 l, pH 5.94, (in mmol/day) Ca 5.4, Mg 4.4, Na 189, K 58, NH_4^+ 23, P 20, Ox 0.33, Cit 2.6, SO42- 16 and UA 3.2), recurrent CaOx stone-formers (SF) (volume 1.4 l, pH 5.94, Ca 8.4, Mg 3.8, Na189, K 58, NH_4^+ 23, P 29, Ox 0.67, Cit 1.5, SO42- 16 and UA 4.5) and recurrent mixed CaOx/CaP SF (volume 1.4 l, pH 6.55, Ca 8.4, Mg 3.8, Na189, K 58, NH_4^+ 23, P 29, Ox 0.67, Cit 1.5, SO42- 16 and UA 4.5) [2].
Results: A summary of the RSS values of CaOx and CaP at different points along the renal tubule of N and SF is shown in the Table 1. (u = undersaturated; + weakly supersaturated; ++ moderately supersaturated; +++ highly supersaturated with crystallisation; ++++ grossly supersaturated with marked crystallisation; PT proximal tubule; DLH descending limb; ALH ascending limb; DT distal tubule, ECD, MCD and LCD early, mid and late collecting ducts). In N, tubular fluid is undersaturated with both CaOx and CaP throughout most of the renal tubule becoming only slightly supersaturated in the loop of Henle and in the late collecting duct. In recurrent Ca SF, it is mildly supersaturated with CaOx in the loop and increasingly supersaturated through the CD. In addition, urine from SF is moderately supersaturated with CaP in the loop and increasingly supersaturated through the CD, particularly in the mixed CaOx/CaP SF.

Table 1 Supersaturation of tubular fluid throughout the nephron

Salt	Subjects	RSS in PT	RSS in DLH	RSS in ALH	RSS in DT	RSS in ECD	RSS in MCD	RSS in LCD	RSS in Urine
CaOx	Normals	u	u	u	u	u	u	+	+
	CaOx SF	u	+	+	u	+	++	+++	++++
	CaOx/CaP SF	u	+	+	u	+	++	+++	++++
CaP	Normals	u	+	u	u	u	u	+	+
	CaOx SF	u	++	+	u	u	u	+	+
	CaOx/CaP SF	u	++	+	u	u	++	+++	++++

Conclusion: The model provides a useful tool (a) to predict the risk of crystallisation at all points along the renal tubule, (b) to correlate with patterns of stone-formation and nephrocalcinosis observed *in vivo* and (c) to understand better the underlying pathophysiology of nephrolithiasis.

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PP-070 A renal crystal clearing mechanism in rat and man

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Introduction: The kidney possesses a number of defense mechanisms against nephrocalcinosis by preventing intratubular crystal formation and adherence. Failure of these mechanisms, which act prior to crystal adhesion, results in intratubular nephrocalcinosis.
Objectives: To investigate renal handling of adhered crystals.
Methods: In the current study we investigated whether and what kind of post adhesion crystal handling mechanism might be available. Therefore the fate of luminally adhered crystals was investigated by quantifying the number and morphology of crystal containing tubules in rats at day 2, 5, 10 and 25 after arrest of a 4-day ethylene glycol (EG) administration period on Von Kossa and PAS/PCNA stained renal sections. In addition, Von Kossa stained renal biopsy sections of patients with nephrocalcinosis of different etiology (acute phosphate nephropathy, primary hyperoxaluria, preterm neonates and transplant patients) were included for morphological evaluation of the crystal containing tubules.
Results: In rats, after arrest of EG-administration the extent of nephrocalcinosis decreased from 364 crystal containing tubules (range: 2–1326) at day 2 to 1 (range: 0–4) at day 25. During the recovery period following EG-administration, 5 different types of crystal containing tubules/sites were identified: (a) normal tubules containing intraluminal intact crystals either lying free in the lumen or adjacent to the epithelium, (b) tubules with intact crystals adhered to flattened regenerating epithelial cells, (c) tubules with intact adhered crystals overgrown by the tubular epithelium, (d) interstitial degraded crystals and (e) granulomas. The relative frequencies of the phenotypes A ($12.5 \pm 2.0\%$) and B ($54.5 \pm 5.2\%$) at day 2 decreased to zero levels by day 25. Concomitantly, the relative frequencies of phenotypes C and D increased consecutively, reaching up to 41.4 ± 10.2 and $46.3 \pm 14.2\%$ by day 10, respectively. The few remaining crystal deposits present at day 25 either were accompanied by a low-to-moderate OX-1 and ED-1 positive cellular infiltration. In man, all four pathologies showed epithelial crystal overgrowth independently of the underlying disorder or the nature of the crystals.
Conclusion: The quantified consecutive changes in frequencies of the different crystal associated tubular phenotypes during the EG-recovery phase, together with morphological changes of the crystals and the concomitant disappearance of renal crystal deposits, quantitatively corroborate, for the first time, the existence of an important and active renal crystal clearing mechanism (in both rat and man) in which adherent crystals are translocated to the interstitium by epithelial overgrowth and subsequently are degraded. The biological mechanism underlying crystal degradation is topic of current research.

OP-051 Oxalate handling is altered in rats with metabolic acidosis

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Introduction: Previous studies have indicated that urinary oxalate excretion is reduced in rats with metabolic acidosis (MA), however, the mechanism by which this occurs is unclear.
Objectives: Because of the emerging role of the large intestine in compensatory enteric elimination of oxalate, we addressed the question of whether the reduction in urinary oxalate induced by MA was correlated with changes in colonic transport of oxalate.
Methods: MA was produced by providing rats with drinking water containing 0.28 M NH_4Cl and 5% (w/v) sucrose for 14 days and untreated rats served as controls. At the end of the treatment period, transepithelial fluxes of ^{14}C-oxalate were measured across short-circuited colonic tissues in Ussing chambers. In addition, since several proteins encoded by the SLC26 gene family are acid-base exchangers in the intestine with a measurable affinity for oxalate, we assessed the abundance of three of these in colonic tissues, including slc26a6, slc26a3, and slc26a1.
Results: Urinary oxalate was measured in both groups and found to be reduced by almost 70% in MA rats compared to controls (from 9.56 ± 0.59 micromoles/24 h to 3.15 ± 0.19 micromoles/24 h, $n = 8$ in each group). However, there were no MA-induced changes in serum oxalate concentrations. Acidosis stimulated a directional change in net oxalate flux across the distal colon from absorption (6.43 ± 0.61 picomoles.cm^{-2} h^{-1}, $n = 22$ tissue pairs) to secretion (-4.58 ± 0.57 picomoles.cm^{-2} h^{-1}, $n = 14$ tissue pairs) and this occurred by way of a significant reduction in the mucosal to serosal flux coupled with a significant increase in the serosal to mucosal flux. We also found that the MA-induced net secretory flux of oxalate was abolished following acute serosal addition of the prototypical inhibitor of the Na^+-H^+ exchanger (EIPA, 50 μM) and the same maneuver enhanced net oxalate absorption across control tissues indicating a coupling of oxalate transport to Na^+-H^+ exchange activity and PH_i regulation in the distal colon.
Conclusion: We conclude from this study that the MA-induced enteric elimination of oxalate across the distal colon leads to the reduction in urinary oxalate excretion. Interestingly, the alterations in colonic oxalate transport in acidotic rats were correlated with a down-regulation of PAT1 protein in colonic tissue but no changes in SAT1 or DRA protein expression were observed.

OP-052 Knockdown of SLC26A6 in Caco-2 monolayers reveals PAT-1 symmetrically mediates apical oxalate exchange

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Introduction: The net transport of oxalate across intestinal epithelia is an important component of oxalate homeostasis: increasing net secretion and/or decreasing net absorption can reduce urinary oxalate levels in animal models. Members of the SLC26A gene family have attracted attention for their ability to mediate oxalate/anion exchange and as potential regulatory targets. For example, the PAT-1 gene product of slc26a6 appears to be important in the secretion of oxalate in the mouse ileum, since net oxalate secretion is reduced in slc26a6 knockout mice. It is not clear whether this directionality is a property of the slc26a6 exchanger or a consequence of the counter-ion gradients in ileal enterocytes.
Objectives: The principal aim of this report was to test the hypothesis that the multifunctional anion exchanger PAT-1 mediates bi-

directional translocation of oxalate across the apical membrane of Caco-2 cells.
Methods: Caco-2 (C2BBe1) cells were grown on polycarbonate membranes or glass coverslips in the presence or absence of a transfection cocktail containing a siRNA for slc26a6 to reduce the expression of PAT-1. After 6–8 days the coverslip-grown monolayers were used to assess Cl-dependent intracellular pH regulation (Cl-HCO_3 exchange) in BCECF loaded monolayers using ratiometric fluorescence techniques. Monolayers grown on polycarbonate membranes were employed to measure the unidirectional fluxes of ^{14}C-oxalate using standard Ussing chamber techniques in bicarbonate buffers.
Results: Transfecting Caco-2 cells with slc26a6 siRNA reduced slc26a6 protein expression in immunoblots to less than 60% of controls and reduced slc26a6 mRNA expression measured by real time RT-PCR to less than 20% of controls. Knockdown of slc26a6 increased pH_i and depressed Cl-dependent HCO_3 exchange which implies functional alterations in $Cl-HCO_3$ exchange accompany successful slc26a6 knockdown. No net oxalate flux was observed in controls or slc26a6 knockdown monolayers. The unidirectional oxalate fluxes in slc26a6 knockdown monolayers were less than 60% of controls and both the M-S and S-M oxalate fluxes were reduced to the same degree. In control monolayers, mucosal addition of the anion exchange inhibitor DIDS blocked the M-S and S-M oxalate fluxes in a similar manner with an EC_{50} of 6 μM. The DIDS-insensitive fluxes of oxalate (in both directions) remaining after addition of 100 μM mucosal DIDS were not significantly different between control and slc26a6 monolayers and likely represents paracellular oxalate flux.
Conclusion: The unidirectional fluxes of oxalate across Caco-2 monolayers are distinctly symmetric, even though transcellular pathways represent a predominant fraction (>80%) of each unidirectional flux. Of the transcellular oxalate flux, PAT-1 mediates at least half oxalate exchange in both directions. Reducing PAT-1 expression by siRNA methods or reducing PAT-1 functionality with mucosal DIDS significantly reduces oxalate transport, yet these manoeuvres do not distort the symmetry of unidirectional fluxes. If slc26a6 is properly targeted to the apical membrane, these results imply that PAT-1 can mediate apical influx and efflux depending upon the prevailing asymmetries in counter-ion gradients. Hence, identifying slc26a6 solely as a mediator of oxalate secretion is not supported.

OP-053 Prevalence of hyperoxaluria after bariatric surgery

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Objective: Recent investigations have shown increased oxalate excretion in patients who have formed kidney stones after undergoing contemporary bariatric surgery. We performed a study to determine if there is an increased prevalence of hyperoxaluria after such procedures performed in non-stone formers.
Methods: Fifty-eight patients who underwent laparoscopic Roux-en-Y(RY) (52) or biliopancreatic diversion-duodenal switch (DS) (6) collected 24 h urine specimens for 6 months or longer after bariatric surgery. Standard stone risk parameters were assessed. Comparisons were made with a group of healthy non-stone forming adults and stone formers from a commercial database.
Results: The bariatric group had a significantly higher mean urinary oxalate excretion, 67.2 mg/day, compared with controls and stone formers, 34.1 and 37.0 mg/day, respectively ($P < 0.001$). The mean oxalate excretion of those subjected to DS was higher than the RY group; 90 versus 62 mg/day ($P < 0.05$). There was a significant correlation between urine oxalate excretion from the two days of collection but some patients showed significant variability. Seventy-four percent were hyperoxaluric in at least one 24-h urine collection. Twenty-six percent demonstrated profound hyperoxaluria (oxalate excretion >100 mg/day) on at least one occasion. This occurred in 3 of the 6 DS group and 12 of 52 of the RY cohort. Hyperoxaluria was not uniformly expressed.
Conclusions: There is a high prevalence of hyperoxaluria in patients without a history of kidney stones who are subjected to bariatric surgery. A significant proportion of these patients have profound hyperoxaluria which is not uniformly expressed.

OP-054 Nephrocalcinosis and nephrolithiasis in children: is hypercalciuria the most important risk factor?

