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THE PIVOTAL ROLE OF A KIDNEY STONE CLINIC IN THE MANAGEMENT AND PREVENTION OF RECURRENT CALCIUM OXALATE NEPHROLITHIASIS

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MB ChB (Wits), FRCP (London), FCP (SA)

Thesis submitted in fulfilment of the requirements for the degree of

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AUTHOR'S DECLARATION

I declare that this thesis is my own, unaided work being submitted for the Degree of MD at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University. The thesis is based on previously published work and all co-authors have agreed and authorised the use of the relevant articles for this thesis. All publishers have also provided letters of agreement for use of these publications for the MD degree, to fulfil copyright issues. These authorisations and agreements are included as appendices to this thesis.

Signed: _____ on this,

Anthony M Meyers

the _____ day of _____ 20 _____ in _____ .

DEDICATION

I dedicate this work to my family and mentors:

- My wife Jennifer Meyers
- Professor Thomas Bothwell (posthumously)
- Professor Rosemary Ryall and Dr Kumar Grover

I thank all my family and colleagues for their support through my many years of full and part-time work.

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- Professor Peter Cleaton-Jones for all his encouragement and excellent help.
- To my wonderful team of co-workers and especially Dr Natalie Whalley, Mrs Maria Martins and all of our dieticians.
- To Professor James Gear who was one of the main founding members of the South African Renal Society as well as the National Kidney Foundation of South Africa.
- To Prof Ben Goldberg (posthumously) who was instrumental in helping me to establish the Johannesburg Stone Clinic in 1977. He remained a major mentor in my whole nephrology career.
- Professor Rosemary Ryall and Dr Kumar Grover who were jointly responsible for my in-depth stone research training whilst on a 5-month sabbatical in Adelaide, Australia.
- Prof FJ Milne – previously Chief Physician at Coronation Hospital, thereafter academic head of Medicine and subsequently Dean of Faculty of Health Sciences, University of the Witwatersrand. I thank John for his many years of valuable support and outstanding advice.
- I would like to thank all the urologists who referred their patients to a stone clinic especially Drs J Szpytko (posthumously), P Porteous, K Fisher, A Ahmad, P Reyneke, P Harden, M Cohen, D Cilliers, W Vickers, J van der Merwe, L Gellman, Prof Haffejee and others.
- Dr Caroline Dickens for her expertise and assistance with the statistical analysis of data presented in the thesis.

- To Mr Fanie du Toit for his highly skilful typing, figures and table planning and most of all, for his patience in putting together the final publication.
- I acknowledge with sincere thanks the several grants awarded from the National Kidney Foundation of South Africa which contributed significantly to the funding of the research which lead to the publications presented in this thesis.
- I would like to thank Prof Allen Rodgers for our past collaboration.
- Mrs Vivienne Stevens for secretarial assistance.

PRESENTATIONS ARISING FROM THIS STUDY

1. Presentation on “There is a stone in my heart - Associations of kidney stones and cardiovascular disorders” on 04 March 2019 at 17h30 to the Cardiologists Journal Club at the Morningside MediClinic. The Journal Club is a monthly meeting for both paediatric and adult cardiologists and is CPD accredited.
2. Presentation on “Management of calcium oxalate stone formation – the role of nephrologists” on 10 October 2019 at 17h20 at the 1st Biennial SA Renal Society Update Course held at the Wits Donald Gordon Medical Centre.
3. A presentation entitled “Management of recurrent CaOx and CaP nephrolithiasis; a challenging medical problem” was presented on Friday 15 November 2019 at 8h00 at the Department of Internal Medicine, Charlotte Maxeke Johannesburg Academic Hospital.
4. The training of potential members for the Aerospace Medical Committee under the auspice of the South African Civil Aviation Authority on potential kidney related disorders and included specific reference to nephrolithiasis. The training was presented at the aviation training venue, OR Tambo Airport, Kempton Park on 18 February 2020 at 15h30.

ABSTRACT

Introduction

Nephrolithiasis occurs in 12 to 15% of males and 5 to 6% of females with frequent recurrence in about 50% of them. The prevalence of stone disease is unknown in the South African black population and is of low frequency. The commonest type of nephrolithiasis in the South African population is calcium oxalate stones. This thesis involved the study of the epidemiology, causes, pathogenesis and management of kidney stones in patients with recurrent calcium oxalate stones.
Dedicated stone clinics indicate that there is a 30% lower mortality rate accompanied by an increase in patient satisfaction, improved recruitment of staff and improved morale.

Patients and methods

Seven previously published studies, which form the basis for this Doctor of Medicine degree, were analysed; six in-vivo and one laboratory study. Three of the studies, analysed the urinary metabolites in a large number of black and white male and female normal controls. Stone risk factors were studied in a total of five papers, also in a large number of patients of comparative cohorts with nephrolithiasis. Standard work-up protocols included dietary, anthropomorphic, serological, 24-hour urinary excretion of mineral and electrolyte measurements. Special crystallographic techniques were used in the paper presenting the role of cystine in calcium oxalate stone formation. The metastable limits of urine were measured using various quantities of cystine in pooled urine, in spun and filtered as well as ultra-filtered specimens. In addition, particle numbers, diameter and volume were measured. Promotion of calcium oxalate formation was measured in the specimens using various concentrations of cystine.

Results and discussion

Hypocitraturia was present in ~50% of white stone patients; black controls excreted much less citrate than whites, 50% of whom fell into the hypocitraturic range. Therefore, two separate controls for blacks and whites were formulated and validated

by statistical evidence and accepted as “good laboratory practice”. Oxalate excretion in controls and stone formers, whether of black or white ethnicity, male or female, was similar. Both black normal controls and stone formers excreted less calcium than their white counterparts. No blacks had calcium phosphate calculi; 20% of the whites did. The role of calcium phosphate excretion was not assessed in the work-up protocols. However, in a “post-hoc” exercise, useful data on urinary metabolites, which included analysis differentiating between the ethnic groups as well as controls versus stone formers, was extrapolated from the relevant published papers. The presence of hyperurcosuria with hyperoxaluria or hypercalciuria was present in whites only. However, due to the invariable presence of hypernatrituria, hypercalciuria cannot be accepted as truly non-dietary from these and most other reported studies. The in vitro laboratory experiment demonstrated the ability of cystine to promote calcium oxalate stones and was an entirely original postulate at the time of publication. The urinary metastable limit did not change with the varying cystine levels, while particle diameter and volume increased significantly and proportionately in a dose responsive manner. The highest dose of cystine increased the calcium oxalate level by 52%. The results were confirmed in 14C-oxalate experiments as well as scanning electron microscopy, neither of which revealed any cystine crystals. Thus, adding cystine to undiluted urine results in marked calcium oxalate precipitation. This study also demonstrated many of the latest and advanced techniques in the study of crystal formation science known at the time. Two of the papers dealt with medical management of nephrolithiasis. The first clearly demonstrated the outstanding efficacy of potassium citrate in the successful long-term prevention of recurrent stones in these patients. Likewise, the use of indapamide clearly outperformed hydrochlorothiazide therapy in patients with hypercalciuria. Significantly superior efficacy and safety parameters were found with indapamide which is clearly the drug of choice.

Conclusions

Hypocitraturia was present in ~50% of white stone formers and considered to be pathogenic. The presence of hypocitraturia in the South African black population, together with their low prevalence of nephrolithiasis warrants further research. ix

A dedicated renal stone clinic is advocated; such an institution would provide high quality care of patients, coupled with a high research output; all of which strongly supports the creation of a dedicated Kidney Stone Institute.

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ABBREVIATIONS

ADPKD	Autosomal Dominant Polycystic Kidney Disease
BMI	Body Mass Index
Ca	Calcium
CaOx	Calcium Oxalate
CaOxSF	Calcium Oxalate Stone Formation
CaOx/PSF	Calcium Oxalate and/or Calcium Phosphate Stone Formation
CaP	Calcium Phosphate
CaPSF	Calcium Phosphate Stone Formation
CKD	Chronic Kidney Disease
CTS	Computed Tomography Scan
ESKF	End Stage Kidney Failure
ESWL	Extracorporeal Shock Wave Lithotripsy
FGF-23	Fibroblast Growth Factor 23
GAGS	Glycosaminoglycans
GFR	Glomerular Filtration Rate
GIT	Gastro Intestinal Tract
HCTZ	Hydrochlorothiazide
IBD	Inflammatory Bowel Disease
IND	Indapamide
K	Potassium
K-Cit	Potassium Citrate
Mg	Magnesium
MSK	Medullary Sponge Kidney
MCP-1	Monocyte Chemotactic Protein-1
NaCl	Sodium Chloride

OPN	Osteopontin
P	Phosphate
PPI	Proton-pump Inhibitors
PTH	Parathyroid Hormone
RCaOxSF	Recurrent/Recurring Calcium Oxalate Stone Formation
RCaOx/PSF	Recurrent/Recurring Calcium Oxalate and/or Phosphate Stone Formation
ROS	Reactive Oxygen Species
RP	Randall's Plagues
RSF	Recurrent stone formers
RTA	Renal Tubular Acidosis (dRTA = distal RTA)
SF	Stone Formation
THP	Tamm-Horsfall Glycoprotein
TmPO4/GFR	Maximum rate of renal tubular reabsorption of phosphate to glomerular filtration rate
TRPV-5	Transient Receptive Potential Channel V5
UTI	Urinary Tract Infection
Vit C	Vitamin C
Vit D	Vitamin D
Vit D3	Vitamin D3

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CHAPTER 1

INTRODUCTION TO NEPHROLITHIASIS

1.1 EPIDEMIOLOGY AND BASICS OF NEPHROLITHIASIS

1.1.1 Stone epidemiology in historical times

Renal calculous disease was known as far back as 5,000 years before Common Era (BCE) [1] and was well known in the days of Hippocrates (±400BCE). At this time and for the next 200 years, many unskilled “stone practitioners” were called upon to surgically remove kidney and bladder stones from patients who were in agony from their disease. These unfortunate victims of literally barbaric surgeons (as many of them were barbers by trade) led in ±180CE to a phrase enunciated in the Hippocratic Oath namely that “I will not cut, even for the stone…”. [2] Stone disease is still one of the commonest conditions in medical practice and is associated with considerable morbidity, chronic kidney disease (CKD) and even occasional end stage kidney failure (ESKF). [3, 4, 5]

From a South African perspective, the earliest epidemiological and clinical reports on nephrolithiasis could be attributed to Monte Modlin from the University of Cape Town in his publications during the 1950’s and 1960’s. In a notable study (1957), he presented the data on 520 patients with special reference to the patterns of recurrence. [6] He reported that 24% of these stone formers (SF) had bilateral calculi and stone recurrence was found in 50 % of the patients. These rates of stone formation (SF) were similar to the epidemiological data reported by others in the 1980’s and 1990’s. In 1966 Modlin was the guest Hunterian Lecturer and delivered his talk to the Royal College of Surgeons of England entitled “The aetiology of renal stones: a new concept arising from studies on a stone-free population”. [7] His main theme was to compare the dietary calcium and sodium intake in blacks and whites. He demonstrated large differences between the dietary intake between the two groups (black subjects having considerably lower dietary calcium) whilst the black subjects excreted a mean of 292milli-equivalents of sodium (approximately 17grams of salt per day – i.e. 3 ½

times higher than the current recommended healthy salt intake levels - see para. 3.2.1.1, p. 15. This, Modlin concluded, protected the black population from SF – a concept at complete odds with accepted modern criteria.

A further pivotal feature of both normal and stone forming urines was presented in this seminal paper. [7] Modlin showed clear differences in the 24-hour urinary citrate levels between black and white controls as well as SF. Whites had significantly higher citrate levels than blacks. Similar findings from the published papers constituting this thesis are presented later on.

In those early pioneering days, however, Modlin brought much respect and admiration to the South African nephrolithiasis research and to whom all subsequent South African nephrolithiasis researchers owe a debt of deep gratitude.

1.1.2 Stone epidemiology in recent years

In 2001 nephrolithiasis was estimated to occur once in a lifetime in 12 to 15% of males and 5 to 6% of females with frequent recurrence in about half of these individuals. [8]

The prevalence of stone disease in the South African black population is unknown. In terms of the commonest type of nephrolithiasis i.e. Calcium Oxalate (CaOx), it would appear, from personal experience and communication with urologists, to still be rarely encountered in the black population, but with westernization of the diet, an increase could be anticipated. Genetic influences play an important role in CaOxSF. Apart from the well-known inherited causes of CaOx stones (e.g. Dent's disease, renal tubular acidosis (RTA) and primary oxaluria), there is good evidence to show that African Americans exhibit lower urinary calcium excretion than whites. South African data on the lower urinary calcium excretion in the black population was documented in the studies presented in Chapter 6 of this thesis and later confirmed by studies such as Charlton et al (2005) [9] and Rodgers et al (2006). [10] Also, the risk of kidney stones in African Americans is lower than that in whites. [11-13] Additionally a higher bone mass and lower incidence of osteoporosis-related fractures are found in African Americans compared with whites, due to the significantly lower urinary calcium

excretion in the white population. [14-18] The genetic explanations for the above mechanism have been recently elucidated and are ascribed to various genetic polymorphisms in the distal tubular transient receptor potential vanilloid 5 (TRPV-5) and vanilloid 6 calcium reabsorption mechanisms. [19-21]

In stark contrast to the earlier prevalence figures there has been a marked increase in the global incidence of nephrolithiasis which parallel those seen in the increase of CKD. A study by Tasian et al (2016) [22] stated that the most notable changes were recorded in children i.e. in 10% of 0-9-year olds and a large increase of 23% in the 10-19-year-old age group, as illustrated in figure 1.1.

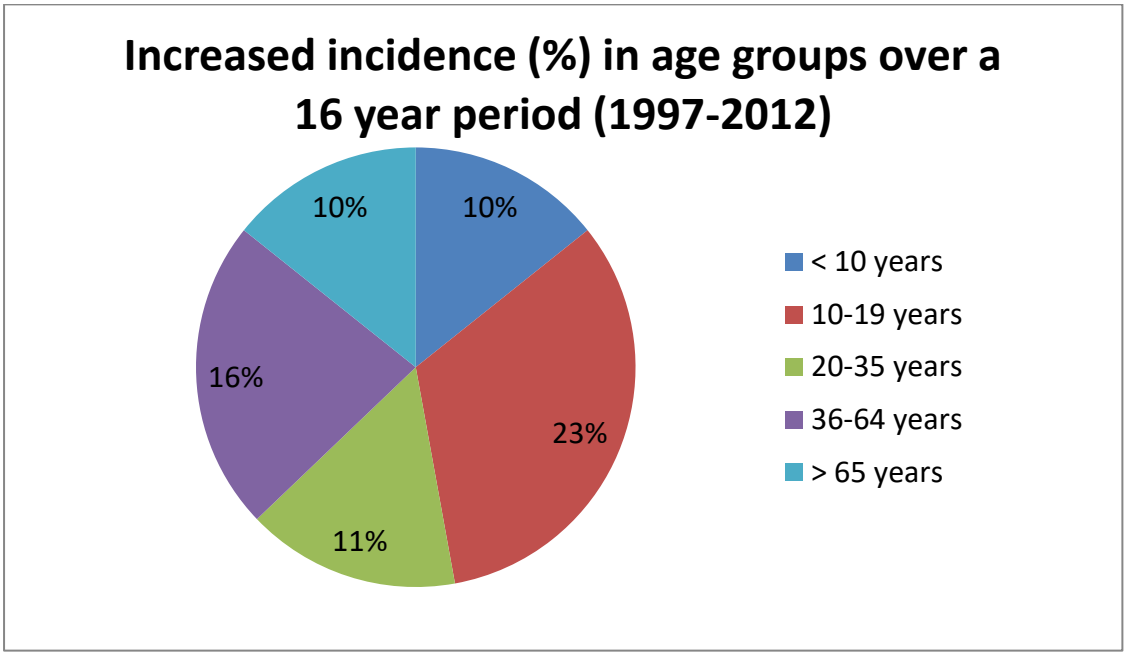


Figure 1.1: Changes in incidence of stone formation according to age groups

Notes:

- *Increase in all age groups – most in 10-19 y (23%)*
- *Mean incidence in Afro-Americans = 15% (stone type not specified).*
- *No data in South African blacks. CaOx calculi still rare (±1%).*

Data extracted from: Tasian GE et al (2016) [22]

Examples of the reasons for the increases are climate change due to global warming and newly formed populations living in arid areas of many drought-stricken regions. [23, 24] A further and most important reason was found to be excessive oral antibiotic exposure particularly in children as discussed later (see para. 3.4.2, p. 33). [25]

1.2 BASIC PATHOGENETIC INFORMATION ON NEPHROLITHIASIS

1.2.1 Stone classification

The commonest types of kidney stones are shown in table 1.1.

Table 1.1: Classification of stone types

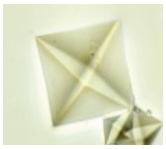
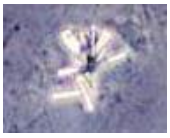
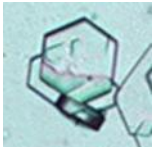
Stone type	Crystals	Urine chemistry	Frequency
1 Calcium oxalate monohydrate	Dumbbell 	Hypercalciuria	50 %
Calcium oxalate dihydrate with calcium phosphate	Envelope 	Hyperoxaluria Hypocitraturia	45% (mixed)
2 Calcium phosphate	Wedge or rosette prisms 	Hypercalcemia Hypocitraturia Urinary pH > 7	5%
3 Struvite or magnesium-ammonium-phosphate	Coffin lid / Staghorn 	High levels of ammonium and biurate	10%
4 Uric acid	Rhomboid / Footballs 	Hyperuricosuria Urinary pH < 5.5	4%
5 Cystine	Hexagonal 	Cystinuria	0.1 – 0.3%

Table adapted with permission (see p. 230) from Pfau and Knauf (2016) [26]; Additional information from: Williams CP et al (1996) [27]; and, Alelign and Petros (2018) [28]

The classification of kidney stones is based on a semi-quantitative estimation and is of considerable value in the diagnosis and management of all patients. Although a reasonably accurate assessment of stone type may be possible by an experienced urologist by direct observation, it is mandatory that in frequent recurrence of the commonest stones, CaOx and calcium phosphate (CaP), an accurate in-depth assessment is required. This can only be achieved by estimating all the minerals present. For example, in CaOx calculi, the diagnosis requires ≥50% of the stones to comprise of CaOx (i.e. mono- or dihydrate crystals). Also, a change of a stone type from e.g. CaOx to CaP (defined as ≥50% of P crystal), makes it likely that the patient was on excessive or unnecessary alkali therapy (e.g. potassium citrate (K-Cit)). CaOx, CaP or mainly urate stones are often seen in secondary intestinal causes of nephrolithiasis. In short, both stone and mineral analysis whenever possible is mandatory and should be acted upon as indicated. [29, 30]

1.2.2 Causes of nephrolithiasis

These have been discussed in several of the publications presented in this thesis. However, in the context of the thesis and before discussing other pathogenic mechanisms, it is important to elaborate on the physico-chemical laws of thermodynamics as related to SF as well as to give a brief summary of all other risk factors related to RCaOxSF. It is important to emphasise that this is a summary only and for detailed presentation of the molecular mechanisms of SF, reference is made to the excellent review by Aggarwal et al (2013) [31] as well as others. [32-36]

1.2.3 General factors related to nephrolithiasis

An approach to major “general” risks for the formation of kidney stones is presented in a schematic format in figure 1.2. Although aberrations in any one or more of these factors may play a role in nephrolithiasis, they interact with one another (e.g. the hypercalciuria of primary hyperparathyroidism or the hyperoxaluria of Crohn's disease and similar disorders).

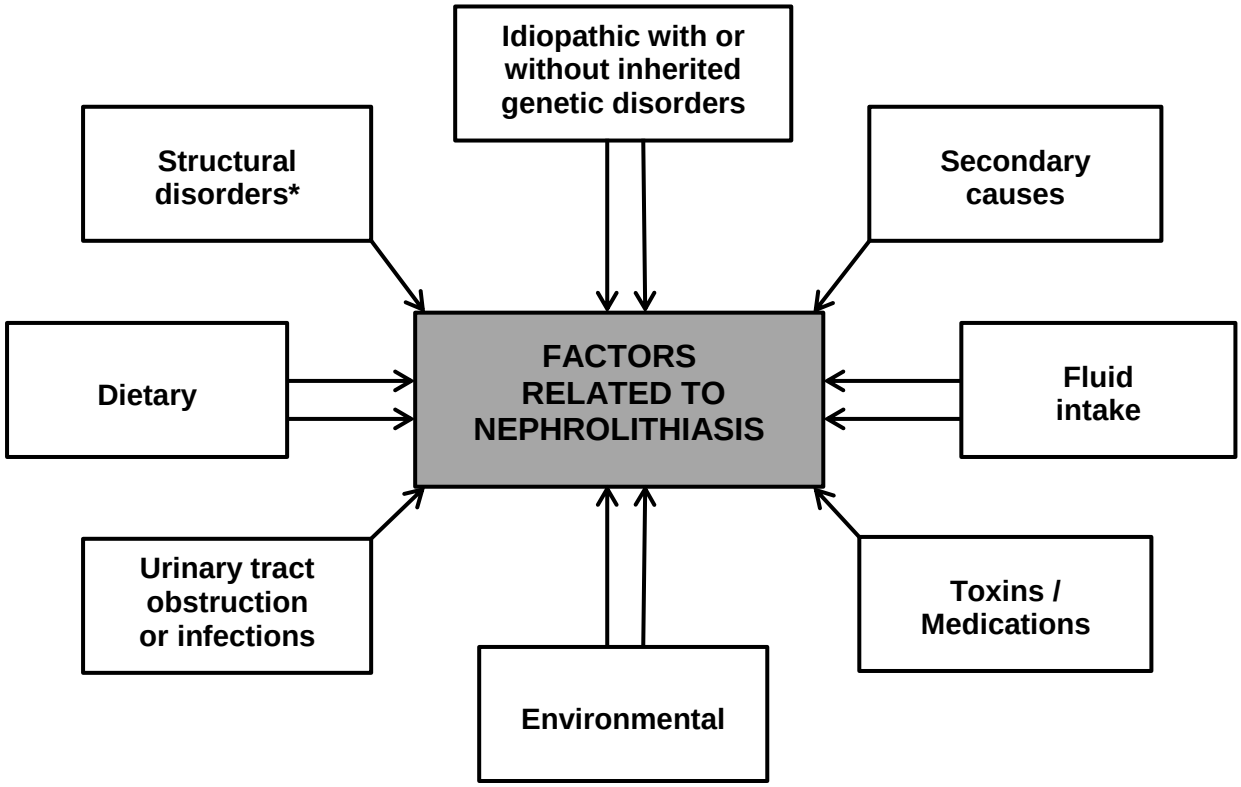


Figure 1.2: The major general risk factors in the development of kidney stones

**e.g. Medullary sponge kidney, horseshoe kidney and autosomal dominant polycystic kidney disease.*

The clinical aspects of each type of kidney stones are summarized in table 1.2, while important secondary causes are presented in table 1.3.

Table 1.2: General aspects related to the formation of different types of renal calculi

Stone type	Urinary risk factor	Clinical aspect
Calcium oxalate monohydrate	Hypercalciuria	Idiopathic Salt excess Hyperparathyroidism Vit D excess Sarcoid and other granulomas Genetic (e.g. Cushing's disease, Dent's disease and similar hypercalciuric disorders)
Calcium oxalate monohydrate	Hyperoxaluria	Genetic hyperoxaluria Idiopathic Bowel pathology Excess Vit C
Calcium phosphate		As for Calcium oxalate monohydrate Distal renal tubular acidosis Drugs e.g. Acetazolamide, Topiramate
Struvite	Ammonium (NH_4) & Bicarbonate (HCO_3)	Urinary tract infections (urea splitting organisms)
Cystine	Cystinuria	Genetic (all 4 subtypes)

Data sourced from: Goodyer et al (1998) [37]; Coe et al (2016) [38]; Alelign and Petros (2018) [28]; Gambaro et al (2004) [39]; Strauss et al (2016) [40]; Worcester and Coe (2008) [30]

Table 1.3: Important secondary causes of nephrolithiasis

Cause	Biochemical change
Primary hyperparathyroidism	Hypercalcaemia / Hypercalciuria
Sarcoidosis	Hypercalcaemia (10%), Hypercalciuria (80%)
Other granulomas	Hypercalcaemia, Hypercalciuria
Genetic: Primary hyperoxaluria (all three subtypes) Cystinuria Tubulopathies Type I Renal tubular acidosis Dent's disease	 Hyperoxaluria (>1000µmol/24-hour) Cystine and other dibasic aminoacidurias Hyper / normocalcaemia, hypomagnesaemia, hypocalciuria, hypomagnesuria Hypercalciuria, hypocitraturia, Systemic acidosis, Urine pH >5.3
Bowel disorders: Inflammatory bowel disease especially: Crohn's disease Other chronic diarrhoeas Post bariatric surgery	 Hyperoxaluria, hypocitraturia, hyperuricaemia, hyperuraemia, low urine volume, hypomagnesaemia and uria
Cushing's Syndrome (primary or post steroid ingestion)	Hypercalcaemia / Hypercalciuria

Data sources from: Goodyer et al (1998) [37]; Coe et al (2016) [38]; Alelign and Petros (2018) [28]; Gambaro et al (2004) [39]; Strauss et al (2016) [40]; Worcester and Coe (2008) [30]

CHAPTER 2

AIMS AND OBJECTIVES OF THE THESIS

2.1 PRESENTATION AND REVIEW OF PREVIOUS RESEARCH

For this thesis the first aim and objective was to present previously published research in relation to the following aspects:

a. Presentation and review of the seven papers submitted for assessment in this thesis

b. To assess the strengths and important contributions to stone disease as well as to highlight shortcomings (i.e. protocol errors, data errors, recent knowledge and understanding of nephrolithiasis).

c. From all of the above, to formulate future ways forward in the management of calcium oxalate nephrolithiasis.

2.2 ESTABLISHMENT OF A NEPHROLITHIASIS INSTITUTE

Secondly, the aim and objective to establish a nephrolithiasis institute is presented in accordance to the following:

a. What are the postulates behind the notion of the necessity to form a stone clinic?

   Firstly, to identify what type of kidney stones should or should not be referred to a stone clinic.

   Secondly, to assess the need for a highly cost-effective management plan for these patients.

Thirdly, to determine the role of these clinics as academic training areas for urological and nephrological trainees and other vocations such as dieticians.

CHAPTER 3

PATHOGENETICS OF NEPHROLITHIASIS

3.1 STANDARD THERMODYNAMICS OF NEPHROLITHIASIS

Physico-chemical laws illustrate that there are two major thermodynamic concepts associated in nephrolithiasis i.e. crystal aggregation and agglomeration. [41]

The following explanation of crystallization and agglomeration presents simplified versions of highly complex processes. The original of these figures were presented as part of the Arthur Landau Lecture in 2000. [42] These simplified explanations are easy to remember and give enough of the basic facts of the processes for the purpose of this thesis. More detailed descriptions of these physico-chemical processes can be found in the literature. [43-46]

3.1.1 Crystallization

Nephrolithiasis is a multistep process comprising nucleation, crystal growth, aggregation (see figure 3.1) and then agglomeration (figure 3.2) after which the agglomerate adheres to the renal epithelial cells. [26, 43-47] Each of these steps is believed to be influenced by a range of renal and/or urinary factors, some promoting and some acting to prevent nephrolithiasis.

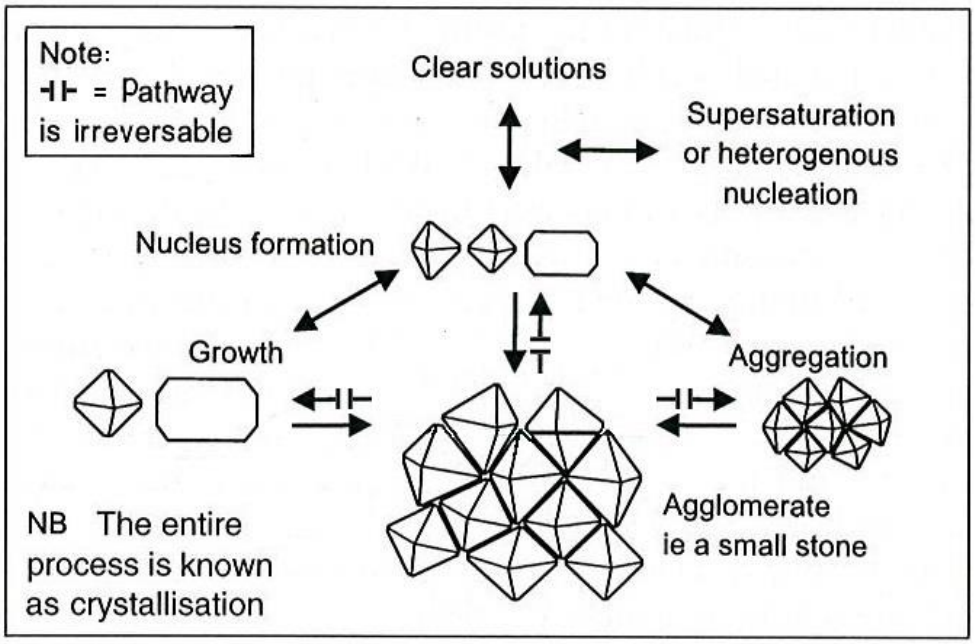


Figure 3.1: The process of crystallisation

There are three steps in the process of crystallisation. Before a stone is formed either nuclei or aggregates of nuclei must stick together. This is the process of crystallisation and depends on the composition and concentration of urinary solutes. Then they must enlarge to form an agglomerate (i.e. a micro-calculous). This process is ordered by the chemical process of "epitaxy" (sticking together) in stone former urines. Calcium oxalate stones can also form in association with "hyperuricosuria"; when epitaxy is absent "salting out" is the mechanism.

Adapted with permission (see p. 231) from Meyers et al (2001) [42]

3.1.2 Agglomeration

Saturation levels of ionic products play an important role in the formation of stones as can be seen in figure 3.2.

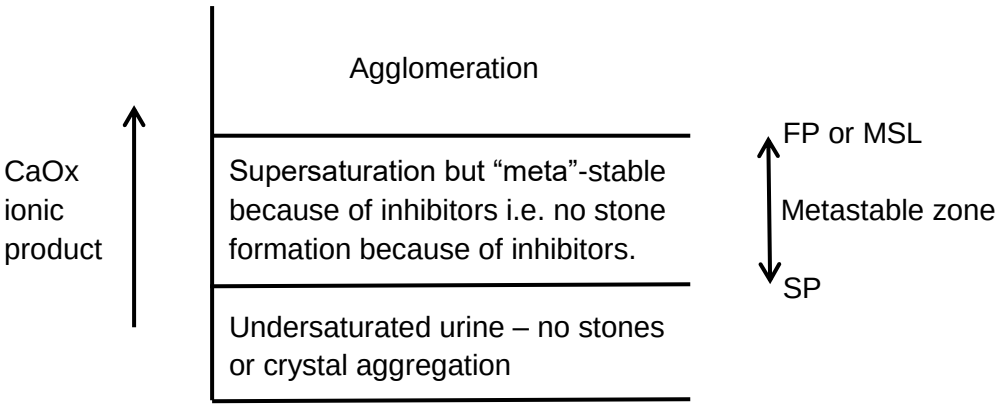


Figure 3.2: Precipitation of calcium oxalate crystals with stone formation in either whole urine or a similarly manufactured solution.

CaOx = Calcium Oxalate; SP = Saturation product; FP = Formulation product; MSL = Metastable limit
***Note**: MSL can also be lower in acid urines (pH < 5.5). This is because of the ability of uric acid to cause precipitation of CaOx by the technique of "salting out" even in the presence of adequate levels of other inhibitors.*

Adapted with permission (see p. 231) from Meyers et al (2001) [42]

3.2 ELECTROLYTE AND METABOLIC FACTORS

The promotors and inhibitors of SF are listed in table 3.1 followed by a more detailed presentation of the role and pathogenesis of a selection of some of these factors, as well as a discussion of some of the associated metabolic aspects.

With respect to electrolyte and metabolic factors, diet is often the most critical factor contributing to the promotion or inhibition of SF and many aspects of diet in relation to nephrolithiasis are well documented. [32-36, 48, 49] A study performed in SA by Rodgers et al (2002) [36] on the effects of different diets on urinary risk factors for CaOx nephrolithiasis in different race groups, concluded that:

> *"renal handling of dietary calcium and oxalate in South African black and white subjects is different and may explain the different stone incidence in the 2 race groups."*

Recent articles, one on the role of bacteria [50] and the other on the role of proton-pump inhibitors (PPI) [40], although showing a strong propensity in the promotion of nephrolithiasis, suggest that further investigation is needed to confirm the findings. Schwaderer and Wolf (2017) [50] stated that:

> *"Bacteria have long been recognized to contribute to struvite urinary stones; however, the role of bacteria in the development of the more common calcium oxalate (CaOx) and calcium phosphate (CaPhos) stones has not been extensively investigated".*

They concluded in their study that:

> *"Selective aggregation to some crystal types seen in urinary stone disease (USD) patients, increased crystal clumping in the presence of bacteria, bacteria-induced lower urine citrate levels and increased CaOx deposits and stone matrix protein expression when bacteria are present as opposed to CaOx deposits alone are initial findings that might provide the basis to begin to unravel the reasons for the clinical USD-bacteria associations."*

In a study by Alelign and Petros (2018) [28]' it is also stated that these stones occur

> *"among patients with chronic urinary tract infections that produce urease, the most common being Proteus mirabilis and less common pathogens include Klebsiella pneumonia, Pseudomonas aeruginosa, and Enterobacter. Urease is necessary to split/cleave urea to ammonia and CO2, making urine more alkaline which elevates pH (typically > 7). Phosphate is less soluble at alkaline versus acidic pH, so phosphate precipitates on to the insoluble ammonium products, yielding to a large staghorn stone formation. Escherichia coli is not capable of splitting urea and is not associated with struvite stones."*

With reference to the PPI, Makunts et al (2019) [51] stated that:

> *"Recent studies have raised concern over associations between PPI use and acute kidney injury, chronic kidney disease, end-stage renal disease, and electrolyte abnormalities."*

They report that:

> *"Our results indicate significant increase in nephrolithiasis reports"* with an odds ratio of 2.8 and they state that *"Nephrolithiasis finding is of particular interest since it has been associated with AKI, CKD, and ESRD progression but* (taken in context) *it only accounts for a small subset of renal injury cases".*

Table 3.1: Promotors and inhibitors of stone formation

Promotors	Inhibitors

• Low urine volume
  ○ Failure of adequate intake
  ○ Global and regional climate change
• Calcium (including the role of sodium)
• Urate
• Oxalate
• Low urinary pH
• Cystine
• Antibiotics
• Bacterial products (not for recurring calcium oxalate stone formation)
• Proton-pump Inhibitors
	Inorganic:
	• Water
	• Magnesium
	• Pyrophosphate
	• Citrate
	
	Organic:
	• Nephrocalcin
	• F-1 fragment of prothrombin
	• Sulphated glycosaminoglycans
	• Tetrahydropalmatine (Tamm-Horsfall protein)

Adapted with permission (see p. 232) from Bihl and Meyers (2001) [8]

Further discussion on the role of inorganic promotors and inhibitors of nephrolithiasis follows.

3.2.1 Urinary Calcium

3.2.1.1 The role of sodium in the magnitude of urinary calcium levels

Excess dietary salt is one of the most important contributors to significant hypercalciuria. [52, 53] The fact that not all individuals who consume large quantities of salt acquire CaOx renal calculi possibly relates to other factors such as genetic influence, the presence of normal citrate levels, absence of a persistently acid urine (pH of <5.6-6.0) or increased levels of both inorganic and organic inhibitors.

Figure 3.3 presents a schematic diagram of the relation between urinary sodium (which reflects dietary salt intake) and the excretion of urinary calcium (Ca) as presented by Blackwood et al (2001). [54] Seven hundred and forty-three patients; 277 whites and 227 blacks, and 239 Asians, collected 24-hour urine specimens and 690 (250 whites, 213 blacks and 2180 Asians) collected fasting urine specimens for the measurement of sodium and calcium. There were no statistically significant differences in the slopes of the regression lines between the three ethnic groups in either figure a or b. Extrapolating from this data it is possible to use fasting specimens

for estimation of the urinary calcium to sodium excretion rather than 24-hour urinary specimens. However, in this thesis the preferred measurement was the 24-hour urinary specimen measurements. The reasons for this are discussed under the relevant sections (see Table 8.1, p. 148; and Appendix H, p. 206).

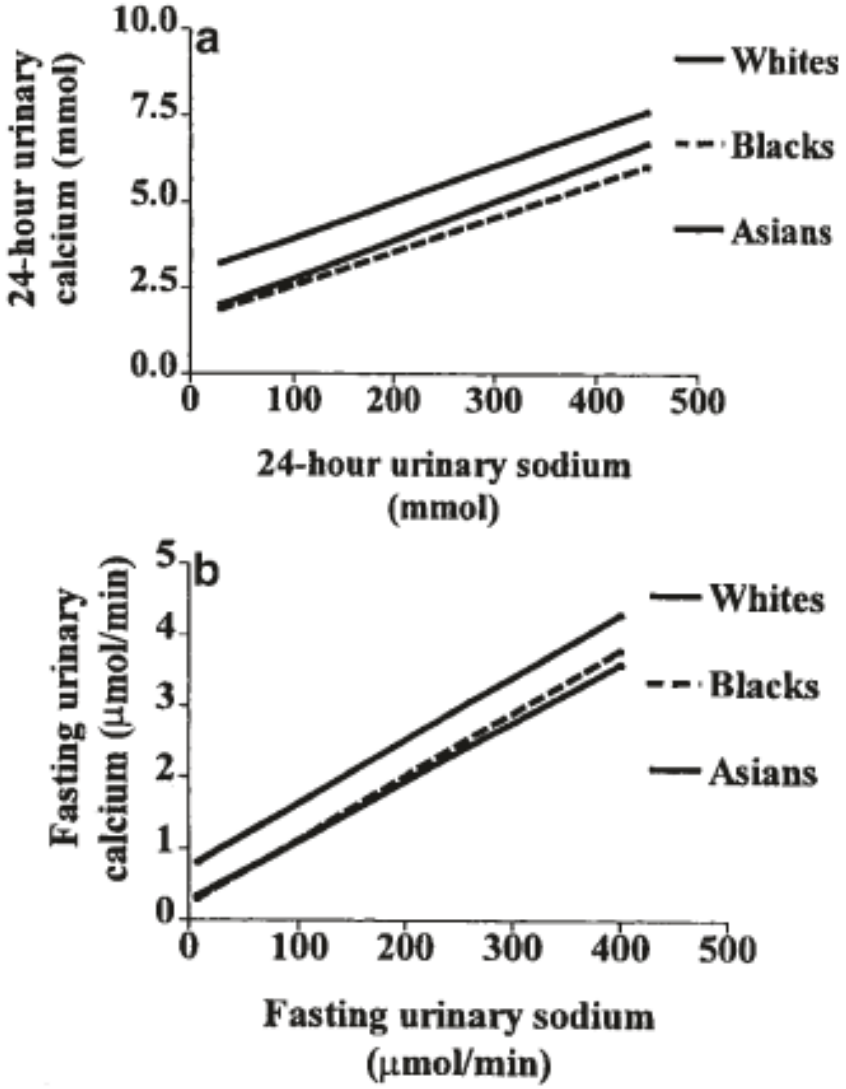


Figure 3.3: The relationship between calcium and sodium

Relationship between (a) 24-h and (b) fasting UCa with UNA after adjustment for age, body mass index, gender and blood pressure in three ethnic groups. The lines represent the estimated regression lines for each ethnic group. Note: In both a and b the regression line for whites are the top lines.

Reproduced with permission (see p. 233) from Blackwood et al (2001) [54]

Further evidence of the relationship of urinary sodium and Ca excretion in RCaOxSF is presented in figure 3.4. However, as discussed above, these were calculated on 24-hour urinary measurements.

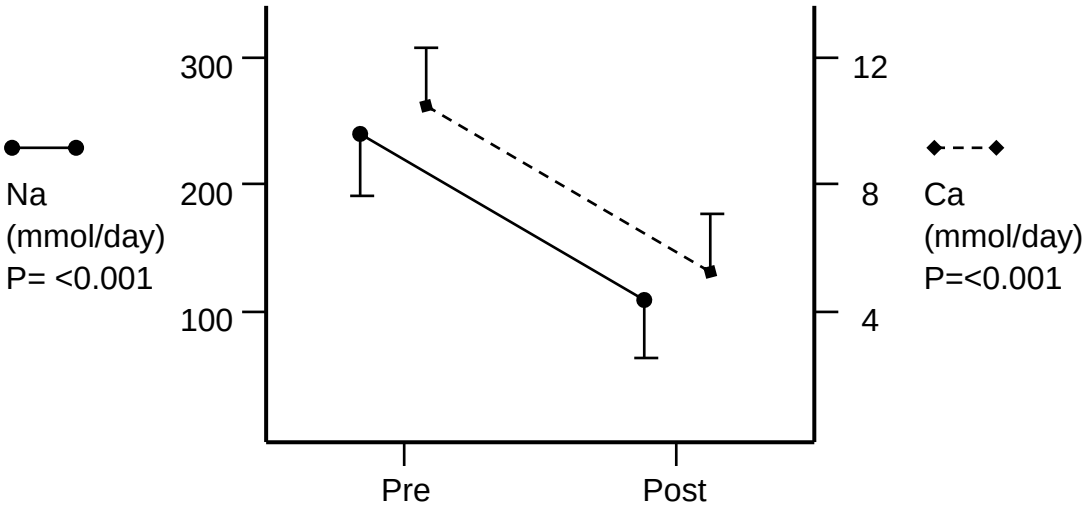


Figure 3.4: 24-hour urine sodium and calcium in recurrent calcium stone formers pre and post standard (i.e. 'healthy') sodium diet (100mmol/day).

Reproduced with permission (see p. 231) from Meyers and Martins (2001) [42]

3.2.1.2 Dietary proteins and hypercalciuria

a. High protein intake especially red meat resulting in hypercalciuria from the following mechanisms:
   - Increase in acid production results in Ca "leaching" out from bones.
   - In addition, acid urine forms a sulphate Ca complex in the proximal convoluted tubule and is excreted unchanged. [55]

b. High protein diets result in hyper-filtration with raised glomerular filtration rate (GFR) so that more Ca is delivered to the distal tubule and excreted. [55]

c. Protein has a direct effect on proximal convoluted tubule, decreasing the reabsorption of sodium chloride which is accompanied by hypercalciuria. [55-57]

d. High dietary protein intake causes increased urine uric acid and together with a low urine pH produces CaOx stones via the "salting out" mechanism – a frequent occurrence (see later). [56, 57]

e. Extracellular volume expansion resulting in increased GFR leading to the increased delivery of Ca to kidney tubules causing hypercalciuria. [55-58]

3.2.1.3 The role of carbohydrate in the excretion of calcium

a. Excess carbohydrate ingestion causes hypercalciuria via a well described decrease in distal tubular Ca reabsorption as well as augmented intestinal uptake. [59-62]
b. Hyperinsulinuria (metabolic syndrome, obesity, diabetes) also contributes to hypercalciuria. [63]

3.2.1.4 The role of urinary phosphate excretion in nephrolithiasis

A brief description of phosphate (P) metabolism, especially the renal handling, is appropriate for the complete understanding of how P is involved in stone pathogenesis.

Eighty percent of the total body inorganic P is ultra-filterable with 20% being protein bound. The other 20% of P is organic and occurs in cells and cell membranes as phospholipids. Eighty percent of the ultra-filterable P is filtered by glomerular filtration and enters the proximal convolute tubule. Fifty percent of the ultra-filterable P is dissociated as follows: H_2PO_4 : HPO_4 = 1 : 4. The other 47 % of the ultra-filterable P in the urine occurs as salts of $NaPO_4$, $CoPO_4$ and $MgPO_4$. The ratio of H_2PO_4 to HPO_4 in the urine is dependent on urinary pH. In acid urine HPO_4 prevails. Of importance is the fact that PO_4 is the "major urine buffer" and accounts for most of the titratable acid production.

Also, of note is the fact that the solubility product of Ca x P is "exceeded" in normal urine and so there are many modifying factors necessary in the urine to prevent CaPSF. [64] Another fact to remember is the P related tubular functions controlling the tubular maximal reabsorption rate of phosphate to GFR (TmPO4/GFR). Urinary P excretion depends on P intake and output (i.e. direct P excretion and fractional excretion) and the nett effect of P balance is parathyroid hormone (PTH) regulated. [65] Practically, a 3g oral load of P is administered to calculate the TmPO4/GFR and approximately 35% of the filtered load is the normal excretion figure. A high dietary Ca load would decrease the fractional excretion of P by forming non-absorbable CaP complexes in the bowel. Large amounts of Ca orally result in secretion of PTH and a

decrease in the urinary P excretion. However, large amounts of dietary calcium form non-absorbable calcium phosphate complexes which may actually lower the absorption of calcium and phosphate causing secondary stimulation of PTH secretion. [65]

Öhman, Larson and Tiselius (1992) [66] were amongst the earliest workers to study the effects of urinary P excretion in the formation of CaOx or CaP stones. They found no influence on urinary P excretion rates if the stones were of "pure" CaOx (i.e. < 0.1% of P). However, the presence of significant quantities of P, either as mixed CaOx/P stones or pure CaP stones had a higher excretion of Ca (but not of oxalates) and the importance of the urinary P excretion in this group was thought to be significant and indicated "*a more complex aetiology than was present for pure CaOxSF*". [66]

The second group of researchers that looked at the role of P excretion in CaOx and CaPSF who also had hypercalciuria, was the group of Williams et al in 1996. [27] They included an assessment of the influence of $1,25(OH)_2D_3$ in the stone patients. Williams and his group were the first to clearly propose that renal stone patients had an overall greater phosphaturia proven by demonstrating a lower TmPO4/GFR in these patients i.e. they interpreted their findings as having shown an "inappropriate P excretion". Prié et al in 2001 [67] performed a similar experiment which confirmed the findings of Williams et al but added nothing new.

In a seminal paper, Rendina et al (2005) [68], has shown a clear-cut and highly significant increase in P excretion in 22 CaSF with a P leak and compared them to SFs with no P leak, and to controls. The important difference in Rendina's cohort study was that the 22 patients with a P leak all had mild hypophosphatemia whereas the other two groups did not. There was a strong inverse association between FGF-23 and serum P ($r=-0.784$, $p<0.009$) and the rate of tubular P reabsorption ($r=-0.791$, $p<0.008$). Interestingly the levels of $1,25(HO)_2D_3$ showed no significant difference between the three groups. This is difficult to explain, but possibly the differences were "relative" e.g. the PTH could have been expected to be higher than the P leak group but unfortunately was not measured in the study.

In 2015 Taylor et al [69] measured associations between plasma levels of calcitriol, fibroblast growth factor 23 (FGF-23), PTH, Ca and P in the role of stone disease. After adjusting for many comorbidities and confounding factors, they reported that high levels of calcitriol produced a higher rate of SF "*independent of other factors*" – except that there was a significantly elevated FGF-23 level in their patients. They indeed discussed the many drawbacks to the study which were probable technical and measurement errors and also the fact that none of their patients studied had any data available on the type of stone that they passed. Similarly, there were no data available on the urinary excretion on any micro or macro stone promotors or inhibitors. Furthermore, not mentioned in their paper but extrapolated from earlier literature, was the fact that Taylor and his group did not isolate stone patients with actual hyperphosphatemia (and in this context about 20% of patients presenting would be expected to be hyperphosphataemic). Thus, these patients were missed in the general group they studied and could also be a confounding factor in the conclusions of their own study.

The protocol format to be employed in future studies on the role of P in CaOx/CaP nephrolithiasis should mimic the format as used by Gianninni et al [70] in their paper on various forms of hyperoxaluria. However, instead of “oxaluria”, the P studies would be grouped as “*hypophosphataemic patients, hypophosphataemic with hypercalciuria patients or no metabolic change patients*”. These studies could sub-divide the first two groups into the sub-classes of hypercalciuria if sufficient patients were recruited for the study.

All of the above papers provide supportive data for the further study of P. The data from three papers discussed in this thesis also supports the importance of P and is presented in Chapter 6 (see para. 6.2.1.2.2(iv), p. 132; and para 6.2.1.3, p. 135).

3.2.2 The role of magnesium in nephrolithiasis

Urinary magnesium (Mg) is a potent inhibitor of oxalate crystallisation and agglomeration. However, neither hypomagnesaemia nor hypomagnesuria has been detected in the urine of past or more recently performed work-ups of recurring CaOxSF

in adults in the Johannesburg Stone Clinic (personal observation). In spite of this finding, there are two potential concerns. Firstly, chronic Mg deficiency is difficult to detect because only 1% of total body Mg resides in the extracellular fluid compartment and measurements of serum Mg will therefore be normal. [71] Secondly, if present, total body Mg deficiency poses some potentially serious health hazards especially to the central nervous system and to cardiac function (both abnormalities of cardiac contractivity and/or cardiac conductivity may be affected). The dietary requirement of Mg is 6-15mmol/kg (130-350mg) and modern diets are only just sufficient to supply this. The absorption of Mg is stimulated by calcitriol ($1,25(OH)_2D_3$) and its renal reabsorption by PTH. [72] Practically speaking from the necessity to measure serum and urinary Mg excretion, it is advisable only if the following conditions exist:

- Vit D3 deficiency in adults
- Malabsorption states in children and adults
- Any cause of persistent hypercalciuria
- Poorly controlled diabetes
- In children the most frequent cause of hypomagnesaemia is found in those with secondary causes of nephrolithiasis e.g. Dent's disease and other tubulopathies, distal type I renal tubular acidosis (RTA) and Bartter's syndrome. [73]
- Many other inherited disorders are associated with hypomagnesaemia and possible SF. [74] They can be classified into four groups: hypercalciuric hypomagnesaemias, Gitelman-like hypomagnesaemias, mitochondrial hypomagnesaemias and other hypomagnesaemias [75]

Therefore, the measurement of serum and urinary Mg is only indicated in a few clinical conditions in adults but, for completeness' sake should be included in a routine work-up of all recurrent SF (RSF).

3.2.3 Oxalate

- Excessive protein intake causes generation of glycolate – a precursor of oxalate. [42]
- Consumption of foods high in oxalate: Ceylon tea, nuts, beetroot, spinach, chocolate, rhubarb, parsley, cocoa, wheat-germ (present in so-called healthy

whole-wheat bread), brown rice and strawberries. These can, if taken in excess, result in dietary hyperoxaluria purely from excessive intake of food high in oxalate content. Many other substances with variable oxalate content cannot be avoided in a balanced diet. [76, 77]

- Dietary deficiency of Ca i.e. <600mg elemental Ca intake per day. This is a commonly seen problem encountered in modern day living and often goes together with poor sunlight exposure (e.g. office bound workers), leading to low levels of calciferol resulting in secondary hyperparathyroidism. Low levels of Ca in the diet results in less oxalate being bound in the small intestine, so more is absorbed from the diet resulting in hyperoxaluria. [53]
- Ingestion of high doses of Vit C (1,000 – 2,000mg/day) anti-cold remedies. Previously it was thought that the "hyperoxaluria" was a falsely high reading due to methodologically impaired oxalate measurement in the presence of Vit C – and is proven now not to be so. Chemiluminescent spectroscopic methods have shown excessive oxalate excretion after Vit C ingestion. [78-80] Thus it can be concluded that the Boehringer oxalate kit method (generally used earlier and also in the studies presented in this thesis) was in fact correct.
- Some select populations have suggested that a rare cause of inherited mild metabolic hyperoxaluria is the reason for the absence of anaerobic bacteria in their gastro intestinal tract (GIT) called oxalobacter formigenes which normally metabolises colonic oxalate while chronic antibiotic consumption may also result in the absence of oxalobacter formigenes. [81-83]
- Hyperoxaluria also is commonly found in patients with inflammatory bowel conditions. [84-86]
- The rare primary autosomal recessive condition of hyperoxaluria and oxalosis. [87]
- Secondary hyperoxaluria such as seen after bowel resection and bariatric surgical procedures – which also may result in chronic renal failure from acquired hyperoxaluria. [88]

3.2.4 Citrate

In most cases the actual cause of hypocitraturia is not known. However, the following conditions are known to be associated with hypocitraturia:

- Excessive acid intake with high levels of meat protein ingestion.
- Diets low in potassium (K). [89]
- Any causes of primary RTA.
- Drug induced RTA [90]
    - Acetazolamide
    - Topiramate (Topamax) often used in control of severe migraine.

Hypocitraturia is the commonest cause of metabolic derangement found in CaOxSF patients. It works not only by preventing crystal growth but also by reducing the super-saturation of CaOx due to its binding to Ca. [91] However, there must be other reasons for hypocitraturia as suggested because the black population without renal calculi and normal renal function were hypocitraturic. [92] This needs further investigation. The most likely aberration in this situation was suggested to be as a result of abnormal renal tubular transportation of citrate. [90]

3.2.5 Pyrophosphate

Although reduced levels of pyrophosphate have been reported in RCaOxSF, [91, 92] normal concentrations of urinary pyrophosphate are so low that it was thought to be of no significant benefit. However, even minute levels of pyrophosphate when added to crystallisation studies have been shown to significantly inhibit precipitation. Therefore, future studies would need to be performed (but is of low priority and not a recommendation in this thesis) to test the practical benefit of oral pyrophosphate preparations for the prevention of CaOx nephrolithiasis. The ability to achieve any clinical benefit form pyrophosphate administration however, remains highly doubtful because of the alterations in its structure during the digestive processes. [94]

3.2.6 Uric acid

There are compelling reasons to present a detailed discussion on the role of uric acid in the genesis of RCaOxSF. Demonstration of these factors is essential to prove the importance of agents lowering urinary uric acid levels in the treatment of these patients. These factors are:

a) How the earlier experiments discussing the role of epitaxy [95-97] as the mechanism relating to the formation of urinary uric acid induced CaOx crystallisation in patients with “hyperuricosuria” were false and that it was the salting–out mechanism of uric acid that resulted in the precipitation of CaOx crystals (see below). [56, 57, 98-101]
b) Presentation of incontrovertible proof that treatment with Allopurinol is highly successful in preventing CaOxSF in patients with hyperuricosuria. [97, 102-108]
c) Presentation of a few papers not in agreement with the current accepted therapy. [109-112] Of note, these papers fail to produce sufficient scientifically validated conclusions of the accepted ideologies (see below).

Previous studies on the mechanism and proof that uric acid can precipitate CaOx stones included some pitfalls. In 2002 and 2003, Grover et al produced incontrovertible evidence that the older methods of examining inorganic and organic urine via crystallisation and precipitation experiments did not relate to the in vivo states. [56, 113] The in vitro experiments performed by Coe (1975) [95] and Pak (1978) [105] using simulated urine, demonstrated that epitaxy was not present in the uric acid induced CaOx stone producing experiments. Therefore, the lack of epitaxy suggested to them that CaOx crystal formation in these experiments was an in vitro phenomenon because of the lack of epitaxy. Grover et al in 2002 [113] produced incontrovertible evidence that in vitro experiments of SF were invalid. They showed that the experiments had to be done on whole undiluted urine. He then went on to prove that after the specimens were spun and filtered which removed the organic inhibitors such as glycosaminoglycans (GAGS), Tamm-Horsfall glycoprotein (THP), F1 fragment of prothrombin, albumin, epitaxy was not present but in fact the mechanism of CaOx crystallisation after experiments using increasing concentrations of uric acid, precipitated the CaOx crystals via the method of salting out. [98] There are two further points of interest: Firstly, it is known that urinary macromolecules do not play a role in the precipitation of uric acid induced CaOx. Secondly of interest is that as far back as in 1971 Kallistratos and Timmerman [114] produced evidence highly suggestive that salting out was the mechanism of CaOx precipitation. This seminal work went unnoticed for about 25 years. The work of Grover then gave credibility to prove that in patients with hyperuricosuria, salting out of CaOx could and does occur. He also showed in experiments using Allopurinol to prevent this effect, that the size of the

crystals was much smaller than in non-treated controls. It is well-known that the size of crystals forming within the renal tubules is of critical importance in intra-tubular SF. [98]

There is a large body of research papers in favour of using allopurinol in patients with recurrent non-gouty nephrolithiasis whether or whether not accompanied by hyperuricaemia or hypercalciuria but in the face of hyperuricosuria [97, 115] These papers antedated the Framingham heart study in 1999 which failed to find an association between serum uric acid and cardiovascular disease or death. [115] Also in spite of the seemingly conclusive work by Grover et al (1994, 2006) [116, 117] other workers doubted the value of allopurinol in the prevention of RCaOxSF in the above setting. Curhan and Taylor (2007) [112] argued against the prevailing believe that hyperuricosuria caused CaOx precipitation and stated (quite correctly) that "…*in addition the current definitions of normal levels for urinary factors need to be re-evaluated*."

Ettinger (1986) [97] did show benefit with the use of Allopurinol but argued that it had nothing to do with its urinary uric acid lowering properties. The explanation given by him was that Allopurinol acted as an anti-oxidant thereby inhibiting CaOxSF. In the consensus statement presented by Gambaro et al (2016) [111], it was agreed that Allopurinol had a role to play in inhibiting CaOxSF but often had to be given together with other agents for the treatment of either hypercalciuria, hypocitraturia and hyperoxaluria. However, they did comment that future studies on the use of Allopurinol in these subjects were warranted. They also mentioned that studies on the definition of hyperuricosuria are also warranted. Current definitions for hyperuricosuria (most commonly quoted) are presented in the appendix presenting normal values – see Appendix A, p. 192.

Having debated the matter positively and negatively for the use of allopurinol in both nephrolithiasis and cardiovascular disease prevention, the pendulum has now swung entirely to strong positive support for the use of allopurinol which has been extensively and skilfully presented in an article titled “Co-morbidities in patients with crystal disease and hyperuricaemia” by Sattuei et al (2014). [118] Not only is it now proven that by lowering the serum uric acid level, cardiovascular disease is prevented, but

also that insulin resistance and metabolic syndrome is reversed while marked vascular endothelial protection also occurs. Indeed, the extensive literature reviewed in the Sattuei paper also demonstrated that by reducing serum uric acid non-gouty CKD progression is markedly slowed (even in non-nephrolithiasis patients). Large clinical trials on these issues are presently underway and so, even in the presence of experimental proof, the actual overall consensus for the use of Allopurinol will inevitably be delayed until the new trial results become available. [118]

Another clinical scenario is worthy of mention. It is known that many RSF do not drink enough fluids despite advice. Because of this, it would not be surprising to expect periods of dehydration during a 24-hour period. Therefore, theoretically, concentrated urine could become “hyperuricosuric” which could accurately be termed “periodic hyperuricosuria”. It would seem possible to investigate these patients who might well also benefit from Allopurinol and might well form a considerable percentage of patients with recurrent CaOxSF.

3.2.7 Cystinuria

The pathogenesis of this condition is beyond the scope of this thesis. However, cystine was found to act as a risk factor for the formulation of CaOx stone precipitation in a few patients with cystinuria via the method of salting out. [119]

To summarize up to this point, it would seem that the more we deviate from a traditional balanced diet with recommended intake of protein, Ca, salt, fruit and vegetables but which is “supplemented” in many modern diets with rich fast foods and artificial drinks, the more we increase our risk for nephrolithiasis – and the risk for a lot of other life-style dreaded diseases. If only modern populations could eat a little of everything and a lot of nothing, many diseases would be prevented.

3.2.8 The role of overweight, obesity and exercise in the pathogenesis of nephrolithiasis

Co-existing causes or associations of either CaOx, CaP, struvite or symptomatic gout and uric acid stones have been excluded from this discussion i.e. the discussion is on weight increase alone. The types of stones referred to in these overweight individuals therefore are mainly CaOx and occasionally CaP.

Several studies have shown an association of overweight and obesity with CaOx stone disease. [120-123]

Wrobel et al (2012) [124] showed in RCaOxSF a differing urinary lithogenic pattern with increasing weight. However, there was no relation of weight increments resulting in an increase in stone recurrence. In 2018, Aune et al [125] showed that the relative risk of getting a stone significantly increased with weight increase. They found a 6 and 12% increase in stone frequency per 5kg increase in weight and weight gain respectively, while the risk of stone disease was 16% higher in diabetic vs non-diabetic stone patients. Interestingly there was no association with exercise although the results were suggestive of a moderate benefit from exercise. However, there may be an association between SF and recurrence in the super-fit and long-distance athletes as compared to the usual non-obese SF (personal observation). The reason for this might lie in frequent periods of mild dehydration with low urine flow rates and, as another example, in patients with high intake of protein shakes.

An important work published by Taylor and Curhan in 2006 studied the effects of body size on the electrolyte composition of 24-hour urine samples. [126] They showed significant association between body mass index (BMI) and urine Ca excretion which was explained by three factors: a) an increase in animal protein intake resulting in hypercalciuria; b) an increase in salt intake resulting in hypercalciuria, and; c) an increase in uric acid excretion associated with both uric acid or CaOxSF.

There are two final points to mention in the discussion of nephrolithiasis in obesity. The first is to mention two excellent overviews on obesity and stone disease. In the first paper by Carbone et al (2018) [127], all of the associated diseases such as gout, diabetes mellitus, insulin resistance, metabolic syndrome, hypertension and all the additional co-morbidity and mortality figures from these disorders, were discussed. (See para. 5.6.1, p. 57 and para. 5.6.2, p. 57) The second paper by Taylor et al (2005)

[128] discussed exclusively the role of diabetes mellitus and nephrolithiasis. The second point is to stress the possibility of childhood antibiotic abuse associated with kidney stone disease which is possibly also associated with obesity; further research is required to endeavour to prove or refute this postulate.

3.2.9 Kidney stones and cardiovascular disease

A distinction is made between cases (a) with hyperuricaemia (with or without gout) and (b) without hyperuricaemia.

(a) The causal relationship between vascular damage (especially coronary and cerebral arterial vasculature) and hyperuricaemia has been extensively studied and proven. [129] This relationship either with or without gout produces even more vascular damage in the presence of hypertension, diabetes and dyslipidaemia. The mechanism for vascular damage in patients with hyperuricaemia is due to the inflammation mediated, uric acid driven blood vessel changes in the coronary circulation, the central nervous system circulation and possibly in the kidney, where inflammatory molecules and free radicals may accelerate interstitial fibrosis and therefore may be related to progression of CKD. [129] Although the cause and effect of hyperuricaemia and hyperuricosuria appears to be controversial, important previous work on this point seems to have been forgotten. As far back as 1985 Nath et al [130] and later in 1991 Nath with the same co-workers [131] reported the defect in renal ammonia production in patients with any form of chronic renal failure. The defect was failure of trapping of ammonia in the renal medulla despite an enhanced ammonia genesis. This increased the concentration of ammonia in the renal cortex and this compensatory enhancement of ammonia production in the cortical tissue contributed significantly to progressive renal injury in patients with existing renal insufficiency. A low urinary excretion of ammonia underlies the inappropriately low pH and uric acid nephrolithiasis associated with gout. This same mechanism of stone production has been found in autosomal dominant polycystic kidney disease (ADPKD). [132] It seems logical therefore to recommend that hyperuricaemia and hyperuricosuria which is not associated with gout, should be further studied. Nevertheless, in patients with nephrolithiasis and hyperuricaemia, dietary control, cessation of smoking, and excellent control of all

other associated diseases are mandatory. In addition, management of hyperuricaemia with allopurinol administration is essential (see sections 3.2.6(b), p. 24 and 5.2.6, p. 55). [133]

(b) Kidney stones without hyperuricaemia are also a well-known risk factor for coronary heart disease and strokes. [134, 135] This still applies even after correcting for confounding issues such as obesity, metabolic syndrome, diabetes, hypertension and dyslipidaemia. The presence of CKD stage 3a or less has been shown to be an important additional factor in these patients. [134, 135]

3.3 ANATOMICAL FACTORS

3.3.1 The role of Randall's plaques and papillary calcification in the pathogenesis of nephrolithiasis

There are approximately 9 to 17 papillae in each kidney. Flat and conjoined papillae are the commonest structures, mostly found in the lower pole but also one or two in the upper polar region of the kidneys. Between these are several conical shaped simple papillae. Stones formed on flat surfaces tend to grow larger and are more likely to remain in situ whereas stones forming on the conical structures (see figure 3.5) are smaller and tend to detach causing acute episodes of colic, haematuria or urinary obstruction. This basic knowledge helps to understand the pivotal role Randall's plaques (RP) play in SF.

In a seminal publication by Alexander Randall in 1937 during the Annual Oration of the Philadelphia College of Surgery first delivered his plaque theory in a work entitled "The origin and growth of renal calculi" [136] which was further elaborated upon in 1940. [137] Briefly this hypothesis pointed out that about 14-20% of normal persons had one or occasionally two RP in either one or both kidneys. However, persons with nephrolithiasis have ≥5 in both kidneys. This data was obtained mainly from autopsy studies. [136]

The plaque concept was lost for nearly 60 years. It is beyond the scope of this thesis to elaborate upon the myriad of all the possible stone pathogenic mechanisms related

to RP which have been published. However, a simplified sketch of the anatomical changes is shown in figure 3.5.

The most informative articles explaining the formation of RP were published after the year 2000. [138-143] In addition, information on the formation of stones on RP in children is highlighted in a recent publication by Bouchireb et al. [144]

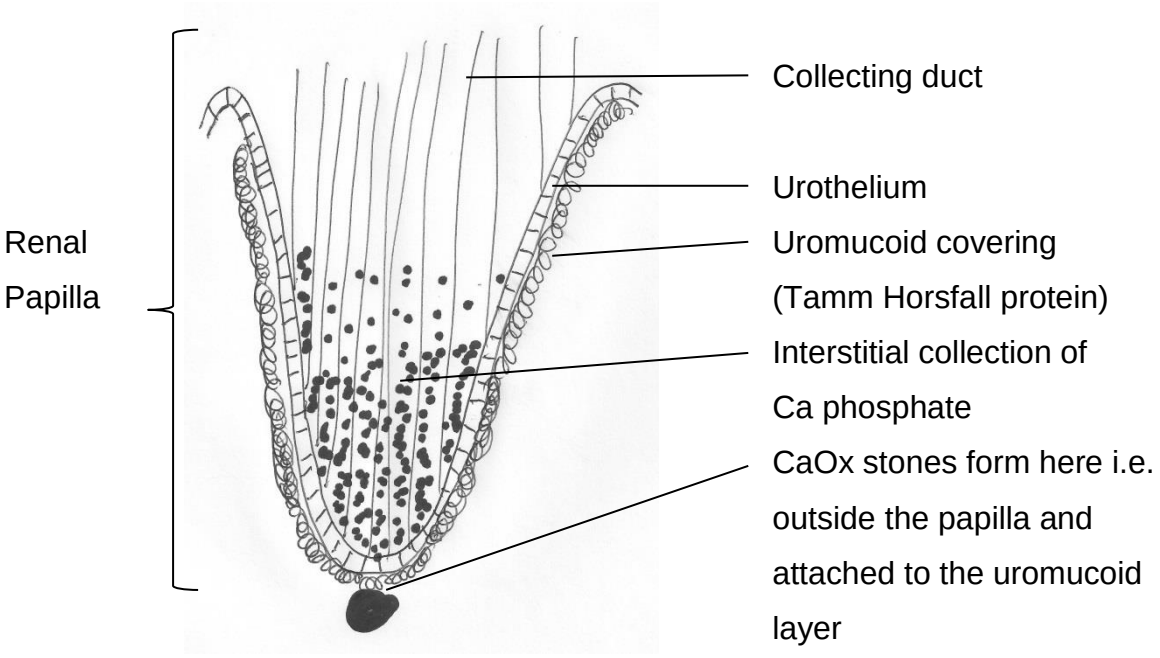


Figure 3.5: Schematic drawing of a Randall's plaque

A new concept in the formation of CaOx /P calculi formed in the ducts of Bellini and associated with RP were presented by Khan et al (2016) [143] as shown in figure 3.6.

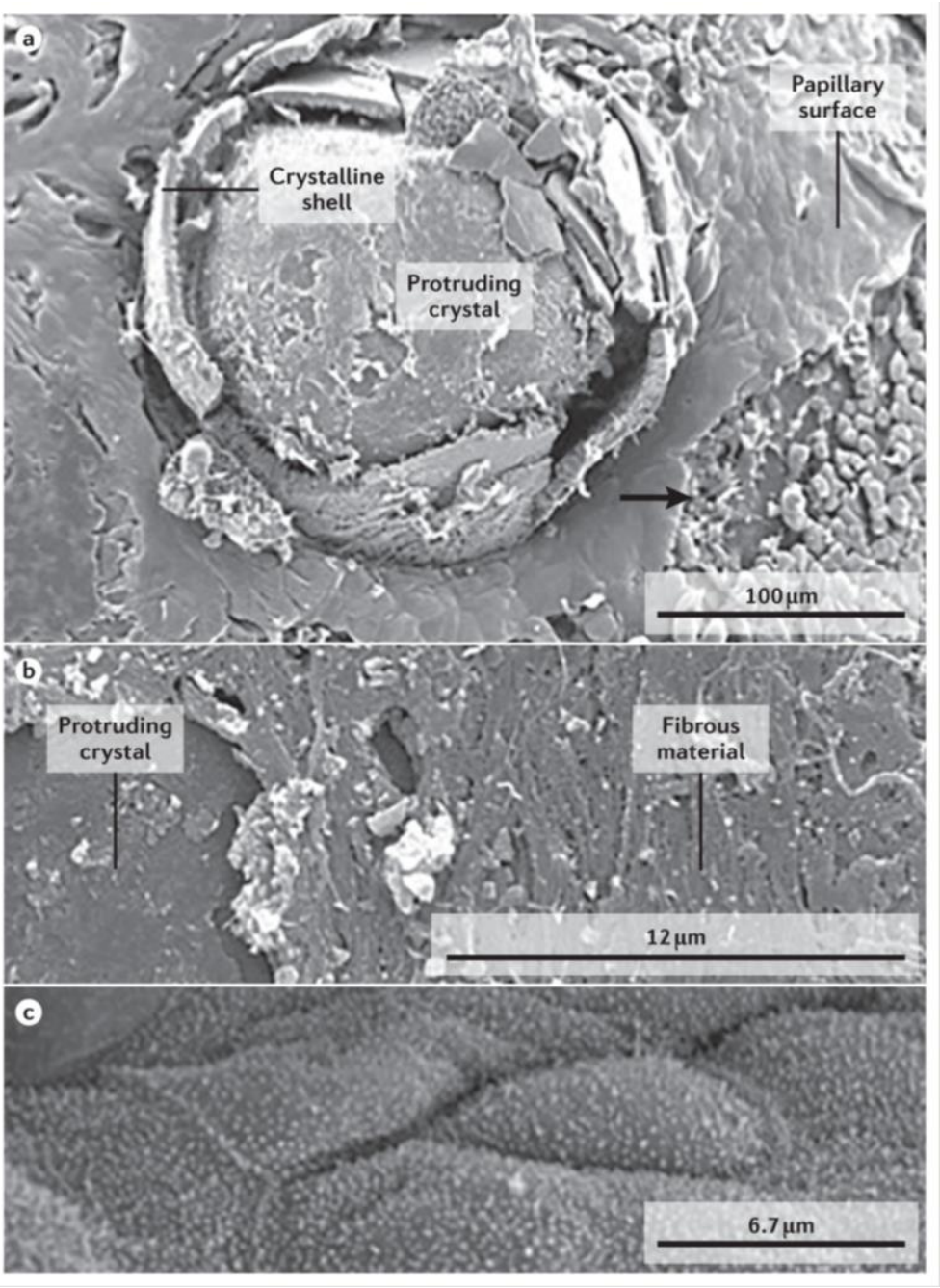


Figure 3.6: The renal papillary surface of a calcium oxalate monohydrate stone former examined using scanning electron microscopy.

a) A crystalline entity is protruding through the papillary surface, causing epithelial cells to be pushed apart (arrow). The protrusion is covered with fibrous material and surrounded by a layer of crystalline shell. b) Fibrous material covering the protrusion. c) An intact portion of the papillary surface epithelium.

Reproduced with permission (see p. 234) from Khan SR et al. 2016 [143]

The finding that many patients with SF have been diagnosed as having nephrocalcinosis after non-contrast computed tomography scan (CTS) is a cause for concern as many radiologists are not reporting some of these cases as RP. Millar et al (2010) [145] have eloquently distinguished between nephrocalcinosis and RP in their paper entitled "Nephrocalcinosis redefined in the era of endo-urology".

Chi and Millar (2012) [146] have recently pointed out a cause for concern about the confusion between stones and RP. They stressed to all endo-urologists that even in SF, RP do not necessarily lead to SF and certainly do not represent a large renal calculus. They concluded that we need to keep our patients out of harm's way and know when the calcifications seen on CTS are actually stones or calcified papillae.

It is necessary to identify RP in SF including CaOxSF; CaPSF and Cystinurics. In CaP stones (brushite), the calcification is dense and there is more interstitial fibrosis and scarring than in CaOxSF where interstitial fibrosis is minimal. Therefore, in CaPSF, a reduction in renal function is frequently found and can rarely even result in ESKF. [147, 148]

Finally, because of the close association of multiple bilateral RP with CaOx /P SF, the study of these plaques in the SA black population is highly relevant. If these plaques are not found in the normal as well as the stone forming black population, this would represent one of the explanations as to why Ca stones are still rare in blacks.

3.3.2 Other anatomical factors in the pathogenesis of nephrolithiasis

There are several additional anatomical factors to be aware of:

- Structural abnormalities of the kidney produced by reflux nephropathy or any cause of obstructive uropathy. Here infection would also play an important pathogenic role.
- Gross abnormalities of renal structures occur in ADPKD and this is one of the postulated pathogenic mechanisms of nephrolithiasis in this condition. (see section 3.4.4, p. 37) It occurs in 20% of patients. [149, 150] Very few studies in these patients have been reported. It is mandatory to plan a research protocol

which should not be difficult considering that ADPKD occurs in about 1 in 800 live birth white cases and in 1 in 1,000 in the black population.

- Medullary sponge kidney (MSK): tubular ectasia of the ducts of Bellini causing urinary stagnation and flow problems are the main pathogenic mechanism of nephrolithiasis in this condition but urinary infection and RTA may also occasionally occur. [151]

3.4 RECENT DEVELOPMENTS IN THE PATHOGENESIS OF NEPHROLITHIASIS

3.4.1 Inherited intestinal oxalobacter insufficiency

This is a disease that occurs particularly in children and the earliest research commenced in 2005 by Mittal et al. [82] The condition can be successfully treated by oxalobacter capsules [152] or with probiotics. [153] These substances are registered internationally but is not available in SA in the formulation required, due to lack of oxalobacter detection protocols. This aspect needs further investigation.

3.4.2 Oral antibiotic exposure and nephrolithiasis

As previously mentioned, Tasian et al (2018) [25] studied the association between 12 different oral antibiotics and the presence of nephrolithiasis in 2018. They compared the “misuse” of antibiotics in 25,981 stone patients to 259,797 healthy controls matched by age, sex and practice data. The logistic regression models used were all adjusted for various relevant health encounters, co-morbidities, urine infections and various over-the-counter medications. There was a marked increase in the incidence of stone disease in patients who had received the following antibiotics: sulphas, cephalosporins, fluoroquinolones, the broad-spectrum penicillins and the nitrofurantoin/methenamine combinations. The adjusted odds ratios varied from 1.27 to 2.33. Many of the patients were infants and young children aged 0-5 years but older children and adults also featured. The greatest odds ratios seen were in infants and children up to 5 years, slightly less at 10-year olds and lower rates for adults. The

antibiotics in the total cohort (but especially in the children) were repeatedly administered and often for no definitive indication.

The mechanisms proposed which are especially active in early life, relate to disruption of gut microbiota and to their metabolic activities although the exact pathogenic mechanisms are as yet unknown.

When reviewing the Tasian paper, one area of considerable concern arose. If, as postulated, early life antibiotic abuse resulted in a high level of childhood nephrolithiasis, why was there no mention of the occurrence of early obesity as well, which would have been expected in a proportion of their subjects? Antibiotic exposure together with the increased risk for nephrolithiasis together with childhood obesity has been well documented by other authors. [154, 155] Because of this, the Tasian group was approached and they expressed a keen interest in future research involving both their patients and children in South Africa with the view of resolving this question (see recommendations for future research in para. 8.1.2, point B.i, p.151).

In 2014 Cunningham et al [156] reported upon the prevalence of childhood obesity in the USA as being about 23%. A plethora of papers has shown the association with probiotics, animal husbandry affecting formula infant feeds, and especially antibiotic abuse (both pre-natal and in the first six months of life). [157-160] This was associated with childhood obesity such that if the child was still obese at the age of 5 years, they stood a 5-fold risk of being obese when 14 years old, which would then project into young adulthood. [161-164] By extrapolation, this would be expected to result in a morbid increase in cardiovascular disease, metabolic syndrome, type II diabetes, hypertension with a potential for multiple adverse events on affected end-organs. However, Leong et al in 2017 [165], although acknowledging the adverse effects on the gut microbiome and early onset obesity in animal models, concluded that evidence for this link in humans is still inconclusive and further well-designed clinical studies are required to clarify this association (see para. 8.1.2, point B.i, p.151).

3.4.3 The kidney as a microbiome

3.4.3.1 The renal crystal microbiome in autosomal dominant polycystic kidney disease

In new and pivotal work Torres et al (2019) [166] suggested that, not only the genesis of formation and growth, but also the progression of CKD was related to the deposition of both CaOx and CaP in the tubules of PKD type I and II patients. Several observations suggested the link between APKD progression and renal cyst burden. Firstly, nephrolithiasis is seen in up to 30% of patients with APKD. [149, 167, 168] Patients with nephrolithiasis have a much more severe downhill course than those without. [167-169] Hyperoxaluria is present in 20% and hyperuricaemia and clinical gout in 25% of ADPKD. [169, 170] Hyperuricaemia by itself also correlates with a more rapid downhill course. [171] Torres and his group [166] were able, via a set of intricate and ingenious experiments, to prove the above hypotheses in male Sprague Dawley rats. The creation of a rat polycystic kidney has been widely accepted as an excellent model to study PKD in humans.

Increased water intake coupled with K-Cit administration has been advised as a major therapeutic tool to prevent crystal formation and slow CKD progression. In fact, this therapeutic benefit is also discussed in paragraph 5.2.1 (see p.51) in this thesis. It would seem advisable to also decrease the dietary P load from early stages of CKD in order to prevent CaP crystallization. However, no research on this aspect has yet been done and would be an important topic to study in future (see para. 8.1.2, points A.iii and A.iv, p.150).

3.4.3.2 The kidney as a likely microbiome in integrated “geobiology”

A new technique for investigating the formation and growth of CaOx calculi in situ is referred to as “Integrated Geobiology” i.e. a combination of integrated geology and biology as demonstrated in the study by Sivaguru et al (2018). [167] The composition and potential effects of biomolecules which could potentially be operative in stone dissolution are currently unknown and are being intensely studied. According to the Sivaguru article a variety of factors are involved in SF including protein degradation

products, cellular responses and other factors. Sivaguru went on to state that SF are likely to represent a microbiome.

The technique used in the study assessed the architecture of stone fragments after Extracorporeal Shock Wave Lithotripsy (ESWL) therapy or lithoscopy. The crystalline structure of each stone fragment was examined and polished. Thereafter Arai scan super resolution microscopy was employed. Resolution via this technique employs optical definitions beyond the diffraction limit of light i.e. ±140nm of resolution. Thereafter mineralogical and chemical determinations of each fragment were assessed using energy-dispersive X-ray spectrometry followed by visualisation under the scanning electron microscope as seen in figure 3.7. This relates to the geology aspect of the Sivaguru study. [167]

Using these techniques, the authors were able to demonstrate that after a small stone was formed, part of it dissolved which upon reformation was then slightly larger. This process was repetitive and is in stark opposition to the usually accepted theory that Ca agglomerates once formed cannot dissolve by themselves in the human kidney. This demonstrated an important development in the biological effects of SF and should be interpreted taking into consideration aspects such as the oxalobacter formigenes (see paragraph 3.4.1, p. 33) and gut microbiota as mentioned in paragraph 3.4.2, p. 33 of this thesis.

They conclude that by further experimentation, new therapeutic methods to prevent SF and growth at its earlier stage should be possible. Also, this technique may well be of use and involve many other areas of pathological environments where bio-mineralisation takes place e.g. in CKD vascular calcification and/or atherosclerosis itself. [167]

Although this technique is not currently available in SA, this is a most important subject and not only kidney stone clinics but also nephrology practises should be aware of this research and keep abreast of possible future developments.

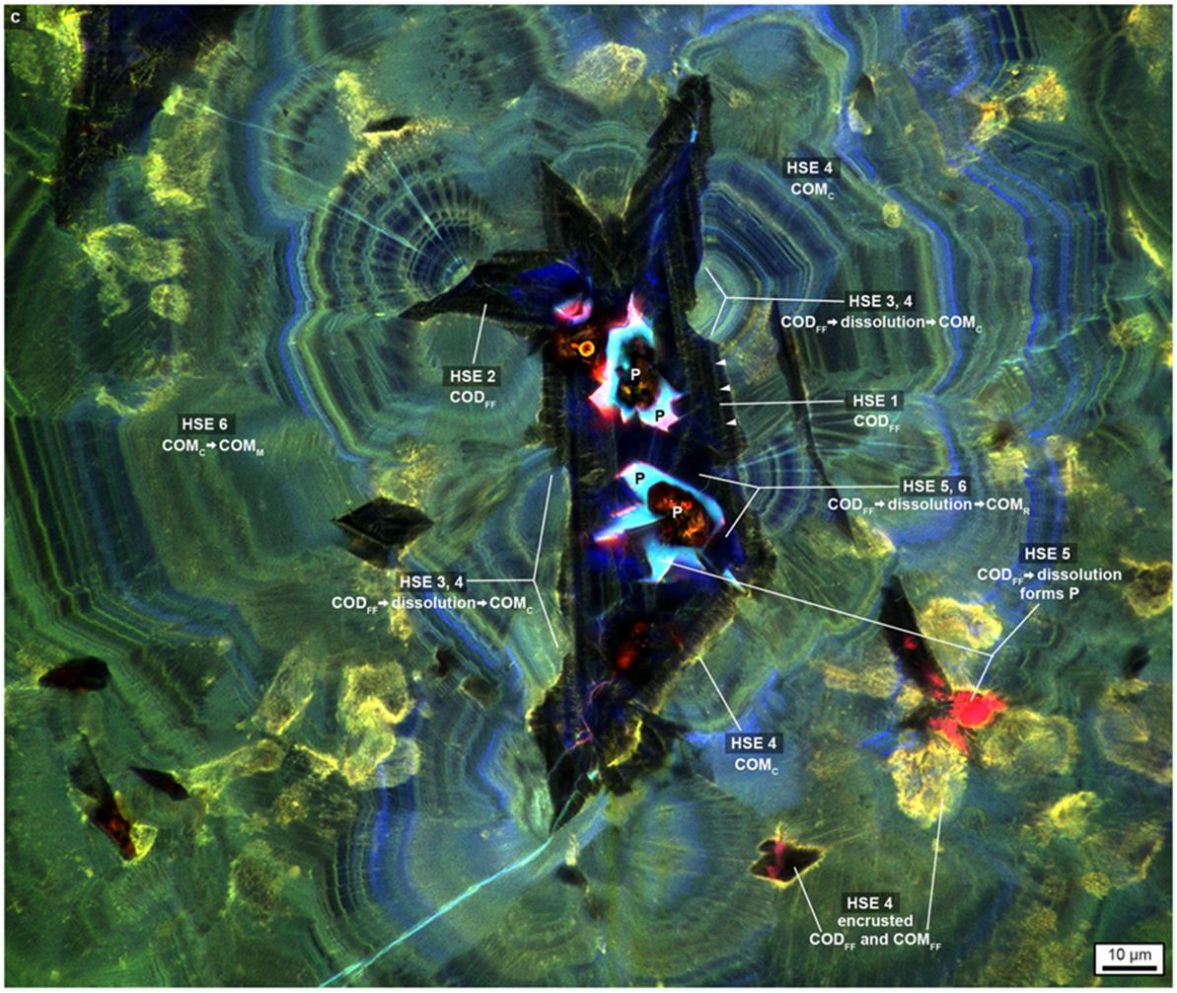


Figure 3.7: Lithoscope image of a kidney stone fragment using the sub-resolution assistant feature (SRAF)

Representative SRAF image composed of merged three pseudo-coloured red, green and blue (RGB) channels. Brightness and contrast of the RGB channel intensities are adjusted to highlight the dark crystalline fabrics.

Reproduced under Creative Commons open access terms from Sivaguru et al (2018) [167]

3.4.4 The role of the transient receptor potential - channel V5 in nephrolithiasis

Prior to the early 1990's, 99% of the filtered Ca was believed to be reabsorbed in the proximal renal tubule. More recently, the transient receptor channels have been described and particularly, the transient receptor potential - channel V5 (TRPV-5) and also channel V6 have been shown to be involved in the reabsorption of about 15% of

filtered Ca. [19, 173, 174] In 2018, Hughes et al [175] described the TRPV-5 channels involved in Ca reabsorption in detail. The site of reabsorption was shown to be the distal convoluted tubule (which is only 3-5 mm long in the human kidney) and the proximal portion of the collecting ducts.

The interaction of two endogenous processes involved with TRPV-5 were studied namely phosphatidylinositol 4,5 biphosphate ($PI(4,5)P_2$) and calmodulin. [175] These substances have been shown to respectively activate or inactivate the process. This results in either increased Ca loss or increased Ca reabsorption.

An interesting point mentioned by the authors is that in African Americans the rarity of RCaOxSF may be partially explained by the fact that a chronic elevation of TRPV-5 stimulation by calmodulin has been found in this population, which could explain lower urinary Ca concentrations.

These and other papers on the TRPV-5 mechanism have provided valuable information which has the potential to guide the way to new anti-stone medications.

In terms of nephrolithiasis the TRPV-5 channels have been involved in three pivotal interlinking areas:

a) Chronic oral fructose ingestion increases the intra cellular level of uric acid as well as Ca which alters the function of the TRVP5 channel resulting in hypercalciuria associated with hyperuricaemia and which often was associated with nephrolithiasis. [176]
b) The effects of excess fructose on the DCT and connecting ducts, causes increased intra-tubular and interstitial production of MCP-1 (monocyte chemoattractant protein-1) with all its inherent dangers. Examples are increased levels of reactive oxygen species, decreased production of vascular nitrous oxide, increase in IL-1, IL-2 and tumour necrosis factor, increase in serum and urine uric acid, increased levels of TGF-1 all producing renal and other organ damage. Again, the predisposing factor is “excessive” fructose uptake leading to obesity and nephrolithiasis. Obesity in its own right without fructose is also complicated by nephrolithiasis and both of these pathogenic entities produce insulin resistance, metabolic syndrome, diabetes II, CKD and nephrolithiasis.

c) A recent development in the function of the TRPV-5 channel related Ca metabolism was the important roles it performed on various genetic polymorphisms in patients with nephrolithiasis. [19; 174-176]
d) Finally, a review of the most recent and relevant data on the TRPV-5 receptor functions in relationship to the pathogenesis of nephrolithiasis was published in the December 2019 edition of Kidney International. [20] However, the role of altered TRPV-5 channels in fructose induced nephrolithiasis vide sigma (3.4.5a), as included in this literature review, was not mentioned in the Kidney International article and, because of poverty of other studies, clearly requires further future research (see para. 8.1.2, point A.v, p. 150).

3.4.5 The role of dietary fructose in stone formation

Modern diets have caused us to *"morph insidiously into a fructose society"*. [176] Fructose metabolism and its effect in the production of the metabolic syndrome, obesity and cardio-vascular toxicity have been well studied recently. [177-179] Although none of these studies referred directly to the occurrence of CaOx renal calculi, other studies have clearly shown that fructose ingestion independently increases the risk of nephrolithiasis. [20, 176-180]

The pathophysiological factors in the production of fructose associated nephrolithiasis are discussed below.

Firstly, an oral fructose load was shown to stimulate increased intracellular acid concentration as shown in figure 8. Another interesting and relevant postulate relates to the study by Cirillo et al. [177] They cultured human proximal tubular cells (HK-2 lineage) and studied the results of fructose on ketohexokinase-dependent production of inflammatory substances in proximal tubular cells. They noted the generation of uric acid within the cells which then induced the production of MCP-1. MCP-1 resulted in the production of several antioxidants causing tubular dysfunction. (See para. 3.4.4(b), p. 38) The concentration of fructose used in this experiment was identical to that which is seen in peripheral blood after a fructose meal. However, they did not further investigate how the increase in intracellular uric acid was disposed of. It would seem

entirely possible from consideration of normal physiology that increase in the intracellular urate level would result in the transfer of uric acid into the peritubular blood supply. The increased uric acid levels in the blood would be conducted to the blood supply of distal tubular and collecting ducts where uric acid re-secretion occurs resulting in hyperuricosuria and all of its attendant dangers. The problem of fructose induced obesity as well as nephrolithiasis relates mainly to studies from first world counties, particularly the USA. However, this hypothesis could easily be tested in vivo and make for a very interesting study in South Africa (see section 8.1.2, point A.vi.c, p. 150). The theory behind fructose induced uric acid leading to CaOxSF is explored in figure 3.8.

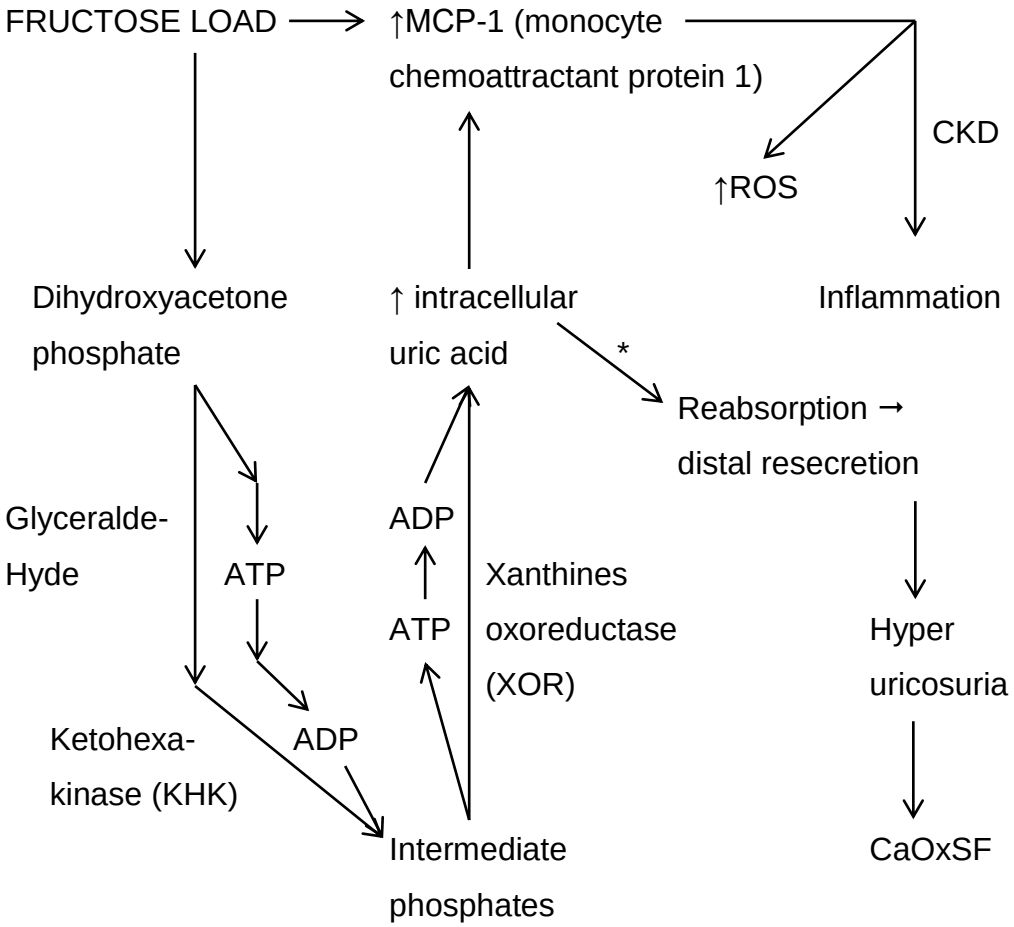


Figure 3.8: Effect of intracellular uric acid in cultured proximal tubular cells after a fructose load

ADP = adenosine diphosphate; ATP = adenosine triphosphate; ROS = reactive oxygen species
** Theoretical possibility. Not yet investigated.*
Note: Production of urate bypasses the usual Embden–Meyerhof pathway (EMP pathway).

Adapted under Creative Commons open access terms from Sharaf El Din et al (2016) [181]

As will be discussed later in the thesis, non-genetic (e.g. dietary fructose overload) or genetic aberrations of the TRPV-5/6 channels were shown to increase the levels of MCP-1. This results in the production of reactive oxygen species (ROS). [151] Therefore, in future, studies should be undertaken to investigate differences between black and white South Africans with and without stone disease by measuring urinary markers of inflammation and ROS. In terms of stone formers, attention should be paid to the eGFR as they may correlate with renal damage.

3.4.6 The role of macro-molecules in nephrolithiasis

A list of the five more important urinary protein macromolecules involved in RCaOx/PSF, their main actions and references are shown in table 3.2 followed by a brief discussion of each.

Table 3.2: Protein matrix modulators of kidney crystallization

Number	Name	Promotors (P) and Inhibitors (I)			References
		Nucleation	Growth	Aggregation	
1	Tamm-Horsfall protein (Uromucoid)	P	-	I/P	32, 33, 48, 49, 139-142
2	Glycosaminoglycans				
	Bicunin	I	I	I	94, 182, 183
	Hyaluronic acid	-	-	I	184-186
	Chondroitin	-	I	I	112
	Sulphate	-	I	-	111, 112
	Heparan Sulphate				
3	Nephrocalcin	I	I	I	161-163
4	Urine prothrombin fragment-1 (UPTF1)*	I	I	I	187-189
5	Osteopontin	I	I	I	31, 190-198

* Previous term for this substance was “crystal matrix protein”

Although thirty macro-molecules were studied and presented by Aggarwal et al (2013) [31] (of which some seem less relevant), only those referenced in this thesis are briefly presented below:

1) Tamm-Horsfall protein: As shown in table 3.2, this glycoprotein can be either an inhibitor or promotor depending mainly on the CaOx crystallisation circumstances in relation to the experiment used to test for this still controversial and complicated phenomenon e.g. the iso-electric focusing point that differs between normals and SF. Further studies are required to elucidate its contribution, if any, to the production of nephrolithiasis and especially to the differences between the urines of normal and stone forming black and white subjects.