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Introduction: Hypercalciuria is assumed to be the most important risk factor for nephrocalcinosis and nephrolithiasis in pediatric patients. However, the impact of other lithogenic factors like oxalate or inhibitory substances like citrate, either alone or as combined secondary risk factors has not yet been evaluated systematically.
Methods: Therefore, we repeatedly performed a detailed analysis of urine excretion parameters in a group of pediatric patients with (1) nephrocalcinosis ($n = 70$, 46 boys, 24 girls, mean age: 6.9 years) and (2) nephrolithiasis ($n = 149$, 90 boys, 59 girls, mean age: 9.7 years) and (3) primary hyperoxaluria ($n = 20$, 12 boys, 8 girls, mean age: 8.2 years). Separation and quantification of urinary oxalate was performed using a Dionex Series Dx500 gradient ion chromatography system. Urinary concentrations of calcium and magnesium were determined by atomic absorption spectroscopy. Citric and uric acid were analyzed enzymatically using the citrate lyase and uricase methods, respectively.
Results: (1) Isolated hypercalciuria was found in 1 patient (1%), isolated hyperoxaluria in 18/70 (26%) and isolated hypocitraturia in 8/70 (11%) children with nephrocalcinosis. Hyperoxaluria and concomitant hypercalciuria was present in 10/70 (15%) patients while hyperoxaluria was accompanied by hypocitraturia in 7/70 (10%) cases. Only in 6 (8%) patients, were all urinary parameters in the normal range. (2) In pediatric patients with nephrolithiasis, isolated hyperoxaluria was the most frequent finding occurring in 49/149 (31%) patients. Hyperoxaluria with concomitant hypocitraturia was found in 23/149 (15%) and isolated hypocitraturia in 21/149 (14%) cases. Only 9 of 149 (6%) children presented with isolated hypercalciuria. In 6/149 (5%) patients, all urine values resulted normal. (3) As expected in primary hyperoxaluria, 9/20 (45%) children presented with isolated hyperoxaluria. In 7/20 (35%) patients, hyperoxaluria was associated with hypocitraturia, in 3/20 (15%) patients with hypercalciuria.
Conclusion: In our patient population of 229 patients with nephrocalcinosis or nephrolithiasis, hyperoxaluria was the most important risk factor for both nephrocalcinosis and nephrolithiasis. In contrast to previous studies, hypercalciuria only played an important role in combination with a second risk factor. However, we found a high incidence of hypocitraturia as well as isolated factor as in combination with hyperoxaluria. Interestingly, also in the subgroup of patients with primary hyperoxaluria, hypocitraturia is a frequent finding as second risk factor. These findings clearly indicate that (1) it is not sufficient to only screen the urine for calcium excretion, (2) that hypercalciuria is not the primary risk factor in a central European population of children and adolescents and (3) that only a detailed

evaluation of urinary excretion parameters will give profound basis of the underlying pathology and provide adequate clues for a successful treatment strategy.

OP-055 Estrogen regulation of renal citrate metabolism

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Introduction: Estrogen status has long been suspected of being etiologically important in the formation of calcium oxalate stones in women. Indeed, it has been demonstrated that estrogen has direct effects at the distal convoluted tubule to increase renal calcium reabsorption and lower urinary calcium excretion. Few studies have examined the role of estrogen on renal citrate excretion but, when performed, have shown estrogen to increase urinary citrate excretion. Because citrate is a potent inhibitor of renal stone formation, understanding the hormonal factors that may regulate its production and secretion are of potential importance.
Objectives: The objective of the current study was to assess the effects of estrogen on renal citrate excretion and on its renal production and transport. This was accomplished utilizing the aromatase knock-out murine model (ArKO) of estrogen deficiency and their wild type littermates as controls. Mice deficient in aromatase have been shown to be estrogen deficient and to display a phenotype consistent with estrogen lack.
Methods: This study was performed in the aromatase-deficient (ArKO) murine model of estrogen deficiency. In this mouse model of estrogen deficiency, female mice lacking aromatase cannot synthesize estrogen from androgen precursors. Wild type (WT) female litter mates were used as the control group. Adult female (5–6 months of age) wild-type ($n = 5$) and ArKO ($n = 5$) mice were placed in metabolic cages for 3 days to obtain 3–24 h urine collections for quantitation of urinary citrate (citrate lyase method) and creatinine. Animals were then sacrificed and blood, kidney, and liver tissue harvested. Tissue was immediately frozen in liquid nitrogen and stored at −80°C. Frozen renal tissue from 3 WT and 3 ArKO mice was pulverized in liquid N2 and homogenized in lysis buffer containing protease inhibitors. Following protein determination, aliquots were subjected to 10% SDS-polyacrylamide gel electrophoresis and subsequent Western blotting using antibodies to murine ATP citrate synthase and the sodium dicarboxylate transporter. Bands were visualized with the supersignal west femto substrate kit and band density quantitated via scanning. Results were expressed relative to tubulin.
Results: Twenty-four hour mean urinary citrate excretion corrected for creatinine (mg/mg) was found to be slightly higher in the ArKO female as compared to WT littermates (9.8 ± 0.5 (SEM) vs 8.1 ± 1.6). This difference was not significant. ATP citrate synthase protein expression was observed to be lower in ArKO mice compared to WT controls (0.625 ± 0.140 vs. 0.704 ± 0.131) while the sodium dicarboxylate transporter protein expression trended upward in the ArKO mice (2.61 ± 0.32 vs. 2.08 ± 0.22). Again, neither of these differences was statistically significant.
Conclusion: Contrary to limited reports for postmenopausal women, we did not observe a significant difference in urinary citrate concentration between estrogen deficient and replete mice nor in the proteins involved in the synthesis of citrate or its transport in the renal proximal tubule. The lack of significant difference may be due to the rather small number of observations in this pilot study. Currently, additional animals are being examined, as well as male WT and ArKO mice. In addition, assessment of ATP citrate lyase expression is also underway in this murine model of estrogen deficiency.

OP-056 Sodium thiosulfate reduces calcium phosphate kidney stone formation in genetic hypercalciuric stone forming rats

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Introduction: Sodium thiosulfate (STS) has been reported to reduce calcium kidney stone formation and be an effective treatment for calciphylaxis in ESRD in anecdotal and uncontrolled trials. However, there has not been a controlled study to determine the effectiveness of STS.
Objectives: Using the genetic hypercalciuric (GHS) rat, an animal model of spontaneous calcium phosphate (CaP) stone formation, we studied the effect of STS therapy on urine chemistries and stone formation.
Methods: GHS rats were fed normal rat chow with or without STS supplementation for 18 weeks ($n = 12$ in each group). STS was dosed to give urine levels similar to those reported in patients treated for kidney stones. Urine was collected at 6, 12 and 18 weeks and urine stone risk factors and thiosulfate excretion was measured. Concentration product ratios (CPR) were measured as a marker of urine supersaturation by adding brushite crystals to urine, mixing for 48 h at a fixed pH and comparing Ca and P concentration products before and after crystal incubation. Upper limit of metastability (ULM) of urine was measured by adding increasing amounts of Ca to the urine until CaP crystallization occurs.
Results: Urine thiosulfate concentration was 3.8 ± 0.2 mM, comparable to levels reported in a prior study in kidney stone patients. Urine Ca and P were significantly higher in the STS treated rats, while urine citrate and volume were lower, all of which would increase urine CaP saturation. Urine pH was lower in the STS treated rats, which would reduce CaP saturation. The CaP CPR was higher in the rats treated with STS but did not reach statistical significance. The CaP ULM and the difference between ULM and CPR, which would offset crystallization risk, also trended higher in the STS treated rats, but did not reach statistical significance. Kidney stone formation was significantly reduced in the STS treated rats. Eleven of 12 untreated rats formed stones (in 15 of 24 kidneys) whereas only 3 of 12 rats treated with STS (in 3 of 24 kidneys) formed kidney stones, $P < 0.002$.

It has been proposed that STS reduces Ca crystallization by forming a soluble complex with Ca. We measured the effect of STS on ionized Ca in aqueous solutions. Addition of STS in concentrations up to 9 mM had modest effect on ionized Ca concentration, similar to that of sulfate but much less than that of citrate. At the concentrations achieved in urine, the effect of thiosulfate on Ca would be small in relation to that of sulfate and citrate in the urine.

Urine measurements	STS	No STS	*P* value
Ca (mg/day)	15.7 ± 0.4	11.5 ± 0.4	<0.001
P (mg/day)	22 ± 1	11 ± 1	<0.001
Citrate (mg/day)	38 ± 2	81 ± 3	<0.001
Volume (ml/day)	37 ± 1	43 ± 1	<0.001
pH	6.31 ± 0.02	$6.72 \pm .08$	<0.001
CaP CPR	4.6 ± 0.8	3.2 ± 0.5	NS
CaP ULM	9.8 ± 2.4	5.3 ± 0.9	NS

Conclusion: In this controlled study of STS used as a treatment for CaP crystallization in the kidney, we show a significant beneficial

effect. The mechanism of action of STS on CaP crystallization still needs to be determined. STS is a promising therapy for kidney stone patients, controlled trials will need to be carried out in humans.

OP-057 Potassium citrate for the treatment of nephrolithiasis associated with Topiramate use

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Introduction: Topiramate (TPM) is a novel neuro-modulatory agent approved for the treatment of a number of neurologic disorders. TPM use is associated with an increased risk of nephrolithiasis, and we have previously shown that TPM-treated patients share overlapping features with patients with distal renal tubular acidosis (d-RTA), including marked hypocitraturia and significantly alkaline urine [1]. In patients with d-RTA, potassium citrate is the drug of choice for the prevention of stones. However, despite the beneficial effects conferred by citrate, there is a concern that potassium citrate may further raise urine pH in TPM treated subjects, which will increase the abundance of mono-hydrogen phosphate, promoting calcium phosphate stone formation.
Objectives: To evaluate the effect of potassium citrate on stone risk parameters in patients treated with Topiramate.
Methods: We studied four TPM-treated subjects before and after 4 weeks of potassium citrate (20 mEq BID) therapy. During the last 2 days of each phase, subjects collected two 24-h urine for stone-risk, acid-base profiles and crystallization parameters. Subjects were studied under a constant metabolic diet with a fixed content of calcium, sodium, phosphorus, potassium and acid-ash content.
Results: No significant difference was noted in serum parameters, including serum creatinine, bicarbonate and calcium (Table 1). Urine potassium increased significantly with potassium citrate use (38 ± 13 to 66 ± 15 mEq/day, $P < 0.005$). Urinary pH increased marginally from 6.63 ± 0.22 to 6.78 ± 0.13 ($P = 0.15$), with a slight rise in urine citrate (185 ± 88 to 199 ± 77 mg/day, $P = 0.6$). Concomitantly, there was a slight drop in urine calcium (218 ± 36 to 189 ± 41 mg/day, $P = 0.15$). Crystallization parameters including relative supersaturation ratio (RSR) for calcium oxalate and brushite did not change significantly.
Conclusion: Judicious use of potassium citrate may be useful as a countermeasure against TPM-associated stone risk. However, the potential role of potassium citrate in modulating stone risk and incidence of nephrolithiasis in TPM-treated patients needs to be further investigated in larger randomized studies.

Reference

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PP-071 Cardiovascular risk and kidney stone disease

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Introduction: Urinary stone disease is associated with several cardiovascular risk factors (excess salt and animal protein, hypertension, metabolic syndrome) and, more recently, the development of stroke.
Objectives: Our objective was to study the frequency of cardiovascular risk factors and cardiovascular events before and after management of urolithiasis.
Methods: We retrospectively collected data from patients born before 1956 and managed surgically or instrumentally for urolithiasis in our establishment in 1994 concerning the frequency of cardiovascular risk factors and the incidence of acute coronary syndrome, stroke or acute lower limb ischaemia before or after treatment of urolithiasis.
Results: Data were obtained from 33 patients, revealing 12 events including 5 previous events (4 cases of acute coronary syndrome, 1 ischaemic stroke) and 7 subsequent events (5 cases of acute coronary syndrome with 1 death, 1 ischaemic stroke, 1 case of acute lower limb ischaemia) an average of 5.7 years after management. These 33 patients had an average of more than two risk factors.
Conclusion: This retrospective study based on a small sample size demonstrated a high frequency of risk factors and cardiovascular events. This correlation needs to be studied in more detail. Urolithiasis could constitute an indirect cardiovascular risk factor dependent on "classical" risk factors, suggesting the need for integrated management of stone patients, in the same way as for patients with erectile dysfunction.