2) Glycosaminoglycans: There are four families of GAGS: a) Hyaluionon/uronic acid, b) chondroitin sulphuric acid/dermatan sulphate, c) heparan sulphate, d) keratin sulphate. [199]

   Bikunin is a member of the kunin c family, which are serum protease inhibitors, and is available in sufficient quantities in human urine to allow testing for inhibitory or promotory properties.

   A study of a mixture of GAGS proteins was performed in SA blacks and whites yielding results that were difficult to explain. This work was published as a congress abstract in Kidney International (see Appendix C, p. 198). In summary this study showed three important facts. Firstly, the nature of the tests and expertise required to perform them was available in SA. Secondly, as the GAGS used comprised of several of the above four mixtures, further work is required to "tease–out" the relevant data which may ensue. Thirdly, the work could be of considerable importance in explaining why renal calculi are so much rarer in the SA black population.
3) Nephrocalcin is not discussed further in this thesis
4) Urinary F1 fragment of prothrombin (Previously called "crystal matrix protein") Much of the important work on this protein, possibly the most potent inhibitor of CaOx crystal growth, aggregation and adhesion of all the macro-molecular modulators was done by Ryall et al (1995, 1999, 2002). [200-202] Work performed by Meyers AM in Ryall's laboratory was presented as a congress abstract (as mentioned above) and is presented in Appendix C, p. 198. Because the substance used was a synthetic commercially available product named prothrombin-X-1 (which contains UPTF1) had albumin as part of the chemical structure, the work was not published in full until the albumin had been removed. The later results were published by Ryall in 1995. [200] Again of importance would be to investigate the stone regulating properties in the black and white SA population using prothrombin X but first removing the albumin as was done by Grover and Ryall. However, this is probably not practically possible in South Africa at the moment.
5) Osteopontin (OPN) which has a physiological role to play in modulating renal inflammation, leucocyte recruitment and cell survival is also important in the generation of nephrolithiasis. It has been called a "molecule for all seasons". [190]

OPN is important in bone mineralisation via its ability to anchor the osteoblasts to bone. [191] It is formed not only in kidney stones but it is also widely found in areas of dystrophic calcification. OPN is actually also synthesised in the kidney and is present in the urine. The effects on its inhibitory properties on CaOx/PSF are well described in several key publications. [31, 192-197] A key publication on the role of OPN as an inhibitor of CaOx crystallisation was published by Thurgood et al in 2012. [198] They found that crystal size decreased in experiments using cultured Mayden Derby canine kidney (MDCK2) cells and that the decrease was directly proportional to the OPN concentration. They suggested that under physiological conditions, OPN was routinely pivotal against SF by inhibiting the growth of CaOx dihydrate (COD) crystals which would result in their increased excretion in the urine and would explain why COD crystals are more prevalent in the urine but less so in the stones of RCaOxSF.

Because of the differences in SF rate between the various population groups of SA, it is important to consider future research along these lines if and when stone research facilities become available (see para. 8.1.2, point A.i&ii&iii, pp.149, 150).

3.4.7 Conclusion: organic, inorganic and other pathogenetic mechanisms responsible for CaOx and P stone formation

Even in 2020 it is still true to state that CaOx (and other) SF is far too complex for simple understanding. As mentioned, SF is a multistep process including nucleation, crystal growth and aggregation as well as crystal adhesion and retention. In spite of literally hundreds of research publications, many aspects of the mechanisms of renal SF remain unclear or unknown. Much more work is still required to elucidate the multiple intrinsic mechanisms of SF which, when known, will undoubtedly lead to the development of novel strategies and new drugs for preventing this disorder.

CHAPTER 4

METHODS RELATING TO ASSESSMENT OF NEPHROLITHIASIS

Several aspects of the methodology involved with the day-to-day management of nephrolithiasis are briefly presented here. These are practical solutions pertinent to the running of a dedicated and well-functioning stone institute. This has been added here to assist with the definition of formation of an ideal stone institute as proposed later in this thesis.

4.1 PATIENT SELECTION

4.1.1 Selection of patients for dedicated kidney stone clinic work-up

Patients with known or suspected CaOx or Ca phosphate (CaP) calculi should only be referred to a stone clinic for full work-up if the clinical setting indicates referral or if the referral is in keeping with various cost-effective criteria. By definition then, it is up to the referring urologist to (a) be cognisant of the condition, (b) to refer patients if and when they consider necessary and (c) if they do not refer patients, to work them up themselves as best they can, remembering the difficulties of urinary chemical sampling as measured by routine urological and laboratory services. Up to now definitions of when and who to refer have been debatable and more rule of thumb than based on scientific evidence. In 1994 Parks and Coe [203] stated that:

> *"…current practice recommends metabolic evaluation of patients who have formed multiple renal stones but not those with a single stone or temporarily remote stones"*.

They argue however that once there is even a single occurrence further stones may well form possibly by leaving nuclei of crystals as residue so that

> *"…the practice of waiting while patients declare themselves multiple SF may not always be the best"*

and thus, not in the patient's best interest.

In 2019, Vaughan et al [204] attempted to scientifically predict those patients who will be more likely to have a second or multiple calculi. Their risk criteria included younger age, male sex, higher BMI, family history of stones, first stone during pregnancy, asymptomatic stone on incidental radiological procedure before first stone episode, suspected stone episode before the actual episode, certain types of stones, pelvic or lower pole imaging, number of stones on imaging (an important point hitherto totally omitted from stone frequency associations) and diameter of largest stone. The risk of recurrence in 5 years has ranged from about 1% to 94%. In spite of many limitations to their study, they concluded that the method of assessment could result in improved clinical care and improve kidney stone prevention. At no time did they give clear advice as to who and when SF should be referred, so a "best guess" scenario still prevails.

4.1.2 Inclusion criteria

Inclusion criteria for the selection of patients:

- All patients who have had two symptomatic calculi over a two-year period; or more than two over a five-year period, especially those with some or many of the "Vaughan" criteria. [204]
- All genetically acquired calculi should be referred to the clinic e.g. cystinuria, Dent's disease and RTA. [205]
- Secondary causes of nephrolithiasis e.g. primary hyperparathyroidism, chronic granulomatous conditions e.g. sarcoidosis, spontaneous and drug induced RTA, inflammatory bowel disease (IBD) and post bariatric surgery.
- RCaOxSF patients with ADPKD. The inclusion of these patients has become even more relevant since the seminal work published by Torres et al (2019) (see para. 3.4.4, p. 37). [166]

4.1.3 Exclusion criteria

The following entities are not considered to be RSF:

- A single or very occasional attack. However, if on presentation, there was seemingly only a single calculus causing the clinical symptoms but on radiological investigation multiple asymptomatic calculi were found (especially if they were bilateral), these patients must be included for work-up. In practice approximately 50% of all CaSF will never have another recurrence.
- Pure or mainly urate calculi.
- Infection-associated calculi.

4.1.4 Special inclusion criteria

Patients are included for work-up in cases with the follow circumstances:

- First stone in a patient ≤19 years of age.
- Pilots, fire-workers and armed forces personnel.
- First stone in pregnancy – all stone work-up recommended postpartum.
- First stone in obese patients especially if diabetic with metabolic syndrome.
- Persons with a single kidney.

These are the criteria being used by the Johannesburg Hospital stone clinic.

4.2 CLINICAL ASSESSMENT

A detailed medical and surgical history is essential and must include clinical findings, risk factors, family and genetic history, history of frequently associated conditions as well as past/present medications. It is also very important to discuss various aspects of nephrolithiasis with patients; presentation of points for discussion is presented in Appendix D (see p. 199).

The diet composition is established with a questionnaire which is then assessed by the clinic dietician (see Appendix E, p. 202). However, it is the task of the doctor to obtain any history of any other dietary risk factors such as so-called “health foods”, complementary medicines, supplements (especially those with mega-doses of Vit C), tonics and body-building protein preparations. Also included is the history of ingestion of drugs such as Vit D3 and other high-risk medications such as drugs causing RTA e.g. acetazolamide (still widely used in the prevention of acute mountain sickness for

those wanting to attain high altitudes) and topiramate (commonly used to control complicated migraine). Many other drugs such as some antiretrovirals can cause excessive crystallization of urinary oxalate. [206]

Also critical to the standard stone history is to enquire about a history of frequently associated conditions such as essential hypertension (including the family history thereof), type II diabetes (which can also be an actual causal factor), dietary conditions e.g. vegan, vegetarian or specific dietary habits, smoking, insulin resistance, metabolic syndrome and gout i.e. all interlinking disorders associated with cardiovascular and renal comorbidity.

4.3 STONE WORK-UP PROTOCOLS

Protocols applicable to nephrolithiasis work-up are included as appendices except for the radiological tests and special urine tests which are not presented in detail as these aspects are beyond the scope of this thesis:

4.3.1 Dietary work-up (as mentioned above)

See Appendix E, p. 202.

4.3.2 Routine blood tests

See Appendix F, p. 204.

4.3.3 Assessment of renal tubular acidosis

See Appendix G, p.205.

4.3.4 Routine 24-hour urine test

See Appendix H, p. 206.

4.3.5 Suggested standard print format for work-up data (for SA laboratory staff)

See Appendix I, p. 209.

4.3.6 Stone analysis / semi-quantitative stone analysis

See Appendix A, p. 192.

4.3.7 Radiological tests

Radiology, especially CTS without contrast is preferred over ultra-sound which often do not show small calculi.

4.3.8 Special urine tests

Specific tests such as crystal saturation measurements, X-ray diffraction crystallography, cryo-electron microscopy and crystal structure matching data and image bases (using saved data and images from confocal microscopic uroscopy) are vitally important, special urine tests used in the clinical investigation of kidney stone formation. [207] However, these processes use expensive apparatus and require highly trained laboratory scientists. Therefore, this service cannot be undertaken by many stone investigators and are only performed for research purposes in highly sophisticated laboratories.

CHAPTER 5

MANAGEMENT OF NEPHROLITHIASIS

Management of nephrolithiasis is discussed in accordance with general and clinical presentations, complications and associations. Detailed management is a highly complex subject and is covered briefly in this thesis. Surgical management and follow-up is also a large and complex issue which is beyond the scope of this thesis, so is only briefly dealt with.

Excellent clinical guidelines for general stone management protocols are available from the European Urology Association group (2016) and the American College of Physicians Clinical Guideline (2013) with updates (2014). [138, 208, 209]

5.1 LIFESTYLE FACTORS CONTRIBUTING TO STONE FORMATION

5.1.1 Fluid assessment

- Fluid intake should be aimed at 2.2-2.5L per day. Patients should be advised to drink more fluids, especially lemon juice, water, coffee, herbal and rooibos tea (but NO Ceylon (black) tea). Avoid various sparkling waters other than soda water as they often contain minerals.
- Although adequate water intake is a standard requirement for anybody with kidney stone disease, the ingestion of large volume of water as a method to prevent SF in idiopathic Ca nephrolithiasis seems obvious. Its huge potential value in stone prevention has only been shown in one 5 year randomized prospective study which was published in 1996. [210] However, there remains one serious drawback to this simple but highly relevant therapy and that is the frequency of non-adherence to adequate fluid ingestion.

5.1.2 Dietary assessment

- Dietary advice and follow-up are discussed in Appendix E (see p. 202) and will not be repeated here except to stress the pivotal role diet plays in prevention of repeated stone attacks. Needless to say, this aim is not easily achievable.

5.1.3 Weight loss when relevant and exercise

Conservative non-pharmaceutical management is essential and focuses almost exclusively on diet, increased fluid intake and exercise. Dietary management is normally associated with ingestion of normal amounts of Ca, salt, reduction of protein (especially red meat) and reduction of dietary oxalate. In the presence of hyperuricaemia and/or hyperuricosuria, allopurinol should be considered. [211]

The evaluation, adjustment and continuous follow-up of lifestyle related factors are essential for all patients. It would seem that the more we deviate from a traditional balanced diet with adequate intake of protein, Ca, salt, fruit, vegetables and supplement it with rich fast foods and artificial drinks, the more we increase our risk for nephrolithiasis – and the risk for a lot of other life-style diseases. If only modern populations could eat a little of everything and a lot of nothing, many diseases could be prevented.

5.2 MEDICATION

5.2.1 Hypocitraturia

The excellent effects of K-Cit on recurrent stone prevention has been well studied and has been fully presented earlier on in the thesis (see para. 3.4.4, p. 37). Figures of greater than 90% decrease in stone recurrent rates has often been found. [212, 213] Of note is that citrate does not lose its stone prevention properties but patients must be fully informed that the preparation has to be taken three times per day.

Preparations such as K Na Hydrogen Citrate (Urolyt-U), citrate plus sodium bicarbonate liquids (Adco Sodasol) and citro-soda should be avoided as they may produce unwanted mineral induced side-effects in some kidney stone patients. To

explain this further, one has to be aware that brushite stones (i.e. CaP stones) are formed in alkaline urines. This has been described before in patients with idiopathic brushite stones or in any case of renal tubular acidosis. The use of preparations such as Urolyt-U which, apart from being very expensive, has sodium in the preparation which increases the urinary Ca and therefore increases the propensity of converting CaOx into CaP stones with their attendant dangers. [214] However the use of Urolyt-U (uroCit-K) in the treatment of uric acid stones is highly effective. [215]

A relatively recent oral product consisting of K-Mg-Citrate is available in many countries and was reported by Ettinger, Pak et al (1997) [216] to be superior in preventing new calculi when compared to standard K-Cit solutions i.e. only 12,9% of subjects on K-Mg Citrate vs 63.6% on standard citrate solutions developed recurrent stones. This is in sharp contradiction to other studies including the citrate paper discussed in this thesis where standard K-Cit solution prevented new SF in more than 90% of patients. [216] Other studies suggesting benefits in various groups of patients also recommended K-Mg Citrate but their protocols were very different from older papers especially in that, instead of using K-Cit solution in their comparative trial they used K chloride solution which, because of its acid profile, would be expected to increase the rate of RCaOx formation. Due to the above criticisms, plus the fact that K-Mg Citrate is not available in SA and that it would cost considerably more that the K-Cit solution provide plausible explanations for deciding against the future use of this preparation – at least for the time being.

In addition, the vague recommendation that all recurrent CaOxSF should be treated with citrate (a common clinical practice) should be avoided because of the possibility of producing brushite stones with all their known problems. [217]

Finally, it has been proposed by Hofbauer et al (1994) [218] that in patients with recurrent CaOxSF plus hyperuricosuria, citrate therapy can be used in prophylactic management instead of allopurinol. This was shown to be beneficial in their prospective randomized study. [218]

5.2.2 Hypomagnesaemia / Hypomagnesuria

As mentioned in the pathogenesis, Mg is a potent inhibitor of SF but deficiency states are very rare in adults but nevertheless must be treated when present. In paediatric SF, Mg deficiency is an important consideration. Therefore, urinary Mg excretion should be part of the routine work-up for both adults and children.

There are many Mg medications available in SA but care must be exercised when choosing the correct formula, for example the Slow Mag tablets consist of Mg Chloride and are not indicated for stone forming patients whereas the capsules contain an amino acid kelate consisting of Mg carbonate, Mg oxide, Mg citrate and Mg glycerate and may be prescribed. However, the use of either Mg oxide or Mg hydroxide is preferable because of cost implications. [219]

5.2.3 Hypernatrituria

As a rough guestimate, about 50% of the stone population with hypercalciuria have significant hypernatrituria. With adequate salt restriction to 4.5grams per day, urinary sodium levels fall to approximately 70-90mmol/day and the hypercalciuria normalizes without further treatment.

5.2.4 Hypercalciuria

Hydrochlorothiazide (HCTZ) requires a dose of 50mg daily for efficient UCa lowering, which produces severe long-term complications e.g. production of diabetes, high cholesterol, high uric acid and low K and should thus be avoided.

Indapamide (IND) is a thiazide-like compound which does not produce a significant diuresis and in a dose of 2.5mg daily, is 4 times more potent and the only side-effect is that of K loss which is off-set by concomitant potassium supplementation (mist pot cit) if used with it. [187] Thus IND 2.5mg per day is the treatment of choice for all patients with idiopathic hypercalciuria. Even in patient who fail to “normalise” their salt intake and remain hypercalciuric, IND is advisable in these circumstances.

However, it is worth noting that until the sodium levels are normalized, the term “idiopathic” hypercalciuria cannot be made with certainty.

The use of IND in these patients has been further confirmed by Borghi et al. [188] Indapamide 2.5mg per day is fractionally more cost effective than HCTZ at 50mg per day. [189] The addition of spironolactone to IND should be investigated in the management of RCaOxSF. Not only may K sparing agents act synergistically with IND (as well as hydrochlorothiazides) to lower urinary Ca, but the K sparing effect of this agent may well result in the production of an increased urinary citrate level. However, renal function should be adequate and future research on this interesting aspect is required (see para. 8.1.2, point A.viii, p. 151).

Note: The postulated existence of “relative” hypercalciuria as described in the pathogenesis section (see para. 3.2.1, p. 15), might require IND if hypercalciuria occurs after the Ca deficient diet has been corrected. However, this postulate remains to be proven and is included in the list of research recommendations for the future (see para. 8.1.2, point A.viii, p.151).

5.2.5 Hyperoxaluria

Management of hyperoxaluria would require restriction of foods high in oxalate as discussed in the pathogenesis section. In summary these restrictions would include:

- Removal of foodstuff high in oxalate;
- Normalisation of Ca intake in line with recommended daily intake guidelines; and
- Removal of agents that precipitate oxaluria (e.g. Vit C).

In certain patients, Pyridoxine in a dose of 25mg BD may help to lower oxalate excretion by inhibiting GIT absorption, but this is much less effective than ensuring normal dietary Ca intake. [42]

It is important to decrease colonic oxalate absorption especially in patients with IBD or bowel reduction operations for weight loss (i.e. bariatric surgery). Oxalobacter capsules have been widely used in these patients but are not available in South Africa. Enteric hyperoxaluria (e.g. Chron's disease, ulcerative colitis and post baro surgery) are the commonest form of acquired hyperoxaluria. In addition to low oxalate diet, a low-fat diet with the ingestion of medium chain triglycerides and cholestyramine should be used for long term treatment. [220]

5.2.6 Hyperuricosuria

Justification for the use of Allopurinol in patients with recurrent CaOxSF and "hyperuricosuria" has been dealt with in detail under pathogenesis and prevention of recurrent SF and has been shown to be highly effective. [101-108] However, in the occasional patient with normouricosuria the addition of allopurinol has also proven to be beneficial. [221] The formation of uric acid calculi in these patients is related to highly acid urines and possibly the presence of >10% of the kidney stone consisting of uric acid. The use of Allopurinol should be avoided in patients with sulphonamide allergy and should be used with care in anyone with a history of allergies. A starting dose of 300mg is recommended and the patient warned that if a rash or any other allergic reaction develops, the preparation should be withdrawn immediately and the patient should contact their doctor. It should also be recommended that Allopurinol allergy can occur even after protracted usage.

5.3 MANAGEMENT OF RARER CAUSES OF NEPHROLITHIASIS IN ADULTS

5.3.1 Cystinuria

Three types of cystinuria are identified but it is beyond the scope of this thesis to discuss it in detail. Treatment of this disorder will however be available at the stone clinic for both paediatric and adult patients.

5.3.2 Familial Hyperoxaluria

Similar to cystinuria mentioned above, the four types of hereditary hyperoxaluria will all be requiring treatment with a high fluid intake plus urinary alkalination (K-Cit) at a stone clinic, but is not further discussed in this thesis. However, there are very recent treatment options which will be assessed in future and added to protocols if applicable.

5.4 SURGICAL MANAGEMENT

Surgical management (e.g. ESWL or LL) is not required if patients are asymptomatic - even in the presence of multiple bilateral calculi. Patients should therefore only seek surgical advice when in doubt.

Other than laser lithotripsy and ESWL, endoscopic procedures are now one of the commonest techniques for symptomatic stone removal. [222] Percutaneous nephrolithotomy is restricted to patients with staghorn calculi or in extremely difficult situations.

5.5 PATIENT EDUCATION, FOLLOW-UP AND REFERRALS

Education of patients includes a full explanation of their stone condition and the consequences of non-adherence to therapy. It must be stressed that therapy, including life-style changes, are lifelong (see Appendix D, p.199).

Follow-ups are required 6-monthly for the first 1-2 years and then annually for life. Failure to do this has resulted in high percentage of non-adherence to therapy and recurrent stone disease. Ideally an individual concerned with ongoing patient care and follow-up should be employed to track patients and arrange appropriate appointments (see also Chapter 7 on a Stone Clinic, p. 138).

Non-contrast CT scans should be included in follow-ups every 3-5 years (2-3 films only).

Relevant referrals (especially for urological procedures) are important in the management of stone conditions.

5.6 MANAGEMENT OF ASSOCIATED CONDITIONS

5.6.1 Obesity, metabolic syndrome and type II diabetes

These are often encountered in CaOxSF. (See para. 3.2.8, p. 26) Frequency and implications of the conditions should form an important topic for future research, especially to assess whether they are implicated in CaOxSF in black patients (see para. 8.1.2, points A(i), p. 149; and B(vii), p. 151).

5.6.2 Hypertension

It is highly unlikely that essential hypertension plays either a pathogenic role or genetic association in CaOxSF. That the association seems to be commoner in recurrent SF has often been commented upon. It needs to be studied further in SA whether hypertension is more common in both black and white SF and if so, are there any causative factors of stone production or are patients with stone risk factors (e.g. obesity, diabetes, etc,) more likely to have kidney stones if also hypertensive? (See para. 8.1.2, points A(i), p. 149; and B(vii), p. 151.)

5.6.3 Dyslipidaemia

Aberrations in low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HPL-C) and ratios occasionally accompanied by hypertriglyceridaemia are commonly found in RCaOxSF and mostly are secondary to many of the associated diseases already discussed. Under these conditions and especially in the presence of hyperuricosuria, both dietary and statin control of the lipid levels are indicated. The target LDL is around 2.5mmol/L or less; however, in the presence of CKD the target is 1.8mmol/L or less, at which level interstitial kidney fibrosis can be avoided, thus preventing further renal function deterioration. [223]

5.7 MANAGEMENT OF OTHER IMPORTANT COMPLICATIONS

5.7.1 Osteoporosis

The frequent occurrence of osteoporosis in recurrent CaOxSF has been well reported. [224-226] The association is not surprising, given the fact that many young people (especially office workers) have significant lower dietary Ca intake and considerably lower sunlight exposure resulting in Vit D3 (Calciferol) deficiency; some are frankly osteopaenic in the absence of other factors e.g. alcoholism, smoking, age and gender. In addition, a common finding of a high salt intake in SF plus the hypercalciuric effect of high salt diets results over time in a negative Ca balance and osteoporosis.

Furthermore, it should be noted that there is even an increased risk of fracturing in patients with nephrolithiasis [227], so that adequate bone work-up studies should be carried out when indicated.

5.7.2 Pregnancy

The following is a brief summary of 8 essential guideline points:

1. As far as is possible, pregnancy should be planned in patients with RCaOxSF.
2. They should, if required by their attending urologist, be stone free because of the seriousness of possible stone passage during pregnancy.
3. If patients are on either maintenance therapy on thiazides or IND, these should be avoided until the post-partum period.
4. If on allopurinol, this agent should be terminated as some animal studies have indicated foetal toxicity. [228]
5. Urinary tract infection is common in pregnancy in patients with recurrent nephrolithiasis and must be carefully monitored and managed.
6. The care team should include, in addition to an informed obstetrician, both the attending urologist and kidney stone nephrologist if possible. Monthly visits are guided by the clinical situation but must be more frequent in stone patients, especially if they have the metabolic syndrome, or are diabetic or have secondary causes of RCaOxSF.

7. Of paramount importance is the necessity to always remain well hydrated aiming at 2,5 litres (at least) of water per day.
8. Although not studied, it would seem desirable that pregnant woman should either commence with or remain on a three times per day appropriate dose of K-Cit with careful monitoring of the urinary pH.

5.7.3 CKD and nephrolithiasis: Classification and brief review

There are 5 groups of conditions in which stone diseases are complicated by varying degrees of CKD. The first 4 are secondary conditions well-known to often occur with kidney damage and therefore are only listed for the sake of classification clarity. The fifth condition complicated by CKD concerns the usual day-to-day stone forming population comprising of mainly idiopathic CaOxSF. This group which forms, by far, the largest section of patients with nephrolithiasis, is the main subject of this thesis and therefore warrants an in-depth review.

In these groups of conditions however, CKD is associated with various general or secondary causes of nephrolithiasis. These groupings are as follows:

1. Examples of such conditions include hereditary genetic conditions such as primary hypertension, cystinuria, Dent's disease, other related tubulopathies and distal renal tubular acidosis. Secondary causes include the hypercalcaemias and/or hypercalciurias such as primary hyperparathyroidism, sarcoidosis, Cushing's disease or steroid administration, other medications causing obstruction or metabolic changes e.g. RTA.
2. CKD associated with various GIT conditions such as Crohn's disease, [229, 230] ulcerative colitis and other chronic diarrhoeic states and post bariatric surgery.
3. CKD associated with anatomic and functional urinary tract conditions including spinal cord injuries. Examples of these are infective struvite/staghorn calculi, calculi in MSK, reflux nephropathy and post obstructive renal scarring.
4. CKD associated with nephrolithiasis induced by obesity in the absence of other associated conditions such as metabolic syndrome, type II diabetes, hypertension and dyslipidaemia.

5. CKD is one of the commonest complications seen in usual day-to-day clinical care of patients with RCaOxSF. The most likely mechanisms of CKD in these patients (i.e. in the absence of secondary or associated conditions) include complications of urological ureteric stone procedures, percutaneous nephrolithotomy, urological placement of stents and even secondary to ESWL or laser procedures. [111]
6. It is worth noting that in some patients who are frequent RCaPSF, mild reductions in renal function associated with more intense interstitial fibrosis in the areas where RP plaques occur. However as discussed previously in this thesis, even in these patients, progression to ESKF is rare unless they have undergone frequent repeated urological procedures.

CHAPTER 6

PREVIOUSLY PUBLISHED WORK

It must be stressed that the candidate (Prof AM Meyers) were not the first author in many of the presented papers. The reasons for this and the percentage contribution towards the protocol construction, the practical work as well as the writing of these papers are present in a formal letter in Appendix J.2, p. 218.

The thesis concisely and constructively reviews a selection of the research work published between 1990 and 2002 and highlights shortcomings in study design, pathogenic mechanisms and therapeutic knowledge as is now evident at the current time.

Although the discussion of the selection of the published papers deal mainly with RCaOxSF (i.e. so-called "primary or idiopathic CaOxSF" requiring detailed discussion of pathogenic mechanisms), it is also appropriate to briefly list the causes of secondary CaOxSF as presented earlier in this thesis (see table 1.3, p. 8). As mentioned, approximately 80% of all recurrent calculi are due to this entity. Although the mechanisms of SF may be mentioned in the discussion of these publications, going into detail is beyond the scope of this thesis.

This chapter of the thesis relates to 7 previously published papers (1990-2002) carried out at the Stone Clinic of the Johannesburg Hospital. These 7 presentations form the mainstay of the thesis in that they demonstrate the kind of patients with a broad spectrum of stone pathogenetic factors who attended the clinic and also clearly demonstrated the ability of the clinic to perform cutting-edge research projects covering patients with a whole range of RCaOxSF groups and sub-groups. As mentioned, the papers highlight what was known at the time and with hindsight, have demonstrated many shortfalls due to either new knowledge brought in by a plethora of recent research or an actual defect in the research protocols which were employed.

From these aspects recommendations for future research are given. Also, a number of other aspects of either less urgency and/or more complicated and less possible research are listed.

Most importantly, the research in these seven publications was able to point out defects in knowledge at the time they were performed and was able to come up with a set of laboratory “norms” both in black and white subjects. From this work it was possible to accurately extrapolate normal urinary mineral levels which were compared with abnormal test results. Even with this information there are still shortcomings which point to the requirement of urgent future research.

The discussions of these publications are arranged in relevant hypothetical, clinical, pathogenic and management categories in an orderly fashion. Seven publications are presented and because they are all inter-related and tell a story, it was impossible to leave any of them out from the presentation of the thesis. The discussions of these publications are divided into 3 distinct groups. Group 1 (2 papers) mainly concerns the inhibitors of CaOxSF but also other information on CaOx precipitator sub-groups. Group 2 (3 papers) mainly consists of CaOx crystal promotion but with some inhibitor data. Group 3 (2 papers) are mainly concentrated on the management of RCaOxSF.

6.1 PRESENTATION OF THE SEVEN PREVIOUS PUBLICATIONS

Discussion of the previously published papers are presented in the associated groups.

6.1.1 Group 1; 2 papers with main theme: Stone inhibitors

6.1.1.1 Publication 1 [94]

Laminski NA, Meyers AM, Kruger M, Sonnekus MI, Smyth AE. Prevalence of hypocitraturia and hypopyrophosphaturia in recurrent Ca SF: As isolated defects or associated with other metabolic abnormalities. Nephron 1990; 56:379-386. https://www.karger.com/Article/Abstract/186179

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Nephron 1990;56:379–386

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Prevalence of Hypocitraturia and Hypopyrophosphaturia in Recurrent Calcium Stone Formers: As Isolated Defects or Associated with Other Metabolic Abnormalities

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Key Words. Citrate · Pyrophosphate · Recurrent stone formers · Calcium calculi

Abstract. Several underlying metabolic abnormalities may be present in patients with recurrent calcium calculus disease (RCCD). The aim of this study was to determine the prevalence of deficiencies of 2 well-known potent inhibitors of crystal formation and growth, citrate and pyrophosphate, in the various metabolic subgroups and as single defects. In 107 patients with RCCD, urinary citrate was significantly decreased in all metabolic subgroups with 49% of patients having hypocitraturia (2.53 ± 1.19 mmol/24 h) versus controls (3.44 ± 0.96 mmol/24 h; $p < 0.001$). Reduced pyrophosphate : creatinine ratios were present in all the patient subgroups, and 48% of all patients had reduced ratios (1.68 ± 1.68 vs. 3.10 ± 2.66 in controls; $p < 0.01$). There was no correlation between citrate and pyrophosphate concentration. Isolated hypocitraturia was found in 11.2%, reduced pyrophosphate : creatinine ratios as the single defect in 11.2% and a combination of both in 12.1% of patients. Thus inhibitor defects play an important role in patients with RCCD and frequently occur as isolated biochemical defects.

Introduction

The prevalence of idiopathic nephrolithiasis in Caucasians has been given as 6–15% in males and 3–5% in females [1], and repeated attacks occur in 40–50% of these. Recurrent calcium stone formation has been associated with various metabolic subgroups: the hypercalciurias (dietary, resorptive, absorptive and renal), hyperoxaluria and hyperuricosuria associated with calcium stones. It is well established that there are potent inhibitors in the urine which delay or prevent formation of the crystalline component of urinary calculi [2]. Among these are citrate and pyrophosphate. Citrate reduces urinary saturation of stone-forming calcium salts (calcium oxalate and calcium phosphate) by forming complexes with calcium and lowering the amount of free calcium ions available for stone formation [3]. Direct inhibition of nucleation, crystal growth and aggregation of calcium salts is found [4, 5]. Hypocitraturia has been reported as affecting 15–55% of patients with recurrent calculi [2, 6–12] and is regularly present in patients with calcium oxalate stones secondary to malabsorption syndrome [13]. Hypocitraturia is also a feature of renal tubular acidosis [14, 15], urinary tract infections [16] and thiazide-induced hypokalaemia [17]. In other patients the cause is not known and usually occurs in association with other biochemical disorders. Pyrophosphate, a low-molecular-weight polyanion [18], delays various processes involved in stone formation, such as epitactic and heterogenous nucleation, crystal growth and aggregation [19]. These effects appear to be related to the binding of pyrophosphate onto the crystal surface [19, 20]. Urinary pyrophosphate has been reported to be decreased in stone formers by some investigators [21, 22] but not by others [23, 24].

Little is known about the actual prevalence of these disorders in the various metabolic subgroups, and even less is known about whether these inhibitor defects can occur as isolated abnormalities [6–12, 22–24]. Finally scant publications exist on the entity of hypocitraturia, hypopyrophosphaturia or combinations of the two as isolated defects in these patients. The aim of this study then was to determine the prevalence of deficiencies of these two inhibitors in the various metabolic subgroups or as single defects in patients previously diagnosed as 'nil metabolic change' or 'dietary nephrolithiasis'.

Table 1. Clinical data in controls and various subgroups

	Controls (n = 32)	NMA (n = 24)	DHC (n = 26)	AHC (n = 29)	RHC (n = 18)	HU (n = 3)	HO (n = 7)
Age, years							
x̄	40.78	45.67	40.27	44.45	41.7?	35.33	54.29
SD	9.83	10.66	11.42	11.35	18.45	5.13	11.40
Range	26–63	26–64	21–65	24–70	14–75	31–41	38–66
Sex, ♀ : ♂	11:21	0:24	9:17	10:19	6:12	0:3	2:5
Weight, kg							
x̄	71.97	81.23	79.19	72.61	79.06	70.33	74.00
SD	12.17	13.48	16.53	12.64	13.74	7.02	12.37
Serum K^+, mmol/l							
x̄	ND	4.15	4.28	4.19	4.30	4.53	4.20
SD		0.31	0.31	0.33	0.40	0.31	0.40
Serum creatinine, µmol/l							
x̄	89.0	98.7	90.1	86.3	94.8	97.7	99.3
SD	13.0	12.8	9.4	13.6	12.5	16.3	21.4
Creatinine clearance; ml/min							
x̄	ND	106.0	115.7	117.6	103.4	120.7	99.6
SD		20.9	29.9	24.4	21.7	26.9	18.5

Values are presented as group means (SD). ND = Not done.

Table 2. Dietary analysis in controls and various subgroups

	Controls	NMA	DHC	AHC	RHC	HU	HO
Phosphate, mg/day							
x̄	ND	1,368.32	1,399.95	1,170.65	1,195.75	1,179.00	1,319.50
SD		442.57	522.62	428.85	522.81	41.72	679.53
Calcium, mg/day							
x̄	699.08	755.10	1,381.48	760.65	856.33	682.50	875.33
SD	431.25	261.34	723.01*	237.72	207.13	193.04	211.06
Oxalate, mg/day							
x̄	142.27	165.10	157.46	172.05	251.57	229.00	218.33
SD	103.33	106.85	114.33	149.14	149.82	26.87	106.63
Sodium, mg/day							
x̄	2,509.83	2,543.05	3,149.89	2,962.14	2,958.14	3,108.50	3,530.50
SD	965.27	1,218.01	1,482.33	1,623.80	1,682.25	135.06	1,513.92

Values presented are group means (SD). Statistical significances from control values are designated by *$p < 0.01$. ND = Not done.

Methods and Patients

Methods

Detailed dietary assessments of calcium, phosphate, sodium and oxalate intake were performed by our dietician. Calcium : creatinine ratios were determined before and after calcium loading as described by Pak et al. [25]. The tests were performed on a 'normal' and after a diet of 400 mg calcium and 100 mmol sodium. The short acid load test was carried out if fasting urinary pH was greater than 5.3 [26]. At least two 24-hour urine samples were collected from each subject, and all treatment was withheld for 2 weeks prior to the collections. Samples were collected without preservatives, kept refrigerated and delivered directly to the stone laboratory. The 24-hour urinary content of calcium, phosphate, uric acid, creatinine, sodium, potassium and total volume were measured using standard laboratory methods. Urinary oxalate was measured using the Boehringer Mannheim oxalic acid kit. Urinary citrate content was measured enzymatically using a modification of the Boehringer Mannheim citric acid kit and was repeated if low levels were recorded. The kit gave < 3% inter- and intra-assay variation [27]. Specimens with bacterial contamination, assessed by measuring nitrite with Dipstix, were discarded. Urinary pyrophosphate was measured enzymatically in the 2-hour fasting specimen of the calcium load test, using a modification of the method of Roullet et al. [28]. This specimen was

Table 3. Urinary chemistries in controls and the various subgroups

	Controls (n = 32)	NMA (n = 24)	DHC (n = 26)	AHC (n = 29)	RHC (n = 18)	HU (n = 3)	HO (n = 7)	Total (n = 107)
Volume, litres								
x̄	1.56	1.60	1.60	1.90	1.93	2.37	2.15	1.78
SD	0.63	0.72	0.68	0.55	0.51	0.26	0.97	0.65
Creatinine, mmol/24 h								
x̄	15.23	16.15	17.48	14.58	15.08	14.27	13.37	15.73
SD	4.41	4.26	6.11	5.72	4.99	1.22	2.74	5.44
Calcium, mmol/24 h								
x̄	4.03	4.55	6.90***	6.95***	8.58***	4.68	3.86	5.90***
SD	1.73	1.38	3.17	2.46	2.66	1.94	1.64	2.83
Phosphate, mmol/24 h								
x̄	26.96	25.59	36.02**	31.06	28.81	31.56	28.29	30.49*
SD	7.76	5.76	12.57	12.31	10.15	6.83	5.65	10.85
Sodium, mmol/24 h								
x̄	133.32	147.45	188.82**	147.80	166.44	146.98	157.27	162.37**
SD	45.87	64.18	78.65	48.72	52.88	45.09	23.84	60.91
Potassium, mmol/24 h								
x̄	70.41	54.34	58.18	60.24	63.32	57.61	75.87	60.06
SD	22.50	17.05	14.80	22.39	23.29	9.01	27.04	19.64
Uric acid, mmol/24 h								
x̄	2.15	3.39	4.35	3.40	3.61	4.22	2.81	3.68***
SD	1.20	0.80	1.88	1.30	1.67	1.70	1.38	1.56
Oxalate, µmol/24 h								
x̄	247.93	296.57	282.40	346.29**	325.06**	314.33	469.71**	323.70***
SD	103.50	106.19	93.39	135.59	126.71	13.58	146.76	122.27

Values are presented as group means (SD). Statistical significances from control values are designated by *p < 0.05, **p < 0.01 and ***p < 0.001.

snap frozen as pyrophosphate was found to be labile when left at room temperature. Correction was made for the concentration effect by determining the ratio of pyrophosphate (µmol/l) to creatinine (mmol/l).

Subjects

One hundred and seven patients referred to the Johannesburg Hospital Stone Clinic for recurrent calcium calculus disease (RCCD) were studied. There were 27 females and 80 males with ages ranging from 14 to 75 years. All patients had normal kidney function, and those with anatomical abnormalities, gout, primary infective stones, hyperparathyroidism, renal tubular acidosis or intestinal pathologies were excluded. Thirty normal healthy volunteers, 11 females and 21 males, ranging in age from 26 to 63 years, served as the control group. Patients were classified according to their calcium : creatinine ratios and other biochemical abnormalities as follows: no metabolic abnormality (NMA, n = 24), dietary hypercalciuria (DHC, n = 26), absorptive hypercalciuria (AHC, n = 29), renal hypercalciuria (RHC, n = 18), hyperuricosuria only (HU, n = 3) and hyperoxaluria without hypercalciuria (HO, n = 7). Hyperuricosuria and hyperoxaluria were often associated with the other biochemical subgroups. The AHC group included type I and type III absorptive hypercalciuria [29, 30].

Definitions: Hyperoxaluria > 350 µmol/24 h (mean ± SD of the control values), hypernatriuria was arbitrarily taken as > 160 mmol/24 h, hypercalciuria ≥ 0.1 mmol/kg/24 h, hypocitraturia < 2.4 mmol/24 h and decreased pyrophosphate : creatinine ratio < 1 µmol : mmol.

Statistical Analysis

Student's t tests, Pearson correlation coefficients, χ^2 tests and Kruskal-Wallis tests for non-parametrical data were used, with the Bonferroni correction for multiple testing where appropriate. All tests were done at the 5% significance level.

Conversion to Mass Units

Serum K 1 mmol/l = 1 mEq/l and serum creatinine 1 µmol/l = 0.0113 mg/100 ml; urine parameters: creatinine 1 mmol/24 h = 0.113 mg/24 h, Ca 1 mmol/24 h = 40 mg/24 h, phosphate 1 mmol/24 h = 0.03 g/24 h, Na and K 1 mmol/24 h = 1 mEq/24 h, uric acid 1 mmol/24 h = 0.168 g/24 h, oxalate 1 µmol/24 h = 0.099 mg/24 h and citrate 1 mmol/24 h = 210.10 mg/24 h.

Results

There was no significant difference in age between controls and patients when compared with either the entire patient group or the individual subgroups. The

Table 4. Urinary citrate and pyrophosphate in all stone formers and controls

	Controls			Total patients		
	male	female	total	male	female	total
Citrate, mmol/l						
x̄	2.30	2.81	2.48	1.56***	1.75**	1.61***
SD	0.80	1.17	0.96	0.90	0.82	0.88
	NS			NS		
Citrate, mmol/24 h						
x̄	3.39	3.52	3.44	2.44***	2.85*	2.55***
SD	1.13	0.54	0.96	1.30	1.13	1.19
	NS			NS		
Ci:cr ratio						
x̄	0.20	0.32	0.24	0.14**	0.24**	0.17***
SD	0.07	0.07	0.09	0.07	0.09	0.08
	$p < 0.006$			$p < 0.005$		
Pyrophosphate, µmol/l						
x̄	7.82	12.45	8.67	6.74	5.25*	6.36
SD	7.12	7.43	7.05	12.23	7.13	11.12
	NS			NS		
Py:cr ratio						
x̄	1.98	4.23	3.10	1.51*	2.16*	1.68**
SD	1.00	3.34	2.66	1.40	2.27	1.68
	NS			NS		

Values are presented as means (SD). Statistical significances from control values are designated by *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Ci:cr = Citrate:creatinine ratio; Py:cr = pyrophosphate:creatinine ratio. There was no significant difference between citrate and pyrophosphate values in males and females in both controls and patients.

male to female ratio in the controls ws 2:1 which compares favourably with the stone formers. Serum potassium, creatinine and creatinine clearance were normal in all subgroups (table 1). All patients acidified their urine normally.

Dietary analysis is shown in table 2. Dietary history was taken on arrival at the clinic, therefore values are prior to any specific dietary therapy. Patients with dietary hypercalciuria had significantly increased calcium intake when compared to the controls (1,381.48 ± 723.01 mg/day vs. 699.08 ± 431.25 mg/day; $p < 0.01$). No other dietary differences were found.

Calcium excretion in the DHC, AHC and RHC groups was significantly greater than in the controls (6.90 ± 3.17, 6.95 ± 2.46 and 8.58 ± 2.66 mmol/24 h, respectively, vs. 4.03 ± 1.73 mmol/24 h; $p < 0.001$). The DHC group had higher phosphate excretion (36.02 ± 12.57 vs. 26.96 ± 7.76 mmol/24 h; $p < 0.01$) and a higher sodium excretion (188.82 ± 78.65 vs. 133.32 ± 45.87 mmol/24 h; $p < 0.01$) than controls presumably reflecting a higher dietary intake. Oxalate excretion was significantly increased in AHC, RHC and HO groups (346.3 ± 135.6, 325.1 ± 126.7 and 469.7 ± 146.8 µmol/24 h, respectively, vs. 227.93 ± 114.71 µmol/24 h; $p < 0.01$) when compared with controls (table 3). Many of the metabolic subgroups showed mixed abnormalities, e.g. hyperoxaluria was found in 3/26 patients with DHC (12%), 11/29 patients with AHC (38%) and 6/18 patients with RHC (33%); Hyperuricosuria without hyperuricaemia was found in 12/26 patients with DHC (46%), 10/29 patients with AHC (35%) and 6/18 patients with RHC (33%); hypernatriuria was found in 8/26 patients with DHC (31%), 6/29 patients with AHC (21%), 6/18 patients with RHC (33%) and 1/7 patients with HO (14%). Hyperoxaluria was found in 20%, hyperuricosuria in 27% and hypernatriuria in 20% of all patients.

Citrate excretion, concentration and citrate:creatinine ratios were significantly lower in the stone patients than in the controls (2.55 ± 1.19 vs. 3.44 ± 0.96 mmol/24 h; 1.61 ± 0.87 vs. 2.48 ± 0.96 mmol/l and 0.17 ± 0.08 vs. 0.24 ± 0.09 citrate:creatinine, respectively; $p < 0.001$ for each; table 4). Pyrophosphate was only significantly different when expressed as the pyrophosphate:creatinine ratio (1.68 ± 1.68 vs. 3.10 ± 2.66; $p < 0.01$). There was no

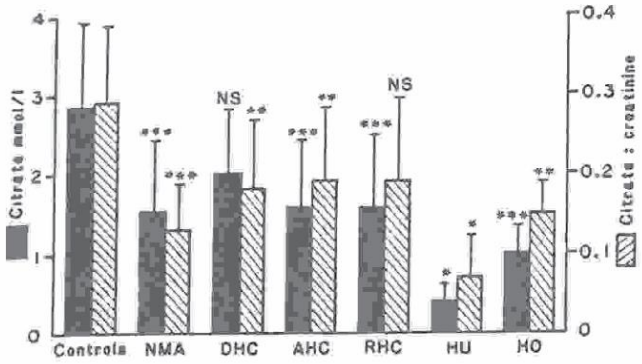

Fig. 1. Urinary citrate (mmol/l) and citrate : creatinine ratios (means ± SD) in controls and various patient subgroups. Statistical significances from control values are designated by $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

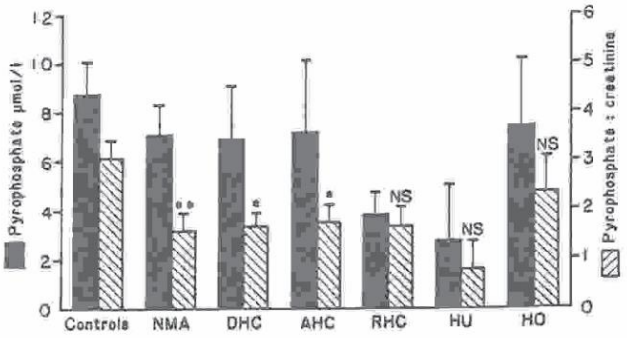

Fig. 2. Urinary pyrophosphate (µmol/l) and pyrophosphate : creatinine ratios (means ± SEM) in controls and various patient subgroups. Statistical significances from control values are designated by $*p < 0.1$ and $**p < 0.05$.

significant difference between citrate and pyrophosphate values in males and females in both controls and patients (table 4). Forty-nine percent of all patients had hypocitraturia, and 48% of all patients had reduced pyrophosphate : creatinine ratios.

Means and SD of citrate (mmol/l) and citrate : creatinine ratio in controls and the various subgroups are shown in figure 1. Citrate is significantly lower in all subgroups of stone formers (fig. 1). Sixteen of twenty-four patients with so-called NMA (67%), 7/26 patients with DHC (27%), 14/29 patients with AHC (48%), 8/18 patients with RHC (44%), 4/7 patients with HO (57%) and all 3 of the patients with HU had hypocitraturia either alone or together with reduced pyrophosphate : creatinine ratios. Pyrophosphate (µmol/l) showed a statistically significant positive correlation with creatinine (mmol/l) in both controls and stone formers ($r = 0.59$ and $r = 0.61$, respectively; $p < 0.001$). Means and SEM of pyrophosphate (µmol/l) and pyrophosphate : creatinine ratio in controls and various subgroups are shown in figure 2. Reduced pyrophosphate : creatinine was present in all the subgroups. Pyrophosphate : creatinine ratios in the so-called NMA group were significantly different from controls ($p < 0.05$). Patients with DHC and AHC showed a trend towards decreased pyrophosphate : creatinine ratios. Eleven of 24 patients with NMA (46%), 13/26 patients with DHC (50%), 14/29 patients with AHC (48%), 9/18 patients with RHC (50%), 3/7 patients with HO (43%) and 1/3 patients with HU had reduced pyrophosphate : creatinine ratios alone or combined with hypocitraturia. These tests were often repeated and always showed the same results.

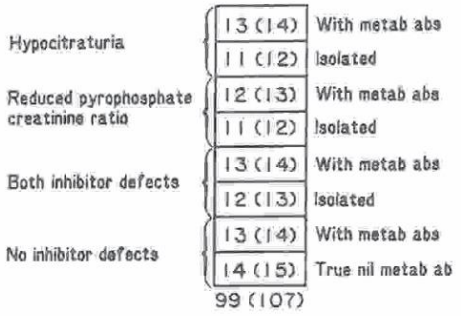

Fig. 3. Frequency of inhibitor defects in all patients occurring either in isolation or in combination with other metabolic abnormalities. Values given as percent, numbers of patients in parentheses; metab ab(s) = metabolic abnormality(ies).

Therefore, of the patients previously classified as NMA prior to the study, 8 had hypocitraturia, 3 had reduced pyrophosphate : creatinine ratios and 8 had both inhibitor defects. Of patients classified as DHC (essentially NMA when on diet) prior to the study, 4 had hypocitraturia, 9 had a reduced pyrophosphate : creatinine ratio and 5 had both inhibitor defects. Thus pure hypocitraturia was found in 11.2% ($n = 12$), pure reduced pyrophosphate : creatinine ratio in 11.2% ($n = 12$) and the combination of both in 12.1% ($n = 13$) of patients with recurrent renal calculi. In other words, fully one third of the patients had inhibitor defects as isolated abnormalities. Figure 3 summarizes these data by depicting the frequency of inhibitor defects either as an isolated finding or associated with the metabolic subgroups.

Discussion

Hypocitraturia and hypopyrophosphaturia are well-recognized inhibitor defects and have been extensively investigated in patients with hypercalciuria, hyperoxaluria or hyperuricosuria [6–12, 22–24]. In our patients, hypocitraturia was present in 57% of patients with HO, 47% with AHC, 44% with RHC and in all 3 patients with HU. Schwille et al. [8] similarly found citrate excretion decreased in all subgroups of stone formers but only significantly so in the normocalciuric subgroup and in patients with calcium oxalate or uric acid stones. In addition the normocalciuric patients had decreased serum potassium and hypomagnesuria. In our study, 3/14 patients in the AHC subgroup had normocalciuria with hypocitraturia ($p < 0.1$), but unlike Schwille et al. [8] none had decreased serum potassium which may partially explain the hypocitraturia in their patients. In total, 49% of our patients had hypocitraturia, and 23.3% of patients had hypocitraturia as an isolated defect or in combination with decreased pyrophosphate : creatinine ratios. Rudman et al. [9] found hypocitraturia in 50% of idiopathic calcium oxalate stone formers, and in 13%, hypocitraturia was the only defect. However, only 15 patients were studied. Nicar et al. [6] similarly found hypocitraturia in 55% of stone formers and in 12% as an isolated defect. François et al. [11] reported a prevalence of hypocitraturia of 27.5%, with 7.5% of patients having isolated hypocitraturia. Hyperuricosuria associated with hypocitraturia was found in 20% of their patients. Only 3 of our patients had pure hyperuricosuria, and all had hypocitraturia. Not all researchers have reported an association with hyperuricosuria [31]. Menon and Mahle [7] found hypocitraturia in 15% of calcium oxalate stone formers, with 13% as an isolated defect.

Conversely, some investigators [31, 32] have questioned the importance of citrate in recurrent calcium stone formers. Parks and Coe [32] concluded that hypocitraturia is not a consistent feature in men with recurrent calcium stones but that women with recurrent stones excrete less citrate. Hosking et al. [31] found hypocitraturia in 29% of patients: however, 11/35 had urinary pH > 5.4. Maximal urinary acidity was not performed, so that renal tubular acidosis as a cause of hypocitraturia could not be excluded.

We found no difference between citrate excretion in men and women in the controls or patients. Although citrate excretion was generally higher in women, it was not significantly so. This is in agreement with Nicar et al. [6] and Menon and Mahle [7]. Welshman and McGeown [10] and Schwille et al. [12] found that normal young women had statistically significantly increased urinary citrate excretion. They attributed this to higher oestrogen levels in premenopausal women. We found a ratio of citrate (mmol/l) in patients to citrate (mmol/l) in controls of 0.54 which compares favourably with the ratios reported by François et al. [11] of 0.56 and Schwille et al. [12] of 0.50. Thus stone formers' urine is less concentrated with respect to citrate. This is not due to increased fluid intake, as urinary volumes in our patients were not increased when the individual groups or the entire group were compared with controls.

The reasons for hypocitraturia in our patients are not known. None had either complete or incomplete renal tubular acidosis [14], hypokalaemia [17] or urinary tract infections [16]. All treatment was withheld 2 weeks prior to the study so that agents such as thiazides could not be implicated as a cause of reduced urinary citrate excretion [17]. Citrate is taken up by a tricarboxylate carrier in the proximal tubule of the nephron [15]. It could be postulated that some stone formers may have a more active carrier system resulting in increased cellular uptake of citrate. Rudman et al. [9] found increased citrate reabsorption in patients as compared with controls (96 vs. 82%). More studies are needed to confirm this result. Decreased urinary magnesium has been implicated as a cause of hypocitraturia. In the presence of reduced magnesium excretion, less citrate is complexed in the renal tubules resulting in increased uptake of citrate by tubular cells and decreased urinary excretion [13]. Hypomagnesuria as a cause of hypocitraturia could not be definitely excluded in our patients. However, none of them had conditions associated with hypomagnesuria or hypomagnesaemia so that this would seem unlikely.

Reduced pyrophosphate : creatinine ratios were found in 50% of patients with RHC, 48% of patients with AHC, 43% of patients with HO and in 1 patient with HU. In total, 49% of our patients had reduced pyrophosphate : creatinine ratios, and 23.2% had reduced ratios as an isolated defect or in combination with hypocitraturia. Unlike other investigators [24] we found a pyrophosphate defect in all age groups and not only restricted to the fourth decade. The different method used for determining pyrophosphate and its measurement in 2-hour fasting urine and expression as pyrophosphate : creatinine ratio may explain this finding. Schwille et al. [24] only found decreased pyrophosphate excretion in 24-hour urines of men > 40 years old. They also measured it in 2-hour fasting urines and found no significant difference between men and women of 20–40 years or > 40 years in

controls or recurrent stone formers. In contrast, we found decreased urinary pyrophosphate (μmol/l) in 2-hour fasting urine in both female and male stone formers aged 20–40 years versus controls (2.61 ± 2.38 vs. 10.55 ± 6.44, $p < 0.001$, and 3.90 ± 2.95 vs. 9.40 ± 7.46 $p = 0.01$, respectively). There was no significant difference between men and women controls aged 20–40 or >40 years or when groups were taken together. Other investigators have reported decreased pyrophosphate in normal younger and older women (differences to men approx. 25 and 65%, respectively) and increased excretion with age in both men and women [24]. Mechanisms responsible for controlling pyrophosphate excretion are not known. Pyrophosphate excretion is directly related to the dietary intake of orthophosphate but not pyrophosphate, as they are completely hydrolysed in the intestine so that urinary pyrophosphate is totally endogenously derived [33]. If anything, our patients had a relatively high urinary phosphate excretion reflecting mainly a high protein intake.

We found no correlation between citrate and pyrophosphate, unlike Schwille et al. [24], probably as our citrate was measured in 24-hour urine and not in the fasting urine.

In conclusion, a total of 49% of patients had hypocitraturia, and 48% had hypopyrophosphaturia (reduced pyrophosphate : creatinine ratio). Of these, 11.2% had isolated hypocitraturia, 11.2% had reduced pyrophosphate : creatinine ratios only and 12.1% had a combination of these two as the only defect. Thus, inhibitor defects play a far more important role in patients with recurrent renal calcium stones than originally thought and appear to form important subgroups in their own right.

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6.1.1.1.1 Key aspects

This paper represents seminal work published on the urine levels of two important inorganic stone inhibitors namely citrate and pyrophosphate. They were measured in multiple 24-hour urine samples only from white subjects while on their usual diets. Measurements were made in 32 normal controls and 107 RCaOxSF who consisted of various causative risk factor sub-groups namely those with "nil-metabolic change", the hypercalciurias (dietary, absorptive and renal), "hyperuricosurias" and hyperoxaluria.

6.1.1.1.2 Discussion:

The definition of normal levels of urinary citrate excretion found in this study have been used by the Johannesburg Hospital stone clinic ever since publication of this study. Although citrate excretion in black subjects were not measured in black subjects in the study, it was measured in two subsequent studies. [92, 231] The numbers of subjects studied and the number of 24-hour specimens obtained for the study of citrate per subject, required statistical validation to obtain clinically normal values. Therefore, by combining these three studies [92,94,231], statistically validated normal levels were obtained which can be used in the South African black and white populations (see

para. 6.2.1, p. 123). The problems which existed in all three are dealt with in the limitations and recommendations described below.

The measurement of pyrophosphate involving very costly and intricate technology, although demonstrating hypopyrophosphataemia, failed to deliver any feasible practical value in stone management. Its role as an important inhibitor of CaOxSF in vivo remains highly unlikely as it is only excreted in minute quantities. Also, any oral pyrophosphate preparation would not be possible because of the rapid GIT hydroxylation into phosphate and water.

Another observation from the study relates to a faulty definition in the various subgroups of hypercalciuria. From the results of the urinary sodium levels it became evident that many patients had not restricted their salt intake making it impossible to assess whether this was dietary hypecalciuria or true hypercalciuria. This observation is also dealt with in the limitations and recommendations below.

6.1.1.1.3 Study limitations

As eluded to above, the limitations of this study were as follows:

1. Citrate was not measured in black subjects;
2. More patients should have been included in the study cohort;
3. The presence of hypercalciuria could not be assessed because the dietary sodium intake was not standardized.

6.1.1.1.4 Recommendations for future research

1. Twenty-four-hour urinary citrate levels from black, white and Indian stone formers should be measured from multiple centres in various climate regions of South Africa.
2. At the same time as the citrate levels are assessed, hypercalciuria and its subgroups should be measured taking care to ensure that the measurements are carried out in patients on a standard sodium intake of 5g of salt per day.

3. Of practical importance, the standard work-up should in future include 2 x 24-hour urine citrate evaluations. This process has already been introduced in the Johannesburg Hospital Stone Clinic.

6.1.1.2 Publication 2 [92]

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Lithogenic risk factors in the urine of black and white subjects

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Objective To identify biochemical and dietary factors which may play a role in the low incidence of stone formation in the black South African population.

Subjects and methods The study included 31 semi-urbanized black and 29 urbanized white subjects. The protocol and modern laboratory techniques used to assess recurrent stone formers were followed. Urinary sodium, potassium, creatinine, calcium, phosphate and urate levels were measured, and urinary citrate, oxalate and cystine assessed.

Results Black subjects ate a diet significantly higher in sodium ($P<0.04$); there was no difference in serum levels but urinary sodium was significantly higher ($P<0.001$) in black than in white subjects. Urinary potassium, calcium, citrate, phosphate and cystine were all significantly lower in black than in white subjects ($P<0.001$ for the first four and $P<0.03$ for cystine).

Conclusion Certain intrinsic factors in South African black subjects may account for their lower frequency of stone formation than in white subjects. Of these, the very low urinary calcium, decreased urinary cystine and different interactions between sodium and calcium/cystine are probably important.

Keywords Epidemiology, ethnic differences, black, white, control, urolithiasis

Introduction

Calcium-containing renal calculi, 40–60% of which are recurrent, occur in 15% of white men and 5% of white women. Forty years ago urolithiasis was virtually unheard of in recently urbanized black South Africans [1,2]. Recently, there has been a slight increase in the incidence in urban blacks, but stones still occur in <1% of the population [3]. Previously they were predominantly of struvite and occurred secondary to bilharzia or urinary tract infections, and were sometimes found in the bladder [1]. Upper urinary tract calcium oxalate stones are now the most common type found in the urban black population of South Africa [4]. Many researchers have speculated whether the difference in prevalence is a question of racial immunity, dietary differences, a disparity in fluid intake, handling of solute or the presence or absence of promoters and inhibitors of renal stone formation. In an attempt to identify the biochemical and dietary factors which may play a role in the low incidence of stone formation in an increasingly urbanized black population, we studied urine biochemistry in the two ethnic groups, incorporating modern laboratory techniques and a protocol used to assess recurrent calcium stone formers.

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Subjects and methods

Eighty normal volunteers (40 black and 40 white) entered into the study, but because some provided incorrect 24 h urine collections, did not comply or withdrew, 31 (19 women, 12 men) recently urbanized, black, light-industrial factory staff and 29 (17 women, 12 men) urbanized white hospital staff were eventually included. A normal physical examination excluded any possible illness. All had normal renal function, were normotensive, on no medication and had no family history of stones.

A simplified dietary questionnaire, specially designed by a qualified dietician, was completed by the researchers after questioning the subjects. This enabled an assessment of calcium, sodium and oxalate intake in each group, by analysing the dietary history using a computer program 'Dietary Manager' (Programme Management, South Africa). Two midweek 24 h urine samples were collected by each subject, the first while on their usual diet and the second after consuming a diet containing 100 mmol Na and 800 mg Ca for 7 days. However, compliance with this diet was suspect, as the 24 h urinary sodium level remained high in the black subjects and increased in the white men, rendering these results unassessable.

The 24 h urinary volume, sodium, potassium, calcium, phosphate, creatinine and urate levels were measured using standard laboratory methods. Urinary citrate was measured using a modification of the Boehringer

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Mannheim citric acid kit [5]. Specimens with bacterial contamination, assessed by measuring nitrite using Dipstix (Combur 9, Boehringer Mannheim, Germany) were discarded. Urinary oxalate was measured using the Boehringer Mannheim oxalic-acid kit (cat. no. 755699). Urinary cystine was measured quantitatively by reducing cystine to cysteine, using sodium sulphate [6]. On the eighth day after the special diet, calcium:creatinine ratios were assessed both after fasting and after a 1 g load of calcium (in a synthetic breakfast, Precision LR, Roussel Laboratories Pty Ltd, South Africa), according to the method of Pak *et al.* [7] and a fasting blood sample was drawn. Serum calcium, ionized calcium, creatinine, phosphate and urate levels were measured using standard laboratory methods. Calcitriol was measured using a radioactive receptor assay (IncStar, Stillwater, MN, USA, cat no. 60065). Urinary pyrophosphate was measured enzymatically in the sample collected after 2 h of fasting for the calcium:creatinine ratio, using a modification of the method of Roullet *et al.* [8].

The values of the variables were expressed as the mean (SD) and evaluated using unpaired *t*-tests, Wilcoxon rank scores and Spearman correlation coefficients, with $P<0.05$ taken to indicate significant differences.

Results

Demographic data, dietary and urinary analyses are shown in Table 1. There was no significant difference in calcium or oxalate intake in black and white subjects. The black subjects ingested significantly more sodium than whites ($P<0.04$). When categorized by sex black men and women tended to ingest more sodium than white men and women ($P<0.1$). Black men ate less calcium than black women ($P<0.02$) and white women ($P<0.04$), and tended to eat less than white men ($P<0.1$). Black women ingested more oxalate than white women ($P<0.04$). The 24 h urine analysis results for black and white subjects on a free diet are also shown in Table 1. There was no significant difference in the volume, creatinine and urate excretion rate. Urinary sodium was significantly higher in black subjects, whereas urinary potassium, calcium, citrate, phosphate and cystine were all significantly lower in black subjects. In black subjects there was no significant correlation between any of the 24 h urine components. In white subjects there was a significant correlation between sodium and calcium, ($r=0.58$, $P<0.002$) and a trend ($P<0.08$) between sodium and cystine. White men excreted more cystine than black women ($P<0.02$) and white women excreted more citrate than black men or women ($P<0.005$). Black women excreted more sodium than white men or women ($P<0.001$). White men excreted more phosphate than white women or black men and women ($P<0.001$). Whites excreted more potassium than blacks ($P<0.005$). Black men excreted less calcium than white men or women ($P<0.001$).

There was no significant difference in fasting serum calcium, ionized calcium, phosphate, and calcitriol in the two groups. Serum creatinine and urate levels were significantly greater in the white than in the black subjects ($P<0.001$ and $P<0.03$, respectively). In black and white subjects, respectively, the mean (SD) calcium:creatinine ratios after fasting were 0.041 (0.029) and 0.043 (0.023), and after a calcium load were 0.131 (0.08) and 0.143 (0.07); there was no significant difference between these ratios in the two groups. The ranges for the ratio in fasting urine were 0.01–0.14 in blacks and 0.01–0.10 in whites. After a 1 g load of calcium the ranges were 0.02–0.28 in blacks and 0.04–0.34 in whites. In addition, there was no significant difference in fasting pyrophosphate:creatinine ratios in black and white subjects.

Discussion

Previously, the cause and composition of urinary stones in white and black South Africans differed [1] but they are now remarkably similar [4]. Many authors attribute the low incidence of urolithiasis in the black population to dietary habits [1,2]. It has been reported that they have a decreased intake of calcium and protein, both risk factors for stone formation [1,2]. Robertson postulated that a diet high in animal protein is the most important factor in the formation of calcium stones [9]. Biochemically, a diet high in animal protein increases the urinary excretion of calcium, oxalate and uric acid, and reduces the excretion of citrate [10]. In the present study, black men tended to (significantly) ingest less calcium than whites and black women, and black women ingested more oxalate than white women. Unfortunately, the analysis of protein intake was beyond the scope of the dietary analysis undertaken in the study, but low urinary phosphate excretion (Table 1) suggests a lower protein intake in the black subjects, as urea, the urinary indicator for protein consumption, was not measured.

Modlin [11] reported extensively on the differences in 24 h urinary composition in normal black and white subjects, finding that blacks had significantly greater volumes, osmolality, ionized calcium and sodium levels than whites, and that their calcium, phosphate and citrate levels were significantly lower than in white controls. On a free diet, black volunteers in the present study excreted significantly more sodium and significantly less calcium, potassium, citrate, cystine and phosphate; there was no difference in urine volume, urate or oxalate (Table 1).

Table 1 Demographic data, dietary analysis and urine results in black and white subjects

	Black		*White*			
Mean (SD)	*Women*	*Men*	*Women*	*Men*	*Black*	*White*
Number	19	12	17	12	31	29
Age (years)	26.5 (7.9)	32.2 (10.2)	28.4 (11.0)	32.3 (14.0)	28.7 (9.2)	30.0 (11.0)
Height (m)	1.59 (0.07)	1.65 (0.07)	1.64 (0.07)	1.78 (0.09)	1.62 (0.07)*	1.69 (0.10)‡
Weight (kg)	63.4 (11.5)	64.8 (10.5)	60.2 (8.8)	75.3 (7.6)	63.9 (13.2)	66.11 (11.1)
BMI	24.8 (3.8)	23.1 (5.4)	22.8 (3.5)	23.1 (2.0)	24.1 (4.6)	22.9 (3.0)
Dietary levels (mg/day)						
Na	3478 (1979)	3956 (2136)	2505 (1103)	2989 (1323)	3699 (2026)*	2709 (1200)*
Ca	836 (400)*	475 (262)*	809 (486)*	810 (589)	677 (386)	809 (513)
Oxalate	332 (185)*	334 (427)	182 (155)*	220 (207)	333 (332)	195 (171)
24 h urine samples						
Volume (mL)	1190 (444)	1041 (361)	1309 (365)	1294 (533)	1132 (413)	1302 (444)
Creatinine (mmol)	11.72 (2.77)	12.46 (3.76)	10.24 (2.01)	15.53 (3.46)	12.0 (3.1)	12.8 (3.9)
Sodium (mmol)	185.3 (60.5)[b]	153.0 (63.6)	112.0 (40.7)[b]	113.0 (41.6)[b]	173.0 (63.0)	113.0 (40.0)‡
Potassium (mmol)	41.8 (16.4)[b]	51.2 (14.4)[b]	77.8 (26.1)[b]	66.1 (14.5)[b]	45.5 (16.2)	73.4 (22.0)‡
Calcium (mmol)	1.89 (1.24)	0.96 (0.52)[c]	3.39 (2.30)[c]	3.63 (1.81)[c]	1.53 (1.10)	3.51 (2.04)‡
Phosphate (mmol)	18.64 (6.51)[c]	17.00 (4.33)[c]	23.40 (6.29)[c]	31.48 (15.7)[c]	18.0 (5.7)	27.3 (12.2)‡
Oxalate (μmol)	244.1 (127.3)	239.2 (134.5)	193.9 (78.0)	280.7 (163.7)	272.0 (197.0)	236.0 (132.0)
Urate (mmol)	1.75 (0.76)	1.06 (0.72)	1.58 (0.80)	1.58 (1.12)	1.48 (0.80)	1.58 (0.95)
Citrate (mmol)	1.51 (0.70)[b]	1.23 (1.19)[b]	3.46 (0.96)[b]	2.32 (1.02)	1.39 (0.92)	2.79 (1.12)‡
Cystine (mg)	42.05 (22.6)[a]	48.32 (19.6)	61.7 (28.3)	82.6 (44.2)[a]	44.4 (21.4)	71.7 (37.6)*

BMI, body mass index, kg/m^2. Unpaired *t*-tests: *$P < 0.05$, †< 0.01; ‡< 0.001. Wilcoxon scores. [a]$P < 0.02$, [b]$P < 0.005$, [c]$P < 0.001$.

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Urinary calcium was extremely low in black men when compared with the white men and women (Table 1): urinary calcium in black women was lower, although not significantly so, than the values in whites. The calcium intake in black men was significantly lower than in black and white women, and tended to be less than in white men, although the calcium intake of black women was similar. In black women, more calcium may be bound to oxalate in the gastrointestinal tract, resulting in less calcium and oxalate being absorbed and relatively less calcium and oxalate excreted in the urine. Conversely, the black men would have been expected to have high urinary oxalate levels, because less calcium was available to bind oxalate; this was not the case. In a recent study, 23 black male stone-formers with similar calcium intakes (mean 644.2, SD 397 mg/24 h) to the present black volunteers had a mean (SD) urinary calcium excretion of 3.65 (2.01), a value similar to that in the white men and significantly higher ($P<0.001$) than in the normal black men in the present study. Thus it would appear that the low urinary calcium level is protecting the black population from stone formation (unpublished data).

Urinary citrate was significantly higher in white subjects than in black ($P<0.001$); values were similar to those found by Modlin [11] (2.79 vs 2.46 mmol/day in whites and 1.68 vs 1.39 mmol/day in blacks, respectively). The reason for the decreased urinary citrate in black subjects is unknown. A high-protein diet which results in increased production and excretion of organic acids, and increased proximal tubular reabsorption of citrate, is unlikely to be the cause [10]. Decreased urinary magnesium can result in hypocitraturia because magnesium chelates citrate and prevents its absorption in the proximal tubule [12]. As urinary magnesium was not measured in this study, it is not possible to totally exclude magnesium as a cause for hypocitraturia in the black subjects. Potassium depletion, with the resultant movement of H^+ ions into cells and decrease in intracellular pH, increases citrate reabsorption in the proximal tubules [12]. Serum and red blood cell potassium, as well as faecal potassium, were not significantly different in similar normotensive black and white subjects, so potassium depletion seems unlikely [13]. Urinary citrate was significantly higher in the white women than in the black men and women. It has been reported that women have a higher excretion of citrate, possibly caused by hormonal differences [14].

Urinary sodium is directly related to dietary intake and except where sweat losses are significant, 24 h urinary sodium is an accurate measure of minimal dietary sodium intake in normal people [10]. Black subjects had a significantly higher sodium intake than whites ($P<0.04$). Urinary sodium was significantly higher in black subjects than in whites ($P<0.001$) but both had much lower levels than those reported by Modlin [11] (172 vs 292 mmol/24 h and 112 vs 213 mmol/24 h, respectively). Black women excreted significantly more sodium than both white women and men ($P<0.005$). In contrast, Cohen *et al.* [15] found no significant difference in sodium excretion between black and white subjects. It is well known that by increasing salt intake and thus urinary sodium excretion, urinary calcium increases linearly [16]. The presumed mechanism is the depression of tubular reabsorption of calcium. Furthermore, increased dietary sodium has been shown to increase calcium absorption from the gastrointestinal tract [17]. Thus high urinary calcium levels would be expected in black subjects, rather than the significantly lower excretion ($P<0.001$) found here. Modlin [11] found a significant correlation ($P<0.01$) between sodium and calcium excretion in black and white subjects. In contrast, in the present study, there was a correlation between urinary sodium and calcium only in white subjects ($P<0.002$).

The lack of correlation in the black subjects may be explained by the low calcium intake in black men, as this correlation disappears in states of chronic calcium deprivation, because calcium is reabsorbed in preference to sodium. Modlin [11] postulated that there is an inverse relationship between the urinary Na:Ca ratio and the risk of forming renal stones, proposing that the high Na:Ca ratio constitutes a physiological inhibition mechanism for crystallization. Even if an insoluble compound of calcium is formed in the urine, the concentration of sodium relative to calcium prevents further deposition by epitaxy. Rodgers and De Klerk [18] supported Modlin's hypothesis by showing, on scanning electron microscopy, profuse sodium deposits in the urine of black men and women. They examined the urine of six black stone formers and found no deposits, concluding that when urolithiasis occurs in blacks, it does so by the same mechanism as in any other ethnic group. We do not consider this hypothesis valid, as white stone formers on low-salt diets have lower urinary sodium levels, with a resultant decrease in urinary calcium excretion and stone formation, and high sodium excretion appears to favour stone formation. Modlin stated that the low urinary calcium level in blacks is more complex than a simple dietary relationship [11]. In the present study, there were no differences in serum calcium, ionized calcium and calcitriol between the groups. In addition, the calcium:creatinine ratios after fasting and after a calcium load were no different. However, had these tests been performed on the subjects' free diet, increased fibre and oxalate ingestion by blacks may have decreased calcium absorption and led to calcium:creatinine ratios after a Ca load lower than those found in white subjects.

However, urban black subjects ingest a diet similar to or lower in dietary fibre than do white subjects, and therefore this argument for low calcium absorption in the blacks is flawed [19]. The tendency to ingest increased oxalate in black subjects ($P<0.09$) would decrease calcium absorption in the upper urinary tract because the oxalate binds calcium. At sites too distal for calcium absorption (e.g. colon), we postulate that oxalate is broken down by the anaerobic bacteria *Oxalobacter formigenes*, and thus oxalate degradation products and increased calcium would be excreted in the stool. This would result in decreased calcium and oxalate absorption, and thus urinary excretion. However, with the test performed as described by Pak *et al.* [7], the calcium:creatinine ratios fell within their reported normal range, and thus calcium handling by the gastrointestinal tract and kidney in blacks and whites appear to be similar when ingesting the same food. However, the tubular interactions between sodium and calcium may differ.

The decreased daily potassium excretion in black subjects found in the present study is similar to that found by Cohen *et al.* [15], i.e. 45.5 mmol in blacks and 73.4 mmol in whites, vs 40 mmol in blacks and 71 mmol in whites [15]. It was concluded that the lower urinary excretion reflected a decreased dietary intake, as was probably the case in the present study. Urinary potassium was significantly lower in black men and women than in white men and women ($P<0.005$).

Significantly less urinary cystine was excreted by blacks than by whites ($P<0.03$) on their free diet. We have found that cystine added to urine results in increased precipitation, growth and aggregation of calcium oxalate crystals (unpublished data). Therefore, decreased cystine excretion in black subjects may be another factor in their lower risk of urolithiasis. Cystine is highly insoluble, thus urinary cystine concentration is reduced by drinking large amounts of water and its solubility increased by alkalinizing the urine [20]. However, urine volumes were not significantly different in black and white subjects. A correlation between urinary sodium and cystine has been reported [21]. Thus a higher cystine excretion would have been expected in black subjects because of their increased salt intake and urinary sodium level. Therefore, as with calcium, black subjects seem to have different tubular interactions between sodium and cystine. In whites, sodium tended to correlate with cystine ($P<0.08$) but there was no correlation in black subjects.

Although oxalate intake was significantly greater in the black women than white ($P<0.04$) and total dietary oxalate tended to be higher in blacks than whites ($P<0.09$), their urinary oxalate excretion was similar to that of white women and lower than for white men. Thus oxalate is either complexing calcium in the gut and less oxalate is absorbed, or the black population have increased quantities of *O. formigenes* which degrades oxalate in the gut and thus prevents its absorption, as discussed previously. Pyrophosphate excretion was no different in black and white subjects, thus a protective role for this inhibitor in black controls seems unlikely.

Thus, the black population is at less risk of forming stones because of their very low level of urinary calcium. In addition, low cystine excretion may further protect against urolithiasis. The lack of interaction between sodium and calcium, and sodium and cystine, would lower their risk for forming stones. The hypocitraturia present in black subjects should theoretically increase their risk for forming stones, but still they do not; this remains an enigma. Finally, organic inhibitors of calcium oxalate precipitation, e.g. glycosaminoglycans, Tamm-Horsfall protein and crystal matrix protein, as yet unstudied in black South Africans, may play an important role in preventing stones in this group of people [22–24].

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6.1.1.2.1 Key aspects

Paper 2 represents work published on SF risk factors as seen in normal black and white volunteers in South Africa. One of the main aims of the study was to present metabolic stone risk factors (both inorganic inhibitors and promotors) in two cohorts from the normal black and white South African populations. The objective was to obtain a base-line “data-bank” on urinary inorganic risk factors which may be responsible for RCaOxSF in two different ethnic groups in SA i.e. blacks where stone occurrence is still <1% of the population and in whites where the incidence is reported as between 12 and 14% in males and 5 to 6% in females. Data of this nature had never been assessed before and as such, represented findings at the cutting edge of stone research at that time.

6.1.1.2.2 Discussion

Forty years ago, nephrolithiasis was virtually unheard of in recently urbanized black subjects. [232, 233] Recently there has been a slight increase in the incidence of SF in urban blacks but nephrolithiasis still occurs in <1% of this population. [234] In addition, previous stones in the black population were predominantly struvite and were found in the pelvis or the lower urinary tract. [232] Upper urinary tract CaOx stones are now the most common type found in the South African blacks. [235]

On assessing the 24-hour urinary profile of black stone formers in this study, citrate excretion was shown to be significantly lower in normal black women and men vs their white counterparts. Of importance is that the excretion of citrate was shown to be lower in black and white men rather than women which is the usual finding and which has been frequently reported from previous studies. [123, 236-238] Of interest is that Modlin in his study in 1967 [7] also found significantly lower urinary citrate levels in the black normal subjects and stone formers as compared to whites. It is apparent that, from the frequency of hypocitraturia found in black subjects, they should in fact be forming more calculi than in their white counterparts. One of the reasons postulated is that black subjects, in spite of being on a higher sodium intake, excreted significantly less calcium in their urine than their white counterparts. Again, the same observation was made by Modlin in his paper. [7]

Because the citrate levels between black and white normals showed no significant differences in either the normal mean excretion rate including the normal ranges (in both sexes), a single norm has been used since then. However, in order to establish a laboratory validated accurate 24-hour urine citrate level the results of composite citrate measurements in all of the relevant studies will be depicted at the end of the group presentations (see para. 6.2, p.123).

It would seem logical to postulate that an important reason for the rarity of CaOx renal calculi in black subjects lies in genetic discrepancies in the DCT Ca sensing TRPV-5 receptors favouring significant Ca reabsorption in the black population. [239] (See para. 1.1.2, p. 2.)

In spite of the findings discussed above, standardisation of salt intake must be achieved in any population group studied. In fact, if this were not achieved in the stone work-up protocol, the urinary calcium could be interpreted as possibly being dietary in origin and would be classified therefore as dietary hypercalciuria. If this was so the use of medical work-up tests could be labelled as a “clinic effect” as was suggested by Hosking et al (1983). [240] The dilemma continues. From a practical point of view, it would seem logical to assess the patient on his/her normal and usual diet. Which is correct? Patients would not be likely to tolerate the notion of performing multiple tests on different dietary regimes. In fact, the sodium problem is further compounded. Is the importance of recommending a sodium intake relative to body mass? Is a normal sodium intake for a 72kg man the same as for one weighing 45kg or one weighing 90kg?

The difficulties of estimating sodium are well illustrated in the paper by Campino et al (2016) [241] in their article entitled "Usefulness and pitfalls in sodium intake estimation”. These authors point out how impossible it has more recently become because of the variety of sodium in all foodstuffs other than completely fresh produce. Indeed, many items do not even contain the amount of salt e.g. spices, tinned foods, take-aways, etc. All these facts go to making accurate sodium intake estimation a most difficult. In addition, it must be checked that there are no GIT problems, no undue

sweating e.g. from severe exercise, etc. and most importantly no snacks during the collection period. Espeland et al (2001) [242] states that 24-hour collections are tedious and expensive. However, in a stone clinic where it is imperative to record several 24-hour urine volumes in all patients, the exercise is far from tedious. In fact, in good hands it is accurate and has become a routine procedure n stone workup of inestimable value. It is noted from the Campino article that results were much more reliable in children than in adults.

These are all problems that require elucidation but from a practical point of view a stone clinic should repeat 24-h urinary excretion on strict dietary sodium control wherever relevant.

Of note is that oxalate excretion was not different between the two groups and from this data and other data, the mean and normal range of urinary oxalate excretion was estimated for the first time in SA. The mean (SD) and range for 24-hour oxalate excretion was 242µmol/24-hour (range 115-374) in blacks and 238µmol/24-hour (range 142-335) in whites. As there were no significant differences between sexes or ethnic groups, for practical reasons, the Johannesburg Hospital Stone Clinic has used a normal mean level mean of approximately 250µmol/24-hour. In addition, because it is almost impossible to estimate a lower level of normal oxalate intake, the clinic considered it empirically practical to add another 100µmol/24-hour to the mean, yielding an upper limit of normal daily oxalate excretion of 350µmol/24-hour. This corresponds reasonably well to the figures used by the referral laboratory. Likewise, there was no difference in the urinary uric acid excretion. Another interesting finding was the significantly lower urinary cystine excretion in the black population which would lower their risk for forming stones. This point is further emphasized in the summary of the reviewed papers (see para. 6.2, p. 123).

6.1.1.2.3 Study limitations

In part these are similar to the previous study (see para. 6.1.1.1.3, p. 71).

1. Although study protocols specified standard diets (i.e. including protein, carbohydrate, fat and daily mineral requirements), it was clear from the results that

frequent non-adherence occurred making accurate assessments impossible. Of special relevance was non-adherence to the correct quantity of salt in the diet.

2. In addition, standardization of fructose intake was not mentioned. Ingestion of≥50g per day of fructose may result in hypercalciuria and occasionally also hyperuricosuria.
3. Again, studies on both urinary inhibitors (citrate) and promotors were in many respects inadequate in quantity (e.g. numbers of black patients and controls) and quality in terms of poor adherence to protocols.
4. In addition, the Indian population which were not included in these protocols.
5. Also, the studies which were carried out were all done on local urban residents.
6. Patients with inappropriately high levels of 24-hour urinary phosphate in various sub-types of hypercalciuria as described by Williams et al (1996) [27] was never performed in any of the papers in this thesis even though the measurements were estimated.

6.1.1.2.4 Recommendations for future research

1. Repeat the studies taking more care that the patients adhere to the standard dietary requirements. A skilled and well-informed dietician should monitor this recommendation especially the sodium allowance not being in excess of 2,000mg per day.
2. As per protocol the first two urines are always performed on the patient's normal home diet. If in the dietary assessment the dietary fructose level exceeds 20g per day on a chronic basis, the tests must be repeated after dietary advice.
3. The study of additional number of black and white normal controls as mentioned above but also to include Indian subjects.
4. To perform the same studies in the same control cohorts but in peri-urban and in rural areas and in various geographical localities of the country.
5. Whilst studying patients in the above mentioned four categories the assessment of relative or inappropriately high P excretion and its effect on urinary CaOx and CaP excretion must be carried out. In addition to identify other sub-groups in this category, serum levels of both $1,25(OH)_2D_3$ as well as phosphatonin (FGF-23) will be included. [68-70]

6. Significantly lower urinary cystine levels in black subjects was found in this study. On this basis it was logical to postulate that these significantly lower cystine levels may be one of the reasons that the black population form less CaOx calculi than the whites. The pathophysiological explanation of this phenomenon was presented in the cystine paper (see p. 100).

6.1.2 Group 2; 3 papers with main theme: Stone promotors

6.1.2.1 Publication 3 [182]

Laminski NA, Meyers AM, Kruger M, Sonnekus MI, Margolius LP. Hyperoxaluria in patients with recurrent CaOx calculi: dietary and other risk factors. Br J Urol 1991 Nov;68(5):454-458. https://www.ncbi.nlm.nih.gov/pubmed/1747716

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Hyperoxaluria in Patients with Recurrent Calcium Oxalate Calculi: Dietary and Other Risk Factors

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Summary—The presence of mild hyperoxaluria in recurrent calcium oxalate stone formers is controversial. The aim of this study was to identify recurrent stone formers with mild hyperoxaluria and to classify them further by assessing their response to a low oxalate diet. In addition, the prevalence of other risk factors for stone formation in this group of patients was investigated. A total of 207 consecutive patients with recurrent renal calculi were screened and 40 (19%) were found to have mild hyperoxaluria. Of these, 18 (45%) responded to dietary oxalate restriction by normalising their urinary oxalate. The remaining 22 patients were classified as having idiopathic hyperoxaluria and were subdivided into those in whom urinary oxalate excretion was consistently elevated in all specimens measured and those in whom the elevation was intermittent in nature. Dietary oxalate restriction had a partially beneficial effect in lowering oxalate excretion in the patients with persistent hyperoxaluria. No difference in urinary oxalate excretion was found after dietary restriction in the patients with intermittent hyperoxaluria. Other risk factors, including dietary, absorptive and renal hypercalciuria and hypocitraturia, were documented, the prevalence of which (65%) was not significantly different from that (62.5%) found in 40 age- and sex-matched calcium stone formers without hyperoxaluria. The prevalence of hyperuricosuria was significantly greater in patients with hyperoxaluria when compared with stone controls. Further studies are required to elucidate the underlying mechanisms of hyperoxaluria in recurrent stone formers.

Oxalate, an end product of ascorbate and glyoxylic acid metabolism, is derived from dietary and endogenous sources (Broadus and Thier, 1979). Primary hyperoxaluria is a rare genetic disorder of glyoxylic acid metabolism characterised by excessively high oxalate excretion (>1000 μmol/24 h), calcium oxalate nephrolithiasis, nephrocalcinosis and eventual rapid progression to end stage renal failure (Watts, 1988).

We have defined mild hyperoxaluria as urinary oxalate excretion >350 μmol/24 h but without systemic consequences. Mild hyperoxaluria has been associated with diets low in calcium, high in oxalate or those containing excessive amounts of ascorbate. It has also been reported consequent to the steatorrhea which has followed small bowel disease, ileal resection and jejunoileal resection (Smith *et al.*, 1972; Dobbins and Binder, 1977; Coe, 1983).

The occurrence and pathogenesis of mild hyperoxaluria (MHO) in idiopathic calcium stone disease are controversial. Urinary oxalate has been variably reported to be normal (Bach *et al.*, 1979; Galosy *et al.*, 1980) or raised (Marshall *et al.*, 1972; Robertson *et al.*, 1978, 1980).

The hyperoxaluria has been attributed to intestinal hyperabsorption or increased renal clearance of oxalate (Pinto *et al.*, 1974; Hodgkinson, 1978; Marangella *et al.*, 1982).

The aims of this study were to identify recurrent stone formers with hyperoxaluria, to classify them further by assessing their response to a low oxalate diet and to determine dietary and other metabolic risk factors. The association and prevalence of these other abnormalities have not been adequately documented in patients with MHO. Risk factors

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were considered to be hypercalciuria induced by excessive calcium and/or sodium ingestion which normalised on dietary manipulation; absorptive or renal hypercalciuria as assessed by abnormal calcium: creatinine ratios (Pak *et al.*, 1975) and hypocitraturia (< 2.1 mmol/24 h) (Laminski *et al.*, 1990). In addition, hyperuricosuria (defined as > 4.4 mmol/24 h) was considered to be a risk factor.

Patients and Methods

A group of 207 consecutive patients with recurrent calculi attending the metabolic stone clinic at the Johannesburg Hospital were screened for hyperoxaluria. A detailed dietary assessment of calcium, oxalate and protein ingestion was obtained by our dietician. Dietary calcium, oxalate and protein excess was defined as > 1000 mg/day, > 200 mg/day and > 1.25 g/kg/day respectively. Forty patients with MHO were identified and classified as follows: dietary hyperoxaluria (DHO, n = 18) defined as patients whose urinary oxalate returned to normal on a low oxalate diet; idiopathic persistent hyperoxaluria (PHO, n = 10), patients whose urinary oxalate was persistently raised and, although it decreased, failed to return to normal on diet; idiopathic intermittent hyperoxaluria (IHO, n = 12), patients whose urinary oxalate was intermittently raised, diet having no effect.

Two control groups were chosen: 40 age- and sex-matched recurrent calcium stone formers without hyperoxaluria formed the stone control group (SC) and 56 normal controls without stones formed the normal controls (NC). Patients with gout, purely infective stones, obstructive uropathy or renal tubular acidosis were excluded. No patient had any gastrointestinal pathology. In addition, none of the patients was on sodium cellulose phosphate, thiazides or ingesting large amounts of vitamin C.

Standard laboratory methods were used to measure 24-h urinary sodium, calcium, phosphorus, creatinine and uric acid. Urinary citrate was measured enzymatically using the Boehringer Mannheim citric acid kit (Warty *et al.*, 1984). Urinary oxalate was measured enzymatically using the Boehringer Mannheim oxalic acid kit (Cannon *et al.*, 1975). Calcium: creatinine ratios were performed according to the method of Pak *et al.* (1975). The tests were performed on the patient's usual diet and repeated on a low oxalate diet; 24-h urine collections were carried out in each patient from 4 to 8 times. Renal calculi were analysed using a standard qualitative wet chemical technique.

Statistical evaluations were made using paired on non-paired Student's t tests, chi-squares (χ^2) with Fisher Exact corrections and correlation coefficients where indicated.

Results

Of the 207 patients studied, 40 (19.3%) had MHO. There was a significantly greater oxalate intake in the DHO and PHO groups than in the SC or NC groups ($P < 0.03$). There was no significant difference in oxalate intake in the IHO group compared with either group of controls (Table 1). Urinary oxalate excretion in μmol/24 h was significantly increased in all groups of patients with hyperoxaluria, *i.e.* DHO, PHO or IHO versus SC and NC ($P < 0.001$) (Table 1). The PHO group had the highest urinary oxalate excretion, and although their urinary oxalate decreased when placed on a low oxalate diet, it still remained higher than that of any of the other groups ($572.93 \pm 132.68 - 445.05 + 88.70$) ($P < 0.03$) (Table 1). This presumably reflected the fact that the initial dietary intake of oxalate was higher in these patients. When placed on dietary oxalate restriction, only DHO patients lowered their oxalate excretion to within the normal range ($420 \pm 71.5 - 261.44 \pm 27.71$) ($P < 0.0005$). In the IHO group, oxalate restriction had no effect on oxalate excretion ($344.71 \pm 32.51 - 329.00 + 35.27$) (NS). The Figure demonstrates the intermittency and lack of correlation with diet of the hyperoxaluria in 2 of these patients. No difference was found between oxalate intake or urinary oxalate excretion in the SC versus NC groups.

There were no significant differences in the prevalence of dietary abnormalities, hypocitraturia or abnormal calcium: creatinine ratios between the stone formers with MHO or the stone controls (Table 2). Hyperuricosuria was found in 12 patients with MHO but only in 3 stone controls ($\chi^2 = 6.45$, $P < 0.001$).

There were no significant differences in the number of calculi passed per year between the MHO patients, *i.e.* DHO, idiopathic hyperoxaluria (PHO and IHO) and the SC group (1.15 ± 0.95, 0.88 ± 0.72 and 0.94 ± 1.10 respectively).

In 11 patients with DHO 18 calculi were analysed; 61% of these were calcium oxalate, 17% were calcium phosphate and 22% contained other mixtures. Twenty-three calculi were analysed in 13 patients with idiopathic hyperoxaluria (PHO and IHO); 39% of these were calcium oxalate, 39% were calcium phosphate and 23% contained other mix-

Table 1 Daily Oxalate Intake and 24-h Urinary Oxalate Excretion

Group	No.	Oxalate intake (mg/24 h)	Urinary oxalate (μmol/24 h)		
			Usual diet		Oxalate restriction
DHO	18	224.96* ±117.08	420.90‡ ± 71.50		261.44* ± 27.71
				$P<0.0005$	
IHO	12	132.58 ± 90.78	344.71‡ ± 32.51		329.00‡ ± 35.27
				NS	
PHO	10	231.44* ± 99.02	572.93‡ ±132.68		445.05‡ ± 88.70
				$P<0.03$	
SC	40	145.38 ±107.23	215.39 ± 70.32		ND
NC	56	141.20 ±105.39	208.60 ± 89.93		ND

Values expressed as means ± SD. Statistical differences from controls using non-paired *t* test are shown, $*P<0.03$, $‡P<0.001$. DHO = dietary hyperoxaluria. IDO = intermittent idiopathic hyperoxaluria. PHO = persistent idiopathic hyperoxaluria. SC = stone controls. NC = normal controls. ND = not done.

tures. Twenty-four patients in the SC group had 32 stones analysed, of which 72% contained calcium oxalate, 16% were calcium phosphate and 13% contained other mixtures. All patients who formed a calcium phosphate calculus had also formed 1 or more calcium oxalate calculi.

There was no difference in the prevalence of hypertension or family history of renal calculi in the stone patients with hyperoxaluria and those without. There was an increased prevalence of diabetes mellitus in the stone formers with MHO (8 patients) compared with the stone controls (1 patient) ($\chi^2=6.13$, $P<0.014$). Only 1 patient with intermittent and 2 with persistent hyperoxaluria had an increased daily protein intake. There was no correlation between protein intake and oxalate excretion in patients with MHO ($r=-0.01$, NS).

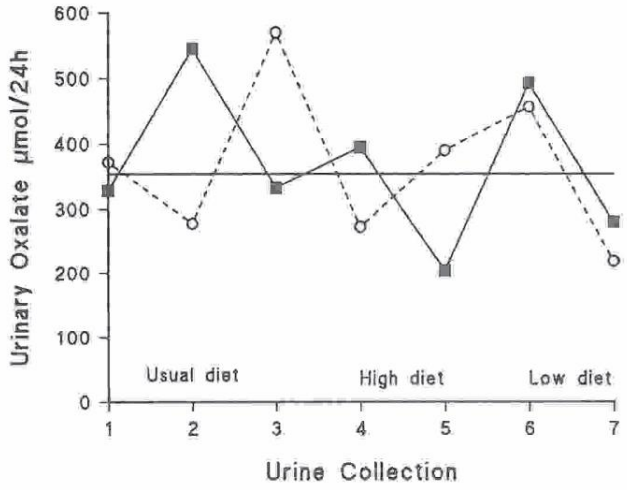

Fig. Pattern of urinary oxalate excretion in 2 patients with intermittent hyperoxaluria on usual, high and low oxalate diets. Twenty-four hour urine collections 1, 2 and 3 were on usual diet, 4 and 5 on high oxalate diet and 6 and 7 on low oxalate diet. ■——■ = patient PS. ○- - - -○ = patient LT.