Table 1 Serum and urinary biochemical parameters

Parameter (Units)	TPM Before K Citrate (Mean ± sd)	TPM + K Citrate (Mean ± sd)	*P*-value (paired *t* test)
Serum potassium (mmol/L)	4.4 ± 0.3	4.2 ± 0.3	0.43
Serum HCO_3 (mmol/L	25.0 ± 1.9	25.0 ± 1.3	0.98
24-h urine pH	6.63 ± 0.22	6.78 ± 0.13	0.15
24-h urine potassium (mEq/day)	38 ± 1 3	66 ± 15	<0.005
24-h urine citrate (mg/day)	185 ± 88	199 ± 77	0.64
24-h urine calcium (mg/day)	218 ± 36	189 ± 41	0.15
24-h urine oxalate (mg/day)	31 ± 2	33 ± 4	0.85
24-h urine phosphorus (mg/day)	528 ± 226	519 ± 96	0.78
RSR Calcium oxalate	5.6 ± 1.6	6.2 ± 1.0	0.42
RSR Brushite	1.7 ± 0.7	2.1 ± 0.8	0.27

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PP-072 Psycho-vegetative stressors: metabolic and clinical study on the risk of calcium oxalate (CaOx) urinary stone formation

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Objectives: The causes of CaOx stone formation are multifactorial. There are indications of the influence of psycho-vegetative factors, whose reaction situation should be studied with regard to metabolically significant risk situations of urinary stone formation.
Methods: A total of 25 control subjects (58 years; m/f 20/5); 23 idiopathic CaOx stone formers (88% recurrent stone formers; 55 y; m/f 21/4) were studied for 4 days of standard diet without stone metaphylaxis; diagnostic of blood values and urine collected over 24 h—calculation of CaOx risk indices (activity product $AP = 1.9 \times (Ca^{0.84}) \times (Ox) / (Cit^{0.22}) \times (Mg^{0.12})$ x $(Vol^{1.03})$ [parameter values in mmol/d]; relative supersaturation [RSS; mmol/l]). Personal stress questionnaire (Trier Inventory of Chronic Stress [TICS]). Evaluation of the sympathetic/parasympathetic reaction state (pupillometry/heart beat variability, …). Median and mean value comparison—Mann–Whitney *U* test respectively. Student's *t* test; correlation according to Spearman.
Results: The risk formula signal for the CaOx stone formers vs. healthy test persons is an increased lithogenic risk (Table 1). For CaOx stone formers, the stress questionnaires showed sufficient correlation to simultaneously experienced stress and stone episodes (75%) as well as to metabolic lithogenic risk (RSS; AP)—including increased chronic stress due to e.g. "poor need satisfaction" as one of the characteristics of the TICS ($r = 0.598$, $P < 0.01$; $r = 0.527$, $P < 0.05$). This patient group also showed psychovegetative symptoms with important relationships to: increased sympathetic reaction state at eyes—chronic stress ($r\tau = 0.473$, $P = 0.010$; $r = 0.436$, $P = 0.018$); exhaustion of the parasympathetic nervous system—stone formation risk (RSS; AP) ($r = 0.631$, $P < 0.001$; $r = 0.426$, $P < 0.04$).
Conclusion: As stress response in the complex condition of "metabolic syndrome", lithogenically significant urine parameters in CaOx stone formers can reveal trends in the sense of an increased stone formation risk. In this patient group the study findings of the psycho-vegetative reaction situation are in clear correlation to chronic stress. We conclude that Inclusion of preventive "psychological hygiene" is important in the metaphylaxis of these patients!

Table 1 Group comparison of the risk indices

Risk Indices	Control mean ± SD	CaOx mean ± SD	*P* value
RSS (EQUIL2)	5.0 ± 2.3	7.2 ± 3.7	<0.01
AP (TISELIUS)	0.9 ± 0.4	1.7 ± 1.4	<0.01

PP-073 Hypocitraturia, but not hypercalciuria, is the characteristic abnormality in patients with urolithiasis associated with long-term use of glucocorticoids

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Introduction: Glucocorticoids are used for various medical conditions because of their diverse pharmacological effects. The administration of glucocorticoids is considered to be a risk factor for urinary stone formation due to hypercalciuria. However, little has been reported on urolithiasis associated with the long-term use of glucocorticoids.
Objectives: The purpose of this study was to clarify different clinical features of urolithiasis in patients with or without long-term use of glucocorticoids.
Methods: We studied 26 patients with urolithiasis who had been treated by long-term glucocorticoids for various systemic diseases (Steroid group). As a control, 41 patients with calcium stones, who were matched by age, sex and body weight, were recruited (Control group). Patients in the Control group had no history of specific diseases causing urolithiasis, such as primary hyperparathyroidism or Cushing's syndrome. Collection of 24-h urine samples was performed under a free diet setting and urinary parameters related to urolithiasis were compared between the two groups. Ion-activity product indices of calcium oxalate and calcium phosphate were calculated from urinary chemistries. Stone composition was analyzed by FTIR if the stones were passed spontaneously or removed.
Results: In the Steroid group, 19 of 26 patients had one or more collagen diseases as underlying causes (systemic lupus erythematosis in 9, rheumatoid arthritis in 6, Sjögren's syndrome in 4, and dermatomyositis in 2). Nine patients in the Steroid group underwent an ammonium chloride acid loading test and all of these patients were diagnosed as having distal renal tubular acidosis (dRTA). Patients in the Steroid group had been treated with Prednisolone for a mean of 7.92 years until the first stone episode (mean dose of 9.25 mg/day). In 61.5% of the Steroid group patients, the stone was composed of pure calcium phosphate or predominantly calcium phosphate. On the other hand, in the Control group, only 2.4% of the patients had calcium phosphate as a predominant stone composition. The prevalence of hypocitraturia (below 320 mg/day) was significantly higher in the Steroid group (69.6%) than in the Control group (34.1%). Moreover, urinary citrate excretion was significantly lower in the Steroid group than in the Control group (224.3 vs. 413.5 mg/day). However, there was no significant difference in the incidence of hypercalciuria (above 4 mg/kg/day) and in the level of calcium excretion between the two groups; 11.5% and 147.2 mg/day in the Steroid group and 22.0% and 177.9 mg/day in the Control group. Urine pH was significantly higher in the Steroid group (6.58 vs. 6.38). There was no significant difference in ion-activity product indices of calcium oxalate and calcium phosphate between the two groups.
Conclusion: The present study suggests that patients with long-term use of glucocorticoids and urolithiasis are likely to have hypocitraturia, but not hypercalciuria, that is associated with a higher urine pH and predominantly calcium phosphate-containing stones. Stone formation in this specific group may be affected by underlying disease itself or disorder in the acidification of urine.

PP-074 The relationship between urinary sodium and calcium excretion in women with idiopathic calcium nephrolithiasis and controls

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Introduction: The prevalence of hypercalciuria is higher in calcium stone formers rather than controls. It is known that a direct

relationship between urinary sodium and calcium exists and that a high salt intake inhibits tubular calcium reabsorption.
Objectives: To evaluate the relationship between urinary calcium and sodium in normotensive women with idiopathic calcium nephrolithiasis (ICN) in comparison with a control group.
Methods: We evaluated 420 ICN women with normal blood pressure (age 40 ± 14), without conditions associated with calcium nephrolithiasis (e.g., primary hyperparathyroidism, RTA, IBD etc) or medical therapy and 290 matched healthy women (age 41 ± 13). Two 24-h urine samples were obtained to assess urinary stone risk factors.
Results: Both in ICN women and in controls a linear correlation between urinary sodium and calcium exists (Pearson's $R = 0.476$; $P < 0.001$ and R = 0.431 $P < 0.001$). However, the slope is significantly higher in ICN group (0.9588 vs. 0.6772; $P = 0.02$); this means that for the same urinary sodium level, calcium is higher in ICN.
Conclusion: Women with ICN show a greater "sodium sensitivity" in comparison with healthy subjects. This aspect may in part explain the higher prevalence of hypercalciuria and it may involve many genetic and environmental factors not completely understood. Further studies are needed.

PP-075 Role of overweight/obesity on the metabolic risk factors for renal lithiasis

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Introduction: In recent decades, there has been an increasing prevalence of urolithiasis in many western countries and the same time there has been an increasing progression of obesity that has reached epidemic proportions.
Objectives: The aim of the present study was to assess the influence of overweight/ obesity on the metabolic risk factors for renal stone formation.
Methods: We studied 799 renal stone formers (462 men and 337 women) who came to the clinic for metabolic risk factor evaluation. They were all studied with a standard protocol (two 24 h urine collections and serum parameters). They were divided according to their BMI in normal (BMI < 25) overweight (BMI 26–29) and obese (BMI > 30). Low weight individuals were excluded.
Results: Among women 55.2% had normal weight, 25.5 were overweight and 19% were Obese; among men 27.3% had normal weight, 51.7 were overweight and 21% were obese. Age increased significantly with increasing BMI both in men and women. In women there was a significant increase in the excretion of creatinine, phosphorus, uric acid, sodium and oxalate with increasing BMI, but no change was observed in calcium, magnesium, citrate and urine pH. In men there was a significant increase in the excretion of creatinine, phosphorus, uric acid, sodium, oxalate magnesium and citrate with increasing BMI, no change in urinary calcium and significant progressive decrease in urinary pH.
Conclusion: Both in men and women we found a significant increase in the urinary excretion of two promoters of stone formation, uric acid and oxalate but no change in urinary calcium. There was either no change or increase in magnesium and citrate, inhibitors of crystallization, and a significant decrease in urine pH only in men.

PP-076 Clinical and metabolic risk factor evaluation of kidney stone formers between 17–20 years old

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Introduction: Many series have described the metabolic risk factors found in adults and children with renal lithiasis but little is known about the metabolic alterations present in kidney stone formers between 17–20 years old.
Objectives: In this retrospective study we evaluated the clinical presentation, family history and metabolic risk factors present in patients between 17–20 years old with renal stones seen at our clinic between 1994 and 2000.
Methods: We reviewed the charts of 79 patients with kidney stone disease with a mean age of 18.8 ± 1.0 years (43 males and 36 females; male/female ratio1.19/1.0) studied with a standard protocol that included two 24-h urine collections.
Results: The most frequent form of presentation was flank pain (72%), followed by hematuria (38%) and urinary tract infection (34.1%). A positive family history of stone disease in first degree and second-degree relatives was found in 32.9 and 34.1% respectively. Biochemical abnormalities were found in 95% of all cases. Single urine metabolic risk factors were present in 57% ($n = 45$) of patients and multiple metabolic risk factors were present in 38% ($n = 30$) of the cases. Of all urinary metabolic risk factors found, hypocitraturia (alone or in combination) and idiopathic hypercalciuria (alone or in combination) were found in 38 and 36.7%, respectively, while hypomagnesiuria (alone or in combination) was found in 30, 3% of the cases.
Conclusion: Metabolic abnormalities are very frequently found in this particular population with renal lithiasis, comparable to our adult series. Similar to children and different from the adult stone formers, hypocitraturia was found with a similar frequency as hypercalciuria. These patients have very often first or second-degree relatives with stone disease.

PP-077 Is undue acidity in uric acid stone formers caused by a decreased excretion of ammonia or by excessive protein intake?

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Introduction: The major risk factor of uric acid lithiasis is a disturbed acidification of the urine with permanently low pH-levels.
Objectives: The pathogenesis is unclear. Nutritional factors and a genetically determined abnormality in the glutamine metabolism resulting in reduced ammonia excretion are discussed.
The latter might be a potential explanation for the unusually high percentage of UASF (28%) in our region (Upper Franconia).
Methods: In $n = 110$ uric acid stone formers (UASF) the following parameters were measured: age, sex, BMI, recurrence rate; uric acid, calcium, creatinine (blood); uric acid, calcium, ammonia, urea, citrate, pH, volume (24 h urine). Calcium was determined using atomic absorption, creatinine, urea, citrate and uric acid spectrophotometrically, ammonia enzymatically and pH by dipsticks.
Results: A reduced excretion of ammonia was found in 18/110 patients (16%), an increased excretion of urea as a marker of excessive protein intake showed 29/110 patients (26%). There were no significant correlations between the urinary pH and all the other parameters examined.
Conclusion: The disturbed acidification of urine as found in the majority of UASF cannot be explained by decreased ammonia excretion exclusively. The majority of UASF has a normal ammonia excretion. Decreased ammonia levels are found in only 16% of these patients. We could not confirm the results of others finding a

significantly reduced ammonia excretion in UASF. Persistently low urinary pH is not a uniform entity. Other factors as impaired renal function, high intake of flesh and alcohol, disturbed postprandial alkali excretion may also play an important role in the disturbed urine acidification.