Discussion

This study has permitted the definition of 2 groups of patients with recurrent calcium renal calculi and mild hyperoxaluria, namely those whose condition was related solely to diet and those with mild idiopathic hyperoxaluria. The latter group could be further subdivided into patients with either persistent or intermittent hyperoxaluria. The PHO patients had a mild dietary component, without urinary oxalate ever completely returning to normal, whereas diet had no effect on the group with IHO. Thus 19% of recurrent stone formers screened had hyperoxaluria and in 45% of these, dietary oxalate restriction reversed the defect.

The mechanisms of hyperoxaluria in the idiopathic group are not clear. None of the patients had any gastrointestinal abnormalities. MHO is unlikely to be due to a pyridoxine deficiency, which has been reported to cause hyperoxaluria in both man and animals (Yendt, 1983). None of the symptoms of pyridoxine deficiency was present in

Table 2 Other Risk Factors in Patients with Hyperoxaluria

		Abnormal Ca/creat			
Group	*NMA**	*R = renal*	*A = absorp*	*Hypocitraturia*	*Hyperuricosuria*
DHO	7 (39)		1A (6)	9 (50)	4 (22)
Idio HO	5 (23)	4R (18)	4A (18)	7 (32)	8 (36)
SC	15 (38)	3R (8)	6A (15)	17 (43)	3 (8)

Expressed as no. of patients (% patients).
*NMA: No metabolic abnormality, but includes reversible dietary excess, *i.e.* increased calcium and/or sodium intake.
DHO = dietary hyperoxaluria. Idio HO = idiopathic hyperoxaluria (includes intermittent and persistent hyperoxaluria). SC = stone controls.

these patients. Previous investigations in patients with MHO have shown increased xanthuric acid excretion after tryptophan loading which was corrected by pyridoxine, which is indicative of pyridoxine deficiency (Gershoff and Prien, 1960). Since high doses of tryptophan and unreliable methods for xanthuric acid measurement were employed, however, the interpretation of these results is in doubt (Yendt and Cohanim, 1988). Some investigators have suggested that MHO is secondary to absorptive hypercalciuria. Hyperabsorption of calcium results in less calcium to bind oxalate in the gut, so that more oxalate is available for colonic absorption (Marangella *et al.*, 1982). Four of our patients with idiopathic hyperoxaluria had this disorder but 18 did not. Furthermore, 6 of the stone controls also had absorptive hypercalciuria without hyperoxaluria, so this explanation seems unlikely. It would seem possible that renal hypercalciuria could also result in hyperoxaluria. Patients with this disorder leak calcium from the kidney, resulting in parathyroid hormone activation, increased formation of 1,25 $(OH)_2D_3$ and increased absorption of calcium from the gut, theoretically permitting an increase in oxalate absorption. However, although 4 of the patients with MHO had renal hypercalciuria, 14 did not; 3 of the stone controls also had renal hypercalciuria without hyperoxaluria, so making this explanation unlikely.

A high intake of animal protein has been implicated as a cause of mild hyperoxaluria, particularly intermittent hyperoxaluria (Yendt and Cohanim, 1988). Protein intake was increased in only 3/22 patients with idiopathic and in 1/12 with intermittent hyperoxaluria. In addition, no correlation between protein intake and urinary oxalate was found in either the PHO or the IHO patients.

Gill and Rose (1986) described an entity which they called "mild metabolic hyperoxaluria" in which there are modest increases in both urinary oxalate and glycolate. This disorder appears to be a different entity from classical primary hyperoxaluria because of the negative family history, onset in adult life, occurrence predominantly in males and the intermittent nature in some patients. Our idiopathic hyperoxaluric patients may have an underlying metabolic abnormality similar to that described by Gill and Rose (1986). However, we did not measure urinary glycolate. The aetiology of this condition is unknown but may result from slightly decreased activity of the peroxisomal enzyme alanine: glyoxylate transaminase, which is lacking in patients with primary Type I hyperoxaluria. Erythrocyte oxalate flux in these patients may also be abnormal, reflecting an increased oxalate uptake. This abnormality has been reported by Baggio *et al.* (1986) in idiopathic calcium oxalate nephrolithiasis. However, the association of this disorder with elevated urinary oxalate excretion was not reported and so needs to be studied in this group of patients.

Although renal and absorptive hypercalciuria and hypocitraturia could not be incriminated in the causation of the hyperoxaluria, these abnormalities were present in 25/40 patients (63%) and so presented additional risk factors for stone formation. Of interest is the fact that these additional risk factors were present in a similar proportion in the stone controls (26/40, 65%). Hyperuricosuria, which has been shown to salt out oxalate, resulting in calcium oxalate precipitation even at normal levels of urinary oxalate, would constitute an important risk factor in these patients (Grover *et al.*, 1990). Twelve of our patients with mild hyperoxaluria had hyperuricosuria, a significantly increased prevalence ($P < 0.001$) compared with the stone controls, thus greatly increasing their risk of stone formation. In fact, hyperoxaluria as the sole metabolic abnormality was present in only 5/22 patients (23%) with idiopathic hyperoxaluria.

It is noteworthy that a proportion of calculi

analysed consisted of calcium phosphate without oxalate. Thus the formation of such calculi should not necessarily preclude the search for possible MHO. The reason for this is obscure. None of our patients had renal tubular acidosis, hyperparathyroidism or urinary tract infections.

In conclusion, 19% of patients with recurrent calcium stone nephrolithiasis had mild hyperoxaluria defined as urinary oxalate >350 μmol/24 h. In 45% of these dietary manipulation had a beneficial effect. The mechanism of idiopathic hyperoxaluria is unclear. This study suggests that hyperuricosuria might be an important additional risk factor for calcium oxalate nephrolithiasis. However, the exact mechanism by which this occurs has not been addressed in this report.

Further investigations, in which glycolate excretion and oxalate flux are measured, are in progress in our MHO patients and it is hoped that further light will be shed on the mechanism of this interesting and common condition.

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6.1.2.1.1 Key aspects

This publication is aimed at identifying the levels of oxalate in multiple 24-hour urine collections which, if present in excess, represents a major inorganic lithogenic risk factor for CaOx crystal precipitation. A total of 207 consecutive stone patients were evaluated in the study, from which 40 RCaOx stone controls without hyperoxaluria and 40 patients with various sub-types of “mild” metabolic hyperoxaluria were chosen. Patients with genetic causes and secondary causes of hyperoxaluria were excluded as were patients with infective stones, gout, obstructive uropathy and renal tubular acidosis. A cohort of 56 normal controls was included in this study.

6.1.2.1.2 Discussion

This study aimed to assess the presence and lithogenicity of hyperoxaluria (HO) in patients with RCaOxSF. Apart from the dietary varieties of HO (diets low in Ca, high in oxalate or Vit C), the occurrence and pathogenesis of “mild hyperoxaluria” in idiopathic stone disease was and still is controversial. Urinary oxalate levels in these patients have been found to be normal. [243, 244] However, subsequent to this study, the oxalate estimation has been revised and so it was considered of paramount importance to research the topic using updated techniques describing what is called “mild metabolic hyperoxaluria”. In addition, and in retrospect, a very small group of patients in this cohort possibly might have represented patients with HO associated with a genetic defect of the GIT microbiome i.e. deficiency of intestinal oxalobactor formigines associated with normal oxalate handling in the small and large bowel. [82] This defect may also (speculatively) be present after frequent usage of various broad-spectrum antibiotics and needs to be studied further in South Africa. [25]

In addition to oxalate estimations the paper analysed the dietary causes mentioned above as well as other “risk factors” i.e. the concomitant presence of hypocitraturia, hypercalciuria and hyperuricosuria. There were two control groups; 56 normal volunteers and 40 “stone controls” who did not have hyperoxaluria. Controls and patients were whites only. Of the RCaOxSF (40 stone controls), 18 (45%) of the patient cohort had pure dietary HO i.e. after dietary control the oxalate levels normalized. The other 22 (55%) had various causes of HO. To calculate the various non-dietary

subgroups of HO the “true” patient cohort comprised of 22 patients; 12 had so-called intermittent HO and 10 were diagnosed as persistent HO. This subdivision was made on the finding that the intermittent group had some improvement on low oxalate diets which, following the diet was associated with occasional urinary oxalate rates falling outside the norm. An inherent problem with this association was the inability to check the adherence to a low oxalate regimen. In fact, so many foodstuffs contain oxalate that it becomes difficult to administer a true low oxalate diet for patient adherence (see para. 3.2.3, p. 21).

As discussed previously, [83] the association of hyperoxaluria with other subgroups was present. At the time of this paper, all patients were given an oral Ca load in order to diagnose what was then termed “absorptive hypercalciuria”. Eleven absorptive and seven idiopathic or “renal” hypercalciurias were found. As mentioned, this differentiation is at present debatable (see limitations). Several dietary aberrations have been shown, after dietary correction, to reverse this so-called “absorptive” subgroup (see para. 3.2.1.1, p. 15). If the entity of mild hyperoxaluria is equivalent to that which Rose et al (1988) [245] named “mild metabolic hyperoxaluria”, then further extrapolations can be made. By measurement of both plasma and urinary glycolate, raised levels would suggest that neither over-ingestion nor over absorption are the responsible mechanisms [32], but rather a deficiency of the normal GIT microbiome. This possibility needs to be investigated (see para. 8.1.2, point B.iv, p. 152).

Even though the fact that this paper poses more questions than answers, it nevertheless was a novel approach with “cutting edge” research at the time when it was performed and most importantly patient profiles were compared with a very large cohort of prospectively studied normals – a strong positive factor in favour of the protocol.

6.1.2.1.3 Study limitations

1. The methods for oxalate measurements have been altered recently which requires further attention.
2. No mention was made of genetic defects which might have been present in the gut microbiome.

3. The subgroups of hyperoxaluria were divided into intermittent hyperoxaluria and persistent hyperoxaluria which had not been previously mentioned in the literature. These findings may be fallacious (see point 4 below).
4. Neither plasma nor urinary levels of glycolate were measured.

6.1.2.1.4 Recommendations for future research

1. Repeat this study in black, white and Indian cohort with CaOx nephrolithiasis using the updated Boehringer Manheim kit. Care must be centred on checking whether the standard diet conditions have been adhered to.
2. Assessment of the GIT microbiome for oxalobacter formigenes as part of study recommendation in point 1.
3. Measurement of plasma and urinary glycolate as recommended in point 1
4. The use of stiripentol for the treatment of primary hyperoxaluria and CaOx nephropathy. A new anti-epileptic drug (stiripentol) which is used in cases of refractory inherited epilepsy has been shown to inhibit oxalate production by the liver. Trials are underway for its use in primary oxalosis as well as ethylene clycal induced hyperoxaluria. [246] There is still a long way to go with respect to its clinical relevance, but if effective and safe future studies could also target its use in patients with recurrent CaOx nephrolithiasis. The subject has also been well reviewed by Wyatt and Drűke (2019). [247]

6.1.2.2 Publication 4 [231]

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Lithogenic risk factors in normal black volunteers, and black and white recurrent stone formers

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Objective To compare lithogenic risk factors in normal black volunteer men (BN), male black stone formers (BSF) and male white recurrent stone formers (WSF); in addition, the differential diagnoses in the stone formers were compared to determine if the causes of renal stones differed in the two groups.

Subjects and methods The study included 22 BN, 22 consecutive BSF and 122 consecutive WSF seen over a 10-year period. Each subject was assessed by a thorough medical history, dietary analysis and a full serum and urinary biochemical evaluation.

Results Although the WSF were significantly more overweight ($P<0.001$) and obese ($P<0.001$) than the BSF, neither group had values significantly different from those of their respective normal populations. There was no significant difference in serum levels of Ca, phosphorus, ionised Ca, calcitriol and alkaline phosphatase in BSF and WSF, but levels of parathyroid hormone tended to be ($P<0.1$) and calcitriol was significantly higher in BSF than WSF ($P<0.03$). The BSF urinary excretion levels tended to be more like that of the WSF, with the following pattern present: BN < BSF < WSF, except for citrate, where BSF < BN < WSF. No dietary or metabolic abnormality was found in 9% of BSF and 5% of WSF. Pure dietary lithogenic risk factors were present in 9% of BSF and 21% of WSF. The prevalence of metabolic risk factors was similar in the two groups but the distribution differed. Renal hypercalciuria, absorptive hypercalciuria and mild metabolic hyperoxaluria were 2.5–3 times more prevalent in the WSF.

Conclusion Although the urine of BSF tends to be more like that of WSF, the lithogenic risk factors present differed and were less severe. The increase in urinary calcium excretion, coupled with a lower citrate excretion, may be the most important reason for stone formation in the black population. An increased protein intake may also play a role. In this small group of BSF there was no family history of urolithiasis, supporting the view that there may be a more prominent genetic influence in WSF.

Keywords Urolithiasis, black, white, stone formers, lithogenic risk factors

Introduction

Recurrent kidney stones occur in 8–10% of white men; 40 years ago, nephrolithiasis was virtually unknown in the black population of South Africa [1]. Stones were mainly struvite, usually secondary to bilharzia or urinary tract infection, and often found in the bladder [2]. Recently the incidence has increased, but it is still involves < 1% of the population [3]. The stones are now predominantly calcium oxalate (CaOx) and from the upper urinary tract [4]. Thus we compared lithogenic risk factors in urbanized normal black volunteer men (BN), male black stone formers (BSF) and male white recurrent stone formers (WSF). In addition, the differential diagnoses in the stone formers were compared to determine if the causes of renal stones differed in the two groups.

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Subjects and methods

The study comprised 22 black hospital and factory workers engaged in activities that were not labour intensive (BN), 22 consecutive BSF and 122 consecutive WSF, the last two groups being referred over a 10-year period; these groups were compared with each other and with the controls. Only men were included and any patients with hyperparathyroidism, cystinuria, renal tubular acidosis and medullary sponge kidney were excluded.

A thorough medical history was taken in each subject, which included date of the first stone episode and the number of stones previously passed. Each BSF and WSF

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underwent renal ultrasonography and tomography, and excretory urography to determine the number and size of residual stones and to exclude medullary sponge kidney. A detailed dietary history of the intake of sodium (Na), calcium (Ca) and oxalate was taken by an experienced dietician. In addition, the ingestion of protein, phosphate, carbohydrate and fat was recorded in the BSF and WSF.

A full biochemical evaluation was undertaken; briefly, the urinary Ca:creatinine ratio was determined while fasting and after a 1 g load of Ca, as described by Pak *et al.* [5]. Standard laboratory methods were used to measure serum Ca, phosphate, ionized Ca (iCa), creatinine, alkaline phosphatase (ALP) and uric acid. Serum parathyroid hormone (PTH) was measured using a chemiluminescent enzyme assay (cat. no. LKPH1, Immulite Corp. Diagnostics Products Corporation, Los Angeles, CA) and calcitriol was measured using a Nichols Institute radioimmunoassay (cat. no. 40–6090). Two 24 h urine samples were collected, one during a weekend, which highlighted the 'weekend effect' [6] and one over a weekday. Urinary volume, Ca, Na, creatinine, urate and phosphate were measured using standard laboratory methods. Urinary citrate was measured using a modification of the Boehringer Mannheim (Mannheim, Germany) citric acid kit [7] and urinary oxalate using the Boehringer Mannheim enzyme assay (cat. no. 755 699). Specimens with bacterial contamination assessed by measuring nitrite with a dipstick test (Combur 9, Boehringer Mannheim) were discarded. The number of nitrite-positive specimens as a proportion of total urine specimens in BN and BNF were no different to that found in WSF. All available calculi were sent for wet semiquantitative chemical analysis using a Merckognost kit (cat. no. 11003, Merck, Sharpe & Dohme, Darmstadt, Germany).

The following categories were diagnosed:

Dietary: (i) hypernatriuria with concomitant hypercalciuria ($\geq$0.1 mmol/kg/day), i.e. hypercalciuria which normalized on a 100 mmol/day Na diet; (ii) hypercalciuria, i.e. that which normalized on a standard calcium diet (0.8–1.0 g/day); (iii) hyperoxaluria ($\geq$ 350 µmol/day), i.e. that which normalized on a low oxalate diet; (iv) low urinary volume (< 1 L/day).

Metabolic: (i) renal hypercalciuria (RHC), i.e. renal Ca:creatinine ratios and hypercalciuria; (iii) absorptive hypercalciuria (AHC), i.e. absorptive Ca:creatinine ratios with or without hypercalciuria; (iii) mild metabolic hyperoxaluria (MMHo), i.e. hyperoxaluria; (iv) hypocitraturia (urinary citrate of < 2.4 mmol/day). The results were expressed as the mean (SD), with unpaired *t*-tests, chi-squared tests and correlation coefficients, as appropriate, used to assess any differences.

Results

The demographic data are shown in Table 1. There was no difference in age between the BN, BSF and WSF for age at the first stone episode. However, the WSF were significantly older at the first visit to the clinic than both the BN and the BSF. White normal subjects (data from [7]) and WSF were significantly heavier and had a significantly higher body mass index (BMI, weight per height squared) than the BN and BSF. The BMI is the best indicator of ideal body weight; a value of > 27 indicates overweight and > 30 indicates obesity. Table 2 gives the percentages of overweight and obesity in the normal black and white populations in South Africa, compared with the present BSF and WSF [8,9].

Serum results are also given in Table 1: there was no difference in Ca, phosphate, ALP and iCa in BSF and WSF. Although still normal, PTH levels in BSF tended to be higher ($P < 0.1$) and calcitriol significantly higher than WSF ($P < 0.03$). Serum urate in BSF was significantly higher than in BN, whereas serum phosphate was significantly lower ($P < 0.03$). Creatinine and urate were significantly higher in both BSF and WSF than BN.

The 24 h urine results are also shown in Table 1: the urinary volume was significantly larger in both BSF and WSF than in the BN. The urinary Na level was significantly higher in BN and WSF, reflecting a higher intake of salt, as was shown on dietary analysis (Table 1). Calcium excretion was significantly higher in BSF than in BN and approached levels found in normal white volunteers [7] (Table 1). In WSF, urinary Ca was significantly higher than in BSF and BN. Urinary phosphate also showed the pattern WSF > BSF > BN, with significant differences between BN and WSF, and between BSF and WSF. Urinary urate was significantly higher in BSF, with levels approaching those found in WSF. Citrate excretion in BSF was the least of the three groups, although not significantly lower than BN. The WSF had a significantly higher urinary citrate excretion than both BN and BSF. Urinary oxalate excretion also gave the pattern WSF > BSF > BN, with WSF excreting significantly more oxalate than BN ($P < 0.04$) (Table 1), in contrast to dietary oxalate, where WSF had a significantly lower oxalate intake than BN ($P < 0.001$) and tended to have a lower intake than BSF. The WSF ate more Ca ($P < 0.05$) than the BN, although at least 40% of them were eating < 600 mg/day. There was no correlation in BN between urinary Na and Ca excretion but there was a significant correlation in both BSF and WSF. There was no significant difference in protein or phosphate intake in BSF and WSF.

Five stones were available for analysis in the BSF and all contained CaOx; of the 65 stones available for analysis in the WSF, 82% were CaOx and 18% were calcium

Table 1 Demographic data, serum values, 24-h urine values and dietary intake in the BN, BSF and WSF groups

Mean (SD) variable	*BN*	*BSF*	*WSF*
Number	22	22	122
Age, years	32.2 (9.2)	35.4 (8.8)	35.5 (12.2)
Age at first visit	-	38.6 (8.8)*	45.1 (12.3)‡
Weight (kg)	65.2 (13.0)	67.6 (10.1)‡	80.8 (12.6)‡
Body mass index	23.0 (4.7)	23.6 (3.7)†	26.1 (3.8)‡
n (%) overweight	4 (18)	6 (27)	72 (59)
n (%) obese	2 (9)	1 (5)	19 (16)
Serum			
Ca (mmol/L)	2.37 (0.08)	2.35 (0.11)	2.40 (0.12)
Phosphate (mmol/L)	1.00 (0.12)*	0.90 (0.17)	0.96 (0.66)
Ionised Ca (mmol/L)	1.25 (0.04)	1.24 (0.04)	1.23 (0.12)
PTH (pmol/L)¶	3.25 (1.46)	3.53 (1.14)	3.11 (1.43)
Calcitriol (pmol/L)	82.6 (27.8)	93.8 (33.6)*	76.3 (27.1)
Urate (mmol/L)	0.31 (0.05)*	0.36 (0.09)	0.40 (0.09)‡
Creatinine (μmol/L)	87 (10)‡	105 (17)	101 (17)‡
ALP (U/L)	ND	81.4 (25.0)	76.6 (22.6)
24 h urine			
Volume (mL)	1138 (492)*	1582 (735)	1557 (608)†
Na (mmol)	154 (57)	132 (57)†	164 (54)
Ca (mmol) [w 4.03 (1.73)]	1.27 (0.84)‡	3.65 (2.01)‡	5.83 (2.70)‡
Creatinine (mmol)	12.3 (3.4)	14.3 (3.9)	16.0 (5.2)†
Phosphate (mmol)	19.4 (4.6)	24.1 (13.2)‡	34.9 (14.2)‡
Urate (mmol)	2.43 (0.42)*	2.79 (1.11)‡	3.83 (0.97)‡
Citrate (mmol) [w 3.39 (1.1)]	1.45 (1.20)	1.04 (0.70)‡	2.06 (1.13)*
Oxalate (μmol)	233 (124)	306 (151)	318 (140)*
Na vs Ca, *r*	0.30	0.45*	0.40‡
Dietary intake			
Na (mg/day)	3809 (1718)	3183 (1337)*	4437 (2144)
Ca (mg/day)	635.2 (341.1)	644.2 (397.3)	854.1 (460)*
Oxalate (mg/day)	271.2 (120.8)	188.8 (168.8)	131.9 (111)†
Protein (g/day)	ND	99 (28)	113 (39)
Phosphate (mg/day)	ND	1569 (561)	1818 (795)

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$, by *t*-test: when shown in column 1, between BN and BSF; in column 2, between BSF and WSF; and in column 3 between WSF and BN. ¶ 10 of 22 sampled in BN. ND, not done; w, white controls, from [7].

phosphate. There was no significant difference in the number of stones passed per year in the two groups, i.e. 1.09 (1.08) in WSF and 1.1 (0.42) in BSF, where the information was available.

The biochemical evaluation allowed the so-called idiopathic stone formers to be divided into various groups, i.e. dietary, metabolic or mixed dietary and metabolic subgroups [7]. No dietary or metabolic abnormality was found in two BSF and six WSF. One BSF had dietary hypernatriuria with concomitant hypercalciuria, and one had dietary hyperoxaluria. In WSF, the prevalence of pure dietary lithogenic risk factors was greater than in BSF: seven (6%) had a high salt intake with hypercalciuria, seven (6%) had dietary hyperoxaluria, two (2%) had hypercalciuria as a result of high Ca intake, two (2%) had a combination of a high-salt diet and dietary hyperoxaluria, one (1%) had a low urine volume and high salt intake and seven (6%) had a low volume as their only abnormality.

The distribution of metabolic and mixed (metabolic

Table 2 The percentage of subjects with ideal weight, overweight or obesity in the black and white population ($n = 986$), and in the present BSF and WSF

	White		*Black*	
BMI	*Normal*	*WSF*	*Normal*	*BSF*
Ideal	31	25	70	68
Overweight	49	59	22	27
Obese	20	16	8	5

White normal vs WSF, BN vs BSF, no significant difference; white normal vs BN, and BSF vs WSF, $P < 0.001$.

and dietary) lithogenic risk factors in 18 BSF and 90 WSF is shown in Table 3. RHC and AHC were three times as prevalent in WSF than in BSF: MMHo, pure or combined with hypocitraturia, RHC and AHC (total 32, 30%) was also three times as prevalent in WSF. Hypocitraturia was the most common metabolic risk factor in both groups of stone formers. Interestingly, nine of 14 of those with AHC in the WSF also had MMHo and in each of the other metabolic groups, 43–60% had hypocitraturia. In BSF, both patients with AHC and RHC and half the patients with MMHo had hypocitraturia. Six of the BSF had two diagnoses and two had three diagnoses, while 51 (42%) of the WSF had two and 10 (8%) had three diagnoses. Of the six BSF with two diagnoses, five had hypocitraturia as one of the diagnoses.

Discussion

The BSF and WSF were of similar age at passage of their first stone, although the WSF were significantly older at their first visit to the clinic. Three of 22 BSF had a single stone episode and six had many stones in the kidney but had never been aware of passing stones. This second group had experienced ongoing renal-angle pain and when they finally consulted a doctor, renal stones were found. There was no family history of urolithiasis in the BSF, whilst 25% of WSF had such a family history. Although the WSF were significantly heavier and had a greater BMI, a recent survey has shown a similar percentage of overweight and obesity in the normal black and white population in South Africa as found in the present BSF and WSF [8,9]. Thus, overweight and obesity are not contributing to the risk of stone formation. However, the dietary aberrations that lead to overweight and obesity are not necessarily those risk factors found in this group of stone formers. A high salt intake, low fluid intake and high calcium intake (in the form of calcium carbonate) would be risk factors for stone formation, but would not lead to overweight or obesity.

The trend toward a higher serum PTH may possibly be explained by the increased loss of calcium from the kidney, coupled with a lower calcium intake in the BSF than the WSF. A chronic negative calcium balance would tend in turn to stimulate the synthesis of calcitriol. The significantly higher serum creatinine in stone formers compared with sex- and age-matched controls probably reflects a higher protein intake, as well as possible mild kidney damage as a result of the recurrent kidney stones. There was no significant difference in total protein or phosphate intake in WSF or BSF (Table 1) but the recommended protein intake is 1–1.2 g/kg body weight. The WSF were ingesting 1.42 (0.47) g/kg (with 60% eating > 1.2 g/kg) and the BSF were ingesting 1.46 (0.47) g/kg (with 64% eating > 1.2 g/kg). Unfortunately, protein intake in the BN was not measured, but if assumed to be 1.2 g/kg, would have been 65 (16) to 78 (19) g/day, a value that is similar to that found on a recent survey of urban black men, of 60–80 g/day (personal communication, Dr T van der Merwe). Thus the stone formers were ingesting considerably more protein than the normal population. Of the BSF 16 (73%) had a creatinine level of > 95 μmol/L whilst 59 WSF (48%) had creatinine levels of > 103 μmol/L. Normal white male volunteers had serum creatinine levels of 89 (13.0) μmol/L [7].

The 24 h urine values of the BSF in most respects approached the values found in WSF, i.e. the solutes were either similar to the WSF or midway between those of WSF and BN. The only solute not following this trend was urinary citrate, that was lower than in BN. Hypocitraturia was diagnosed using the normal value for white controls (3.39 (1.13) mmol/day [7]) and thus this risk factor as a whole is overestimated in the BSF. The reason for the low citrate is unknown but it was not infection, magnesium or potassium deficiencies [10]. The low citrate levels present in the normal black

Table 3 The distribution of metabolic and mixed metabolic risk factors in individual BSF and WSF

	Risk factors BSF (n = 22)					*WSF (n = 122)*				
Diagnosis	*Hypocit*	*MMHo*	*RHC*	*AHC*	*Diet*	*Hypocit*	*MMHo*	*RHC*	*AHC*	*Diet*
Hypocitraturia	10	1	–	1	–	19	10	4	3	–
MMHo	–	–	–	–	–	–	8	2	6	–
RHC	–	–	–	–	–	–	–	4	–	–
AHC	–	–	–	–	–	–	–	–	1	–
Dietary	4	1	–	–	2	24	2	1	1	26
Hypocitraturia + diet	–	–	1	–	–	–	–	1	–	–
Hypocitraturia + MMHo	–	–	–	–	–	–	-	1	3	–

*Total can be > 100% because many stone formers had more than one diagnosis.

population may be further decreased in BSF by a higher protein intake (already discussed) which decreases citrate excretion. Urinary calcium excretion was significantly higher in the BSF than the BN, even though these groups had a similar calcium intake. Therefore hypercalciuria is probably underestimated in BSF as their calcium was within normal white values. Of interest, although the urinary sodium was lower in the BSF than in the BN, their urinary calcium excretion was significantly higher. The extremely low citrate levels and increased calcium levels may be an important reason for stone formation in the BSF. The increase in calcium may be partly caused by a lower ingestion of oxalate, with less calcium bound in the gut. There was a weak but significant correlation between urinary Na and Ca excretion ($P < 0.04$); this correlation would increase the risk of hypercalciuria when salt intake is high. Thus, although urinary calcium was lower than values found in WSF, it may be an important lithogenic risk factor in BSF at this level. Although urinary oxalate level was higher in the BSF than BN, with a value similar to that in the WSF (Table 1), the BSF were ingesting significantly more oxalate in their diet. Therefore it could be postulated that the black population may have a protective mechanism in the gastrointestinal tract which helps to reduce the absorption of excess dietary oxalate. More calcium may be bound to oxalate, preventing calcium and oxalate absorption in the upper intestinal tract. We postulated that a colonic anaerobic bacteria breaks down oxalate in a site too distal for calcium absorption [11]. If anaerobes were more active or more plentiful in the black population, the result would be an increase in oxalate breakdown products and higher calcium excretion in the stool, and consequently, lower absorption and thus lower urinary excretion of calcium and oxalate [11]. This postulated mechanism could be a factor in explaining the lower incidence of stones in the black population. Oxalate is considered a more important risk factor than calcium in the formation of CaOx stones [12]. Mild metabolic hyperoxaluria as a lithogenic risk factor is much less common in BSF (9%) than WSF (26%), even though more oxalate is ingested, which supports our hypothesis. Others [13] have shown that *Oxalobacter formigenes* colonies are present in the stool of humans. Urinary phosphate and uric acid were higher in the BSF, probably through an increased protein intake (discussed above). This increase in protein ingestion could be contributing to their stone formation as, biochemically, a high protein diet increases the urinary excretion of calcium, oxalate and uric acid, and reduces the excretion of citrate [14].

Dietary factors were less prevalent in BSF than in WSF (9% vs 21%); the prevalence of metabolic risk factors was similar in the two groups but the distribution was markedly different. RHC, AHC and MMHo were two to three times as common in the WSF (Table 3), possibly because the absorption of calcium and oxalate was lower, as discussed. Hypocitraturia, present in 17 of the 22 BSF, was the most common risk factor found, but as stated, is probably over-diagnosed. The reason for hypocitraturia in BN and stone formers is unknown; in WSF, hypocitraturia alone or combined with either hypercalciuria and/or hyperoxaluria plays an extremely important role in their risk for stone formation. This is borne out by the highly successful prevention of recurrence of stone formation in those patients treated with potassium citrate [15]. Some other factor present in BN but absent in BSF might augment hypocitraturia together with increased calcium to be a major cause in the formation of kidney stones in this population. A possible link with urinary pH is presently being investigated.

Therefore, although the urine of BSF is more like that of WSF, the lithogenic risk factors present differ and are less severe. The organic inhibitors, e.g. crystal matrix protein, Tamm–Horsfall protein and glycosaminoglycans, need to be examined in normal and black stone formers to determine whether it is these substances that protect the black population at large, and whether it is a reduction in the level of these substances that leads to stone formation.

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6.1.2.2.1 Key aspects

This paper investigated the lithogenic risk factors in three different cohorts: (a) Twenty-two black normal volunteers, and; (b) twenty-two black CaOxSF plus 122 consecutive white SF referred to the clinic over a ten-year period. Only males were included. Patients with secondary causes of RCaOxSF were excluded. The effects of diet and increasing BMI were also recorded in the three cohorts.

6.1.2.2.2 Discussion

Forty or more years ago, CaOx nephrolithiasis was virtually unknown in the black population of South Africa. [232, 233, 248] Recently the incidence has risen slightly but still occurs in <1% of the population. [234]

Although the study was in most ways similar to other studies presented in this thesis, it differed in that it was, and still is, the only study where CaOxSF has been analysed in black South Africans. Also, all of the 22 black stone formers were males. Furthermore, where chemical stone analysis was carried out, all of the calculi in the black subjects consisted of CaOx only whereas in the white population 82% of the stones were of CaOx and 18% of CaP.

In addition, these 22 men with CaOxSF all had completely negative family histories of any type of nephrolithiasis. The above and other important clinical discrepancies are summarized in point form below

- All CaOxSF occurred in men only
- No stones in the black population were of CaP
- In the black cohort, the existence of a positive family history was conspicuous by its absence
- In the black stone former the urinary P was significantly lower than in white SF
- Coupled with this the plasma calcitriol levels were significantly lower in black SF compared with white SF
- In turn, the serum P in the black SF was also significantly lower than in black controls

Apart from comparison with the African American population, the above sequence of clinical and biochemical factors requires an explanation. Reduced levels of calcitriol will result in P "wasting". Both lower urinary P in blacks accompanied by raised FGF-23 plasma levels suggests that the expected FGF-23-driven intrarenal inhibition of calciferol conversion to calcitriol was occurring in these patients. At present this is speculative and awaits to be confirmed.

- Finally, the presence of a low incidence of obesity in the black normal and the black SF as compared to whites suggests that fructose-driven obesity and nephrolithiasis is not an important factor in South African black SF.

Therefore, apart from the Vit D / FGF-23 aspects the only other and probably the most important factor is the presence of various phenotypic polymorphisms in the TRPV-5 distal tubular calcium sensing chromosomes as has been well described in Afro-Americans. [249] (See p. 2.)

6.1.2.2.3 Study limitations

1. Variability in adherence to standard dietary requirements were also present in this study. The matter has been dealt with in the previous papers presented and so further discussion is not required.
2. Epidemiological data on CaOx /CaP SF in the South African black population is of paramount importance. In spite of the study being over a ten-year period and being the first of its kind in South African blacks, similar data is also not available from the African-American population in the USA.
3. Assessment of relative hypophosphatemia and FGF-23 estimations were not available at the time that these experiments were performed. In addition, these patients have all been lost to follow-up.
4. Dietary fructose intake was not assessed.
5. TRPV-5 polymorphisms were unheard of at the time of this experiment and so was not assessed.

6.1.2.2.4 Recommendations for future research

1. This study must be repeated taking care to adhere to and to monitor standard dietary requirements.
2. The study must encompass data from multiple sites, urban, peri-urban and rural if possible.
3. The study must be geared to investigating the possibility of “relative hypophosphataemia” using fractional excretion of P as previously described (see para. 3.2.1.4, p.18). [68]
4. Likewise, the study must endeavour to perform genetic experiments on the TRPV-5 gene as previously discussed (see para. 3.4.5, p. 39).

In conclusion the above recommendations are of paramount importance to be studied as the South African black population is progressively changing from a well-balanced and healthy diet to the more harmful “Western” type of lifestyle which is so prevalent in developed countries.

6.1.2.3 Publication 5 [119]

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CYSTINE: A PROMOTER OF THE GROWTH AND AGGREGATION OF CALCIUM OXALATE CRYSTALS IN NORMAL UNDILUTED HUMAN URINE

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ABSTRACT

Purpose: Many variables are known to be associated with the formation of calcium oxalate stones. We noted that on analysis a number of patients with calcium oxalate calculi also had cystine in the stones. Some but not all of these patients showed urinary cystine excretion slightly above the normal limits, resembling heterozygous carriers. This finding raised the question of whether some recurrent stone formers may be at risk for calcium oxalate calculi when they excrete cystine in above normal concentrations.

Materials and Methods: Pooled urine obtained from 3 pairs of age and sex matched controls was independently analyzed. Each urine sample was divided into spun and filtered, and ultrafiltered urine. A Multisizer II (Coulter Electronics Ltd, Beds, England) was used to measure particle number, diameter and volume. The metastable limit of each specimen was determined. Promotion activity was measured in spun and filtered, and ultrafiltered urine using 3 concentrations of cystine (80, 160 and 320 μmol./l.). Results were confirmed by measuring the incorporation of ^{14}C-oxalate into the crystals. Scanning electron microscopy was performed to study further the agglomerates as well as exclude cystine crystals. Each experiment was repeated 6 times. Crystalline material was collected for x-ray powder diffraction analysis.

Results: The urine metastable limit did not change with increasing cystine concentrations. Particle diameter increased significantly from 10.6 μm. in ultrafiltered urine alone to 11.6 and 13.5 μm. ($p < 0.05$) at 160 and 320 μmol./l. cystine, respectively. In addition, particle volume also increased proportionally in a dose response manner to cystine concentration. The dose of 320 μmol./l. cystine increased the crystal growth rate 52%. ^{14}C-oxalate experiments confirmed these results. Scanning electron microscopy at 500× magnification revealed no cystine crystals in any experiments performed. Furthermore, x-ray powder diffraction analysis of samples revealed that experimentally determined parameters matched reference values for calcium oxalate trihydrate but not for cystine, again confirming absent cystine in the samples.

Conclusions: Adding cystine to undiluted human urine resulted in the marked enhancement of calcium oxalate crystal precipitation. When considered with the finding of cystine in calcium oxalate stones in the noncystinuric population, this result implies that urinary cystine may be a risk factor for calcium oxalate calculi. Cystine was not observed in any calcium oxalate crystals, suggesting that the mechanism of crystal formation was a salting out effect.

KEY WORDS: urine, kidney, kidney calculi, calcium oxalate, cystine

The formation of cystine stones is usually attributed to excessive renal excretion of this extraordinary insoluble substance. However, it is not well appreciated that patients with cystinuria sometimes have stones composed of a mixture of calcium oxalate and cystine or even occasionally pure calcium oxalate.[1,2] In 1942 Lehmann and Pollock first suggested that cystine may form insoluble complexes with calcium salts in the test tube.[3]

We became interested in this possibility when we noted that small amounts of cystine were occasionally present in calcium oxalate calculi in a few of our patients who attend the clinic. In addition, we also noted that 5 of the 28 patients with homozygous cystinuria (cystine excretion greater than 250 mg./gm. creatinine or 1,800 μmol. daily) had stones containing small amounts of calcium oxalate and a further 2 had passed at least 1 pure calcium oxalate stone. From these observations we postulated that moderately elevated urinary cystine, as in individuals heterozygous for types II and III cystinuria with a cystine excretion of 100 to 200 mg./gm. creatinine or 750 to 1,500 μmol. daily, may be a risk factor for calcium oxalate stone formation. In normal controls cystine excretion is less than 100 mg./gm. creatinine or 750 μmol. daily. Furthermore, we wondered whether the level of cystine that may be present in concentrated urine in normal individuals is sufficient to precipitate calcium oxalate. To our knowledge there is no proof that calcium oxalate may be precipitated by cystine in human urine. We determined the effect of small quantities of cystine on the growth and aggregation of calcium oxalate crystals in undiluted human urine, and elucidate the possible mechanisms of cystine induced crystallization.

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METHODS

Urine collection and preparation. We collected 24-hour urine samples without preservative from 6 healthy controls with no personal or family history of renal calculous disease. All had normal renal function according to serum creatinine

and the Cockroft formula.[4] Specimens were refrigerated during collection. Aliquots were stored and sodium, calcium, oxalate, creatinine and osmolality were measured. Sodium was measured by the indirect ion selective electrode method[5] and calcium was determined by the o-cresolpthalein complex method[6] using kits (Roche Molecular Biochemical, Indianapolis, Indiana). Creatinine was measured by the Jaffé colorimetric method[7] (Roche Molecular Biochemical). Oxalate was measured enzymatically[8] using a 591-D diagnostic kit (Sigma Chemical Co., St. Louis, Missouri) and osmolality was measured using an Osmette II osmometer (Osmette Precision Systems, Inc., Ossining, New York).

Urine was centrifuged at 8,000 rpm for 15 minutes at 20C to remove Tamm-Horsfall protein, as previously described.[9] Supernatant was filtered through a 0.22 μm. Millipore filter (Millipore, Bedford, Massachusetts) and divided into 2 portions. The first half was stored as spun filtered urine. Spun filtered urine still contained most of the macromolecules and served as the positive control in our experiments. The second half was ultrafiltered using a Prep/Scale – TFF 6 feet^2 cartridge (Millipore) with a relative molecular mass of 10 kDa. (ultrafiltered urine). This technique allows the ultrafiltration of ±2 l. urine in 15 to 20 minutes. Absent protein was confirmed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Thus, in ultrafiltered urine the organic inhibitors were removed and it served as the negative control in our experiments. Spun filtered and ultrafiltered urine specimens were stored at 4C and then used for crystallization studies.

Metastable limit determination. The calcium oxalate metastable limit was determined based on the method of Ryall et al.[9] Briefly, 20 ml. samples of spun filtered urine were titrated with increasing concentrations of 0 to 120 μmoles. sodium oxalate and incubated at 37C for 30 minutes. Particles greater than 2 μm. are detected by the Multisizer II and by examining the samples under light microscopy. As defined by Ryall et al, the metastable limit is the minimum amount of oxalate necessary to induce nucleation (that is particles greater than 2 μm.) detectable by the instrument.[9]

Experiment 1 effect of cystine on metastable limit. Spun filtered urine was divided into 4 aliquots. One sample was treated as the control, while to the other 3 cystine was added in increasing concentrations of 80, 160 and 320 μmol./l. These concentrations were chosen because they are within physiological limits. The metastable limit of each sample was then determined in triplicate, as described.

Experiment 2 effect of cystine on growth and aggregation kinetics of calcium oxalate crystals. The experimental method for measuring growth and aggregation in undiluted urine was based on the method of Ryall et al.[9] In this method a standard oxalate load equivalent to 30 μmol./100 ml. urine in excess of the metastable limit is added to the urine specimens. Volume and size of the crystals and aggregates are measured at 15-minute intervals using the Multisizer II counter. Cystine (80, 160 and 320 μmol./l.) was added individually to 3, 100 ml. aliquots of ultrafiltered urine at these concentrations. The same volumes of pure spun filtered, and pure ultrafiltered urine served as the positive and negative controls, respectively. Spun filtered urine was considered the positive control since it contains all macromolecules except for Tamm-Horsfall glycoprotein, which is almost completely removed during centrifugation and filtration. On the other hand, ultrafiltered urine has no macromolecules but contains all molecules less than 10 kDa. and, hence, serves as the negative control. Urine was incubated for 120 minutes at 37C. Samples were checked for crystals by light microscopy. This procedure was repeated 6 times per experiment.

Experiment 3 ^{14}C-oxalate analysis. Aliquots of the same spun filtered and ultrafiltered urine were used. Urine samples in this experiment were treated identically to cold urine in experiment 2. To each urine sample 3.125 μCi./100 ml. ^{14}C-oxalate were added with the sodium oxalate load. Urine was incubated for 120 minutes and at 15-minute intervals 2 ml. aliquots of urine per flask were filtered over a 0.22 μm. syringe filter. Filtrate (1 ml.) was added to 10 ml. Ultima Gold LLT scintillation fluid (Packard, Bioscience Meridan, Connecticut) and counted for 10 minutes in a 2200CA Tricarb Liquid Scintillation System (Packard). This method was based on the series of Grover et al.[10] This experiment was performed twice and the mean coefficient of variance was determined. Measuring the incorporation of ^{14}C-oxalate into crystals enabled comparison with the results of experiment 2.

Experiment 4 scanning electron microscopy. A modified version of the method of Grover et al was used for preparing samples.[11] Aliquots (2 ml.) of 120-minute specimens from experiment 2 were filtered (0.22 μm.) and prepared for scanning electron microscopy. Filters were dried overnight at 37C, mounted on aluminum stubs and coated with gold for 5 to 6 minutes using an IB-3 ion coater (Eiko, Eiko Engineering, Ltd., Mito City, Ibaraki, Japan). Stubs were viewed using an S-570 scanning electron microscope (Hitachi, Hitachi, Ltd., Tokyo, Japan) at an operating voltage of 10 kV.

Experiment 5 x-ray diffraction analyses. At the conclusion of these experiments and after collecting samples for scanning electron microscopy the remainder of the urine was filtered (0.22 μm.). Filter paper was air dried and crystalline material was collected for x-ray powder diffraction analysis using an x-ray powder diffractometer (Phillips, Hamburg, Germany). This experiment was performed to test for a cystine nucleus in the crystals precipitated in the previous experiments.

Statistical analysis. The mean growth rate plus or minus standard deviation was determined per experiment. Two-sample t tests were done in samples. We used Statgraphics statistical software (Plus*Ware Product, STSC, Inc., Rockville, Maryland).

RESULTS

Effect of cystine on the metastable limit of urine. The metastable limit was determined in spun filtered samples only and it was assumed to be the same as in ultrafiltered samples. At the concentrations tested cystine did not affect the metastable limit in any urine samples. Figure 1 shows that the same amount of exogenous oxalate was required to initiate crystallization irrespective of the amount of cystine present. However, after nucleation began the quantity of particles increased with increasing cystine concentrations. In addition, after nucleation occurred crystals started to aggregate and, hence, there was a decline in crystal number at 90 μmol. oxalate. Figure 1 shows a typical example of the 3

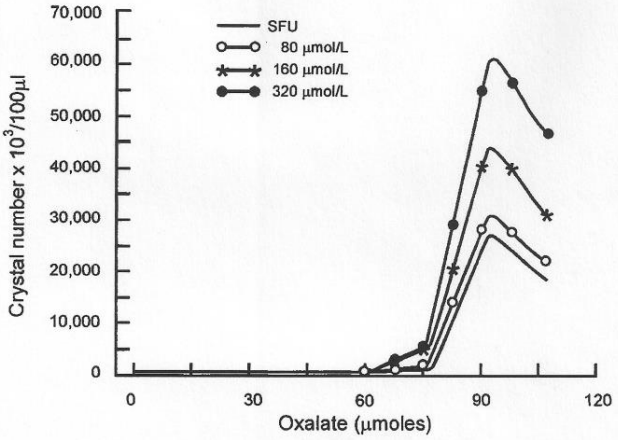

FIG. 1. Effect of cystine on urinary metastable limit. *SFU*, spun filtered urine.

experiments performed. Because the metastable limit was unaltered in the presence of cystine, an identical oxalate load was added to all samples to induce calcium oxalate crystallization.

Effect of cystine on particle volume and size. Figure 2 shows the dose response effect of cystine on calcium oxalate particle volume in ultrafiltered urine during the 2-hour incubation period. In control ultrafiltered urine volume deposition commenced after an initial 15-minute lag phase. In all samples containing ultrafiltered urine with added cystine there was an immediate increase in volume deposition but differences in volume only became apparent at 30 minutes. Notably the curves for the lower concentrations of 80 and 160 μmol./l. cystine were almost parallel, began to plateau at 105 minutes and were not significantly different at 120 minutes. At 45 minutes there was a sudden increase in crystal volume in urine containing 320 μmol. added cystine and this curve did not plateau at 120 minutes. Crystal volume was smallest in ultrafiltered urine alone and increased proportionally in a dose response manner according to cystine concentration. The mean volume of crystalline material deposited after 120 minutes in ultrafiltered urine alone was 33, 360 $\mu m.^3/\mu l.$, while corresponding values in ultrafiltered urine with 80, 160 and 320 μmol./l. added cystine were 40,970, 42,190 and 49,020 $\mu m.^3/\mu l.$, respectively. These values represent crystal volume increases of 22.8%, 26.5%, and 46.9%, respectively, relative to the ultrafiltered control. Figure 2 also shows the increase in the crystallization rate during the 2-hour incubation period. The gradient of the linear section of the curve was used as an index of crystal growth. In the urine sample shown adding 320 μmol./l. cystine increased the growth rate 46% compared with ultrafiltered urine. The lower concentrations of added cystine also increased the growth rate but to a lesser degree.