PP-078 Hypercalciuria in infants with urinary tract infection

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Introduction: The aim of the paper was to assess disorders of calcium-phosphorus metabolism in infants with urinary tract infections (UTI).
Objectives: The study encompassed 128 infants aged 1–11 months, referred to the Nephrology and Hypertension Out-Patient Clinic of the Children Memorial Health Institute because of UTI.
Methods: In all cases a detailed medical history was obtained, concerning familial incidence of urolithiasis and clinical symptoms preceding the diagnosis of UTI. Urinary tract sonographic studies were obtained and calcium-phosphorus metabolism was assessed. Arterial blood pressure, renal function and acid-base balance were normal in all children observed.
Results: The incidence of UTI was not gender-dependent. A positive family history of urolithiasis was present in 40% of infants with UTI. Hypercalciuria was detected in 70.5% of boys and in 55% of girls. Furthermore, 78% of infants with a positive family history of urolithiasis had an elevated calcium/creatinine ratio
Conclusion: Standard diagnostic work-up in infants presenting with UTI should include assessment of calcium-phosphorus metabolism. Further management should include measures designed to prevent the development of urolithiasis.

PP-079 Baseline measurements of bone formation and bone resorption markers in South Africa's two population groups

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Introduction: Throughout life bone is in a dynamic state of continuous resorption and formation in a process known as bone remodelling. Bone remodelling, or turnover, consists of two opposing activities: the breakdown (resorption) of old bone by osteoclasts, resulting in the release of minerals and a transfer of calcium from bone fluid to the blood, and the formation of new bone by osteoblasts. Bone turnover can be assessed by measuring levels of various biochemical markers which are released into the blood or urine [1]. Since urinary calcium is a key factor in the pathogenesis of kidney stones, bone turnover is a potential risk factor for this disease. In South Africa the incidence of stone disease and osteoporosis is rare in the black group but occurs in the white group to the same extent as in other western societies [2]. Therefore baseline levels of bone turnover markers are of interest.
Objectives: To determine urinary deoxypyridinolines (DPD) (bone resorption marker) and serum osteocalcin (OC) (bone formation marker) levels in subjects from both race groups in South Africa.
Methods: The concentration of urinary DPD and serum OC in 8 black and 8 white healthy South African males was determined using competitive ELISA kits (Metra Biosystems, USA) with 96 well plates already coated with deoxypyridinoline and osteocalcin, respectively (Table 1).
Conclusion: Individual bone turnover measurements and their means indicate that urinary DPD levels were generally higher in blacks, while serum osteocalcin OC levels were generally lower in blacks than whites at baseline, but these differences were not statistically significant. Further studies involving larger cohorts of subjects are required to confirm these findings. Nevertheless, these initial observations are counterintuitive as they suggest that bone resorption is higher and bone formation is lower in the black group.

Table 1

Subject	DPD (mmol/l) Blacks	DPD (mmol/l) Whites	OC (ng/l) Blacks	OC(ng/l) Whites
1	9	3	0	14
2	3	6	6	11
3	9	1	6	10
4	11	3	13	9
5	3	10	12	27
6	2	6	9	14
7	6	5	11	15
8	17	3	19	14
Mean	7.5 ± 5.1	4.6 ± 2.8	10.4 ± 4.7	14.2 ± 5.7

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PP-080 Urinary risk factors of stone-formation in idiopathic calcium urolithiasis

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Introduction: In the Guidelines on Urolithiasis of the EAU, recommendations were made for the metabolic evaluation of patients with different categories of calcium urolithiasis (CU). However, on the basis of these recommendations the study of only a few parameters of urinary stone risk factors may be suitable for clinical purposes but we suggest the need for carrying out of more detailed investigations on patients for the purpose of scientific investigation.
Objectives: To study the condition of urinary stone risk factors in patients with idiopathic CU and compare them with the different categories of CU.
Methods: Our study consisted of 121 patients and 10 healthy persons (control group). The criteria for inclusion were patients having 3 categories of CU: So, Sres and Rmo. The criteria for exclusion from the study were: primary hyperparathyroidism, renal tubular acidosis and urinary tract infection. Ninety-five (78,5%) patients had calcium oxalate (CaOx) stones (consisting of CaOx and calcium phosphate (CaP) salts). One (58,7%) of the patients composed the group of So category, 12 (9,9%)—the group of Sres one and 38 (31,4%)—the group of Rmo category. The clinical investigations included ultrasound and X-ray examination of the urinary tract, the measurement of serum creatinine, albumin, calcium, magnesium, phosphate, uric acid and the measurement of calcium, magnesium, phosphate, uric acid, oxalate, citrate, creatinine excretion in 24-h urine samples, urine pH, the volume of daily urine (diuresis).

Table 1 Parameters of mineral metabolism

Daily excretion in urine	Healthy subjects ($n = 10$)	Patients with ICUL ($n = 123$)	Great Britain	Italy	Germany
Ca (mmol/24 h)	2.38 ± 0.26*	4.804 ± 0.228	5.3 ± 0.54	5,.25 ± 0.26	6.22 ± 0.35
P (mmol/24 h)	10.51 ± 1.23*	12.42 ± 1.209	–	–	–
Mg (mmol/24 h)	4.17 ± 0.15*	3.679 ± 0.129	–	–	4.68 ± 0.16
UA (mmol/24 h)	2.68 ± 0.45*	3.45 ± 0.12	–	–	3.71 ± 0.13
Ox (mg/kg/24 h)	0.37 ± 0.05*	0.811 ± 0.131	0.46 ± 0.09	0.52 ± 0.09	0.43 ± 0.03
Cit (mmol/24 h)	3.045 ± 0.18*	1.828 ± 0.233	0.84 ± 0.12	0.95 ± 0.15	–
Urine pH	6.28 ± 0.14*	5.43 ± 0.22	5.65 ± 0.23	5.56 ± 0.53	6.16 ± 0.06
Urine volume (diuresis)	1524.3 ± 124.2*	934.3 ± 122.2	1353.4 ± 110.4	–	–

Results: The urine volume of patients in all the groups was significantly lower than that of the control group, as well as the degree differentiated in groups Sres and Rmo as compared with the group So. Furthermore, the daily excretion of magnesium in urine was significantly decreased in group Rmo (3.05 + 0.18 mmol/24 h) as compared with groups So, Sres (3.73 + 0.22, 3.59 + 0.21 mmol/24 h). In patients of all 3 groups, the daily excretion of citrate in urine was decreased as compared with that of the control group ($P < 0.05$) and also, this degree in group Rmo significantly differed from groups So, Sres. Such parameters as daily excretion of calcium, oxalate, phosphate, uric acid in urine and urinary pH in all 3 groups there was a significant difference with control group ($P < 0.05$), but there was no difference among the groups of So, Sres, Rmo categories ($P > 0{,}05$).
Conclusion: The patients of all 3 categories: So, Sres and Rmo have more urinary stone risk factors. But those changes in patients of category Sres and, particularly, in patients of category Rmo are more significant in comparison with category So (in view of daily urine volume, daily excretion of magnesium and citrate in urine). Thus, the presence of these urinary stone risk factors suggests the need for their correction in patients with categories So, Sres and Rmo in idiopathic CU.

PP-081 Metabolic evaluation of the patients with idiopathic calcium urolithiasis

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Introduction: The metaphylaxis of urolithiasis remains a major challenge in urology. The incidence of recurrent calcium oxalate calculi formation was 10% after 1 year, 35% after 5 years and 50% after 10 years.
Objectives: To perform metabolic evaluation of the patients with idiopathic calcium urolithiasis (ICUL) and to compare these findings with data from similar studies in the literature.
Methods: We studies 123 patients with non-complicated ICUL on the basis of Recommendations of ABEUR Conference (Manheim, 1999). X-ray analysis of urinary calculi passed spontaneously —112 (91,1%) and removing by surgery—11 (8.9%) showed that in 78.3% of cases the stones were composed of CaOx, in 8.7%—CaP, and in 13%—CaOx and CaP. Cases of urinary tract infection and primary hyperparathyroidism were excluded. Main measurements included urinary excretion of total calcium (Ca), inorganic phosphorus (P), magnesium (Mg), uric acid (UA), oxalate (Ox), citrates (Cit) and urine pH.
Results: In the patients with ICUL in contrast to control there was a significant increase in the urinary excretions of Ca, P, UA, Ox ($P < 0{,}05$) and lower excretions of inhibitors of stone formation—Mg, Cit; There was a reduction in diuresis and urine pH (Table 1). The comparison of the data in ICUL and in healthy subjects revealed increase in excretion of urine Ca 2 times (by 101,6%), urine Ox excretion—2,2 times (by 118,9%), uric acid by 28.7%, reduction of Cit excretion—by 67%, Mg—by 13.3%, pH by 17.6%, diuresis by 63,1%. Then we have compared our results with data of investigations of Robertson et al (Great Britain), Trinchieri et al (Italy) and Siener et al (Germany). Comparison of the results showed that the daily excretion of Ca, Ox and Cit as well as urine pH and diuresis showed statistically significant differences. In contrast to other countries for patients with ICUL of our region there were characteristically more significant differences in the daily excretion of urine Ox and daily diuresis, reduced excretion of lithogenesis inhibitors—Mg and Cit. This was associated with factors including volume of water digested, food ingredients and climate. However, it should be emphasised that for patients with ICUL there were characteristic changes of similar parameters in all countries studied.
Conclusion: The differences in urinary parameters for stone formation were characteristic for patients with ICUL in different countries. On the basis of these results the choice of metaphylactic measures for the correction of the above-mentioned differences in mineral metabolism, urine pH and urine volume may be different in each region.

PP-082 Study of urinary crystals in diabetic subjects (type I)

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Introduction: Diabetes mellitus constitutes a current problem in public health because of its frequency and the gravity of its complications. The prevalence of urolithiasis in the diabetic population was estimated recently at 21%, almost double that of the prevalence in the general population. The high frequency of uric acid stones in patients with diabetes has been the subject of several studies in this field. The aim of our work was to measure crystalluria and to determine the most frequent crystalline forms among type I diabetic patients (insulin-dependent), since crystalluria provides clinicians with an indication of the risk of stone formation. Crystalluria could be harmful to the kidneys of these patients and is an indication that preventative measures should be taken against the formation of stones.
Methods: The study includes 116 patients with type I diabetes. For each patient, an average of three early morning urines were analyzed for crystalluria. These samples were examined using optical microscopy for both qualitative and quantitative analysis of crystalluria. The samples were analysed when taken and also preserved at +4°C for 48 h before further examination. In addition, a measure of urinary pH was made at the time of the microscopic examination.

Results: The frequency of crystalluria in all the samples measured at the time of sampling and at +4°C respectively was 21.0 and 39.4%. These values were approximately three times lower than those observed in patients with stones. Calcium oxalate crystals predominated in the urines of both sexes with a frequency of 79.5% at the time of sampling and 84.6% after 48 h at +4°C. The frequency of uric acid and urate crystals was 22.0% at the time of sampling: these crystals were three times more common among women than among men (16.5% against 5.5%).
Conclusion: Studies of crystalluria in patients with type I diabetes showed that calcium oxalate dihydrate (Weddellite) was the most common type of crystal with a frequency of 64.5% follow-up by calcium oxalate monohydrate (Whewellite; 15.0%). In general, the crystallization of uric acid and urates, confirmed the high rate of the frequencies of the complex crystals of amorphous urates and of uric acid (22%). This was not the case in patients with stones. The high percentage of the uric acid and urate crystalluria in diabetic subjects in general and for females in particular shows that women are more likely than men to develop uric acid or urate stone formation. This alerts clinicians and experts in the field to treat this kind of patient in order to eliminate or reduce the risk of crystalluria and uric acid and urate stone formation.

PP-083 Levels of sex hormones in urolithiasic patients

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Introduction: The multifactorial nature of the etiology of urolithiasis makes it difficult for the real cause of stones to be identified. The factors involved include urinary supersaturation, nutrition, anatomical defects and hormonal factors.
Objectives: This research was performed with a view to evaluate the levels of the hypophyseal, ovarian, and testicular hormones in stone patients coming from Unidad de Nefrologia del Servicio Autónomo Hospital Universitario "Antonio Patricio Alcalá" in the city of Cumaná.
Methods: The study consisted of 60 blood samples from stone patients and from apparently healthy individuals These were subsequently separated and the concentrations of estradiol, testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) measured using an electrochemoluminiscence assay.
Results: The *t*-Student statistical test identified highly significant differences for estradiol and non-significant differences for the other hormones between the two groups. Furthermore, the multifactorial ANOVA statistical test was applied to evaluate variations of these hormones according to the age and sex of the stone patients, and highly significant differences were found according to sex in the estradiol and testosterone hormones and very significant differences for FSH and LH. Significant differences were also found for FSH and LH according to age.
Conclusion: These results suggest that sexual hormones play an important role in the processes of supersaturation and precipitation of crystals in the urinary tract and therefore in the stone-forming process.

PP-084 Cystinuria: the South Indian experience

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Introduction: Cystinuria is reportedly a rare condition affecting the stone patients in India. No significant report is available in the literature on this problem from India.
Objectives: This paper attempts to assess the relevance of cystine metabolism in the stone population reporting to a hospital in South India.
Methods: A total of 2,800 urine samples from 1300 patients attending the urinary stone clinic during the period 2004–2008 were assessed for cystinuria by performing the nitroprusside test on the early morning urine and random samples on the day of attendance. Urinary deposits were studied in all the patients. Stones retrieved from 800 stone patients were analysed qualitatively and by Fourier transmission infra red spectroscopy.
Results: Cystinuria was identified by the nitroprusside test in only three patients. They did not show cystine crystals on repeated examination. None of these patients showed cystine crystals. Three patients out of the 1300 showed presence of cystine crystals in their urine deposits. None of these six patients had stone passage or retrieval. Fourier transmission Infra red spectroscopy of the stones retrieved from 800 patients showed presence of cystine in 19 (2.375%). None of the patients with cystine in the stones had either cystine crystals in the urine or positive nitroprusside test for cystine. All six patients who had either positive cystine or cystine crystalluria and the 19 patients with cystine positive stone analysis had other biochemical abnormalities. They were medically managed with appropriate biochemical corrective chemotherapy and their stone problem was under control. Specific treatment with D-penicillamine was not administered to the patients in view of the high cost, non-availability and possible toxicity. All the patients were advised purine restriction in the diet.
Conclusion: It is concluded from the study that cystinuria is a rare metabolic entity in South India. It exists in only a small percentage of stone patients. None of the patients considered above had intractable stone disease not amenable to the usual modalities of chemotherapy and chemoprophylaxis.

PP-085 Comparison of urinary oxalate assessment between six international reference laboratories

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Introduction: Intraclass correlation of oxalate measurement of 10 urine samples in duplicates.
Objectives: We first evaluated intra-laboratories reliability by analysis of the duplicates. Intraclass correlation (ICC) showed variability between 0.808 (95% confidence interval: 0.427–0.948) and 0.998 (95% confidence interval: 0.994-1), with lower values for HPLC-based methods.
Methods: We then compared laboratories between them using ICC (Table 1) and limits of agreement. Among laboratories that run the oxalate oxidase method, the highest reliabilities were obtained between labs B, C, and E, while lab A exhibited very low correlation with the three other labs, suggesting problems with sample handling. Among HPLC-based methods, lab D showed better ICC than lab C or F. In general, HPLC-based methods showed more variation between them and compared to oxalate oxidase kit.

Table 1

	HPLC-Lab D	Based Lab F	Methods Lab C	Oxalate Lab A	Oxidase Lab B	Methods Lab C	Lab E
HPLC based methods							
Lab D	1.0						
Lab F	0.6679	1.0					
Lab C	0.7843	0.4721	1.0				
Oxalate oxidase methods							
Lab A	0.2659	0.2674	−0.0508	1.0			
Lab B	0.9523	0.6433	0.7922	0.1664	1.0		
Lab C	0.9183	0.5541	0.7266	0.1682	0.9553	1.0	
Lab E	0.9376	0.5811	0.7762	0.1902	0.9799	0.9803	1.0

Results: In conclusion, urinary oxalate measurements by oxalate oxidase method showed better ICC and limits of agreement compared to HPLC-based methods, if samples were handled properly. HPLC-based methods showed more variation. In order to compare urinary oxalate values between labs, our data urges the need for a standardization of the method of measurement.

PP-086 Problem of analysing cystine stone by FTIR

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Introduction: Cystine stones are produced by an inherited (genetic) disorder of the transport of amino acid cystine that results in excess of cystine in the urine (cystinuria). Cystine calculi in urinary tract present a significant problem in patients. We have recorded that cystine calculi are very uncommon in our region. Cystine crystals are unusually identified in the urinary deposits. The problem of recognizing cystine by Fourier Transmission Infrared Analysis (FTIR) as a component in mixture of stones is significant. The problem is compounded by the similarity of wavelengths of cystine with that of whewellite and uric acid.
Objectives: The objective of this paper is to elucidate the problems of identifying cystine in stone analysis and identifying a solution to get over this deficiency.
Methods: Out of 1,300 urinary stones analysed by ordinary wet chemical methods and infer red spectroscopy, 30 stone samples which were reported to have cystine peaks in significant numbers were selected. These samples were powdered, mixed with potassium bromide, made into pellets and taken up for FTIR. The wavelength patterns were scrutinized comparing with the peaks obtained by the reference standards of cystine. Spectra were also obtained from pure cystine. Comparison of spectra with those of whewellite and uric acid was performed. Then the samples were taken for SEM-EDAX. The samples were made conductive by gold sputtering and fed into JEOL JSM 35 C SEM machine. Morphology was recorded by taking photographs. Further elemental distribution analysis (EDAX) was carried out to identify the elemental composition.
Results: Of the 30 samples taken up for FTIR analysis, all showed spectra identifiable with the reference peaks for cystine. However, when these peaks were compared with those of whewellite and uric acid, all the stone samples showed duplication of peaks for whewellite and uric acid and whewellite. The pure cystine spectra showed identifiable peaks are in the range of 3,026, 1618.28, 1,485, 846.75 cm^{-1} etc. (from the standard spectrum of pure cystine). All the analysis findings were then correlated with EDAX findings. On doing EDAX, we could separately find out the components present in a mixture. Three stones contained elemental pattern to fit with those of cystine.
Conclusion: Even though it is difficult to find out the presence of cystine molecule in FTIR, it is possible to recognize it through EDAX and will be possible to confirm the presence of cystine in mixed urinary stones.

PP-087 Stone symptoms and urinary deposits

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Introduction: There is a general belief that urinary stones are always associated with symptoms such as pain, dysuria and haematuria. Many patients stop medical treatment when they are symptom-free and return with excruciating pain, dysuria and haematuria either alone or in combination. There are patients who present with end-stage renal failure because they had no symptoms till late on in the progress of the disease.
Objectives: The objective of this study was to determine stone activity in an individual patient by assessing the urinary deposits at the time of the visit to the stone clinic and correlate them with the presence or absence of symptoms at that time.
Methods: A total of 418 patients who attended the stone clinic in 2007 with proven urinary stone disease, including stones, colic and crystalluria were studied. Presence or absence of symptoms at the time of presentation was recorded. A minimum of two samples of urine were collected (early morning and random) to assess the presence and extent (1–5) of urinary deposits namely RBC, PC, COM, COD, uric acid and phosphate. The scores obtained were correlated with the presence or absence of symptoms by logistic regression.
Results: Of the 418 patients studied, 238 had symptoms and 180 had no symptoms. The total score of the patients with symptoms was 1,759 (with a mean of 7.39 per patient) against the total score of 1,327 in the patients without symptoms (with a mean of 7.37 per patient). This difference was not statistically significant. The total values and mean of the patients with symptoms and without symptoms, respectively, for the individual deposits were RBC 561 (2.35) versus 390 (2.16), PC 732 (3.07) versus 556 (3.08), COM 160 (0.67) versus 133 (0.77), COD 245 (1.03) versus 171 (0.95), Uric acid 18 (0.07) versus

19 (0.10) and phosphate 113 (0.48) versus 87 (0.48). Of these the RBC, PC, uric acid and phosphates were not significantly different between the two groups. However, the presence of COD was significantly higher in patients with symptoms $P < 0.05$) and COM was significantly higher in patients without symptoms $P < 0.05$).
Conclusion: It is concluded that the presence or absence of symptoms does not alter the presence and extent of urinary deposits in the urinary stone patients. COD was more common in symptomatic patients and COM was more common in the asymptomatic patients. This contrast could be due to the morphology of the COD crystal which is bipyramidal and produces injury to the urothelium whereas COM is dumb-bell shaped and produces less injury and fewer symptoms.

PP-088 Insulin resistance increases the risk for kidney stone formation in Otsuka Long-Evans Tokushima fatty rats, a type 2 diabetes mellitus model

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Introduction: Epidemiological studies indicate that obesity increases the risk for kidney stone formation. We here investigated whether insulin resistance associated with adiposity affects the risk for kidney stone formation using rat model of metabolic syndrome.
Methods: (1) 4-week-old male Otsuka Long-Evans Tokushima Fatty (OLETF) rats (n = 18), animal models of type 2 diabetes, and Long-Evans Tokushima (LETO) rats ($n = 18$), as controls, were given standardized diet and free access to water. Urine pH and 24-h urine chemistry were measured every 2 weeks. At 10 weeks of age these rats were divided into 3 groups ($n = 6$ per groups), respectively, that were treated with vehicle or oral administration of 3 or 10 mg/kg/day pioglitazone, an agent that improves insulin resistance. (2) 8-week-old male OLETF and LETO rats were subjected to lithogenic diet of 1% ethylene glycol as a drinking water for 2 weeks. Calcium oxalate (CaOx) crystal deposits in the kidney were determined by histological examination and measurement of renal calcium contents.
Results: (1) At 4 weeks of age no differences in urinary parameters were observed between OLETF and LETO rats. After 6 weeks of age OLETF rats showed significantly lower urine pH (6.37 vs. 7.10, $P < 0.01$) and citrate (10.1 vs. 37.3 mg/day, $P < 0.05$) as well as elevated fasten serum glucose. Administration of pioglitazone to OLETF rats for 4 weeks reversed urine pH and citrate to the similar levels in LETO rats. (2) Ethylene glycol-induced renal crystal deposits in OLETF rats were significantly greater than those in LETO rats as evidenced by renal calcium contents (13.5 vs. 8.1 mg/g tissue, $P < 0.05$).
Conclusion: The results indicate that insulin resistance causes low urine pH and decreased excretion of urinary citrate and that diabetic rats are prone to form CaOx stones. Thus, insulin resistance might contribute to the increased prevalence of nephrolithiasis in obese patients.

PP-089 Arteriosclerotic diseases increase the risk for uric acid stones

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Introduction: An increased prevalence of urinary stone disease has been reported in patients with obesity, type 2 diabetes and hypertension, collectively termed metabolic syndrome (MetS). Since insulin resistance, characteristic of MetS, is reported to result in low urine pH through impaired renal ammoniagenesis, it is hypothesized that MetS should favor the formation of uric acid (UA) stones.
Objectives: The aim of the present study is to clarify the distribution of the stone composition in patients with MetS and determine which components of MetS might favor UA stone formation.
Methods: We retrospectively reviewed the charts of 467 consecutive patients whose renal or ureteral stones were analyzed. Calcium oxalate and calcium phosphate were grouped into the single category of calcium (Ca) stones ($n = 384$), and stones containing uric acid (UA) were categorized as UA stones ($n = 48$). Infection stones and cystine stones were excluded form the study. The distribution of Ca and UA stones were analyzed in relation to age, sex, body mass index (BMI), presence of the diseases related to MetS (lipid disorders, hyperuricaemia, type 2 diabetes, arteriosclerotic diseases).
Results: Of 432 patients with Ca and UA stones, mild obese (BMI $\geq$ 25 kg/m^2) was observed in 177 (44.4%), lipid disorders in 171 (39.6%), hyperuricaemia in 112 (25.9%), type 2 diabetes in 75 (17.4%), arteriosclerotic diseases in 175 (40.5%). Overall, 335 patients (77.5%) had at least one of these disorders. The distribution of stone composition was not associated with BMI, lipid disorders and

Table 1 Relative risk of having UA stones

Variables	*n*	Ca stone (%)	UA stone (%)	*P**	RR for UA	95% CI	*P***
Age							
<56	211	93.8	6.2	<0.01	1		<0.01
56≤	221	84.2	15.8		2.9	1.5–5.6	
Sex							
Female	199	95.0	5.0	0.01	1		0.02
Male	313	86.6	13.4		2.9	1.2–7.1	
Hyper-uricaemia							
No	320	92.2	7.8	<0.01	1		<0.01
Yes	112	79.5	20.5		3.1	1.7–5.6	
Arterio-sclerosis							
No	222	92.2	7.8	<0.01	1		<0.01
Yes	177	84.0	16.0		2.3	1.2–4.2	

*P** Chi-square test, *P*** logistic regression model

type 2 diabetes. While, the proportion of UA stones was significantly higher in the elderly, men, patients with hyperuricaemia and arteriosclerotic diseases (Table 1).
Conclusion: The results indicate that metabolic syndrome is strongly associated with urinary stone disease and that arteriosclerotic diseases as well as hyperuricaemia are the strong predictors for UA stones. It is proposed that UA stone patients should be screened for arteriosclerotic diseases.