Figure 3 shows crystal volume and crystal size distribution in the samples after 120 minutes of incubation measured using the counter. Adding cystine caused a dose response shift in crystal size to greater diameters. Mean crystal diameter in ultrafiltered urine containing no cystine was 10.6 μm., which increased steadily to 11.2, 11.6 and 13.5 μm. at corresponding cystine concentrations of 80, 160 and 320 μmol./l. At the lower dose of 80 μmol./l. the increase was not significant ($p = 0.12$) but at the higher doses of 160 and 320 μmol./l. the increase was significant ($p < 0.05$). Figure 3 shows only 1 of the 3 experiments performed. However, results were similar in all 3 experiments (table 1).

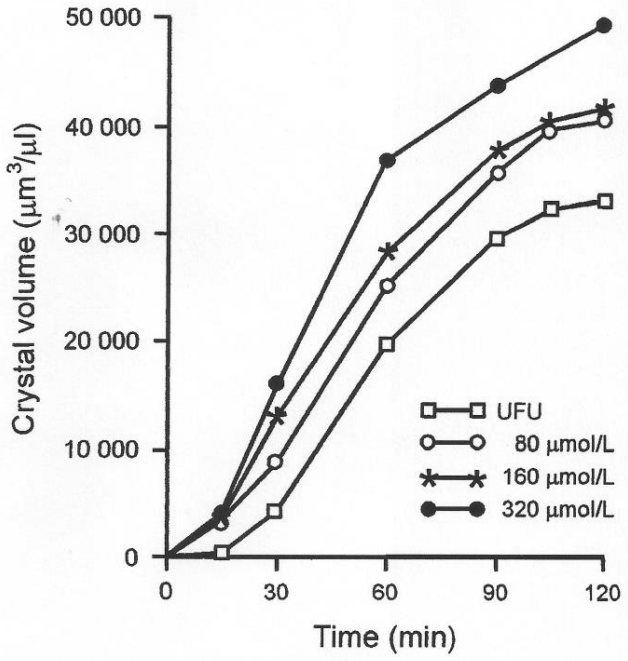

FIG. 2. Change in total volume of calcium oxalate crystal particles greater than 2 μm. after 30 μmol. oxalate load above metastable limit during 2-hour incubation. *UFU*, ultrafiltered urine.

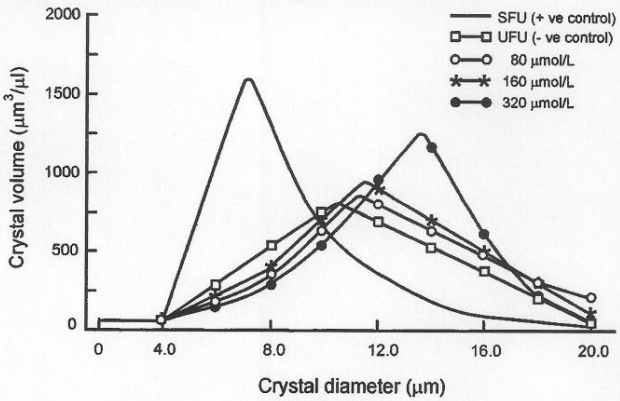

FIG. 3. Particle size distribution 120 minutes after oxalate loading in ultrafiltered urine (*UFU*) alone and in aliquots of ultrafiltered urine spiked with 80, 160 and 320 μmol./l. cystine. *SFU*, spun filtered urine. + *ve*, positive. − *ve*, negative.

Measurement of calcium oxalate deposition by ^{14}C-oxalate. Figure 4 shows the percent decrease in soluble ^{14}C-oxalate during the 120-minute incubation period. To normalize data values are presented as the amount of ^{14}C-oxalate remaining in solution relative to time zero. The rate of disappearance of ^{14}C-oxalate from solution increased as the concentration of added cystine increased compared with ultrafiltered urine alone. At 120 minutes a mean of 61.7% of ^{14}C-oxalate remained in solution in the ultrafiltered sample with no added cystine. Corresponding values in the ultrafiltered sample containing added cystine at final concentrations of 80, 160 and 320 μmol./l. were 60.4%, 59.5% and 56.7%, respectively. ^{14}C-oxalate experiments showed that the mass of calcium oxalate precipitated increased with increasing concentrations of cystine. These findings add further evidence to support the notion that cystine acts as a promoter of calcium oxalate crystal precipitation (fig. 2).

These findings were confirmed by scanning electron microscopy. Figure 5 shows typical crystals isolated after 2 hours of incubation from spun filtered and ultrafiltered urine, and ultrafiltered urine supplemented with 80 and 320 μmol./l. cystine. In all cases calcium oxalate monohydrate and calcium oxalate dihydrate crystals were observed. Figure 5, *A* shows spun filtered urine in which individual crystals were observed but no aggregates were noted. Crystals from ultrafiltered urine with no added cystine were slightly aggregated (fig. 5, *B*). Also, the size of individual crystals did not differ much at the lower concentration of added cystine (fig. 5, *C*) but it was slightly greater at 320 μmol./l. The addition of cystine to ultrafiltered urine greatly increased the degree of crystal aggregation. At 320 μmol./l. scanning electron microscopy revealed a large tight aggregate that can be described as a small calculus (fig. 5, *D*). Therefore, the variation in modes of the size distribution curves was clearly attributable to increased crystal aggregation (fig. 3).

X-ray diffraction analysis. In this method 2 parameters were determined from the x-ray diffraction pattern, namely interplanar spacings within the crystal structures in Å and the relative intensity. The crystal composition was then deduced by matching measured parameters against published reference parameters for different compounds.[12] Hence, this method is often referred to as fingerprint analysis.

TABLE 1. *Change in mean particle diameter and volume in all experiments performed*

Experiment No.	Spun Filtered Urine	Ultrafiltered Urine	Cystine (μmol./l.)		
			80	160	320
		Mean particle diameter change (μm.)			
1	7.2	10.6	11.2	11.6	13.5
2	7.3	10.9	11.4	11.9	12.1
3	6.8	11.2	12.4	12.4	12.9
		Mean particle vol. change (μm.³/μl.)			
1	158,531	77,231	79,508	93,763	125,054
2	124,288	70,757	97,133	99,096	119,570
3	57,695	50,056	76,961	75,270	78,702

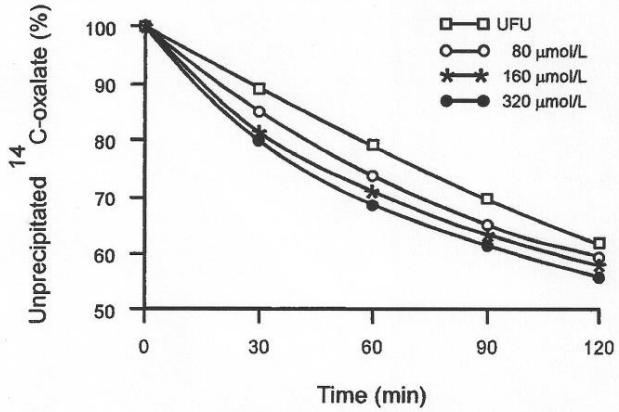

FIG. 4. By ^{14}C-oxalate measured calcium oxalate deposition in ultrafiltered urine (*UFU*) supplemented with 0, 80, 160 and 320 μmol./l. cystine.

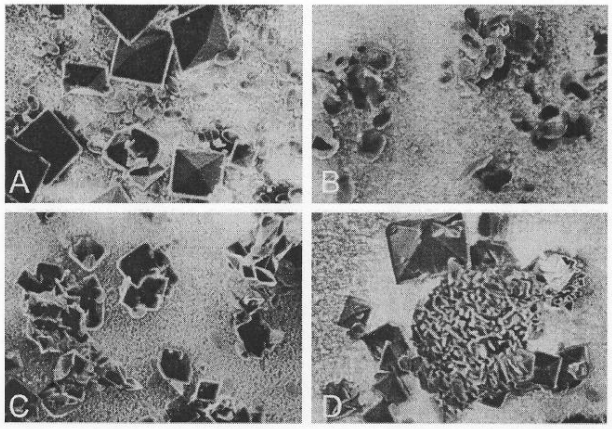

FIG. 5. Scanning electron micrographs (×500 magnification) of crystals precipitated during 2-hour incubation. *A*, spun filtered urine. *B*, ultrafiltered urine. *C*, aliquots of same ultrafiltered urine supplemented with 80 μmol./l. cystine. *D*, aliquots of same ultrafiltered urine supplemented with 320 μmol./l. cystine.

Table 2 lists experimentally determined parameters for the crystalline material obtained in experiment 5 of our study. Reference values for cystine and calcium oxalate trihydrate are also shown. Notably experimentally determined parameters matched those of calcium oxalate trihydrate but not those of cystine, confirming the presence of the former and the absence of the latter substance.

DISCUSSION

Our study clearly demonstrates that cystine in the concentrations used (80, 160 and 320 μmol./l.) promoted calcium oxalate crystal precipitation in a dose dependent manner. We

TABLE 2. *Experimentally determined spacing and relative intensity of ultrafiltered urine plus 320 μmol./l. cystine*

Spacing (Å.)	Intensity
Cystine reference parameters	
4.70, 4.68, 4.63	100
3.13	30
2.71, 2.70, 2.68	32
Experimentally determined parameters	
5.45	100
4.91	31
3.73	15
3.60	54
3.55, 3.39, 3.31, 3.23	12
2.83, 2.76	72
2.62	19
Calcium oxalate trihydrate reference parameters	
5.50	100
4.99	60
3.75	20
3.62	60
3.58, 3.35	10
3.26	20
2.84	100
2.77	80
2.64	40

noted an increase in mean crystal size as well as in mean crystal volume (table 1). Increased crystal size may have been due to growth, aggregation or the 2 conditions. Cystine in the doses used increased average crystal size from 10.6 to 11.2, 11.6 and 13.5 μm. at cystine concentrations of 80, 160 and 320 μmol./l., respectively (fig. 3). This finding clearly demonstrates the promotory effects of cystine on crystal growth or aggregation.

Crystal aggregation is well recognized to be a crucial step in the formation of calculi. To prove that aggregation has occurred it is required to demonstrate that the increase in crystal volume is real. The counter estimates particle size based on the volume of electrolyte it displaces as it traverses the counting orifice.[13] It cannot account for differences in particle density or intracrystalline spaces in a loosely aggregated cluster of particles. This situation would be recognized by the counter as a large solid particle, thereby, overestimating the volume of crystalline material in suspension. To assess whether particle volume recorded by the counter in the presence of cystine reflects the amount of solute deposited and is not due to loose aggregates, counter results must be compared to ^{14}C-oxalate deposition in experiment 3. Figure 4 shows the time course of the disappearance of ^{14}C-oxalate from the supernatant of urine samples during the 2-hour incubation. Increased cystine concentration increased the binding of radioactive oxalate to calcium oxalate crystals, thereby, decreasing the amount of ^{14}C-oxalate in the supernatant. This finding shows that the dose dependent increase in crystal volume recorded by the counter at increasing concentrations of cystine may have been attributable to the increased deposition of calcium oxalate. Briefly, growth and aggregation occurred in this experiment and calcium oxalate deposition was promoted in proportion to the prevailing concentration of cystine.

Cystine in this study did not alter the metastable limit of

urine, namely the minimum amount of oxalate required to induce detectable nucleation by the counter. However, after nucleation began the quantity of crystals increased in a dose dependent manner with increasing doses of cystine (fig. 1). This finding suggests that after nucleation begins the rate of crystallization increases in response to increasing concentrations of cystine. This result was further supported by our results showing increased crystal volume during the 2-hour incubation period, indicating a rapid rate of crystallization in the samples containing cystine compared to urine with no cystine added (fig. 2).

Thus, we postulated that calcium oxalate precipitation did not occur by the process of heterogeneous nucleation with subsequent epitaxial growth, but rather by a salting out process, that is the reduction in solubility of a substance due to the addition of another substance to the system. Further evidence supporting this theory was the fact that no cystine crystals were present in any experiments performed on light or scanning electron microscopy. Although this theory is highly speculative it was further tested by subjecting samples to x-ray crystallographic analysis. The results of this study clearly revealed absent cystine in the samples tested (table 2).

Experimentally determined parameters did not match those of cystine but those of calcium oxalate trihydrate. Calcium oxalate may be present in 3 forms in urine, namely calcium oxalate monohydrate, calcium oxalate dihydrate and calcium oxalate trihydrate. Calcium oxalate trihydrate represents the first phase that may precipitate and it is the most kinetically favored type of calcium oxalate. It is then transformed into a more thermodynamically preferred form, usually calcium oxalate monohydrate. Calcium oxalate dihydrate is thermodynamically less stable than the monohydrate form.[14] X-ray powder diffraction also detected calcium oxalate monohydrate and calcium oxalate dihydrate crystals in the samples analyzed. This finding was confirmed by scanning electron microscopy (fig. 5).

Grover et al reported that increased urate concentrations promote nucleation (urate significantly decreases the metastable limit), growth and aggregation of calcium oxalate crystals in undiluted human urine.[11,15] In addition, they concluded that induced calcium oxalate crystallization was not caused by heterogenous nucleation but rather by salting out. Our study differed from urate induced calcium oxalate precipitation, in that nucleation was not affected. Our findings confirm the earlier study of Pak and Fuller showing that cystine was incapable of causing heterogeneous nucleation of calcium oxalate.[16] Based on our results it is not possible to identify the exact thermodynamic mechanism by which cystine exerts its effect.

Empirical observations have previously linked cystine to CaOx urolithiasis.[1,2] Resnick et al reported that heterozygosity for cystinuria seemed to be a risk factor for calcium oxalate stone formation.[1] When assessing the excretion of amino acids in relation to calculous disease, McGeown noted that cystinuria was a common finding in patients with stones.[17] In addition, it has always been puzzling why stones do not form more often in black individuals, particularly since citrate excretion is so low in the normal black population.[18,19] In a previously published study we observed that normal black volunteers excreted significantly less mean cystine plus or minus standard deviation than normal white individuals (44.4 ± 21.4 versus 71.7 ± 37.6 mg. daily, $p < 0.05$).[18] Thus, cystine may be important as a promoter of calcium oxalate crystallization in individuals with mildly elevated concentrations of this substance. It may also be the case in heterozygous cystinuria carriers or even in idiopathic calcium oxalate stone formers with concentrated urine, that is low 24-hour urine volume and increased solute concentration. Together with other predisposing factors, such as low citrate concentration, high urate or oxalate concentration, or renal leakage of calcium this result would increase the likelihood of calcium oxalate crystallization. It may be an explanation for the presence of cystine in calcium oxalate calculi in some patients at our clinic and may also explain why some of our patients with cystinuria pass pure calcium oxalate calculi.

CONCLUSIONS

Our findings show that cystine is a potent promoter of the growth and aggregation of calcium oxalate crystals in vitro and it supports evidence previously linking stone formation to cystine in the urine.[1,2,17] Thus, elevated cystine in the urine of stone formers may have a critical role in promoting the formation of calcium oxalate crystals and, thereby, renal calculi in these patients.

G. L. Norman, Electron Microscopy Unit, Department of Anatomical Pathology assisted with the samples.

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6.1.2.3.1 Key aspects

This paper demonstrates that cystine is a promotor of RCaOxSF (rather like uric acid is also a promotor of CaOxSF. This was related to any form of metabolic cystine. The study was most important for demonstrating the ability of the stone clinic at that time to investigate crystallization promotors using the most advanced stone research techniques available.

6.1.2.3.2 Discussion

The research was based on observations of previous work where it was noticed that some of the patients at the Johannesburg Hospital referred to the stone clinic for control of cystinuria, had small amounts of CaOx in their stones. [94, 231] This phenomenon has also been reported upon several times before. [250-252] Also but of much more clinical and academic relevance was the observation that some patients who were RCaOxSF had small amounts of cystine in their CaOx stones. [92, 231] The aim of this study therefore was to investigate the clinical, epidemiological, pathogenetic and chemical reasons which were associated in mixed CaOx-cystine SF. Resnick et al (1979) [251] had also noted that in patients with cystinuria, occasionally some of the stones were comprised of cystine plus CaOx or, even occasionally pure CaOx. [250] Even as far back as 1942, Lehmann et al [253] suggested that cystine may form insoluble complexes with Ca salts in vitro. From theirs and other observations the postulate therefore was that the moderately elevated urine cystine levels which may be found in individuals heterozygous for cystinuria may place these individuals at risk for RCaOxSF.

This study contains all the methodological checks and balances required to prove the original hypothesis which was successfully accomplished. The highest concentration of crystals produced in this experiment consisted of CaOx trihydrate. This is an unstable precursor which soon converts to the di- and eventually monohydrate which is the commonest crystal form in CaOxSF. Of note is that, in spite of the trihydrate being the most plentiful of the CaOx crystals in the experiment, the crystals found also contained some di and mono hydrate indicating that the conversion process had already started. These findings were confirmed by scanning electron micrographs as

presented in figure 5 on p. 320 of the publication (see presentation of the paper, p. 104 in the thesis).

Finally, the study did show that CaOx crystal nucleation was not affected by cystine which differs from the true “salting-out” mechanism seen with urate on CaOxSF. So, in conclusion, from the results the precise thermodynamic mechanisms by which cystine exerts its effect is not known and is possibly best termed as a “salting-out like” effect.

From a practical aspect, why is this knowledge of importance in patients with RCaOXSF?

a) Mostly, under which circumstances do cystine stones occur? They are most likely to be found in patients who are heterozygotes for any of the three types of hereditary cystinuria.
b) Other non-cystinuric patients with mild elevations of urinary cystine. It should be pointed out that methionine, cystine and cysteine provide the sulphur for sulphur containing proteins e.g. for co-enzyme A and for taurine synthesis, but how this would alter the plasma concentration of cystine is not yet known.
c) It may also be possible that normal RCaOxSF whose urine either contains slightly increased concentrations of cystine or, more likely, are “chronically dehydrated” and therefore concentrated - a not uncommon finding in idiopathic RCaOxSF - are possible causes of this phenomenon.

As a “guestimate” the prevalence of this important CaOx stone promotor could be in the region of ±5% of all CaOxSF. The calculations here are based on the three types of autosomal dominant inherited cystinuria (i.e. ~1,5% of all stones) plus another 1,5% of “other” causes i.e. 5% - rare but an important group to diagnose especially because, in these circumstances, the stones can be prevented by a) increasing urine volume, b) decreasing dietary salt which results in a significant lowering of urinary cystine excretion and c) by urinary alkalinisation with K-Cit to increase the solubility of cystine.

6.1.2.3.3 Study limitations

1. The study is labour-intense, extremely expensive and required a large array of complicated and expensive equipment
2. At least two experimental clinical scientists are required to perform the experiment.
3. As to the limitations of the actual study itself there were none to be found.

6.1.2.3.4 Recommendations for future research

There are four critical points to consider in future:

a) All urologists must be educated and encouraged to send their patient's stones in for the laboratory analysis (see para. 1.2.1, p. 4).
b) Dedicated laboratories and dedicated stone clinics are essential.
c) Further research in this area would be well worth-while, for example: In the SA black population it is known that there are less CaOx stones. To add to this list is the finding that cystine excretion in the SA black population is considerably lower than in whites. [81, 221] (See para. 8.1.2, point C.i, p. 153.)
d) Write a review on this topic for publication, to highlight the topic which is not well recognised globally.

6.1.3 Group 3; 2 papers with main theme: Stone management

6.1.3.1 Publication 6 [187]

Martins MC, Meyers AM, Whalley NA, Margolius LP, Buys ME. Indapamide (Natrilix): the agent of choice in the treatment of recurrent renal calculi associated with idiopathic hypercalciuria. Br J Urol 1996 Aug;78(2):176-180. doi: 10.1046/j.1464-410x.1996.00633.x https://www.ncbi.nlm.nih.gov/pubmed/8813907

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British Journal of Urology (1996), 78, 176–180

Indapamide (Natrilix): the agent of choice in the treatment of recurrent renal calculi associated with idiopathic hypercalciuria

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Objective To compare the hypocalciuric and potential side-effects of hydrochlorothiazide (HCT) to indapamide (IND; a thiazide-like drug) in patients with idiopathic hypercalciuria.

Patients, subjects and methods Twelve patients with recurrent renal calculi and renal hypercalciuria were studied using a randomized double-blind cross-over protocol. In addition, because the side-effects of HCT are attenuated using small doses, the hypocalciuric effect of 12.5, 25 and 50 mg daily was assessed in six normal subjects.

Results There was a significant reduction in urinary calcium excretion using both agents, with a mean (SD) of 6.06 (2.68) and 3.37 (2.00) mmol/24 h using IND, and 5.58 (1.98) and 3.93 (2.35) mmol/24 h using HCT, before and after treatment ($P < 0.05$). The treatment was not sufficiently prolonged to assess adequately all the side-effects of either agent but both produced a similar decrease in serum potassium, whilst HCT significantly increased the mean (SD) serum urate levels, from 0.34 (0.09) to 0.43 (0.08) mmol/L ($P < 0.01$). There was also a significant decrease in mean (SD) urinary citrate excretion in those receiving HCT, from 1.41 (1.05) to 1.00 (0.71) mmol/24 h ($P < 0.001$), but not in those receiving IND, from 1.19 (0.71) to 1.18 (0.79) mmol/24 h. Although there was a hypocalciuric effect with doses of 12.5 and 25 mg HCT, it was sub-therapeutic when compared with the dose of 50 mg.

Conclusion At a daily dose of 2.5 mg, IND is at least as effective as 50 mg HCT in controlling hypercalciuria. Because of its safety profile and lack of effects on urinary citrate excretion, this agent should be the preferred therapy for patients with idiopathic hypercalciuria and recurrent renal calculi.

Keywords Recurrent stone formers, hypercalciuria, indapamide, hydrochlorothiazide

Introduction

The incidence of idiopathic nephrolithiasis in caucasians has been reported as 11% in males and 3.2% in females [1]. Repeated attacks occur in about half of these patients. Recurrent calcium stone formation has been associated with various metabolic abnormalities, of which the hypercalciurias comprise an important subgroup. About 16% of all the patients attending our metabolic stone clinic have been diagnosed as having renal hypercalciuria, an abnormality which cannot be corrected by diet alone [2]. For over two decades, this disorder has been treated with hydrochlorothiazide (HCT) in a dose of 25–50 mg per day, depending on whether amiloride was incorporated or not [3,4]. However, potentially serious side-effects have been reported when thiazides in these doses are used for prolonged periods, which include the emergence of diabetes mellitus, hyperlipidaemia, hyperuricaemia and hypokalaemia [5–7]. Indapamide (IND), structurally a thiazide-like drug, at a daily dose of 2.5 mg, has been shown previously to cause none of these complications other than hypokalaemia [8–11]. Moreover, IND at this dose is not natriuretic, either in the short or long term. [12]. The effects of IND on the excretion of calcium are largely unknown. Two studies have mentioned the hypocalciuric effects of IND when used at a daily dose of 2.5 mg [13,14].

It has become standard practice to use low and even ultra-low doses of HCT (25 mg or 12.5 mg daily) for the control of hypertension. Side-effects are attenuated at these doses, whilst little of the hypotensive potential is lost [5,10]. Thus, the aims of this study were to compare the efficacy and side-effects of these two agents in the treatment of patients with so-called idiopathic 'renal' hypercalciuria and to determine dose-response relationships to assess whether 'safe' doses of HCT were equipotent with 2.5 mg of IND for hypocalciuric potential.

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Patients, subjects and methods

Study 1

Of 20 patients with recurrent renal calculi and renal hypercalciuria (RHC) who were suitable for the study, only 12 agreed to undergo the arduous protocol. Patients with any degree of renal functional impairment, renal tubular acidosis, or who were receiving drugs such as beta-blockers and calcium-channel antagonists, were excluded from the study.

Two consecutive 24 h urine samples were collected after 10 days on a controlled daily diet containing 120 mmol sodium, 100 mmol potassium, 800–1000 mg of calcium and 1 g/kg body weight of protein. Patients with a urinary calcium excretion of >0.1 mmol/kg/day were considered to have hypercalciuria. RHC was distinguished from absorptive hypercalciuria by determining the calcium:creatinine ratio as described by Pak *et al.* [15].

The protocol consisted of a randomized double-blind cross-over study with four phases (Fig. 1). During the 3-week induction and 'washout' periods, all previous treatment was withheld. During the two 3 month drug phases, 50 mg HCT or 2.5 mg IND were administered daily. Lipid, carbohydrate, electrolyte and red blood cell cation levels were determined, with other routine evaluation tests for stones, at the end of each phase of the protocol. Each patient acted as their own control.

Standard laboratory methods were used to measure the 24 h urinary levels of calcium, creatinine and uric acid, and the levels of plasma calcium, creatinine, uric acid, fasting lipids, fasting blood glucose and glycosylated haemoglobin. Urinary citrate and oxalate levels were measured enzymatically using the appropriate Boehringer Mannheim kit [16,17]. Urinary plasma, and red blood cell sodium and potassium, were measured using flame photometry [18] and magnesium was measured using atomic absorption spectrophotometry [19].

Study 2

The dose-response relationship was determined using 12.5, 25 and 50 mg of HCT in six normal volunteers matched for age and sex and who were maintained for 10 days on the standard diet. The subjects had no history of renal calculi, medical or surgical illnessess and all had normal GFRs. Each subject then collected a 24 h urine sample after a 1 week induction and then after 1 week on each dose of HCT. The effect of increasing doses of HCT on urinary calcium excretion was then measured. The significance of differences were determined using the Wilcoxon matched-pair signed rank test, the paired *t*-test and the mean percentage change with treatment calculated as;

[(value before treatment − value after treatment)

/(value after treatment)] $\times 100$

Results

Table 1 shows the individual changes in the rate of urinary calcium excretion before and after treatment with 2.5 mg IND or 50 mg HCT. There was a marked decrease at 3 months in the mean urinary calcium excretion using both IND and HCT; IND decreased the rate more significantly than did HCT ($P<0.01$). However, when the mean urinary calcium excretion in the 12 patients was expressed as mmol Ca/24 h, both drugs had a similar hypocalciuric effect ($P<0.005$) and there was no significant difference between the drugs (Fig. 2). When the results were expressed as mmol Ca/g of creatinine, the same significant changes occurred. The mean percentage change of the groups receiving HCT and IND was 72% and 103%, respectively.

The rate of urinary calcium excretion in the HCT dose-response study in six normal volunteers is shown in Fig. 2; although there was a significant decrease in urinary calcium excretion using 25 and 50 mg of HCT ($P<0.05$ and $P<0.001$, respectively), the decrease with 25 mg was sub-optimal and with 12.5 mg did not reach statistical significance. Notably, the change of 70% in the six normal controls receiving 50 mg HCT was the same as that found in the 12 patients.

There were no significant changes before or after treatment with either drug in the levels of plasma Na, Mg, Ca, creatinine, glucose, HbA_1C, cholesterol,

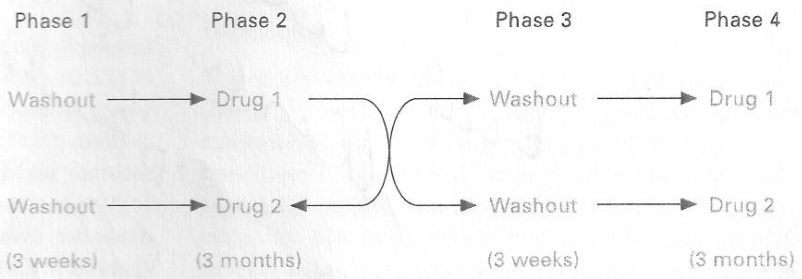

Fig. 1. The double-blind crossover protocol.

Table 1 The individual changes in urinary calcium excretion expressed as the mean of two 24 h urine samples collected before and after 3 months of therapy with daily doses of IND (2.5 mg) and HCT (50 mg)

	Urinary calcium (mmol/kg/24 h)			
	IND		*HCT*	
Patient	*Before*	*After*	*Before*	*After*
1	0.05	0.02	0.06	0.03
2	0.06	0.03	0.08	0.03
3	0.13	0.08	0.12	0.08
4	0.09	0.04	0.06	0.04
5	0.06	0.02	0.07	0.03
6	0.12	0.06	0.10	0.10
7	0.06	0.06	0.06	0.05
8	0.15	0.07	0.12	0.10
9	0.11	0.08	0.07	0.07
10	0.07	0.05	0.07	0.03
11	0.09	0.09	0.09	0.08
12	0.07	0.03	0.08	0.03
Overall mean (SD)	0.09 (0.03)	0.05 (0.03)*	0.08 (0.02)	0.06 (0.03)†

*$P < 0.001$, †$P < 0.005$

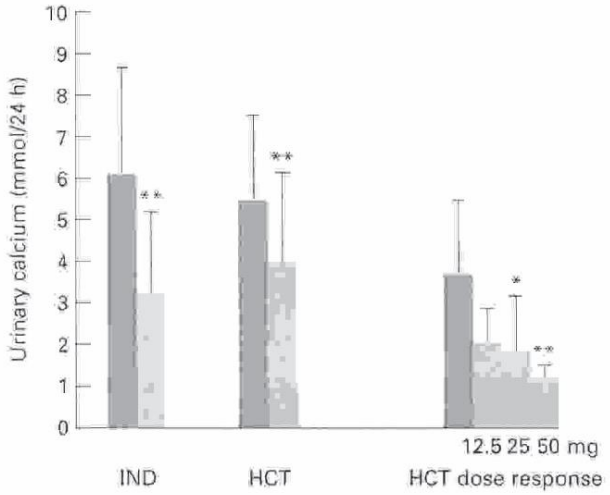

Fig. 2. The mean urinary calcium excretion of 24-h urine samples collected before (dark green) and after (light green) 3 months of therapy with daily doses of IND (2.5 mg) and HCT (50 mg) and the dose-response values for six normal subjects (light red).

triglycerides, high-density lipids and low-density lipids, red blood cell Na, K and Mg, urinary Na, Mg, creatinine and oxalate. Moreover, all these variables were within the normal ranges.

There was a significant decrease in mean (SD) plasma K in patients receiving 50 mg HCT, from 4.01 (0.31) to 3.76 (0.36) mmol/L ($P < 0.005$), and in those receiving 2.5 mg IND, from 4.20 (0.33) to 3.56 (0.40) mmol/L ($P < 0.005$). This decrease correlated with an increase in urinary K output which was still maintained after 3 months. The mean (SD) plasma urate level increased significantly from 0.34 (0.09) to 0.43 (0.08) mmol/L ($P < 0.01$) when using HCT; IND caused a slight but insignificant increase in mean plasma urate from 0.35 (0.06) to 0.39 (0.08) mmol/L. Moreover, the mean excretion of urinary urate decreased significantly with the administration of HCT, from 1.39 (0.89) to 0.75 (0.61) mmol/24 h ($P < 0.05$) but insignificantly during administration of IND, from 1.46 (0.76) to 1.32 (0.74) mmol/24 h. HCT caused a significant decrease in mean urinary citrate excretion from 1.41 (1.05) to 1.00 (0.71) mmol/24 h ($P < 0.001$), which was not evident with IND, at 1.19 (0.71) to 1.18 (0.79) mmol/24 h. Minor side-effects were recorded in two patients (both receiving HCT), i.e. mild weakness and temporary cramps. No patient withdrew from the study and there were no episodes of postural hypotension with either drug. Of the 12 patients, five were recent referrals who had presented with an average of two stones in the year preceeding the initial evaluation. The other seven were already receiving HCT. In the year before commencing IND therapy, three of these developed one new stone each, whilst the others did not. Since starting IND, now representing a 4–5 year period of follow-up, none of the 12 patients have developed any new stones.

Discussion

This study clearly shows that over a 3-month period, 2.5mg IND was at least as effective in reducing urinary calcium excretion as 50 mg HCT. Most of the 12 patients have now been followed for more than one year while taking 2.5 mg IND and the reduction in urinary calcium excretion has been maintained (unpublished results). Several hypotheses have been proposed to explain the

effects of thiazides on calcium excretion but the mechanisms of urinary calcium preservation using IND are largely unknown [13] and were not addressed in this study.

Continued loss of potassium occurred with both HCT and IND, an effect previously well documented with both agents [7,10,11]. However, despite the significant decrease in plasma K, the levels remained within the normal range; no supplementation with potassium was required for patients taking either drug.

This study showed no significant changes in either serum uric acid or glucose levels. Several reports have shown that even after 2 years of treatment with 2.5 mg of IND daily, no changes occurred in lipid, carbohydrate or uric acid metabolism [8,10]. At 50 mg, to a lesser extent with 25 mg and even with 12.5 mg, these side-effects were still recognized with HCT [5,7,11]. However, most studies have indicated that these changes take at least 6 months to become evident. The short period of treatment in this study probably explains some of the negative findings. Despite the present results, a moderate elevation in serum uric acid has rarely been shown but has not resulted in clinical gout [10]. Likewise, hyperglycaemia in the normal population is extremely uncommon but a mild deterioration in carbohydrate metabolism has occasionally been shown in known diabetics [20,21]. Notably, disturbances in the lipid status have never been reported. Nevertheless, despite these complications occurring far less frequently than with thiazides, caution should be exercised when using IND as long-term therapy.

HCT in any dose formulation is approximately one-quarter the price of IND. As 12.5 mg of HCT is relatively safe and because of the flat dose-response curve of thiazides when used in hypertension, it was therefore essential to test the dose response of HCT for urinary calcium excretion. Figure 2 shows clearly that there is a marked dose-response effect with HCT but that in the lower, relatively safe, range the hypocalciuric effect is inadequate.

A significant decrease in urinary citrate excretion occurred in patients taking HCT, an effect which has been well documented [22]. Because urinary citrate is one of the most potent inhibitors of calcium oxalate crystallization, a decrease in citrate excretion due to HCT might well offset some of the benefits. This effect was not seen with IND despite both agents producing a similar degree of hypokalaemia. The effects of chronic hypokalaemia involve the exchange of K^+ out and H^+ into cells, resulting in a net extracellular alkalosis and intracellular acidosis. Citrate is reabsorbed in the proximal convoluted tubule by a citrate/Na co-transport mechanism. Therefore, in situations of intracellular acidosis, citrate will be reabsorbed. Metabolism of one citrate molecule generates three HCO_3^- molecules, which will help to restore the intracellular pH [23]. This is the presumed mechanism of hypocitraturia reported with the use of HCT. Although IND is structurally a thiazide-like drug, in a daily dose of 2.5 mg, no natriuresis was seen either in this study (mean [SD] 148 [70.6] to 152 [41.6] mmol/day) or in studies reported previously [14]. Therefore, it is possible that a low dose of IND is actively inhibiting the Na/citrate co-transporter system in the proximal convoluted tubule and that the sodium so generated is reabsorbed more distally in exchange for potassium. Another less likely theory would be an inhibition of the basolateral Na^+/H^+ antiporter system, which would also result in a decrease in intracellular pH. The mechanisms of both the maintained excretion of citrate and the preservation of urinary calcium need to be elucidated by further studies.

In conclusion, clear recommendations can be made for the long-term use of IND (2.5 mg daily) rather than HCT (50 mg daily) in patients with renal hypercalciuria.

Acknowledgements

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6.1.3.1.1 Key aspects

This paper conclusively demonstrated that IND in a dose of 2.5mg daily was highly effective in controlling patients with RCaOxSF due to primary hypercalciuria. The study was carried out as a carefully controlled, blinded cross-over protocol of 12 patients with 6 patients in the IND group and 6 in the thiazide group with subsequent cross-over (see figure 1, p. 177 in the paper, presented on p. 110 in this thesis). This comparative study was the first of its kind using IND in comparison with thiazide.

6.1.3.1.2 Discussion

For many years HCTZ, usually in a dose of 25-50mg daily, was the treatment of choice to effectively reduce urinary Ca levels in patients with RCaOxSF. Because of poor marketing techniques it took many years before it was realized beyond all doubt that IND, as an agent usually used in the treatment of hypertension was superior to HCTZ both in efficacy and in safety profiles. Thiazides in the doses necessary to control hypercalciuria, can cause diabetes, dyslipidaemia, hyperuricaemia, and hypokalaemia. As discussed in this paper, IND (a thiazide-like drug) in a dose of 2.5mg per day produces none of these, other than mild hypokalaemia. In fact, reports referenced in this paper have shown that after 2 years of IND treatment, no changes occurred in lipid, carbohydrate or uric acid metabolism. Although there were two studies published (as referenced in this paper) on the treatment of RCaOxSF by Borghi et al (1988, 1993) [254, 188] the first was only concerned with reducing Ca excretion and the second was addressed only to the success of reducing actual stone formation. In other words, neither paper compared the potency, efficacy and safety of thiazide vs IND over a long period of time as was the case in this study.

In the first part of the protocol a randomized, double-blind, cross over regime using HCTZ 50mg daily or IND 2.5mg daily was analysed. Careful standardization of dietary requirements was made for all 12 participants. Urinary Ca excretion was calculated as mmol/kg/day. The upper limit of “normal” was calculated as 0.1mmol/kg/day. [138] In addition blood test measurements included duplicates of serum creatinine, Ca, uric acid, fasting lipids and blood glucose and glycosylated haemoglobulin as well as red cell cations. Apart from urinary Ca, 24-hour urine levels were measured for oxalate,

sodium and citrate. Each patient acted as their own control. Highly significantly reductions in 24-hour urinary Ca were shown with both IND and HCTZ with IND being slightly more effective (p value comparing controls to post treatment of IND = < 0.001 vs 0.005 for HCTZ). Plasma K decreased and urinary K increased with both drugs but only HCTZ caused an increase in plasma urate. In addition, urine citrate decreased in subjects on HCTZ but not in the IND group. This effect as reported in the paper, had previously been well documented. [255] Also, the longer-term reduction of plasma K was significantly more accentuated in patients on thiazides and hypokalaemia itself is a known cause of lowering citrate.

The second part of the study compared the dose response relationship between 12.5mg, 25mg and 50mg of HCTZ per day in six normal volunteers matched for age and sex who, prior to the tests, were maintained for 10 days on a standard diet. IND in a dose of 2.5mg per day was compared to the three doses of HCTZ. Not surprisingly the pre-thiazide 24-hour urinary calcium in the six normal volunteers was significantly lower than in the patient study group. Figure 2 in the paper clearly demonstrated the diminishing excretion of Ca with an increase in the thiazide dosage. The “optimal” reduction was seen with the 50mg dose ($p<0.001$) which is the dose of thiazides anticipated to cause marked side-effects as discussed above.

To conclude, this study was a carefully controlled, double-blind, cross-over protocol which clearly demonstrated the, at least equal, to slightly superior effects of indapamide in comparison to thiazide administration. Most importantly none of the serious side-effects caused by thiazides in doses of 25 - 50mg daily were recorded in the IND group.

6.1.3.1.3 Study limitations

1. In the actual study, table 1 better illustrated the far more superior effect of IND versus thiazide. The calculations were bases on the urinary Ca excretion per kg of body weight of each patient per 24 hours i.e. the most scientific method of assessing the actual excretion as to when body weight is not considered. The actual paper failed to recognise and discuss the importance of this aspect.

2. Limitations of pathological Ca excretion can only be made if subjects are on the correct sodium intake. Again, in this study patients did not adhere to this recommendation as discussed in previous papers. However, even in the absence of sodium compliance the study is in no way invalidated.
3. The study did not record the plasma ionized calcium or PTH levels which are necessary to exclude the possibility of “occult” primary hyperparathyroidism e.g. normocalcaemic parathyroidism adenoma. [256]

6.1.3.1.4 Recommendations for future research

1. IND is indicated in patients with idiopathic hypercalciuria and thiazides are contra-indicated. There are two reasons for this contra-indication, firstly the probability of severe side-effects as discussed and secondly the hypocalciuric effect of thiazides tend to decrease with time.
2. An urgent recommendation is that this study should be repeated and publication should be sought in an American journal. This is because IND was not adequately marketed in the USA and so is only rarely used. Support for this also comes from a paper published by Borghi et al (1993) [188] is important. They concluded that “*indapamide could be an interesting alternative to thiazide in the prevention of Ca stones*”.

6.1.3.2 Publication 7 [212]

Whalley NA, Meyers AM, Martins M, Margolius LP. Long-term effects of potassium citrate therapy on the formation of new stones in groups of recurrent stone formers with hypocitraturia. Br J Urol 1996 Jul;78(1):10-14. https://www.ncbi.nlm.nih.gov/pubmed/8795392

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British Journal of Urology (1996), 78, 10–14

Long-term effects of potassium citrate therapy on the formation of new stones in groups of recurrent stone formers with hypocitraturia

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Objective To compare stone formation rates before (as total rates and during remote periods) and after therapy with potassium citrate in patients with hypocitraturia.

Patients and methods The study comprised 15 patients with hypocitraturia only (Group I) and 12 patients with hypocitraturia associated with other abnormalities (Group II), all of whom were recurrent stone formers. Their urine chemistry, including citrate, was measured before and after treatment.

Results In both groups, the urinary citrate concentration increased significantly to within normal limits during therapy with potassium citrate ($P<0.005$). The rate of total stone formation in patients in Group I decreased significantly from 0.7/year before to 0.13/year after treatment ($P<0.005$). The corresponding remote stone formation rate before (0.88/year) was significantly greater than the rate after treatment (0.13/year; $P<0.005$; follow-up 4.6 ± 1.9 years). Patients in Group II showed a similar striking decrease in total stone formation rate, from 1.2/year to 0.08/year after treatment ($P<0.005$). The corresponding remote stone formation rate (1.66/year) before was significantly greater than that after treatment (0.08/year; $P<0.005$; follow-up 4.1 ± 1.6 years). There was a remission rate of 93% for stone formation over the complete follow-up.

Conclusion Potassium citrate appears to be the drug of choice in the long-term treatment of patients with hypocitraturia as it not only decreases the rate of stone formation but also maintains normal citrate levels in the urine.

Keywords Hypocitraturia, nephrolithiasis, potassium citrate

Introduction

Many studies have been performed to examine the efficacy of oral citrate therapy in patients with recurrent formation of calcium oxalate (CaOx) stones. Although urinary citrate level was always increased by citrate therapy [1–8], there have been conflicting results and the objective benefits in preventing the formation of new stones were not always established. A remission rate for stones of 74–99% has been reported in long-term follow-up studies [3–8]. In one of these reports [6], the rate of relapse increased and urinary citrate returned to pre-treatment levels when the therapy ceased. In contrast, Hofbauer *et al.* [1] reported that alkali citrate only decreased the rate of stone formation by 29%, a value which could be attributed to the so-called 'clinic effect'. Leone *et al.* [2] found that the recurrence of stone was reduced significantly in both those receiving potassium citrate or hydrotherapy after 2 years of treatment.

The aim of this study was to determine the long-term efficacy of oral potassium citrate therapy on the rate of stone formation in patients who were recurrent CaOx-stone formers and with either hypocitraturia only or with hypocitraturia and other metabolic abnormalities.

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Patients and methods

Of 275 consecutive patients screened at the metabolic stone-clinic, 130 (47%) were found to have hypocitraturia. Of these, 70 defaulted after diagnosis, 22 were excluded as they had renal tubular acidosis or impaired renal function and 11 were excluded because their follow-up period was inadequate; thus 27 patients comprised the study.

All patients were evaluated as out-patients whilst on their normal diet and off all medication for at least 2 weeks. If necessary, tests were repeated on a controlled diet (including 800 mg calcium and 120 mmol/day sodium). The evaluation included a detailed clinical examination, acid load if the urinary pH was >5.5, renal CT to determine the presence of existing stones and the estimation of calcium:creatinine ratios, as

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described by Pak *et al.* [9]. At least two pre-treatment 24-h urine samples (controls) were obtained from each patient. Samples were collected with no preservative, refrigerated and delivered directly to the laboratory. The volume and 24-h urinary content of calcium, phosphate, uric acid, creatinine and sodium were measured using standard laboratory methods. Urinary oxalate content was measured using the Boehringer Mannheim oxalic-acid kit (cat. number 755699). Urinary citrate was measured enzymatically using a modification of the Boehringer Mannheim citric-acid kit and was repeated if low levels were recorded [10]. Specimens with bacterial contamination, assessed by measuring nitrite with Dipstix (Combur 9, Boehringer Mannheim) were discarded. Patients were only considered to have hypocitraturia if both of the control urine collections contained <2.4 mmol/24 h citrate.

Patients were divided into two groups: Group I consisted of 15 patients whose only defect was hypocitraturia and thus were given only potassium citrate; Group II consisted of 12 patients who had hypocitraturia together with other metabolic or dietary defects and were given potassium citrate plus the relevant therapy for their defect. Of the patients in Group II, three had renal hypercalciuria, three had absorptive hypercalciuria, two had mild metabolic hyperoxaluria [11] and four had 'dietary' hypercalciuria. The two groups were of similar age, sex ratio and follow-up period: patients in Group I had a mean (SD) age of 45.1 (12.5) years, male:female ratio of 2.7:1 and a mean (SD) follow-up of 4.6 (1.9) years and Group II a mean age of 42.0 (11.0) years, male:female ratio of 3:1 and a mean follow-up of 4.1 (1.6) years.

At least two 24 h urine samples (usually four to five) were collected every 6–12 months after treatment and all the tests were repeated. After starting potassium citrate therapy, urine samples were collected at each clinic visit and the pH measured immediately to check compliance: potassium citrate alkalinizes the urine, so that a pH which was often <6.0 at subsequent visits indicated non-compliance.

The dose of potassium citrate given was adjusted to the requirements of each patient, with urinary pH and total citrate acting as regulators. If either of these factors was low, the dose was increased. Patients in Group I received a mean of 30.4 (8.3) mmol/day of potassium citrate: one patient had 17.1 mmol twice daily, five had 12.9 mmol thrice daily, eight had 8.6 mmol thrice daily and one had 4.3 mmol twice daily. Patients in Group II received a mean of 31.5 (9.5) mmol/day of potassium citrate: one patient had 17.1 mmol twice daily, four had 12.9 mmol thrice daily, six had 8.6 mmol thrice daily and one had 8.6 mmol twice daily.

Renal CT was repeated yearly and reviewed to determine the appearance of new stones, the presence of pre-existing stones and the growth of stones. New formations were defined as the spontaneous passage of stones in the absence of pre-existing stones, or with no change in the number and appearance of new calculi on radiographs. The rate of stone formation before treatment was determined as the total stone formation rate (TSFR), calculated as the total number of calculi passed before treatment divided by the total number of years before treatment from the diagnosis of the first stone, and as the remote stone formation rate (RSFR), calculated from the number of calculi passed yearly starting immediately after the diagnosis of the first stone and over periods of the same duration as the treatment period. The post-treatment stone formation rate (PSFR) was calculated as the number of stones passed after therapy divided by the number of years on therapy. Calculi were analysed in 11 patients in Group I and nine in Group II. Results for all variables were expressed as means (SD) and evaluated using the paired Student's *t*-test.

Results

The results from the 24 h urine samples before and after citrate therapy are shown in Table 1: there was no significant difference in urine volume and oxalate excretion in the two groups. Urinary calcium was

Table 1 Urine chemistry of 24-h samples collected before and after therapy with potassium citrate

	Group I (mean [SD])		*Group II (mean [SD])*	
	Before	*After*	*Before*	*After*
Urine volume (L)	1.78 (0.75)	1.79 (0.62)	1.98 (0.49)	1.98 (0.65)
Citrate (mmol)	1.07 (0.51)	2.44 (0.69)†	1.36 (0.46)	2.37 (0.52)†
Calcium (mmol)	4.55 (1.70)	4.60 (1.70)	7.26 (2.70)	5.44 (2.60)‡
Oxalate (μmol)	308.4 (65.1)	329.6 (104.4)	354.1(100.0)	304.7 (103.6)

*Results from individual patients were meaned and the mean of the entire group is given. ‡$P<0.05$; †$P<0.005$, paired Student's *t*-test

significantly reduced after therapy in 10 patients in Group II who were receiving the appropriate therapy for hypercalciuria. There was a significant increase in urinary citrate to within normal levels after potassium citrate therapy in both groups.

Of the 27 patients, 25 (93%) showed no increase in either the size or number of existing stones over the follow-up period (Figs 1 and 2). Most patients (81%) were completely free of stones on CT, and remained so. In Group I, the TSFR decreased significantly from 0.7/year to a 0.13/year (PSFR) ($P<0.005$). Similarly, the RSFR decreased significantly from 0.88/year to 0.13/year (PSFR) ($P<0.005$). The only patient in Group I who continued to form stones confirmed her non-compliance, suspected initially because her urine pH was repeatedly <6.0 (Fig. 1). In patients in Group II, the TSFR decreased significantly from 1.2/year to 0.08/year (PSFR) ($P<0.005$). Similarly, the RSFR decreased significantly from 1.66/year to 0.08/year (PSFR) ($P<0.005$). One of the patients in Group II with concomitant renal hypercalciuria also continued to form stones when treated with either potassium citrate or hydrochlorothiazide alone. However, with a combined therapy, stone formation ceased (Fig. 2). All the other patients in Group II were receiving combined therapy from the initiation of citrate therapy. Table 2 shows the results of stone analysis in 11 patients from Group I and nine from Group II. One patient in Group I and two from Group II also passed a calcium phosphate stone as well as stones containing calcium oxalate.

Table 2 Stones available for analysis from patients in Groups I and II before treatment

	Group I (n)	*Group II (n)*
Patients	11	9
Calculi	16	14
Patients with CaOx stones	10	7
Patients with CaPhos stones	1	2

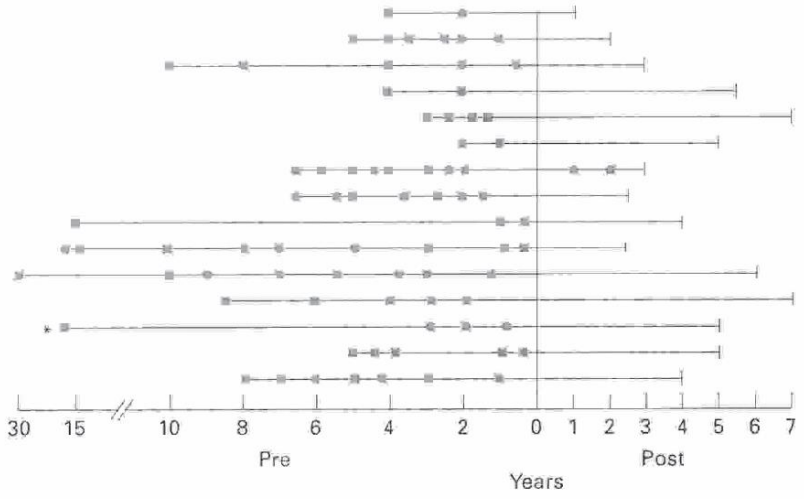

Fig. 1. Stones passed before and after therapy with potassium citrate in patients in Group I. *, Residual stones in kidney.

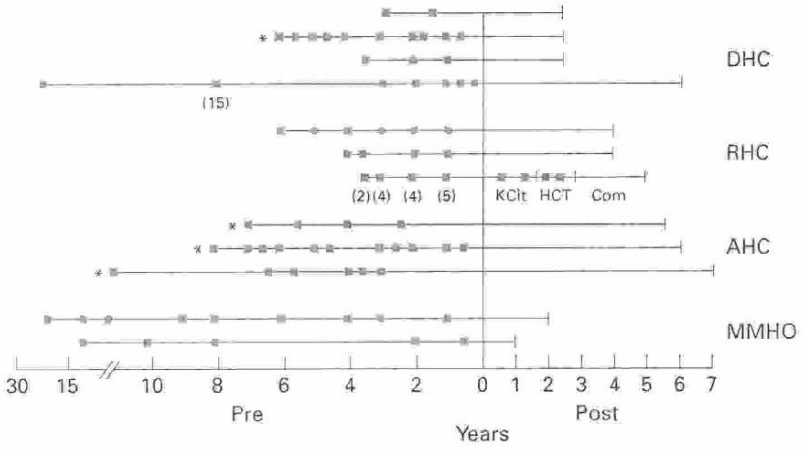

Fig. 2. Stones passed before and after therapy with potassium citrate in patients in Group II. *, Residual stones in kidney: (2 etc), Number of stones passed if > 1; KCit, Potassium citrate; HCT, Hydrochlorothiazide; Com, Combination therapy; RHC, Renal hypercalciuria; AHC, Absorptive hypercalciuria; DHC, Dietary hypercalciuria; MMHO, Mild metabolic hyperoxaluria.

Discussion

Hypocitraturia is a common aberration of normal physiology in CaOx stone formers, affecting from 15 to 55% of patients [10]. Although alkaline citrate therapy has been accepted by many as the treatment of choice in hypocitraturia, controversy still exists as to its efficacy.

Citrate complexes with calcium, thereby decreasing urinary saturation [12], and has been shown to inhibit the aggregation of CaOx crystals [13]. Furthermore, urinary alkalinization by potassium citrate increases the solubility of uric acid and thus prevents the 'salting out' of CaOx by urate [14].

The present results suggest that hypocitraturia was pathogenetically important in the formation of CaOx stones in these patients. Potassium citrate therapy resulted in an increase in urinary citrate to within normal levels, an effect shown by all previous studies of alkaline citrate therapy [1–8]. The citraturic response is mainly due to urinary alkalinization, which decreases citrate reabsorption in the proximal tubules [15]. However, it results partly (8–10%) from the renal excretion of absorbed citrate which has escaped *in vivo* metabolism [16]. Nevertheless, a diet high in citrate has been shown not to influence significantly the levels of urinary citrate [17]. Thus, changes in diet after therapy would not unduly modify the results. Potassium citrate not only increases the urinary pH and citrate excretion but, in contrast to sodium citrate, it also decreases the saturation of CaOx and inhibits the spontaneous precipitation of CaOx [4,8,18]. Sodium citrate increases the sodium concentration of the urine, which in turn may increase urinary calcium excretion [18] and finally, the additional sodium load may aggravate hypertension, a reasonably common condition in stone formers. Thus, those who have administered mixtures of sodium and potassium citrate to their patients may have had less dramatic results because sodium citrate is not as effective in correcting the physiological imbalances found in hypocitraturia.

In the present study, the mean dose of potassium citrate was 31 mmol/day; Leone *et al.* [2] only gave 9 mmol/day, a very low dose, which may explain the lack of a significant difference in the rate of stone formation between those receiving hydrotherapy or potassium citrate. Berg *et al.* [7] found no significant difference in the mean (SD) rate of stone formation [0.59 (0.69) to 0.25 (0.45)] before and after treatment in 17 patients treated with sodium potassium citrate two to three times daily. However, the daily dose in this study was also low (19–27 mmol).

In the present study, with the restoration of normal urinary citrate levels, 14 of 15 patients receiving only potassium citrate and 11 of 12 patients receiving potassium citrate and other therapies, had no further formation of stones. The rate of stone formation was decreased significantly in Group I (by 81% for TSFR and 85% for RSFR) and in Group II (by 93% for TSFR and 95% for RSFR), which are comparable with other published results, e.g. 87% [4], 58% [7] and 86% [8]. In Group II, the decrease in stone formation could have been enhanced by the combination of potassium citrate with the other therapies, highlighted by the result in the patient who continued to form stones, but ceased to do so when given the appropriate combination therapy. Thus, it appears to be important to treat all the specific defects present to obtain a complete response. The present study has one of the longest documented follow-up periods (mean 4.4 years) and some patients were followed for up to 7 years; other studies had follow-up periods of only 1–3.5 years [1,2,5,7].

Unfortunately, the present study did not use a randomized placebo (hydrotherapy) controlled protocol; each patient acted as his or her own control. There are inherent weaknesses in this type of open study and many researchers attribute effects found after therapy to the 'clinic effect'. However, this is unlikely in the present study, as neither diet nor urine volumes differed significantly before and after therapy. In addition, the study included the recorded incidence of stones for up to 20 years before therapy and not just that immediately preceding the treatment. The value of comparing the rate of stone formation remote from the time of evaluation with that after treatment has been shown previously [19]. Bek-Jensen and Tiselius [20] found that with hydrotherapy, the rate of stone formation did not differ from that found long before treatment. Therefore, in the absence of prospective randomized control studies, the rate of stone formation during therapy should be compared with that occurring long before treatment. In addition, the effect was too extreme to be solely a 'clinic effect'. Potassium citrate produced a 93% remission in stone formation, much greater than the 13–84% (mean 54%) reported in various placebo-controlled trials [4].

Potassium citrate was well tolerated and, if the advice to dilute it in a large glass of water was followed, compliance was good. On subsequent follow-up, the few patients who could not tolerate the liquid were given potassium citrate capsules, which gave excellent compliance and the same beneficial effects. Thus, potassium citrate appears to be the drug of choice in the treatment of those patients with hypocitraturia, as it not only decreases the rate of stone formation but corrects the urine citrate chemistry without increasing urinary calcium.

Acknowledgement

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6.1.3.2.1 Key aspects

The aim of this study was to; a) assess the ability of long-term K-Cit to prevent RCaOxSF in patients with pure hypocitraturia and in those who also had other primary metabolic defects, and; b) to assess tolerability and safety of the compound.

6.1.3.2.2 Discussion

Although in previous studies urinary citrate levels always increased with citrate therapy, there has been conflicting data as to whether citrate therapy was successful in the prevention of CaOxSF. [257-262] Hofbauer et al (1994) [263] reported a mild decrease in SF of 29% in pre vs post citrate treatment. This was attributed to the “clinic effect”.

In addition to the above, the strong tendency of South African urologists to use preparations such as "Urolyte-U” or citrosoda instead of K-Cit stimulated the research published in this paper.

6.1.3.2.3 Study limitations

1. The study failed to assess the possibility of K-Cit “overdose” i.e. the production of CaP calculi.
2. “Hypocitraturia” is a common finding in the normal black population. However, no black patients with CaOxSF were included in this study.
3. Likewise, the use of K-Cit was not studied in the Indian population.

6.1.3.2.4 Recommendations for future research

1. The study should be enlarged by assessing the efficacy, or otherwise, of K-Cit in the South African black and Indian populations.
2. A “general” recommendation to all practising urologists and stone clinics is to record the occurrence of CaP stones associated with K-Cit administration. This is where a dedicated stone clinic would be essential to act as the agent collecting such stone data on a national basis i.e. similar to the functions of a registry.

6.2 SUMMARY OF THE MOST IMPORTANT POINTS EXTRACTED FROM THE REVIEWED PAPERS

Several key recommendations arise as a result of this research and these recommendations are presented below.

6.2.1 The establishment of normal levels for urinary metabolite excretion.

The measured levels of metabolite excretions from three of the papers published were collated and reviewed. The data extracted from these studies have enabled the establishment of normal levels of excreted metabolites which are shown below and are expressed as the 24-hour urinary estimations of:

1. Urinary citrate excretion
2. Urinary Ca excretion
3. Urinary Sodium excretion
4. Oxalate excretion
5. Uric Acid
6. Phosphate

Statistical information required in the validation of the data presented was obtained from the methods using "*A robust approach to reference interval estimation and evaluation*" as prescribed by Horn et al (1998). [264] This statistical estimation was also supported by the guidelines and standards of the Clinical and Laboratory Standards Institute. [265] The extrapolated data presented in this thesis confirmed that the sample sizes as well as the methods of presentation complied with the accepted norms and standards of laboratory excellence (see para. 6.2.1.2, p.128).

The extraction of data, validation of the formulae used in the calculations and interpretation of the levels of urinary metabolites, as extracted from papers presented in this thesis, are presented below. Changes to methodology over time might have presented a limitation to the analyses, but provision was made to calculate conversions of measurements into a standard format as presented.

6.2.1.1 Extraction of data from the three published papers

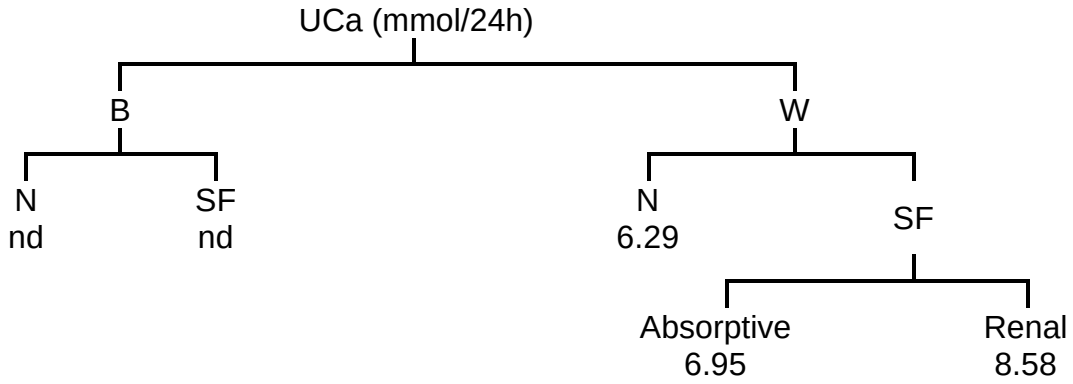
6.2.1.1.1 Urinary minerals

Results of the urinary findings from three of the papers presented in the first part of this chapter are presented in the following figure 6.1.

Abbreviations: B = Black; W = White; N = Normals; SF = Stone Formers; nd = No Data; U = Urinary; NC = Normal controls; SC = Stone controls; BN = Black normal; WN = White normal; HC = Hypercalciuria; HO = Hyperoxaluria (without hypercalciuria); BSF = Black stone formers; WSF = White stone formers; IMHS = Idiopathic mineral hyperabsorptive state.

a) Urines from paper presented as publication 1 (see para. 6.1.1.1 in the thesis, p. 62). [94]

a.1



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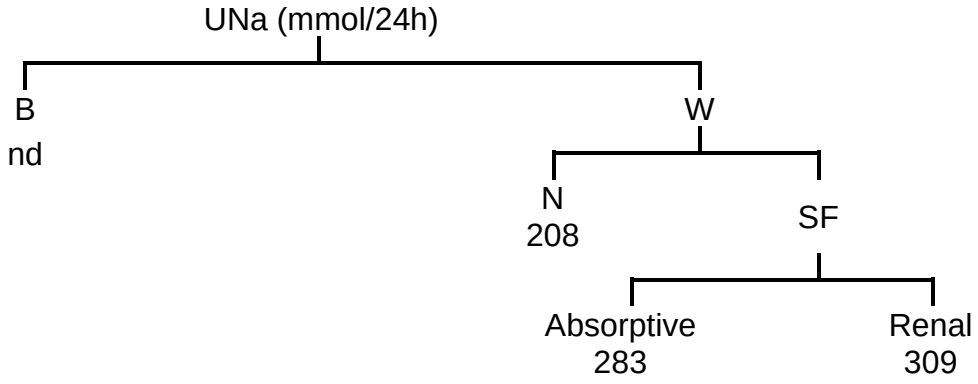

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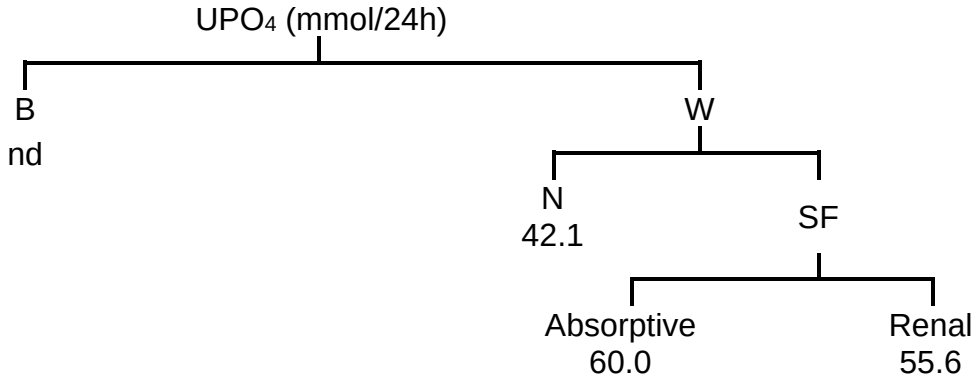

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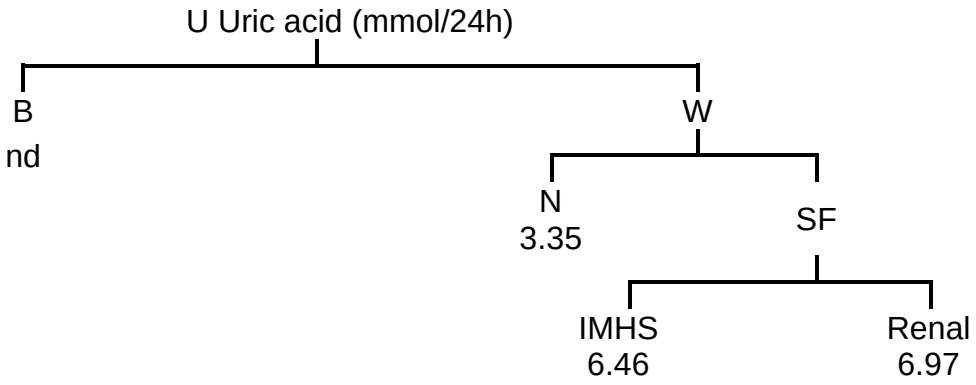

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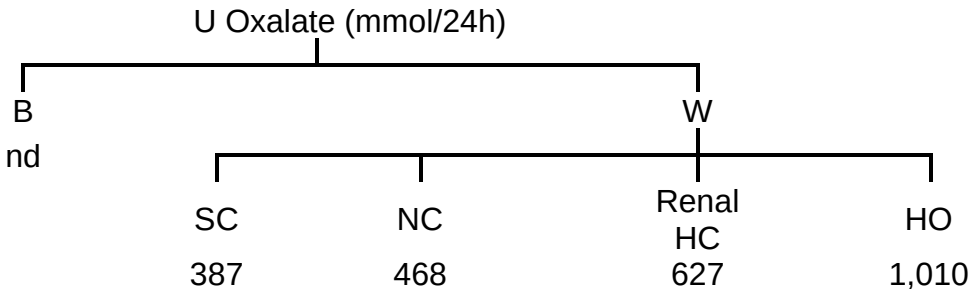
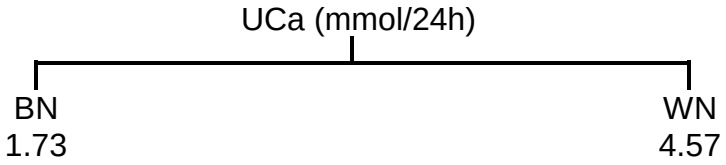


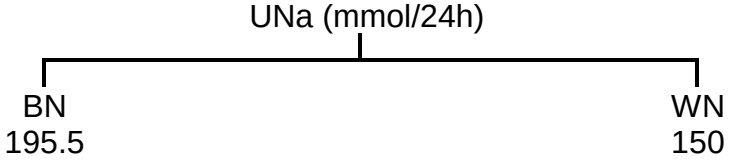
b) Urines from paper presented as publication 2 (see para. 6.1.1.2 in the thesis, p. 72). [92]

Urinary volumes: B = 1.13; W = 1.302

b.1



b.2



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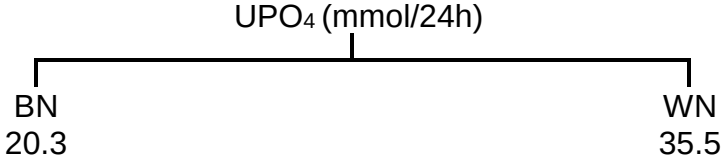

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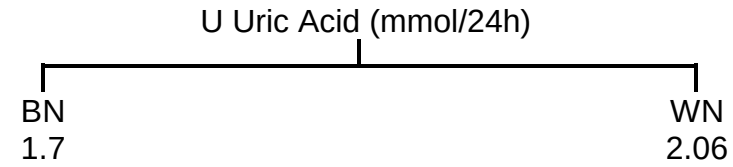

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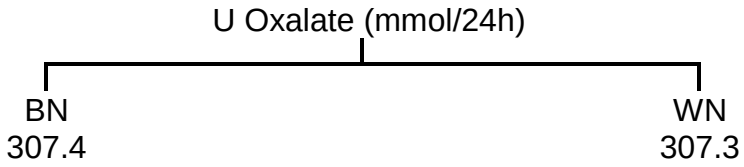
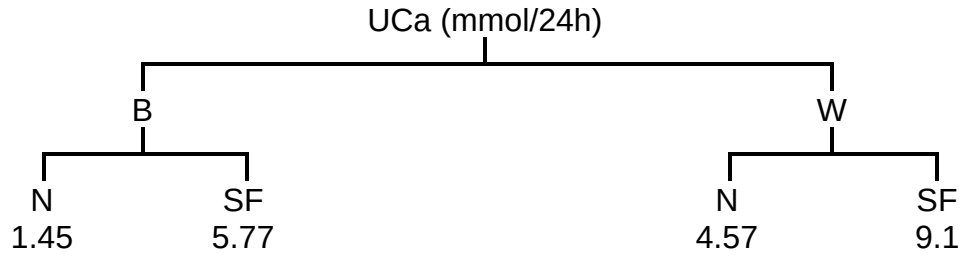


c) Urines from paper presented as publication 4 (see para. 6.1.2.2 in the thesis, p. 91). [231]

c.1



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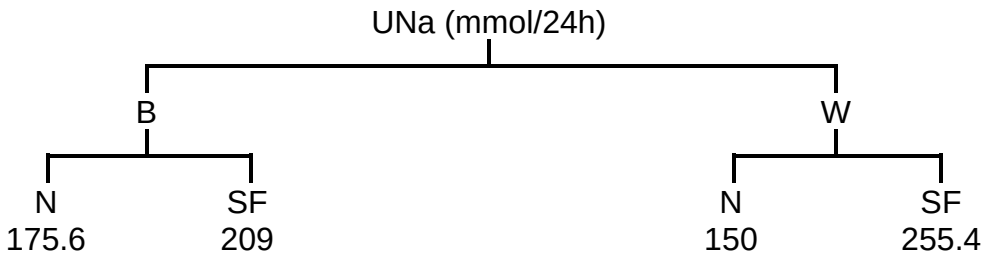
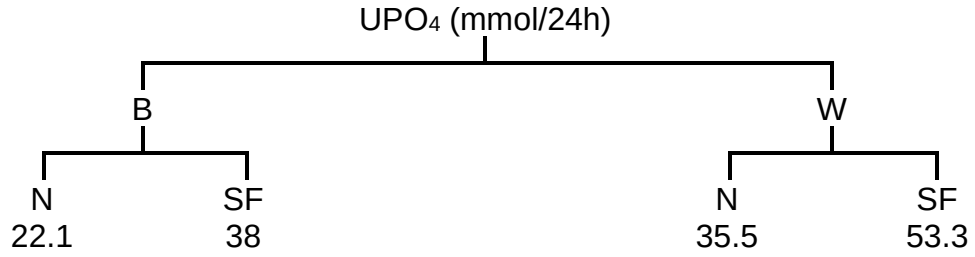
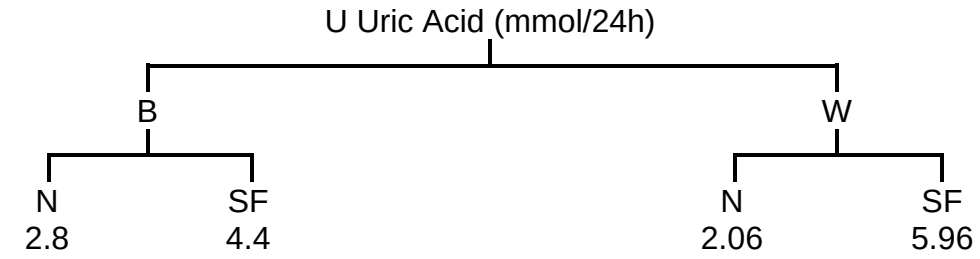


c.3



c.4



c.5

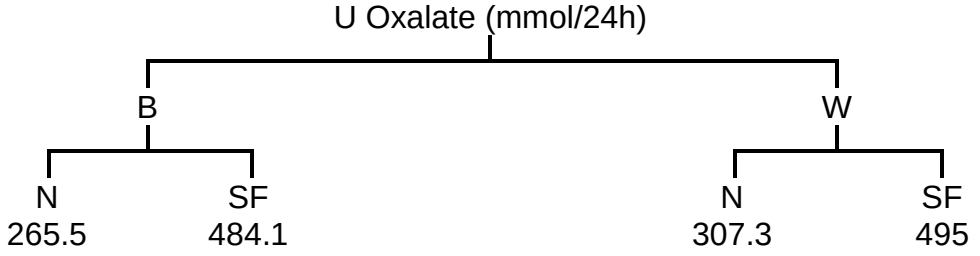


Figure 6.1: Urinary values extracted from three presented papers

6.2.1.1.2 Other metabolic data extrapolated from the three published papers

a) Blood tests (mean):

a.1 Serum uric acid (mmol/dL):

NW = 0.4; NB = 0.31; SFB = 0.36; SFW = 0.4

a.2 PTH (pmol/L):

NW = 3.11; NB = 3.53; SFB = 3.25

a.3 $1,25(OH)_2D_3$ (pmol/L):

NW = 76.3; NB = 93.8; SFB = 82.6

a.4 ∴ Black SF:

i. None had CaP stones
ii. Dietary Ca lower; UCa less →↑PTH
iii. $1,25(OH)_2D_3$ ↑d in BSF >? Due to low diet →↑PTH →↑VitD → stimulating absorption
iv. Uric acid in urine significantly lower in B → less salting out
v. Other: ↑ GAGS; ↑OP; ↑THP production (all to be investigated)

vi. ↑TRPV-5 → ↑ DT Ca reabsorption with lower Ca and uric acid levels in the urine
vii. NB have lower urinary P levels
viii. Above suggest that the B population have normal to decreased FGF-23 levels and a cohort of whites had increased levels. Therefore, a postulated inappropriately raised TmPO4/GFR would only be applicable in WSF on assessment from data in this thesis
ix. A urinary sodium far too high to be able to accurately assess and interpret urinary Ca and urinary P excretion.
x. The 24-hour UP levels were significantly lower in BSF making the dual effect of a low urinary P concentration further protective factor in the BSF (i.e. the P crystal saturation point would not be reached).

Taken as a whole these results which have been extrapolated in a “post-hoc” analysis is most revealing and of pivotal importance in planning the way forward. Because of all of the confusion with standardization and non-adherence to protocols bizarre results were obtained from the urinary sodium levels which negates interpretation and assessment of many factors of stone risk assessment e.g. the hypercalciurias, the hyperoxalurias and the hyperuricosurias.

In conclusion, taken as a whole, in addition to obtaining accurate protocols, measurement of TmPO4/GFR and FGF-23 must be obtained in all future work. In addition to the above, Ca loading must also be performed to study the effects in the different sub-groups.

As mentioned, these suggestions will appear in the way forward section of the thesis and are regarded as a high priority.

6.2.1.2 Validation of the formulae used in the calculations and interpretation of the levels of urinary metabolites as extracted from published papers

Data extracted from studies are combined in order to establish a sufficient number of subjects for statistical validation. Basic information from published papers presented

as number of subjects (n), mean ($\bar{x}$) and standard deviation (SD) were derived from papers 1, 2, 3 and 4 data.

6.2.1.2.1 Formulae used for statistical analysis:

- Formula used to create a pooled mean:

  $\bar{x} = \frac{\sum x}{n}$

  where $\bar{x} = mean\ citrate\ /\ 24h$

  and $\sum = \frac{Sum\ of\ pooled\ data * mean}{number\ of\ readings}$

- Formula used to create a pooled standard deviation:

  $SD = \frac{\sqrt{\sum(\bar{x} - xi)x^2}}{n}$

  where xi = number of data sets

6.2.1.2.2 Application of the formulae to the extracted data

i. Citrate

a) In black subjects:

Females (one paper): n=19; 1.51(0.7)

Males (two papers): n=12; 1.23(1.19)

n=22; 1.45(1.2)

Pooled mean: $\bar{x}\ (black\ males) = \frac{(1.23)(12)+(1.45)(22)}{34}$

= 1.37

Pooled mean: $\bar{x}\ (black\ males\ and\ females) = \frac{(1.51)(19)+(1.23)(12)+(1.45)(22)}{53}$

= 1.42

Standard deviation: *SD (black males)* = $\frac{\sqrt{1.19^2(12)+1.2^2(22)}}{34}$

= 1.2

Standard deviation: *SD (black males & females)* = $\frac{\sqrt{0.7^2(19)+ 1.19^2(12)+1.2^2(22)}}{53}$

= 1.05

b) In white subjects:

Males (two papers): n=21; 3.35(1.3)

n=12; 3.32(1.02)

Females (two papers): n=11; 3.25(0.54)

n=17; 3.46(0.96)

Pooled mean: $\bar{x}\ (white\ females) = \frac{(3.25)(11)+(3.46)(17)}{28}$

= 3.38

Pooled mean: $\bar{x}\ (white\ males) = \frac{(3.39)(21)+(3.32)(12)}{33}$

= 3.36

Pooled mean: $\bar{x}\ (white\ males\ and\ females) = \frac{(3.25)(11)+(3.46)(17)+(3.29)(21)+(3.32)(12)}{61}$

= 3.37

Standard deviation: *SD (white males)* = $\frac{\sqrt{1.13^2(21)+1.02^2(12)}}{33}$

= 1.09

Standard deviation: *SD (white females)* = $\frac{\sqrt{0.54^2(11)+0.96^2(17)}}{28}$

= 0.82

Standard deviation: *SD (white males & females)* = $\frac{\sqrt{1.3^2(21)+ 1.02^2(12)+0.54^2(11)+0.96^2(17)}}{61}$

= 0.98

A summary of the calculated results is presented in table 6.1

Table 6.1: Composite 24-hour urinary excretion rates for black and white subjects from the presented studies:

a) Blacks:

Parameters measured	Females	Males	Both
n	19	34	53
Mean	1.51	1.37	1.42
Standard deviation	0.7	1.2	1.05

b) Whites:

Parameters measured	Females	Males	Both
n	28	33	61
Mean	3.38	3.36+	3.37
Standard deviation	0.82	1.09	0.98

Interpretation:

a) Blacks

Mean citrate higher in females which is contra to what has been previously found Secondly the means for females and males are similar so for practical purposes, males and females are regarded as a single cohort i.e. mean citrate = 1.42mmols per day with standard deviation of ± 1.05 (0.37 to 2.47) and therefore hypocitraturia is defined as the excretion of less <0.37mmols per day for both sexes. (This is one standard deviation below the mean and it is a practical and acceptable statistical principle of interpretation.)

b) Whites

Mean citrate = 3.37mmols per day SD =0.98 (2.47 to 4.27) therefore hypocitraturia for both male and female whites is <2.47mmols per day.

Therefore, the definitions of standard hypocitraturia in the black population are completely different from the white population and the norms and standard deviations for both populations should be expressed independently by the reference laboratory. Because of the recent changes in diets and in socio-economic strata, this work needs to be repeated in the near future (see para. 5.1.2, p. 50).

From the laboratory guidelines it has become advisable to measure urinary citrate more frequently in CaOx stone workup procedures because of intra-individual variations both in citrate and other urine metabolites. [266] For this reason, 24-hour citrates estimations will be made on both samples in the workup protocol.

ii. Calcium and; iii. Sodium

For reasons stated in the reviewed papers standardised methods for the definition of hypercalciuria have not been adequately achieved. The individual has to be on a standardised protein and carbohydrate intake as well as no more than 5.2gms of sodium chloride per day. Even then if the urinary sodium is not less than ±100 to 110mmols per day, dietary instructions must be reinforced and the urinary collection repeated in order to achieve a correct diagnosis of hypercalciurias. Once this has been achieved the various types of hypercalciuria can be further studied, i.e. absorptive, renal (idiopathic) or calcitriol excess; this critical work should take precedence over any other study envisaged.

iv. Phosphate

The importance of measuring urinary phosphate excretion was dealt with in paragraph 3.2.1.4 in the thesis (see p. 18). Important conclusions emanating from papers 2 [92] and 4 [231] in the thesis (higher serum calcitriol levels in black subjects coupled with significantly lower phosphate excretion in black subjects), can now be discussed as an essential task to be included in any future stone work-up protocol in SA. These important conclusions are explained below.

a) Inappropriate phosphate excretion can be detected by measuring the relationship between the 24-hour urinary Ca excretion and the TmPO4/GFR to detect the subgroup with an inappropriately high urinary phosphate level i.e. a phosphate "leak" as described by Williams et al in 1996 [27] Later on (in 2001) Prié et al [67] demonstrated that in addition to the above subgroup who also had high levels of calcitriol, 20% of patients with idiopathic hyperoxaluria (and normal PTH levels) fell into the Williams classification. Even later in 2015 Taylor et al [69] showed that those with Ca stones who also had a slightly raised 125 hydroxy levels and fell into Williams's classification also had elevation of FGF-23 which was implicated in the stone pathogenesis.

   By extrapolation from results in this thesis i.e. the finding of significantly lower excretion of Ca levels in the black population coupled with significantly higher levels of calcitriol and FGF-23 in some white stone formers might be the explanation for a decreased incidence of CaOx and 0% incidence of CaP stones in black SA citizens. To explain this further, in people with low serum levels of FGF-23 the 1-alpha hydroxylase would not be inhibited and either normal or higher levels of calcitriol will then be produced. Apart from providing normal Ca balance (GIT and renal), the direct role of hyperphosphaturia and its direct role in stone formation would be absent. This hypothesis requires verification and could be easily done.

v. Oxalate

Comments on "mild hyperoxaluria" in the genesis of RCaOxSF:

a. Present laboratory normal ranges are not acceptable to diagnose MMHO (mild metabolic hyperoxaluria). These results only state the oxalate excretion in the patients' existing diet. To assess "true hyperoxaluria", the patient needs to be on a standard Ca intake (±1,000gm elemental Ca per day, a standard sodium intake (2,000mg Na per day) and a "low" oxalate diet by removing foodstuffs highest in oxalate. [76, 77] The above diet need only be administered for three days with the 24-hour urine specimen collected on day 4. The studies reported in the thesis [92, 231] attempted to adopt the above dietary advice but urinary sodium levels of ~100mmol/day were not achieved. Nonetheless, as there is no relationship between

sodium and oxalate excretion, the results obtained were acceptable for accurate oxalate interpretation.

b. The following table show composite oxalate levels in black and white normal volunteers from the presented publications.

Table 6.2: Urinary oxalate excretion (µmol/24-hour) after 'standard' diets.

Variables	**Blacks**			**Whites**	
Sex	Female	Male		Female	Male
Study*	2	2	4	2	2
n	19	12	22	17	12
Mean	244	239	271	194	181
SD	127	135	121	78	164

* Refers to the number of the publication as presented in the first section of this chapter.

Because urinary oxalate levels - even on diets, will always be variable, reasonable accuracy can be achieved by the combination of urinary levels of all males and all females of both normal cohorts (both black and white). Also, because the urinary oxalate level in both sexes were similar, both sexes were also combined and averaged, i.e. total average for all 82 normal subjects:

Mean = 224µmol/24-hour

SD = 125µmol/24-hour

Because oxalate is ubiquitous and is present in most foodstuffs, the clinic decided to increase the mean level on normals maintained on low oxalate diets to 350µmol/24-hour. In excess of this level, mild hyperoxaluria can be reasonably diagnosed. To be 100% scientifically accurate, the above work needs to be confirmed by the Na Ox crystallisation tests in vivo urinary specimens spun and filtered 24-hour human urine samples - but is of low priority in the way forward ranking list.

vi. Uric acid

This has been comprehensively presented (see para. 3.2.6, p. 23).

6.2.1.3 Conclusions

In summary with the knowledge and requirements of controlled protein, Ca and sodium intake together with the calculations required to diagnose a phosphate leak, an accurate clinical classification of the idiopathic hypercalciurias can be achieved. These in summary are:

a) Absorptive hypercalciuria:
    a(i) Type I
    a(ii) Type II
    a(iii) Type III

    Type III is the one with the phosphate leak and is due to the decreased renal capacity to reabsorb PO_4 resulting in hypophosphaturia which stimulates the production of calcitriol which will increase intestinal Ca and phosphate absorption and produce hypercalciuria (see para. 3.2.1.c and para. 3.2.1.d, p. 18).
b) Renal hypercalciuria
c) Unknown cause of hypercalciuria
d) Phosphate loss due to increased levels of FGF-23
e) The actual level of urinary PO_4 which may alter the saturation of CaOx crystal physiology.

The recommendations which accrue from the summary of the seven papers and especially from the summary of information to be obtained from phosphate studies, form the basis for a considerable amount of future studies. This again points out the importance of a stone clinic. Another vital part of this research is that it should include studies in black, white and Indian cohorts.

6.2.2 Establishing the incidence of CaOxSF in the South African black population

It has not yet been conclusively established why, even in recent times, CAOXSF is still a rare disease in black South Africans. Postulates are summarized in table 6.3.

Table 6.3: Postulates why calcium oxalate stone formation occurs less frequently in the South African black population

Reasons	Future research
Dietary calcium is low in blacks	To be repeated due to recent changes in diet
Differences in phosphate metabolism and excretion	TmPO4 excretion, calcitriol and FGF-23 tests must be included for assessment
Lower urinary cystine levels	All urinary calculi should be chemically analysed
Possible difference in GAGS and THP metabolism	Both subjects require in-depth study in South Africa
Genetic polymorphisms in TRPV-5 channels in Afro-Americans*	Genetic study highly desirable in South Africa
Fructose gluttony which “switches on” TRPV-5 calmodulin production resulting in hypercalciuria*	Dietary studies in black and white South Africans required **
The GIT microbiome with low levels of oxalobactor formigenes (genetic)	No studies published in either black or white stone formers from South Africa and are urgently required

* *Switched-on TRPV-5 channel results in marked increase in calcium reabsorption*

** *Fructose gluttony is unlikely to be a problem in black SA citizens.*

6.2.3 Further recommendations:

1. Why is the normal rate of citrate excretion so low in black subjects and why do these low normal levels not actually predispose to SF? Possibly there are genetic reasons in the handling of urinary citrate which occur in the presence of other functional defects, which if present, would result in decreased SF in the black population. This needs to be further investigated.
2. The association of RP with CaOx and CaP is well known. However, if it could be shown that RP are a rare phenomenon in the black population, the lower

incidence of nephrolithiasis in the black population would be supported, so population studies on this aspect are indicated (see para. 8.1.2, point B.iii a&b&c, p. 151).

3. Many Indian patients with RCaOxSF attend the stone clinic but studies on normal or abnormal citrate levels have not been performed in this population and should be attended to as a matter of urgency (see para. 8.1.2, point A.ii, p. 149).
4. Finally, as many of our patients (especially our Indian patients) with RCaOx stones are vegetarian or vegan, normal urinary metabolite excretion values should also be studied in this group of patients as a matter of urgency (see para. 8.1.2, point A.ii, p. 149).
5. The dietary factors and genetic related changes in patients with RP were discussed on p.40. Therefore, a strong recommendation is that the levels of inflammation should be studied in normals and patients with RP, in both black and white South Africans. This is especially in view of dietary changes since the date of the studies in both blacks and whites.

CHAPTER 7

FORMATION, STRUCTURE, FUNCTIONS AND NECESSITY OF DEDICATED NEPHROLITHIASIS INSTITUTES IN SOUTH AFRICA

7.1 INTRODUCTION AND LITERATURE REVIEW

An extensive literature search has revealed minimal information on the formation, functions and cost-effectiveness of a stone clinic. It would seem that the concept of a dedicated stone clinic has never previously been published as such, and is of special importance for the detection, correct management and lifelong follow-up of patients with RCaOxSF and CaPSF. This is even more important in low- and middle-income countries, not only from the viewpoint of improving patient care but also regarding the cost efficiency of stone management. The incidence of actual stone disease in various population groups were studied by Friedlander et al [249] in the white, black, Hispanic, and Asian populations in the USA. This epidemiological data contained information on the passage of CaOx calculi, hydroxyapatite/struvite stones as well as uric acid stones. In addition, the data contained calculi from first stone formers and therefore bears less relevance to studies in this thesis. Of interest CaOx stones were found in 53,2%, 52,3% and 45,3% in whites, blacks and Hispanics respectively. Other studies from the USA and around the world have not assessed the incidence of RCaOxSF in black and Asian populations making the necessity of further relevant research in South Africa even more important - not only form the viewpoint of improving patient care, but also from cost-efficiency aspects.

Other than in two papers which presented some of the details of a stone clinic structure, [265, 266] there have been a few articles written by one or more practising urologists or associates advertising their own, usually a single service centre with little or no advice as to any further management after the referral of the initial stone episode. [269-271]

While there are many countries where organised stone clinics and numerous guidelines on the work-up and management of patients with nephrolithiasis exist, few are published in journals of academic excellence and mostly in “promotional style” publications. [269-271]. Charles Y Pak from the U of Texas, Dallas (1996) [215] stated quite clearly his concerns as to why, just because of the new surgical management techniques e.g. ESWL, LL, etc., the need for medical management and research has largely “lapsed” and he urged rapid reform. The important study by Scales et al (2013) [272] emphasised that emergency visits and hospitalisations associated with kidney stone recurrences are very costly and harmful and urged that the problem be addressed. They strongly advocated a clinic approach with appropriate work-up and medical preventative therapy.

Furthermore, there have been many more papers strongly in favour of stone research and management centres and which have proved them to be efficient and cost effective. [240, 273-276] Parks and Coe (1996) [277] showed that a well-functioning stone clinic resulted in a calculated reduction in costs of $3226 per patient per year. More recently Strohmaier (2012) [278] reported on overall patient and cost benefits of medical management of stone disease.

In summary, although there are many papers advertising the medical protocols of individuals or groups of (mainly) urologist interested in stone disease, no-one has ever produced any information on the actual structure or functions of such a facility so that the information given of so-called stone clinics is purely in the form of medical outcomes without this basic information.

7.2 SUGGESTIONS ON STONE INSTITUTE SERVICES AND FUNCTIONS

An important aim of this thesis is to put forward suggestions on the establishment of stone clinics in the major centres of South Africa. This initiative is based on:

i. Ideas gained from forty-two years of running the Johannesburg Hospital stone clinic services

ii. Experience gained by extensive research and presentation of papers at National and many International congresses on nephrolithiasis over many years. The stone clinic at the previously called Johannesburg Hospital was initiated in 1977 and consisted of a nephrologist and occasionally an attending urologist. In the late eighties the clinic expanded to include a urology registrar, a dietician and a clinical technologist who also acted as clinic administrator and eventually two highly trained physiologists who took on the portfolio of stone researchers. The academic achievements of this early clinic form the basis of the published papers as presented in this thesis.

iii. Experience gained from a short sabbatical in a high quality, well known, international stone research unit in Australia (Flinders University, Adelaide, South Australia).

7.2.1 The formation and function of a dedicated stone institute

The following postulates which underpin the notions of the necessity to form a stone clinic follow below.

Firstly, what type of kidney stones should or should not be referred to a stone clinic. Very briefly all patients with multiple episodes of CaOx/P kidney stones (primary and secondary) should be referred.

Apart from primary (idiopathic) causes, there exist a wide range of secondary stone disorders which when managed in a clinic would yield the correct diagnosis with appropriate life-long therapy and in particular therapeutic avenues aimed at prevention of future stone recurrences. A highly cost-effective management plan for these patients is possible. In addition, these clinics must serve as an academic training area for urological and nephrological trainees and other vocations such as dieticians. This will result in better patient outcomes (e.g. for morbidity, less hospitalizations, fewer procedures (not without their inherent dangers), better staff morale and increased levels of both patients and staff content. [279] Lastly, important and relevant research will proliferate after establishment of a stone institute.

Many years of experience in a medically directed stone clinic point to three categories of SF circumstances which need to be discussed when assessing cost-effectiveness of nephrolithiasis management.

1. With first stone formers as an entity of SF, “usual office” handling is advised without the requirement of a detailed stone work-up protocol and without specifically directed medical management.
2. In RCaOxSF the “stone clinic effect” must be firstly evaluated before any specifically directed medical therapy can be considered. In other words, if the patient is reliable in terms of their own healthcare and has been assessed by the clinic that they will continue with regular visits and be adherent to the therapy, then the “clinic effect” treatment is the most cost effective.

   To debate this point further these patients will be compliant with adequate hydrotherapy, with dietary requirements (especially those with the correct daily sodium intake), with avoidance of dietary excesses, with avoidance of excess animal proteins and processed foods especially “take-away” products and with the avoidance of excess fructose intake, then the “clinic effect” approach will be by far the most cost effective.
3. Regrettably and from stone clinic experience, most patients will ignore long-term monitoring and adherence clinic discipline. Here specifically targeted medical therapy will be the most cost effective.

With all of the above considerations it becomes clear that a fully dedicated stone clinic is essential to successfully achieve a regular follow-up and monitoring of its patients. This indeed is one of the most important supporting aspects of such a stone clinic or institute. Previously supported research on cost-effective aspects supports the above postulates.

Finally, it must be remembered neither routine urologic services nor routine laboratory testing (from a practical point of view) are able to provide the level of care as outlined above.

In their 1996 study, Parks and Coe [277] reported that with a prevalence of 8% of nephrolithiasis in the USA the cost was about $5 billion per year. More recently (2014)

Hyams and Matlaga [280] reported on an indirect loss in the USA of $ 775million per year purely from work absenteeism for stone events. Clearly, this indicates the value that a stone clinic would add from a cost-effective aspect alone.

7.2.2 The proposed structure and functions of a stone institute

7.2.2.1 The proposed compilation of the role-players in a Nephrolithiasis Institute (i.e. a stone clinic)

A schematic presentation of the team work required is shown in figure 7.1.