PP-090 Metabolic evaluation of infected urolithiasis

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Introduction: Although the gold standard of infected urolithiasis treatment is the complete elimination of the stone, there remains a difference of opinion regarding the need for metabolic evaluation of such cases. However, metabolic anomalies are present in more than 50% of patients with infected renal calculi. Furthermore, there are differences in terminology: infected renal lithiasis may designate staghorn stones (which may have a variety of composition), urolithiasis secondary to urea-splitting bacteriuria (struvite ± carbonate apatite) or calcium oxalate calculi that have become secondarily infected .
Objectives: In an effort to clarify the problem of infected urolithiasis, we recently reviewed our experience of the metabolic evaluation of patients whose stone analyses reveal the presence of struvite ± carbonate apatite.
Methods: Forty-three patients (27 females and 16 males, mean age 45 year, range from 19 to 76 year) treated for infected nephrolithiasis between 1990 and 2003. Based on stone composition, 19 patients had pure struvite ± apatite and 24 mixed stones (struvite ± carbonate apatite and calcium oxalate). All patients underwent complete metabolic evaluation. Twenty-four hour urine samples were analysed for levels of calcium, oxalate, uric acid, citrate, magnesium and phosphate. Fasting venous blood was analysed for calcium, phosphate, uric acid and creatinine; morning spot urine was collected for urine analysis and urine culture.
Statistical analysis was performed by chi-square test and unpaired *t* test.
Results: The results of 24-h urine studies are listed in Table 1.
Our data show that the urinary calcium, oxalate, and uric acid excretion in mixed stone formers was higher than in those with pure infected stones and was very similar to that observed in idiopathic stone patients. Hypercalciuria was present in 46% of mixed infected patients (Chi-square = 4.70, $P < 0.05$), hyperoxaluria in 38% (Chi-square = 6.88, $P < 0.01$) and hyperuricosuria 33% (Chi-square = 5.73, $P < 0.025$). Urinary excretion of citrate was found low in both groups, without statistically significant difference. Patients with pure struvite ± carbonate apatite stones had risk factors for chronic urinary infections (neurological bladder, urinary diversion and urinary tract anomalies).
Conclusion: Our study demonstrates that patients with mixed infected stones have different clinical and metabolic characteristics compared with patients with pure struvite ± carbonate apatite stones. The infected lithiasis in these patients probably occurs on top of original sterile idiopathic stones. Furthermore, it shows that a complete metabolic evaluation of patients with pure infected stones is not necessary. Hypocitraturia in these patients could be attributed to the presence of chronic bacteriuria. Therefore, our data suggest that patients with mixed infected stones will benefit not only from the usual antibiotic prophylaxis, but also from specific therapies for their underlying metabolic abnormalities.

Table 1 Twenty-four hour urinary parameters (mg%)

	Calcium	Oxalate	Uric acid	Citrate
Pure struvite	204± 92	27± 6.7	465 ±159	216 ± 115
Mixed	304± 87	34± 9.9	620 ±222	244 ±123
P	<0.001	<0.02	<0.02	ns.

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PP-091 Research of significant associations between presence of the Randall's plaques and constitutional or metabolic variables

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Introduction: According to the literature, Randall's plaques are crystal deposits of interstitial calcium phosphate (CaP), localized in the tip of the renal papillae and suggested to be involved in idiopathic calcium-oxalate (CaOx) stone pathogenesis.
Objectives: Aim of this study was to identify possible significant associations between these interstitial crystalline deposits and several constitutional and metabolic variables.
Methods: Twenty-eight renal stone patients underwent ureteroscopy (URS) in 8 cases or percutaneous nephrolithotomy (PCNL) in 20 cases, and renal papillae biopsies in order to obtain papillary specimens for Randall's plaque research. In 27 cases, papillary biopsies were adequate for a correct histological analysis. Detection of the interstitial Ca crystal deposits allowed us to stratify our population into two groups: group A characterized by presence of the Randall's plaques (12 patients, 6 males and 6 females) and group B with no interstitial crystalline deposits (15 patients, 6 males and 9 females). We analyzed in each group the following variables: age; body mass index (BMI); serum creatinine, urea nitrogen, calcium, phosphate, sodium, potassium, chloride and uric acid, glucose, cholesterol and triglycerides, and in urine, pH and specific gravity. Possible associations between the above variables and the presence of interstitial crystals deposits were looked for.
Results: The two groups of patients were similar for gender stratification. Median age was 37.5 years [Interquartile Range (IQR) 31.75–46.75] and 48 years (IQR 41–56) in group A and B, respectively. This difference was statistically significant ($P = 0.037$). Median BMI was at the upper normal limit in both groups, without significant difference. Median values of all laboratory blood and urine parameters were all in their normal range. Only glycaemia was statistically different between patients with/without Randall's plaques at the univariate analysis ($P = 0.03$), being lower in group A. However, the multivariate analysis did not confirm the statistically association between age and glycaemia and the presence of the interstitial Ca deposits (Table 1).
Conclusion: We did not find any significant association between the investigated metabolic and constitutional variables and the presence of the Randall's plaques. Nevertheless, the lower age of patients with Randall's plaques could mirror the increasing occurrence of

Table 1 Multivariate analysis

Variables	*P*	Odds ratio	Confidence Interval (C.I. 95%)
Age	0.124	1.082	0.979–1.197
BMI	0.701	0.954	0.749–1.214
Cholesterolaemia	0.430	1.014	0.979–1.050
Urinary pH	0.657	1.447	0.283–7.399
Serum Calcium	0.120	0.077	0.003–1.946
Serum Phosphate	0.725	1.580	0.123–20.296
Serum Uric acid	0.468	1.518	0.492–4.685
Glycaemia	0.917	1.003	0.946–1.064

nephrolithiasis in younger patients observed over the last three decades, probably due to nutritional habit changes. If this will be confirmed, a role for diet in Randall's plaque formation might be proposed. Larger studies are necessary to confirm this hypothesis.

PP-092 The importance of urinary pH in the genesis of urinary calculi

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Introduction: There has been controversy for many years as to whether or not it is useful to measure urinary pH in the assessment of the risk of forming stones in 24-h urines collected from patients with urolithiasis. Indeed, many Stone Units do not carry out this measurement as part of their routine screening procedure for risk factors for urolithiasis in their stone patients. In this paper, the value of measuring 24-h pH in the assessment of patients with stones is reviewed.
Methods: The role of pH as a chemical factor in the genesis of stones was investigated by considering the effect of pH on the dissociation of uric acid (UA), phosphoric acid and oxalic acid, respectively. The relative importance of pH, calcium and phosphate on the precipitation of calcium phosphate (CaP) and the formation of CaP-containing stones was reviewed. The influence of diurnal fluctuations in urinary pH on the likely dissolution and precipitation of UA and CaP was reviewed. The roles of distal renal tubular acidosis (dRTA) and "Metabolic Syndrome" on urinary pH and the risk of forming CaP and UA stones, respectively were reviewed.
Results: Urinary pH was found to be a critical chemical factor in determining the supersaturation of urine with respect to both UA and CaP. It was also important for determining the risk of forming both UA- and CaP-containing stones. In turn, it was found that metabolic status, diet and diurnal variations all had a role to play in determining urinary pH and the likelihood of forming these types of stones.
Conclusion: It is concluded that it is extremely useful to measure urinary pH in 24-h urine samples since, in idiopathic stone-formers, this provides a good indicator of the acid-base balance of the patient's diet and also defines the most likely type of stone to be formed by the patient. In addition, urinary pH controls the supersaturation of urine with respect to all the stone-forming materials, with the exception of CaOx, and is a major risk factor for stone-formation when it becomes either persistently more acid (as in "Metabolic Syndrome") or more alkaline than normal (as in dRTA or in patients with a urinary tract infection involving a urea-splitting organism). In the case of stones whose solubility is dependent on urinary pH, it also provides a suggested mode of preventative treatment for the medical management of the patient concerned.

PP-093 Role of BMI on lithogenic risk in women with idiopathic calcium nephrolithiasis and controls

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Introduction: Many data suggest a link between body weight and lithogenic risk. BMI, weight and abdominal circumference are independently associated with kidney stone risk, particularly for uric acid nephrolithiasis. However the mechanisms subtended are unknown and data about idiopathic calcium nephrolithiasis (ICN) are unclear.
Objectives: To evaluate modification of urinary stone risk factors in relation to BMI in women with idiopathic calcium nephrolithiasis (ICN) and in a matched control group.
Methods: We evaluated 420 women with normal blood pressure and ICN (age 40 ± 14), without conditions commonly associated with calcium nephrolithiasis (e.g., primary hyperparathyroidism, RTA, IBD etc) or medical therapy and 290 matched healthy women (age 41 ± 13). Both group were divided in 4 class of BMI (Group I BMI < 19; Group II 19–25; Group III 25–30; Group IV > 30). Two 24-h urine samples were obtained to assess urinary stone risk factors.
Results: In ICN women but not in controls we found a linear increase of frequency of hypercalciuria with increasing BMI (Pearson's $R = 0.998$; $P < 0.01$); moreover urinary calcium was significantly higher in ICN rather than controls ($P < 0.0001$; c2 Test). In ICN women when BMI increased, about all urinary stone risk factors were higher, particularly sodium (Group I 123 ± 49 vs. Group IV 200 ± 76, $P < 0.0001$), oxalate (Group I 22 ± 8 vs Group IV 35 ± 24, $p = 0.002$) and phosphorous (Group I 525 ± 169 vs. Group IV 884 ± 288, $P < 0.0001$). In control group uric acid, phosphorous and sulphate increased but calcium, oxalate and magnesium were not different in various class of BMI. Neither in ICN women nor in controls urinary pH significantly changed.
Conclusion: In women with idiopathic calcium nephrolithiasis overweight and obesity are related to linear increase of frequency of hypercalciuria and of many others urinary stone risk factors. In healthy subjects, BMI increase is not associated to calcium kidney stone risk.

PP-094 The hypercalciuria in an experimental model of sodium-sensitive hypertension is related to down-regulation of calcium transport proteins expressed along the renal distal tubule

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Introduction: It has been reported by several investigators that sodium-sensitive hypertension is associated with hypercalciuria. On the other hand the distal tubule is the only nephron segment where trans-epithelial calcium transport is completely sodium-independent. At this level, trans-cellular calcium reabsorption proceeds through a well controlled sequence of events consisting of luminal calcium entry via the epithelial calcium channel (TRPV5), cytosolic diffusion of calcium bound to calbindin-D28 k, and basal–lateral extrusion of calcium through Na/Ca exchanger (NCX1) and plasma membrane Ca-ATPase (PMCA).
Objectives: In the present study we have investigated if the hypercalciuria of the hypertensive Milan strain of rats (MHS) (a well defined model of sodium sensitive hypertension) may be dependent

from alterations of gene expression of the four aforementioned calcium transport proteins.
Methods: Adult MHS rats and age matched Milan normotensive (MNS) rats were studied; mRNA abundance was quantified by real-time quantitative polymerase chain reaction (PCR).
Results: As compared to MNS, urinary calcium excretion (measured by urinary calcium/creatinine ratio) was significantly increased (60%) in MHS. As compared to MNS, in MHS there was a significant decrease in mRNA expression of TRPV5 ($46 \pm 6\%$) ($n < 0.05$), calbindin D28 k ($54 \pm 7\%$) ($P < 0.01$), PMCA ($71 \pm 8\%$) ($P < 0.01$), while NCX1 was unaffected. With respect to calbindin D28 k, the PCR data were fully confirmed by immuno-blotting and immuno-histochemistry experiments performed on renal cortex.
Conclusion: These data demonstrate that in this model of sodium-sensitive hypertension the hypercalciuria is associated with a down-regulation of most of the calcium transport proteins expressed along the distal tubule.

PP-095 Primary hyperoxaluria: case report of a family with clear sex-conditioned penetrance

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Introduction: Primary Hyperoxaluria is a rare autosomal recessive disease with impaired hepatic detoxification of glyoxylate, due to AGT (PHI) or GRHPR (PHII) enzyme deficiency. The conversion of Glyoxylate to Oxalate catalysed by LDH and glycolate oxidase (GO) leads to Ca-oxalate nephrolithiasis, end-stage renal failure and systemic oxalosis. As vitamin B6 -the AGT cofactor- can stabilize and rescue some of the mutated forms of AGT, notably the common Gly170Arg mutation, an early diagnosis can direct responsive subjects to vitamin B6 supplementation. In many cases, liver or combined liver/kidney transplant is necessary to correct the metabolic defect.
Objectives: The object of the study is to describe the clinical variability of a PHI family, in which two female Gly170Arg homozygous sisters are still asymptomatic while two younger males are affected.
Methods: The proband, a 33 year-old male from Albania, first experienced renal colic at the age of 30, with expulsion of a Ca-oxalate stone. Urinary oxalate (UOX) was148 μmol/mmol creatinine (normal: 12–55). He started Vitamin B_6 (600 mg/day) and K^+/Mg^{2+} supplementation, with a good response (UOX after 1 month: 55 μmol/mmol creatinine).
Family history: the proband has two healthy children (4 and 6 years old), three healthy sisters, and a brother affected with recurrent renal stones, who died at age 27 due to complications after a surgical nephrectomy. The proband's father had an episode of renal stones. No consanguinity among parents is recorded. The AGXT gene was analysed by DHPLC and direct sequencing.
Results: Homozygosity for the common Gly170Arg mutation was found in the proband's AGXT gene. Genetic testing of relatives revealed heterozygosis for the mutation in the parents and the two children -as expected- and in one of the sisters. Surprisingly, the other two sisters, 35 and 28-year-olds, proved to be Gly170Arg homozygous despite the absence of any symptoms of hyperoxaluria. Consequently both sisters were treated with Vitamin B6 and K^+/Mg^{2+}. Response data were available for the youngest sister: initial UOX: 59 μmol/mol creatinine; after 2 months of therapy 39 μmol/mol creatinine (normal: 12–55).
Conclusion: The Gly170Arg is a well know mutation associated with a milder, and Vitamin B_6 responsive, PH. In fact, we observed this phenotype in two male brothers of the family, while two elder homozygous females were asymptomatic. This variability might be ascribed to different factors, either environmental, such as different dietary vitamin B_6 intake, or genetic, such as different sex-specific GO expression. Sex-conditioned difference of idiopathic kidney stones is reported in epidemiological studies [1]. It is interesting to note that a similar difference of expression was found in the rat, probably due to sex hormones [2], and recently a higher male basal expression of GO was reported in mice by a microarray study (ratio M/F = 1.42, [3]). It deserves to be investigated whether this holds true also in humans.

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PP-096 The relationship between molecular analysis and ethnic differences of genotype in Japanese patients with primary hyperoxaluria type 2

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Introduction: Primary hyperoxaluria type 2 (PH2), is an inherited autosomal recessive disorder characterized by excessive urinary oxalate and L-glycerate excretion which is caused by mutations in the GRHPR gene encoding the glyoxylate/hydroxypyruvate reductase enzyme. To date, 15 mutations have been identified [1–7]. All mutations reported lead to a loss of enzyme expression or function. Previous studies suggest that there are variations in allele frequency among different populations. The most common of all the mutations is c.103delG in exon 2 which results in a frameshift and induces a premature stop at codon 451. The prevalence of this mutation in PH2 is around 40% [2, 5] of all reported mutations and appears to have originated by a founder of Northern European origin [5]. However, it has not been found in any patient of Asian origin.
Methods: Genomic DNA of 3 patients and 2 pairs of patients was extracted from peripheral blood samples by the QIAamp blood kit (Qiagen) according to the manufacturer' instruction in Japan and shipped on ice to Wake Forest University. Informed consent was obtained from the patients and parents in accordance with Institutional Review Board of Hamamatsu University School of Medicine. PCR was applied to all nine exons with all splice acceptor and donor sites of the patient by using methods previously described [1, 6] and the modified methods and sequenced with an ABI Prism 377 or ABI 310 DNA sequencer. Sequencing results were compared with the established human GRHPR genomic DNA sequence (GenBank accession no. AF146689 and CH471071) using NCBI BLAST alignment.
Results: We have identified a novel mutation (unpublished data). A two nucleotide deletion (c.248_249delTG) in exon 3 results in a premature stop codon at codon 91. This patient 1 is a compound heterozygote for mutations in the GRHPR gene. Another mutation is

missense transition, c.904C > T (Arg302Cys) in exon 9, which mutants had GR activity 5.6% of the wild type control [4]. Patient 2 is also a compound heterozygote. One allele contained missense transition, c.337G > A (Glu113Lys), in exon 4, which has no GRHPR activity. The other allele contained a two nucleotide deletion in exon 8 at codon 288 (c.864_865delTG) [7]. Patient 3 is a homozygote of c.864_865delTG mutation. The c.864_865delTG mutation was previously reported in a Chinese PH2 patient with end stage renal failure. It is, however, interesting that renal function of patient 3 is within normal limit as well as patient 1 and 2. The allelic frequency of c.864_865delTG mutation is 62.5% (5/8) in 3 Japanese and 1 Chinese patient with PH2.
Conclusion: We undertook molecular analysis of three Japanese patients with PH2 and identified a novel mutation. The prevalence of c.864–865delTG mutation in PH2 patients from East Asia is 62.5%. This mutation is a candidate mutation for DNA screening especially in patients of East Asian origin.

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PP-097 Haplotypes in the osteopontin gene associated with nephrolithiasis and bone mineral density

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Introduction: Osteopontin (OPN) is a major component of urinary stone matrix and plays a role in bone resorption. We previously reported strong expression of OPN on renal tubular cells in stone-forming kidney.
Objectives: We investigated polymorphisms of the OPN gene to explore whether these could be used as a gene marker for determining the risk of both urinary calcium stone and osteoporosis.
Methods: A total of 126 patients with urinary calcium stone and 214 healthy individuals were studied. We re-sequenced an entire OPN gene and examined associations with nephrolithiasis and bone mineral density (BMD) measured by DEXA methods. BMD was evaluated by the *Z*-score, the ratio to age-average BMD.
Results: We identified 61 polymorphisms. After a systematic genetic analysis, we found significant associations between two polymorphisms located in the OPN promoter, or two haplotypes, and the risk of nephrolithiasis. The increased-risk association haplotype (G-T-T-G) (odds ratio, 1.676; 95% confidence interval, 1.072–2.621; $P = 0.023$) and the reduced-risk association haplotype (T-G-T-G) (odds ratio, 0.351; 95% confidence interval, 0.179–0.689; $P = 0.0016$) were found. These haplotypes have no association with Z-score of BMD.
Conclusion: Our findings provide potential support for a significant dual association between OPN gene haplotypes and the risk of nephrolithiasis, and present a potential genetic clue to clarify OPN function in the process of stone formation.

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PP-098 Overexpression of the AGT 2 gene in ethylene glycol-treated rats

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Introduction: Ethylene glycol (EG) is a frequently used lithogenic agent. EG caused calcium oxalate crystal deposits in the renal cortex and caused death after over-dosage.
Objectives: In this study, we used methods of proteomics and quantitative ¡Vpolymerase chain reaction (Q-PCR) to investigate the possible over-expression of alanine-glyoxylate aminotransferase 2 (AGT2) gene induced by EG in the kidney of rat.
Methods: We divided the rats into two groups, i.e. control and EG fed group. Rats ($n = 8$) were fed with 0.75% EG in drinking water of the EG fed group. In control group ($n = 8$), rats were fed with normal drinking water. After 4 weeks, rats were killed and kidney harvested. All rats fed with EG had calcium oxalate deposited in renal cortex. The renal cortex was cut into pieces. Proteins were extracted and isolated by 2-D gel electrophoresis. A point of commonly and markedly increased density (3.0 fold) on gel in EG treated group was selected. After in-gel digest, samples were analyzed by the surface-enhanced laser desorption ionization-time of flight (SELDI-TOF) technique. The peptide sequences were analyzed from the data of mass spectroscopy. Proteins were identified from Database Search (SwissProt) on MASCOT server. The identified protein was AGT2 (Mass: 57164, Score: 52). For further detection and confirmation the mass findings, the expression of AGT2 gene between the EG treated and control group was compared by Q-PCR.
Results: AGT2 was identified from SDS-PAGE in EG treated renal samples through SELDI-TOF. The expression of the AGT2 gene in the EG treated group was significantly higher than in the control group.
Conclusion: EG induced the over-expression of AGT2 gene in the rat kidney which was significantly higher than in the control. This expression could be identified from methods of proteomics and Q-PCR. Since AGT2 gene expressed in the epithelial cells of Henle's loop of the rat activates some proteins in the kidney, our results suggest AGT2 may play some role in the stone formation and the nephrotoxic effect of EG.

OP-058 Modification of primers for GRHPR genotyping avoiding allele dropout by single nucleotide polymorphisms (SNPS) and homology sequence

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Introduction: Primary hyperoxaluria type 2 is an inherited autosomal recessive disorder of endogenous oxalate overproduction, resulting in

urolithiasis, nephrocalcinosis, and/or renal failure. The disease is caused by mutations in the GRHPR gene encoding the glyoxylate/hydroxypyruvate reductase enzyme.
Objectives: We developed a GRHPR sequence assay more effectively for clinical use. We re-designed GRHPR primers for polymerase chain reaction (PCR)-based sequence assay based on whole human genome sequence.
Methods: Blood was collected from health volunteers at Hamamatsu University school of Medicine. Genomic DNA was isolated from samples by the QIAamp DNA Blood Kit (QIAGEN). PCR amplified each exon. PCR products were purified using the Wizard PCR Preps DNA Purification System (Promega). DNA sequence was analysis on an ABI 3100 (Applied Biosystems).
Results: SNPs were found in described primers (1) to amplify exon 6 and 8 from NCBI database. To eliminate the possibility of allele dropout, we designed primers avoiding SNP sites. Common (>10% minor allele frequency) 4 SNPs were found between intron E and exon 6. Linkage exists between these SNPs. It shows linkage disequilibrium. As described primers (1) for amplification of exon 4 have many homologous sequences around human genome of GRHPR, we designed new primers for exon 4. Because there is a length disparity on intron H of GRHPR between NCBI sequence data of AF146689 (GRHPR) and CH471071 (human genome), we designed new primer for first reaction primers of each exon 8 and exon 9.
Conclusion: These re-designed primers will be useful to avoid a failure of mutation detection of GRHPR. The approach of designing primer to avoid known SNPs and homology sequences can be generalized to the design of any PCR-based assay and should be employed whenever possible. We need to check the genome sequence before using published primer for genotyping.

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OP-059 Intrarenal gene expression of monocyte chemoattractant protein-1, interleukin-6 and transforming growth factor-beta1 in kidney stone patients

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Introduction: Oxidative stress and inflammation are suggested to play important roles in the pathogenesis of nephrolithiasis. Lithogenic crystals formed in persistently supersaturated urine cause oxidative damage, tubular injury and inflammation in the kidneys of nephrolithic rats. Up-regulations of monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6) and transforming growth factor-beta1 (TGF-b1) in oxalate/crystals-challenged renal tubular cells have been demonstrated.
Objectives: We aimed to investigate the intrarenal gene expressions of MCP-1, IL-6 and TGF-b1 in nephrolithiasis patients and to evaluate whether their expression was associated with renal impairment and oxidative stress.
Methods: Twenty-nine patients with nephrolithiasis who underwent surgical removal of stone were recruited. Renal biopsy from near the stone, blood and 24-h urine specimens were obtained from the patients. Control renal tissues were taken from non-cancerous and cancerous portions of nephrectomy specimens from patients with renal cancers ($n = 6$). Control 24-h urine samples were obtained from 35 health subjects. N-acetyl-beta-glucosaminidase (NAG) activity, proteins and 8-hydroxy-2'-deoxyguanosine were measured in the urine specimens. Corrected creatinine clearance (CCr) was determined to assess the kidney function. The mRNA expressions of MCP-1, IL-6 and TGF-b1 in renal tissue were measured by real time RT-PCR.
Results: Nephrolithiasis patients had significantly greater renal tubular damage and oxidative stress than the healthy controls. Intrarenal mRNA expressions of MCP-1 and IL-6 in stone-adjacent renal tissues were significantly lower than in cancerous renal tissues, but not statistically different from that in non-cancerous renal tissues. The intrarenal mRNA level of TGF-b1 compared among the three sources of renal tissues was not significantly different. In stone-adjacent renal tissues, mRNA levels of MCP-1 and TGF-b1 were significantly higher than that of IL-6, and expressions of MCP-1 and IL-6 were significantly correlated with each other. Histological examination showed that the number of infiltrated leukocytes corresponded well with the intrarenal mRNA levels of MCP-1 and IL-6. Intrarenal mRNA expressions of MCP-1 and IL-6 were inversely related to the corrected CCr, but positively correlated to urinary NAG activity and proteins. The association between intrarenal mRNA expression of the three genes and oxidative stress was not observed.
Conclusion: Nephrolithiasis was associated with low-grade intrarenal inflammation. A greater intrarenal mRNA expression of MCP-1 and IL-6 was associated with enhanced renal impairment. Thus, expression of MCP-1 and IL-6, at least in part, contributed to the progression of nephrolithiasis. A comparable expression of TGF-b1 between in stone-containing kidneys and malignant kidneys suggests that there is an ongoing renal fibrosis in the kidneys of stone patients. The present findings support the hypothesis that inflammatory response and renal fibrosis involved in the pathogenesis and progression of kidney stone disease.

OP-060 Deficiency in tissue kallikrein results in a defect in renal tubular calcium absorption

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Introduction: Renal tubular calcium (RTCa) transport is one of the main factors that determines serum Ca concentration and urinary Ca excretion. The distal convoluted and connecting tubules reabsorb a significant fraction (10%) of filtered Ca. These tubule segments also synthesize in large abundance tissue kallikrein (TK).
Objectives: Tested was the hypothesis that TK is involved in controlling RTCa transport by studying TK (TK-/)-deficient mice on different Ca diets and humans bearing a loss-of function polymorphism of the human tissue kallikrein (TK) gene (R53H) that induces a major decrease in enzyme activity.
Results: Mice studies: On a 0.9% w/w Ca diet, 129 Sv or C57Bl/6 TK−/− mice excreted significantly more Ca in urine than their wild-type (WT) littermates. There was no difference between TK−/− and WT mice for plasma concentrations of Ca, Mg, creatinine, parathyroid hormone, or 1,25-dihydroxyvitamin D. On a low Ca (LCa) diet (0.01% wt/wt), urinary Ca excretion decreased in both TK−/− and

WT mice but still remained higher in TK-/- mice compared with WT. The plasma Ca concentration was unchanged in C57Bl/6 TK−/− mice but decreased significantly in 129 Sv TK−/− mice. Taken together, these data demonstrate that TK deficiency led to impaired RTCa absorption. On the LCa diet, renal TK gene expression doubled in WT mice. TK deficiency had no effect on the renal abundance of distal Ca transporter mRNA.
Human studies : the participants were studied before and during a 2-h infusion of furosemide that functionally excludes the thick ascending limb and increases Ca delivery to distal tubular segments. Urinary kallikrein activity was 50–60% lower in R53H participants than in R53R participants. R53H participants after furosemide infusion had significantly lower serum ionized Ca concentrations than did R53R participants ($P < 0.0001$) and tendency toward higher urinary Ca excretions than did R53R participants. These results suggest in R53H individuals an increase in Ca reabsorption in the thick ascending limb under baseline conditions that counteracts a distal tubular defect that is revealed by furosemide infusion.
Conclusion: In humans, as in mice, TK thus may act as an intrarenal modulator of Ca reabsorption.

OP-061 Bone damage in primary hyperoxaluria type 1: a monocenter study using new bone imaging techniques

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Introduction: Primary hyperoxaluria type 1 (PH1) can be associated with severe oxalate osteopathy leading to bone fractures and pain as well as radiological lesions. Oxalate crystals deposits in bone form rosette-like structures surrounded by granulomatous reaction.
Objectives: The objective of the study was to evaluate new bone imaging techniques in PH1.
Methods: We performed biological and bone investigations in 11 PH1 children (median age 7.2 yrs [1.7–18.7 yrs] and median age at diagnosis 1.5 years [3 month−7.6 years]). Bone imaging consisted of 2D high resolution radiographs with direct digitization (BMA™, D3A Medical Systems) studying bone texture parameters and 3D HR-pQCT (High Resolution peripheral Quantitative Computed Tomography) evaluating bone mineral density and micro architecture. Skeletal age was studied with the MATUROS® system.
Results: Five patients had a history of fracture. Four patients had infantile PH1, with combined liver-kidney transplantation (CLKT) at a mean age of 26 months. Two children were on hemodialysis. Median GFR for children without ESRD was 77 (40–115) mL/min/1.73 m^2. Median plasmatic ox/cr was 0.153 micromol/micromol (0.06–0.43). Bone maturation was accelerated in five patients, four of whom were less than 5 years of age. Two have had a CLKT for infantile PH1; the other three had a normal GFR. There was no evidence for premature pubertal maturation and endocrinological investigations were inconclusive.

In the four patients with infantile PH1, severe bone lesions were found in long bones (cystic bone changes, deformations, dense metaphyseal bands, cortical thickening) and in flat bones (dense circle around ossification nucleus in calcaneus and carpal bones).
Conclusion: Bone damage remains an important problem in PH1 patients despite therapeutic improvement (new tools for managing hyperparathyroidism, earlier diagnosis and treatment). We report here a previously unreported acceleration of bone maturation in some children as well as new morphological bone lesions.

OP-062 Genetic polymorphisms and messenger RNA expression of sodium-dicarboxylate co-transporter-1 in patients with nephrolithiasis

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Introduction: Hypocitraturia is a major metabolic risk factor for kidney stone development. Genetic heritability of urinary citrate excretion has been demonstrated. Reabsorption of citrate takes place in renal proximal tubules, which is principally responsible by sodium-dicarboxylate cotransporter-1 (NaDC-1).
Objectives: We investigated the association of genetic polymorphisms and NaDC-1 mRNA expression with hypocitraturic phenotype in patients with kidney stone.
Methods: The exonic polymorphisms of NaDC-1 gene were screened in 13 patients with nephrolithiasis using direct genomic sequencing method. A missense SNP in exon 12 (I550 V) was identified, rs11567842 (A/G). This SNP was genotyped in 114 nephrolithiasis patients (aged 21–77 years old, 46% males) and 62 healthy controls (aged 20–61 years old, 45% males) using PCR-RFLP. Logistic regression was performed to calculate adjusted odds ratios and 95% confidence intervals. Intrarenal mRNA expression of NaDC-1 was carried out in 29 nephrolithiasis patients using real-time RT-PCR.
Results: The patients excreted urinary citrate significantly lower than the controls. Hypocitraturia (<200 mg/day) was associated with a significantly increased risk of nephrolithiasis (adjusted OR, 6.89; 95%CI, 3.20–14.88). Allele and genotype frequencies of rs11567842 (A/G) SNP compared between nephrolithiasis and control subjects were not significantly different. The prevalence of hypocitraturia in individuals (all subjects) with homozygous GG was significantly lower than those carried A allele. In nephrolithiasis group, the risk of hypocitraturia in homozygous GG patients (adjusted OR: 0.19, 95%CI: 0.06–0.62) was, while in those with heterozygous AG (adjusted OR: 1.47, 95%CI: 0.50–4.34) was not, significantly lower than in those with homozygous AA (adjusted OR: 1.00). However, no association of AA genotype with hypocitraturia in the healthy group was found. A trend of increased intrarenal mRNA expression of NaDC-1 in nephrolithiasis patients with hypocitraturia was observed although it was not significant.
Conclusion: We have demonstrated that the GG variant of rs11567842 SNP in NaDC-1 gene is a protective genotype for hypocitraturia in the nephrolithiasis population. Detection of this polymorphism may be useful for identifying individuals who are at risk of hypocitraturia. An increased expression of NaDC-1 in the kidneys of stone patients might contribute to the low urinary excretion of citrate.

OP-063 GDNF gene variants in medullary sponge kidney disease

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Introduction: Medullary sponge kidney (MSK) is a developmental disorder. To date, no genetic study of MSK has been conducted. We advanced the hypothesis that MSK results from a disruption in the

'ureteric bud-metanephric mesenchyme' interface. GDNF/RET binding is crucial in this stage of renal embryogenesis.
Objectives: Disease-causing mutations/specific polymorphisms of GDNF may be responsible for MSK. We looked for GDNF gene variants in MSK patients, in healthy controls (HC), and in idiopathic calcium stone former (ICN) controls.
Methods: Blood samples for DNA were obtained from 40 MSK pts, 100 HC, and 75 ICN subjects. Two MSK families were also investigated. Both the coding and non coding sequences of GDNF (5 exons) genes were analysed by direct sequencing of GDNF gene PCR products. The frequencies of -45G > C and IVS3 + 18G > A GDNF variations were evaluated using MnlI and AVAI enzymes.
Results: Three different SNPs in heterozygosity were found in MSK patients: −45G > C and IVS3 + 18G > A, never described before, in the 5′UTR region and R93 W, already described, in the coding region. A complex allele constituted by both of the novel 5′UTR variations and a simple allele constituted by the IVS3 + 18G > A intronic variant were identified in 4 and in 2 patients, respectively. Both alleles had a frequency of 0.008 in 200 consecutive HC chromosomes. None of the 75 ICN pts showed the -45G > C or IVS3 + 18G > A variants. On the contrary, the complex allele had a frequency of 0.040 and the IVS3 + 18A allele showed a frequency of 0.020 in the affected individuals. We observed a significant difference in the complex allele frequency between controls (HC and ICN) and MSK patients ($P = 0.035$).
In the MSK families, direct sequencing of all the family members revealed that the GDNF alleles co-segregate with MSK disease.
Conclusion: The discovery of GDNF sequence variants, which are very probably disease causing mutations, in MSK patients and families indicates that MSK may be considered a genetic developmental disorder due to mutations in the GDNF gene.

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OP-064 Glyoxylate reductase deficient mice: a model for primary hyperoxaluria type 2

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Introduction: A deficiency of glyoxylate reductase activity results in primary hyperoxaluria Type 2 and elevated urinary excretions of oxalate and L-glycerate in affected individuals.
Methods: We have examined the phenotype of a knockout (KO) mouse with an inactivating mutation in the glyoxylate reductase/hydroxypyruvate reductase(GRHPR)gene.
Results: Western blot analysis showed a complete absence of GRHPR in all tissues. These mice developed normally and had elevated plasma and urine concentrations of oxalate and L-glycerate. Mean urinary oxalate and glycerate excretion increased 3.5 and 8.1 fold, respectively. Interestingly, one of the 10 KO animals examined to date showed no increase in urinary glycerate excretion. An absence of hyperglyceric aciduria has been reported in PH2 patients. Mean urinary glycolate excretion in KO animals was 7% lower than WT mice. This finding suggests GRHPR activity is not the major source of glycolate in urine. Heterozygote mice show intermediate urinary excretions of oxalate and glycerate, and similar glycolate excretions. Mean plasma oxalate and glycerate were 1.8 and 4 fold higher in KO mice compared to WT animals. Tissue analyses showed glyoxylate levels in the liver and oxalate levels in the kidney of KO mice to be 1.4 fold and 1.5 fold higher, respectively, compared to WT mice. Hydroxyproline feeding (1%) produced 2 fold more oxalate than wild type mice and 6.6 fold less glycolate. In contrast, 1% glycine feeding produced no difference in urinary oxalate excretion between KO and WT animals.
Conclusion: These results indicate that these mice will be suitable models for increasing our understanding of the pathophysiology and the biochemical changes associated with PH2, and are ideally suited for testing novel treatment strategies that limit oxalate synthesis.

Author Index