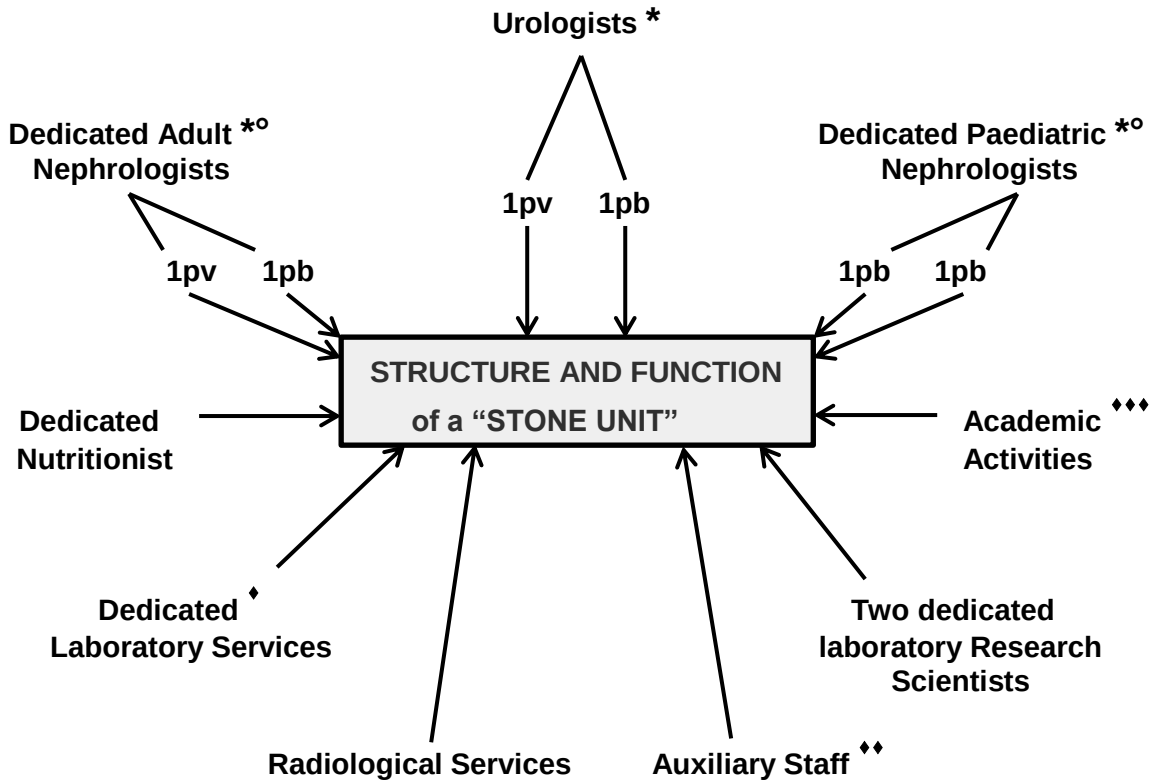


Figure 7.1: Outline of a functioning Nephrolithiasis Institute

pv = private, pb = public

* *Part-time;* ° *Part-time and rotating*

♦ *Includes the reception for specimen preparation laboratory plus a senior pathologist*

♦♦ *Includes a receptionist, unit manager and an administrative assistant*

♦♦♦ *Academic activities:*

- *Teaching protocols and standards; Journal assessment clubs; Enrolled research candidates*

All persons in this working group should have a basic knowledge of the stone protocols and a detailed knowledge of their own academic input. None of the personnel including the nephrologist director have to work full-time in the clinic and time allocation would be dependent on patient load. At the outset the ability to attract research scientists would depend on the quality of the proposed research topics as well as the funds available for the staff and research studies. Periodic group and monthly academic meetings (e.g. journal clubs and case presentations for CPD points) are essential. Academic input is always a priority and several suggested avenues for future research are presented in the conclusion of this thesis

7.2.2.2 Involvement and commitment

The word “dedicated” requires definition. In the first instance, it is unlikely that individual practising urologists, however knowledgeable, are able to give sufficient time from their busy schedules to run their own clinic-style medical management for patients who are frequent SF. In addition, cost effectiveness in this type of private practice would almost be impossible. The dedicated urologist by definition is one with a particular interest in nephrolithiasis and willing to give of his/her time to function efficiently in the clinic. The same applies to the dieticians and they need to update themselves on the plethora of existing literature containing the most recent dietary information relevant to their practice in the clinic. The same applies to the dedicated nephrologists, laboratory and research personal with provision for paediatric services as and when needed. In particular it is the pathology laboratory nurses, technologists and pathologists who will be approached to apply this dedicated service which is so different from routine laboratory protocols. Preparations and measurements of all specimens have to be meticulously carried out. Routine laboratory services are unable to follow all of the necessities required for accurate stone work-up techniques. In addition, the normal ranges of various chemicals and elements do not always apply to clinic norms and neither does the laboratory standard computerised method of reporting comply with clinic requirements. (See Appendix I, p. 260.)

In summary therefore, a dedicated laboratory technologist and knowledgeable stone pathologist are essential for efficient running of clinic protocols.

7.2.2.3 Location

The location of such a clinic should be if at all possible at an academic hospital attached to a university. With the proposed universal health cover, it is anticipated that a senior member of the department of urology will also be involved in the clinic. Kidney stone patients from the non-academic private or public sectors who have either idiopathic or secondary causes of CaOx and CaP stones as well as patients with rare tubulopathies and other metabolic abnormalities should also be referred.

Because it is known that kidney stone disease is still very common and expensive to manage it is believed that the formation of these stone clinics should be, to begin with, firstly in Johannesburg where a "semi stone clinic" already exists to be followed as quickly as possible by such a clinic in Cape Town and Durban.

With time the clinic, if successful, could expand to include interested urologists from various areas within the province who may wish to rotate their duties in the stone clinic service.

7.2.2.4 Educational function of a stone clinic

The educational functions of a dedicated stone clinic include:

- **Patient education**

  Thorough and ongoing patient education is mandatory not only in terms of achieving the goals of the various treatments but is essential in achieving adequate long-term follow-up and adherence to treatment. After the initial work-up the patients are usually seen four months later, then twice a year for one year and then only on an annual basis. Adequate follow-up cannot be entirely reliant on the patient to make all of the necessary arrangements. This underscores the importance and necessity of a stone clinic administrator and an adequate administrative set-up.

- **Education of urologists and nephrologists in SA**

  To support the concept of this thesis, urologist's education is required. It is the intention that on completion of this, it should be circulated to selected groups (e.g.

university departments, South African Renal Society, Urological Society and selected practicing urologists and nephrologists around South Africa).

- **Education of the stone clinic healthcare personnel and service providers**

This would include the education of the stone clinic dietician(s), the laboratory personnel including the pathologist(s), the research scientists (if available) and the attending urologist (paediatric and adult) and the nephrologist (paediatric and adult). Regular academic meetings of all of the above would be recommended e.g. journal clubs, travel to relevant congresses and maintenance of a stone library should be considered.

7.2.3 Development of a stone risk factor index for South Africa

Although not part of the scope of this thesis a review of the literature indicated the importance of a stone risk factor index. [30, 204]

This might be a future consideration, especially for the unique South African mixed population groups with different risk factors. Further discussion is presented in the recommendations that follow (see para. 8.1.2, C.iii, p.152).

CHAPTER 8

SUMMARY AND CONCLUSIONS

8.1 SUMMARY

8.1.1 The published papers

In parallel with the recent global increase in the prevalence and associated mortality from both cardiovascular and renal diseases, so has the incidence of recurrent nephrolithiasis risen. Although not associated with high mortality, renal SF being so common produces considerable morbidity, increased rates of hospitalization, problems from multiple procedures, time off work – all of which add up to considerable high financial burden on the health system. Furthermore, in terms of cardiovascular risk profile, a large proportion of RCaOx/PSF are in that very category themselves and so form part of a continuum of the many vagaries of modern society.

Before summarising the main topics of this thesis (7 papers), it is necessary first to comment upon the many recent developments in the field since these papers were published. New developments in the work-up tests such as, quite simply, how correctly to collect 24-hour urine specimens so as to measure the urinary pH and chemical composition with utmost accuracy (see Appendix I, p. 209). Recent state of the art and important new methods must be the focus of future investigation e.g. measurements of urinary GAGS, THP glycoproteins, urinary crystallization investigative procedures using chemical tests which are then confirmed by x-ray crystallographic and scanning electron-microscopic techniques.

There has been a vast increase in research into the formation, structure and function of RP in the formation of CaOx and other types of stones. No research in SA has ever looked at these aspects but so fundamental are RP to SF that this topic is a priority to investigate especially because of the different population groups in SA.

With all these factors in mind the assessment of each of the chapters presented, research was done on a broad spectrum of both stone risk factors (promotors and inhibitors), of therapeutic medication outcomes and of epidemiological aspects such as SF in SA black and white subjects.

The extensive literature review of modern advances in stone pathogenesis and management enabled relevant critique of the studies presented in the thesis. This exercise has been of inestimable value to shape the structure and function of the proposed stone clinic and to formulate a comprehensive planning of future steps.

Before presenting the final recommendations and conclusions, essential clinical points that are of paramount importance are highlighted in table 8.1.

Table 8.1: Clinical points to remember

Clinical aspect	Points to remember / Interpretation
RCaOx stone presentation	Citrate is NOT the panacea for al CaOx or CaP nephrolithiasis treatment
Total body of Mg deficiency states	Sub-clinical Mg deficiency states are dangerous and found more in children. Also found in patients on chronic loop diuretics.
Use of allopurinol	Is safe and even important in CaOxSF in normo-uricosuria patients
CT scan diagnosis and Endo-uroscopic stone removal procedures	Remember, Randall's plaques are not stones. Patient safety of paramount importance.
Remember all the host of secondary and the large list of drug causes of nephrolithiasis	Varied pathogenesis and treatment. Recognition of and treatment of cause and or discontinuation of offending drug
High salt intake	Of paramount importance in causing RCaOxSF and can result in serious osteoporosis. Can only be diagnosed with 24-hour urine examination
The most important dietary advice to all stone formers (and everyone else)	Eat a little of everything and a lot of nothing Note: When on a weight-reducing diet, this does not apply and only kicks in as maintenance when the target weight has been reached.
General scientific advice behind the overall dietary pattern	Diet imposes patterns of metabolism upon the organism. *

** Gilman J (1951) [281]*

8.1.2 Summary of research recommendations resulting from this thesis

The investigation and management of RCa nephrolithiasis by a dedicated stone clinic requires careful patient selection procedures, a detailed work-up protocol, appropriate treatment and periodic re-examination of the risk factors. In addition, ongoing reassessment of the success of the clinic, including cost effectiveness, is mandatory.

Amongst the most important aspects of future research, is to look at the genetic, epidemiological, pathogenetic and other aspects of nephrolithiasis in the various population groups. Many genetic aberrations have been described in the various forms of nephrolithiasis [282] (excluding the TRPV-5 genetic polymorphisms) which requires in-depth study in South Africa. Some of the suggested research would not be possible at the inception of a stone clinic but will be contemplated once sufficient data has been accumulated. In the short term a few simple, urgently required and easily performed research topics could be chosen from the suggested list. However, the bulk of the research as suggested below would have to be dealt with as seen fit and practical with due prioritisation of the available resources. While some protocols are either drawn up or partially drawn up, some of which may form part of international studies, there are many new protocols that would need to be created if and when the opportunity arises.

However before presenting categories of future stone research a pivotal point must be presented suggesting that all previous drug trials in stone patients have ignored the impact that residual stone disease could have on the trial results. For all future studies, it is essential to know how many residuals but asymptomatic calculi there are (either in one or in both kidneys), as well as their size and position. During the study period “old” stones may be passed either asymptomatically or may produce symptoms which could appear as a lack of efficacy of the treatment. Therefore, at the termination of the trial, repeat scans should be done to assess the number of new stones which may still be present in the kidney, or to note symptomatic episodes of stone disease during the trial which may either present as previous stone passage or new stone passage. This may impact either positively, negatively or not at all on the results but should be included in future trials.

Summary of suggested future research topics grouped and appropriately ranked in terms of priority are:

A. Essential / Urgent

i. Undertake the research on all types and subtypes of stone risk factors assessments using strict and standardized protocols taking into consideration all the shortfalls mentioned in the thesis e.g. black vs white controls and patients with hypercalciuria and mild metabolic hyperoxaluria. Included in this

group but as a separate study should be a multi-centre epidemiological assessment of black patients with RCaOxSF.

ii. Study all risk factors in Asian patients, vegetarians and vegans along with protocols mentioned in the above point.

iii. The role of P in the genesis of RCaOx/PSF. This study requires the estimation of fractional excretion rates of urinary P in black, white and Asian controls and RCaOx/PSF to assess a new but potentially important group of risk factors in the development of nephrolithiasis.

iv. Study of the potential pathogenetic mechanism(s) of RSF secondary to inappropriate hyperphosphaturia. This must be carried out in all populations with Ca nephrolithiasis including control measurements of the fractional excretion of P in all normal ethnic control groups. In addition to this study, the levels of $25(OH)D_3$, $1,25(OH)_2D_3$ and FGF-23 will be measured in all patients and control groups.

v. Study of the phenotypic variants of the distal-tubular Ca sensing channel i.e. the TRPV-5 (i.e. transient receptor potential vanilloid 5) in black, white and Asian RSFs.

vi. Study of function / dysfunction of the TRPV-5 chemical in RSF patients with:
    a. Obesity (metabolic syndrome);
    b. Diabetes mellitus, and;
    c. Fructose “gluttony”.

vii. Study of the role of tubular crystal formation and growth in the genesis of ADPKD. This study outline is briefly summarized in the schematic presentation in figure 8.1.

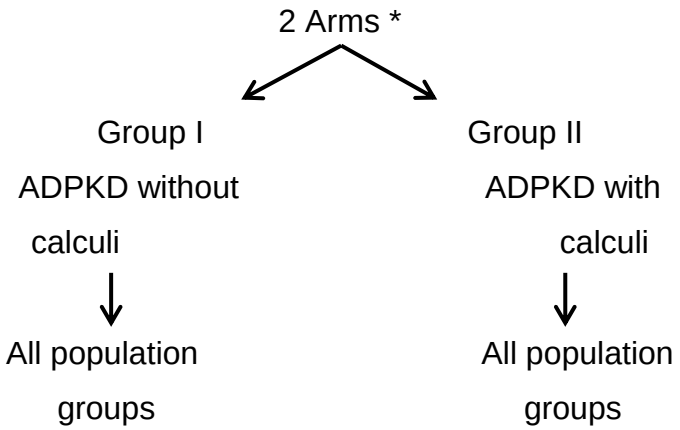


Figure 8.1: Schematic presentation on the study of nephrolithiasis in ADPKD

** Patients will be divided into two groups as shown for:*

- *Adults with ADPKD (Autosomal dominant polycystic kidney disease)*
- *Children (from age ~10 years) with suspected ADPKD*

Notes:

a. If polycystin 1 and 2 levels can be assessed, this will be included.

b. The management of adults and children with potential crystal deposition with or without stones will be assessed.

viii. The study of the use of IND with and without the addition of spironolactone in the management of nephrolithiasis. This is an important study which can easily be accomplished and will compare the effect of the treatment on hypercalciuria in the various population groups. In addition, a sub-group where spironolactone is added to IND will be planned. The main motivation of repeating this study is to endeavour to achieve global consensus of this subject and in particular to stress the hazards of using what we now call "high dose thiazides" for patients with nephrolithiasis.

B. Future nephrolithiasis studies

i. Study in children aged 0-10 years who were "abused" by receiving excessive antibiotics in this period. [25] This study will include aspects of:
   a. Changes in body habitat (weight gain)
   b. Premature obesity,
   c. Premature hypertension; and,

d. Nephrolithiasis,

ii. Studies of lithogenic macromolecules

a. GAGS levels. The GAG targeted would require measurements of urinary bikunin and to assess its role in the protection of SF in the various ethnic groups (patients and controls).

b. Studies on the role of THP using the above parameters

c. Studies of the urinary levels and kidney stone inhibitory properties of OPN - the so-called “molecule for all seasons”, The same parameters stipulated for the GAGS studies apply.

iii. Randall’s plaques

a. Studies in blacks, whites and Asians of material obtained from existing (but retrospectively assessed) x-rays, i.e. CTS/MRI as obtained from public and private units as well as normal black and white controls.

b. These studies should be accompanied (if at all possible) by measurements of urinary GAGS in those who can be traced.

c. In addition, post-mortem material for this type of study should be investigated as a possible way forward and which could be important in shedding light of the function of RP in the various population groups.

iv. Studies of the gut-microbiome

a. Studies in three different circumstances

1. Children who have been exposed to antibiotic abuse (see above)
2. Patients who have nephrolithiasis associated with the abuse of PPI
3. Patients who may have inherited defects in the numbers and function of the GIT oxalobacter formigenes content.

v. Studies of the glycolic acid pathway relating to:

a. “mild metabolic” or other causes of primary, non-inherited hyperoxaluria, and;

b. patients taking mega-doses of vitamin C in the winter to prevent influenza

vi. The role of uric acid in the non-gouty RCaOXSF as well as non-gouty pure uric acid stones.

a. Establishment of normal control uric acids needs to be studied in all population groups as well as those with hyperuricaemia, hypercalciuria, hyperoxaluria and hyperuricosuria.

b. These levels also need to be studied in those with metabolic syndrome, diabetes II and obesity.
c. Links should be recorded with any form of cardiovascular diseases in these patients.
d. Long-term control trial of allopurinol in comparison to placebo (i.e. dietary control) with respect to the efficacy and safety of long-term administration of allopurinol. This needs to be on the recommendation list because of the few papers highlighting the dangers of allopurinol use in these circumstances, especially if not proven to be of significant clinical importance in the treatment of these patients as discussed previously.
e. All cardiovascular comorbidities should be investigated as well as cardiovascular and all-cause mortality data.

C. Other research

i. The role of cystine in CaOx/PSF in black and white South Africans is recommended
ii. Assessment of the cost-effectiveness of the Nephrolithiasis Institute. This study should be commenced 2 to 3 years after the inception of the stone institute. A most important aspect of this research includes the calculation of usual urological stone management in SA (including i.e. hospital admission, surgical procedures, time off work, etc.) vs the dedicated stone clinic protocol as outlined in the thesis.
iii. Another important aspect of research to be undertaken is to look at the creation of a stone risk factor index for SA. This would be a unique exercise for SA and will be contemplated once the other recommended research protocols have been completed. Because the subject has not been dealt with in this thesis a brief summary of the rationale of a stone risk factor index is necessary.

In 2014 Turney et al [283] published the concept of a stone index using seven parameters of a 24-hour urine in 26 patients with RCaOxSF. They looked at seven risk factors in the 24-hour urine samples which included volume, pH, Ca, uric acid, citrate oxalate and Mg. The patients were then stratified into various risk categories and then put onto the appropriate treatment. Repeat

24-hour urines were then collected after 4 months which showed that 65% of them had a marked reduction in their PSF (probability of SF). They cautioned that previous algorithms formulated to adopt a stone risk index had shown contradictory outcomes but pointed out that the reason for this was that different populations had different genetic backgrounds, different dietary backgrounds and often very different environmental factors. Therefore, it would be appropriate to do a risk index for one's own country or area to assess the benefits of management. Criticisms of this article included the fact that they did not measure urinary sodium or K and they did not specify the method of urinary collection and pH measurement. Nor did they follow their patients long enough in order to calculate therapeutic benefit. Nevertheless, the technique needs to be researched further in SA in order to establish a better method of who to include or to exclude in stone work-up. A future stone risk index for SA would be an essential future development.

This extensive list is presented to emphasize the importance of a stone clinic in the rational management of nephrolithiasis which is a common and expensive disease. Obviously, it would be impossible to carry out more than one or two at any given time and so would have to be carefully prioritised.

8.2 CONCLUSION

This thesis was essentially concerned with two main aspects. Firstly, the review of the seven published papers in which the following factors were commented upon:

a. The relevance and importance of the work at that time i.e. all the successes of the work achieved,
b. Included in this aspect of this thesis was the ability to produce a validated list of normal urinary promotors and inhibitors in the various race groups in SA. This came as a result of extrapolation and summation of the data from five of the seven papers which were reviewed
c. To point out the shortcomings of all of the papers presented. This required an extensive literature search on clinical and pathological aspects which has also

been presented. These shortcomings included both protocol irregularities, the necessity of some repeat studies because of this and many other new studies which have not been performed in SA.

Secondly the dire necessity of a kidney stone institute including the structure and function of which is presented in detail which would be the first of its kind to be formally documented.

Finally, a most important responsibility of this thesis is the possibilities it has presented to draw up a teaching manual from the contents of the whole document. Although this does not form part of the thesis, if the concept of a kidney stone institute is accepted and implemented, such a document becomes mandatory and will be implemented as part of the way forward.

A presentation was done at a recent Update Symposium held under the auspice of the South African Renal Society (see list of presentations emanating from this thesis on p. vi), in which the data used was based on many of the aspects of this thesis. Prior to the presentation the following question was asked to an audience of about 80 people which consisted of a mixture of renal fellows as well as senior and experienced nephrologists; “How many of you are interested in the topic of urolithiasis from a nephrology point of view?” There was a zero hand-showing in answer to the question. After the Power Point presentation was completed, the same question was asked. This time 25 hands were raised, both from the fellows as well as the senior practitioners. This was pleasing and bodes well for the creation and implementation of the proposed kidney stone institute.

9 REFERENCE LIST

Note: Although internet links are not required, it is provided in most of the references as an additional tool of reference for the reader.

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10. APPENDICES:

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Note: References in the appendices which are already quoted in the main body of the thesis are referenced using the corresponding number, while additional references are added as footnotes.

APPENDIX A

STONE ANALYSIS / SEMI-QUANTITATIVE STONE ANALYSIS NORMAL VALUES

Normal excretion of various minerals and molecules in the urine are stated below. These levels were originally established from our own published studies. [40, 90, 92, 117, 180, 210, 229]

1. Urine volumes should never be < 2.1 – 2.4L per day in stone formers.
2. Accurate urine pH measurements on 24-hour:
   - Abnormal low ≤ 5.6 – suggests high acid ash (protein) diet
   - Abnormally high ≥ 6.4 – suggests RTA or infection (For RTA type 1 a standard urine pH of ≤5.3 rules out complete RTA.)
   - If urinary pH ≥ 5.4 perform further tests to exclude RTA.
3. Creatinine
   - > 7.4 – 17µmol/24-hour
     Note: < 7.4 found in some vegetarians. Note also: <7.4 is otherwise interpreted as representing a less-than-accurate 24-hour urine collection and would be repeated after discussion with patient.
4. Urea
   - > 16mmol/24-hour in females and 17mmol/24-hour in males represent an unacceptably high protein intake. We also measure 24-hour urine urea levels and make similar comparisons.
5. Sodium
   - ~80mmol/24-hour (i.e. ~5grams dietary salt per 24-hour)
6. Calcium
   - Usual quoted values:
     Males = up to 7.2mmol/day
     Females = up to 6.8mmol/day
   - Ideal as per Tiselius et al (see footnote 3) is 0.1mmol/kg/day of mean “ideal” body weight. (Preferred norm.)
7. Oxalate
   - Normal ranges as expressed by routine laboratory tests (internationally):
     Males = up to 450µmol/day
     Females = up to 400µmol/day
     Note: When 24-hour oxalate levels are measured in both males and females, all controls received ≥1,200mg per day of elemental Ca. The so-called normal levels given in the standard oxalate kit (Boehringer) did not consider normalising their levels for a recommended normal, healthy Ca diet but the Johannesburg Stone Clinic routinely recommends this dietary protocol. Therefore, the calculated accurate range is:
     Males = up to 350µmol/24-hour.

Females = up to 300µmol/24-hour.

These are relevant normals. [23, 24]

8. Citrate
   - Males ≥ 2.46mmol/24-hour
   - Females ≥ 2.36mmol/24-hour
9. Urinary uric acid

Table A.1: Current definitions for hyperuricosuria most commonly quoted:

	Sorensen et al [1]	Fink et al [2]	Tiselius et al [136]	Türk et al [3]
	mmol/24-hour	mmol/24-hour	mmol/24-hour	mmol/24-hour
Males	≥ 3.8 – 3.9	>4.8	4.3	>5
Females	≥ 3.5 – 3.6	>4.5	4.3	>4

10. Cystine
    - The Brand's test (BT) for screening is done by the laboratory.
    - If BT cystine values greater than 242mg/24-hour a diagnosis of cystinuria can be confirmed.
    - For BT values between 188 and 242mg/24-hour a more specific technique would be required to confirm the diagnosis.

Notes:

i. Recently the Ca excretion before and after a Ca load has not been determined because of all the dietary factors explained above. Without standardisation of these factors, especially the salt intake, the Ca load testing was thought to be an unnecessary additional expense.

ii. The urinary pyrophosphate is not routinely measured as this is an extremely difficult and costly test.

iii. The search for CaOx crystals on early morning urines should be carried out. However, this requires patients to pass urine into warmed thermoflasks and bring the specimen directly to the laboratory for microscopy. The problem encountered was that patients given the thermoflasks for these tests either "lost" or "broke" them or the patient was never seen again. This is a useful diagnostic exercise and should be re-introduced with all the necessary checks and balances.

[1] Sorensen CM, Chandhoke PS. Hyperuricosuric calcium nephrolithiasis. Endocrinol Metab Clin N Am 2002; 31(4):915-925. DOI: http://dx.doi.org/10.1016/S0889-8529(02)00038-5 http://www.endo.theclinics.com/article/S0889-8529(02)00038-5/fulltext

[2] Fink HA, Wilt TJ, Eidman KE, Garimella PS, MacDonald R, Rutks IR, Brasure M, Kane RL, Ouelette J, Monga M. Medical Management to Prevent Recurrent Nephrolithiasis in Adults: A Systematic Review for an American College of Physicians Clinical Guideline. Ann Intern Med. 2013;158(7):535-543. DOI: 10.7326/0003-4819-158-7-201304020-00005 http://annals.org/aim/fullarticle/1671716/medical-management-prevent-recurrent-nephrolithiasis-adults-systematic-review-american-college

[3] Türk et al. EAU Guidelines on Urolithiasis - limited update march 2016. European Association of Urology. Accessed online 23 January 2019. https://uroweb.org/wp-content/uploads/EAU-Guidelines-Urolithiasis-2016-1.pdf

APPENDIX B

CORRESPONDENCE

APPENDIX B.1

E-MAIL CORRESPONDENCE ON POSSIBLE FUTURE COLLABORATION ON ORAL ANTIBIOTIC EXPOSURE RESEARCH

National Kidney Foundation of South Africa

From: Tasian, Gregory E <TasianG@email.chop.edu>
Sent: 05 July 2018 10:43 PM
To: Denburg, Michelle R; National Kidney Foundation of South Africa
Subject: Re: stone antibotics

Dear Professor Meyers,

Indeed. Let's set up a time to discuss.

Greg
--
Gregory Tasian MD, MSc, MSCE
Assistant Professor of Urology and Epidemiology University of Pennsylvania Perelman School of Medicine Division of Urology and Center for Pediatric Clinical Effectiveness The Children's Hospital of Philadelphia

On 7/5/18, 3:04 PM, "Denburg, Michelle R" <DENBURGM@email.chop.edu> wrote:

Dear Professor Meyers

My apologies I missed this email somehow. I am cc'ing my colleague and collaborator Dr. Tasian from CHOP Urology. It would be great to find a way to collaborate.

Best
Michelle

Michelle Denburg, MD, MSCE
Assistant Professor of Pediatrics
Division of Nephrology
The Children's Hospital of Philadelphia
Perelman School of Medicine at the University of Pennsylvania
215-590-2449 (phone)
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immediately by sending a return message, and destroy the message you received. Thank you.

From: National Kidney Foundation of South Africa <nkfsa@mweb.co.za>

Sent: Thursday, May 31, 2018 5:47:41 AM
To: Denburg, Michelle R
Subject: FW: stone antibotics

Hi Michelle

As predicted Prof Meyers responded as follows:

Dear Michelle

Thank you for your correspondence. I know that I have several chinks in my armour but up to now I did not realize that my brain is included in an ever lengthening list! The best way of explaining my future most important research priority is to include in this correspondence what I had quoted in my doctorate thesis for your comments.

I would be most grateful for your thoughts and thank you for your time.

Best wishes

Tony Meyers

As included in dissertation submitted to WITS under the title: THE PIVOTAL ROLE OF A KIDNEY STONE CLINIC IN THE MANAGEMENT AND PREVENTION OF RECURRENT CALCIUM OXALATE NEPHROLITHIASIS:

"A seminal paper on the role of "Oral antibiotic exposure and kidney stone disease" has just been published. [23] A cohort of 25,981 children with renal calculi were studied all of whom had recently been exposed to several groups of oral antibiotics. There was a highly significant correlation between oral sulfas, cephalosporins and fluoroquinolones and the advent of stone disease which explained the morbid recent increase in paediatric stone disease being experienced in the USA and elsewhere. This is indeed an area in which our stone clinics could be involved in a South African based study. Valuable information could also be obtained by assessing whether recently given antibiotics to our children of various population groups produced similar or different results. In conclusion such a study is a priority and would be greatly enhanced if we could join forces with CHOP (Children's Hospital of Pennsylvania, USA)."

Kind regards
Fanie

From: National Kidney Foundation of South Africa [mailto:nkfsa@mweb.co.za]

Sent: 30 May 2018 08:09 AM
To: 'Denburg, Michelle R'
Subject: RE: stone antibotics

Dear Michelle

Thank you very much for the e-mail. Apologies but there is a little bit of a misunderstanding - Prof Meyers did not send an e-mail even if he might have had the intention to do so at the time that I printed the copy of the article for him. He did subsequently refer to the article in his current submission of a dissertation to the University of the Witwatersrand with the full reference included.

I will however print your e-mail and give it to him and I am sure that he will respond soon.

Thanks again and kind regards.

FANIE DU TOIT
Administrator

NATIONAL KIDNEY FOUNDATION OF SOUTH AFRICA
Tel.: 011-447-2531, Fax: 086-671-7347
e-mail:
nkfsa@mweb.co.za <mailto:nkfsa@mweb.co.za>
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Website:
www.nkf.org.za <http://www.nkf.org.za/>
Facebook:
Nkfsa (https://www.facebook.com/Nkfsa-National-Kidney-Foundation-of-SA-305907116088545/)

From: Denburg, Michelle R [mailto:DENBURGM@email.chop.edu]

Sent: 29 May 2018 05:40 PM
To: nkfsa@mweb.co.za
Cc: Meyers, Kevin E
Subject: stone antibotics

Hello Dr. Meyers,

Kevin mentioned that you had emailed me regarding our recent publication on antibiotic exposure and kidney stones. I apologize but looked and cannot find any email.
Perhaps you could reply to this email?

Thanks so much and look forward to hearing from you.

Best
Michelle

Michelle Denburg, MD, MSCE
Assistant Professor of Pediatrics and Epidemiology
Division of Nephrology
The Children's Hospital of Philadelphia
Perelman School of Medicine at the University of Pennsylvania
215-590-2449 (phone)
215-590-3705 (fax)

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APPENDIX B.2

E-MAIL ON PUBLICATION OF REVIEW ARTICLE ON CYSTINE

National Kidney Foundation of South Africa

From: National Kidney Foundation of South Africa <nkfsa@mweb.co.za>
Sent: 03 February 2020 09:24 AM
To: 'aua@AUAnet.org'
Subject: Possible review article

The Editor
Journal of Urology

Refer to article: J Urol. 2002 Jan;167(1):317-21.
Martins MC, **Meyers AA***, Whalley NA, Rodgers AL. Cystine: a promoter of the growth and aggregation of calcium oxalate crystals in normal undiluted human urine.

This article forms part of my (Emeritus Professor Anthony Meyers) Medical Doctorate thesis. However, the previously published article has had very few citations on what I consider an important topic. Two other points for consideration are, firstly, no cystine has ever been found in the calcium oxalate calculi in any of the indigenous black patients and secondly, as part of my thesis, I had to assess the published work. With the above considerations, I intend to write an assessment of the article together with our new work on our black patients. In addition I have (as part of the thesis), reviewed the literature on this subject.

Therefore I am asking whether you agree to me sending to your journal my article (when finished) which could take on the form of a review?

Please let me know your decision.

Many thanks.

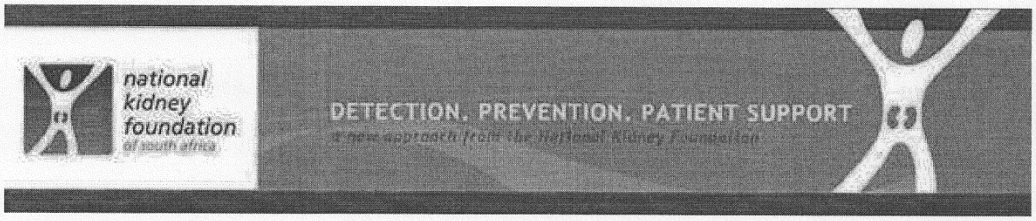
Anthony Meyers
Prof Emeritus WITS
NKFSA Chairperson

*Please note that the original article quoted Prof Meyers as AA Meyers which is incorrect and should actually be AM Meyers (Anthony Molyneux Meyers).

Sent on behalf of Prof Meyers:

FANIE DU TOIT
NKFSA Administrator

NATIONAL KIDNEY FOUNDATION OF SOUTH AFRICA
Tel.: 011-447-2531, Fax: 086-671-7347
e-mail: nkfsa@mweb.co.za
74 St Andrew Street, Birdhaven, Johannesburg, 2196
Website: www.nkf.org.za
Facebook: Nkfsa (https://www.facebook.com/Nkfsa-National-Kidney-Foundation-of-SA-305907116088545/)



APPENDIX C

ABSTRACT FROM THE SOUTH AFRICAN NEPHROLOGY RENAL CONGRES, CAPE TOWN, SOUTH AFRICA, SEPTEMBER 13-17, 1992

Kidney International, Vol. 43 (1993), pp. 1179–1190

Abstracts

South African Society of Nephrology Renal Congress,
Cape Town, South Africa, September 13–17, 1992

expressed as a function of total cell protein (μ/mg), EPO production (ml O_2/kg/min) and maximum workload achieved (W) during maximal exercise increased significantly after the 6-month training program (15.4 ± 2.5 vs. 19.8 ± 2.3 [mean ± SD] ml O_2/kg/min; $P < 0.01$; 57.0 ± 17.5 vs. 78.0 ± 14.7 W; $P < 0.01$); a further 27% increase ($P < 0.01$) was observed after 3 months of EPO treatment. There was a significant correlation ($r = 0.75$ to $r = 0.82$; $P < 0.01$ to $P < 0.001$) between peak muscle strength and peak workload achieved during maximum exercise. At any given workload, ventilation, heart rate, serum lactate concentration were all reduced, as were resting and maximum systolic blood pressures. No changes occurred in hematocrit or hemoglobin with training prior to EPO administration, and no adverse events occurred during or after exercise. EPO treatment increased mean hemoglobin (5.2 ± 0.2 vs. 7.4 ± 1.8 g/dl; $P < 0.05$), and hematocrit levels (15.8 vs. 22.6%; $P < 0.05$) by 43%. We conclude that: (a) exercise training during hemodialysis treatments is safe; (b) muscle strength is a predictor of exercise capacity in renal dialysis patients receiving chronic hemodialysis; (c) exercise training increases physical work capacity and improves blood pressure control; (d) EPO partially corrects anemia and increases physical working capacity; and (e) the mechanism for increased skeletal muscle function in hemodialysis patients is not currently known.

Role of urinary copper and phosphorus in kidney stone formation. *A. Rodgers, L. Barbour, M. Pougnet, C. Lombard, and R. Ryall, Department of Chemistry, University of Cape Town, Cape Town, South Africa.* Past attempts to distinguish between stone formers (SF) and normals (N) on the basis of urinary component concentrations have failed to demonstrate consistent differences between the groups. One avenue not fully explored is that of urinary elements. Since the presence of any element in a kidney stone suggests that it may have fulfilled some determinant role in the stone's pathogenesis, the present study was undertaken to establish whether differences exist between stone formers and controls with respect to their excretion of such elements. Nineteen male calcium oxalate stone formers and 20 healthy male controls participated in the study. Early morning urines were collected for convenience and because they were more likely to reveal differences than 24 hr collections as a result of their more concentrated nature. Subjects collected urines on 3 consecutive mornings (Mon, Tues, Wed) to compensate for random daily fluctuations in urine composition. The concentrations of several elements were determined by inductively coupled plasma atomic emission spectroscopy and graphite furnace atomic absorption spectroscopy. Only those elements which have been detected in stones or urine and for which we have ac- tions of P in stone formers might also be indicative of lower concentrations of pyrophosphate, a recognized inhibitor of calcium oxalate crystallization.

Urinary excretion of glycosaminoglycans in normal Black and White subjects and in recurrent renal stone formers. *A.M. Meyers, N. Whalley, M. Martins, and L.A. Margolius, Department of Nephrology, Johannesburg Hospital and University of Witwatersrand, South Africa.* Renal calculi form via the processes of crystal nucleation, aggregation and growth. Urinary supersaturation and inhibitor deficiencies constitute important risk factors. We and others have shown a pivotal role played by reduction in the urinary concentration of small molecular weight inhibitors, such as, citrate, pyrophosphate and Mg, in the pathogenesis of recurrent renal calculi. In patients with single oxalate calculi, macromolecular weight inhibitors such as glycosaminoglycans (GAG) and crystal matrix protein have been shown to be markedly reduced in concentration. However, much controversy exists about urinary GAG levels in patients with recurrent oxalate calculi. Low, normal or even raised levels have been reported. Additionally, nothing is known about GAG levels in Black normals and recurrent stone formers, and in particular, whether, if the normal population has high GAG levels, this could be protective towards the low incidence of renal calculi in the Black population. For this reason, we have studied urinary GAG excretion in Black and Caucasian normals (Groups C1 & C2 and Black and Caucasian recurrent stone formers (P1 & P2). Aliquots of 24 hour urines and early morning "spot" specimens collected without preservative and on the patients usual diet were stored −20°C. Urine was purified and GAG precipitated with CPC solution. Total GA was measured via a modified method of glucuronic acid determination. Further aliquots were stored for component GAG measurement via HPLC, that is, chondroitin and heparan sulphate and hyaluronic acid.

Collection	Group	*N*	GAG	*P* value
"Spot" mg/g creat	C1	16	4.24 (1.04)	<0.05
	C2	16	2.92 (0.95)	
24 hr mg/g creat	P1	5	9.67 (11.4)	
	P2	29	5.69 (2.4)	NS
Micro-mol 24 hr	C2	17	21.65 (7.3)	<0.05
	P2	29	43.05 (15.5)	

Thus, these preliminary results suggest that GAG levels are significantly higher in Black normals, but that both Black and Caucasian stone formers have, in fact, higher concentrations of urinary GAG than the normal population. The reasons for this are not known. However, more numbers are required, particularly in Black patients, before definite conclusions can be drawn. In addition, "spot" specimens must be validated with 24 hour urines, and HPLC GAG must be analyzed as one or more of the GAG components which could be the critical factor conferring protection.

A clinical trial comparing the treatment of hypercholesterolemia with simvastatin and diet versus diet alone in patients with idiopathic membranous nephropathy: A preliminary report. *B.L. Rayner, M. Byrne, R.* time of stopping the prednisone dose ranged between 20 and 24 mg/day. During the changeover, azathioprine was either started or if the patient was already receiving this agent the dose was doubled from 50 to 100 mg/day. The prednisone dose was transiently increased to between 28 and 32 mg/day, but then rapidly tailed off. By the end of the year the mean prednisone dose was 14 mg/day. By three months following transplantation, the cholesterol had risen from a mean of 5.08 mmol/liter (pre-transplant) to 6.01 mmol/liter ($P < 0.0001$). By 6 months the mean cholesterol had risen even further to 6.30 mmol/liter. By the end of the first year, cyclosporine had been stopped for a period of 6 months and the steroid dose reduced to a mean of 14 mg/day, yet the mean cholesterol level was now 6.42 mmol/liter. Even at 4 years the mean level was still 6.30 mmol/liter despite a low-maintenance steroid dose of

APPENDIX D

PATIENT INFORMATION SHEET

4 lessons to remember and share with the patient:

1. How a stone form.
2. How to prevent any new stones occurring in the future:
    a. Dietary advice
    b. Medication adherence
3. Follow-up tests:
    a. Urine in 2-3 months after therapy started
    b. Annual (occasionally 6-monthly) follow-up visits
    c. Annual follow-up urine and blood tests
    d. Annual either ultrasound or CAT-scan (without contrast) of kidneys to detect either new stones or change in size or position of old ones for the first 5 years. After this only when indicated.
4. Remember follow-up is life-long.

Explanation of points 1 and 2:

1. Formulation of a stone:

a. Urinary indications:

Table D.1: Urinary factors in the formulation of a kidney stone

Excess in the urine of:	Lack in the urine of:
Calcium* Oxalate** Sodium (salt) Uric acid Low urine pH Cystine Bacteria	Urine volume <1.8-2.4L/day Inhibitors of stone formation: • Organic • Inorganic – especially “citrate”

* Upper limit of normal calcium excretion +- 6.8mmol/day in females and 7.2 in males or, 0.1mmol/kg per day of mean value of ideal body weight.

** Upper limit of normal oxalate = 350mmols per day in males and 300 in females

Note: Urinary levels of urea and creatinine are used to roughly assess the amount of protein in the diet – high or low and if less than the lower limit of normal represents strong evidence that the 24-hour urine sample is incorrect.

b. Blood level indications:

Excess or deficiency in the blood in terms of:

- Hypercalcaemia – multiple causes
- Hypokalaemia – diet or multiple causes
- Hyperuricemia – gout or other causes
- Metabolic acidosis – renal tubular acidosis

2. Prevention

a. Dietary

- Avoid high protein diet
- Avoid or minimize red meat
- Avoid a high carbohydrate/sugar diet
- Avoid foods high in oxalate
- Ensure a normal Ca diet (i.e.>1.2g per day)
- Avoid unnecessary supplements:
    - Vitamins (especially Vit C and Vit D);
    - Tablets high in Ca; and
    - “Health” foods high in Ca

The most important dietary advice relates to excessive salt intake. The more salt the more Ca is lost in the urine with the production of CaOx stones.

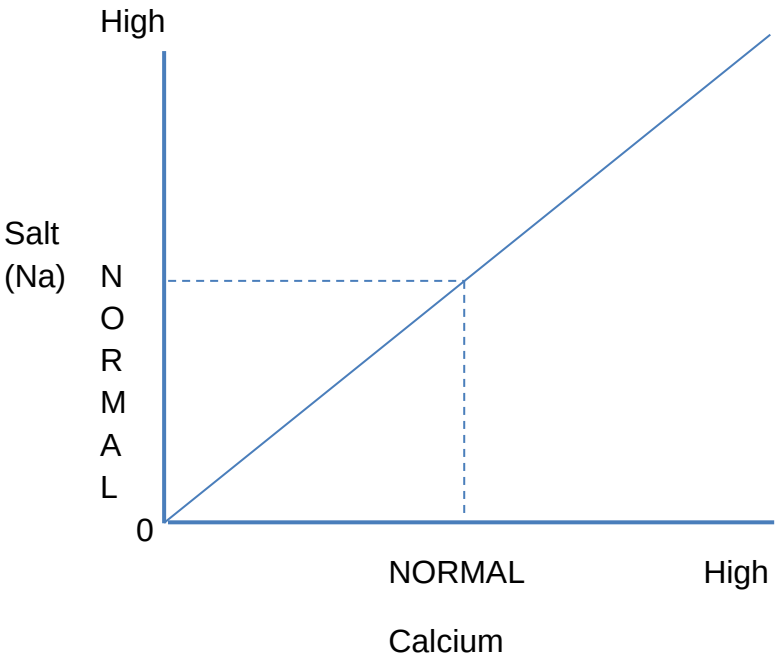


Figure D.1: The Salt : Ca ratio

Interpretation of the graph:
Ingestion of <5g of NaCl/day will result in a maximum of Na- excretion of 80mml Na/24-hour. Excessive salt intake accounts for 80% of hypercalciuria. Therefore, limitation of salt intake will benefit the patient in 3 ways:

- No new stones
- Blood pressure control
- Cardiac health

b. Medication advice

i. Has to be life-long otherwise the stones will occur again.
ii. If the cause of your kidney stones is either CaOx or CaP, please remember to always take potassium citrate liquid and not other preparations such as Uralyte U
iii. The potassium citrate solution has to be taken 3 times per day (with meals). If taken less, the urine citrate levels fall again and, even in this short space, you could begin to make another new stone. It only takes 2 hours to make a new stone in the kidney tubules (see reference 1)
iv. If you are put onto Allopurinol to lower urine uric acid please remember 3 important points:
    - All patients must start with a low dose (100mg/day) going up to 300mg per day at 2-monthly intervals.
    - If you develop any rash, stop immediately and consult with your doctor.
    - Never take Allopurinol with a sulphur containing diuretic e.g. HCTZ, Indapamide. Cases of either serious skin rashes, some of them with blisters and hypotension can occur.

APPENDIX E

DIETARY ASSESSMENT AS REQUIRED BY THE STONE CLINIC DIETICIAN

Renal stone clinic patient dietary information sheet:

Food intake diary:

- Please complete the form to determine the dietary risk factors for stone formation.
- Please write down everything that you eat and drink.
- Write down the time at the start of each meal, snack or drink.
- Try to describe the food as accurately as possible e.g. a sandwich is actually three or four foods: bread – wholemeal, white, thick or thin; spread – butter, margarine or low-fat spread – cheese and lettuce etc.
- In addition, all spices added must also be indicated.
- Describe the portions as a weight where possible or in household measures e.g. slices, tablespoons etc.
- Only record food that is eaten (e.g. not what is left on the plate).
- If you are on any supplements, vitamins or medication, please indicate the brands, how many you are taking and when they are taken.
- Remember this includes salt, pepper, spices, condiments, etc., etc., etc.
- EVERYTHING THAT YOU EAT OR DRINK!

Please note:

- The form must be completed on the following days:
    - A normal weekday (to evaluate the diet on a normal weekday)
    - The Sunday of urine collection (to evaluate the diet on a weekend day and compare results with urine results)
    - Monday of urine collection (to evaluate the diet on a normal weekday and compare results with urine results)
- Please bring the completed form with you to your consultation with the dietician

Table E.1: Example of a dietary information sheet

Please tick the applicable box: **Normal weekday** ☐ **Sunday** ☐ **Monday** ☐

Meal	Food	Amount	Cooking methods	Spices/ Flavourants/ Sauces added	Time eaten	Where eaten
Breakfast						
Snack						
Lunch						
Snack						
Dinner						
Snack						

Table E.2: Example of a meal filled in on the dietary assessment form:

Please tick the applicable box: **Normal weekday** ☐ **Sunday** ☒ **Monday** ☐

Meal	Food	Amount	Cooking methods	Spices/ Flavourants/ Sauces added	Time eaten	Where eaten
Breakfast	Egg Bacon White toast Flora Light margarine	1 large egg 2 rashers 2 slices 1tsp on each slice	Scrambled Fried Toasted	Salt (a pinch), pepper (a pinch), Parsley (1/2 tsp)	8:00	At home
Snack						
Lunch						
Snack						
Dinner						
Snack						

APPENDIX F

ROUTINE BLOOD TESTS

Included in the list of routine blood tests for urolithiasis management (diagnostic and follow-up) are:

- FBC (Full blood count)
- Platelets
- ESR (Erythrocyte sedimentation rate)
- CRP (C-Reactive protein)
- Albumin
- Urea
- Serum Creatinine
- Electrolytes
- eGFR (estimated glomerular filtration rate)
- Ca (Calcium)
- PO4 (Phosphorous)
- PTH (Parathyroid hormone)
- AST (Aspartate aminotransferase)
- GGT (Gamma Glutamyl Transferase)
- Fasting lipid profile
- FBS (Fasting Blood Sugar (Glucose))
- TSH (Thyroid Stimulating Hormone)
- Vit D (25(OH)D (25-Hydroxy Vitamin D))
- Uric Acid
- Serum Magnesium (not commonly done in SA)
- ABG (Arterial Blood Gas) (only if required)
- Other tests (to be specified at request)

APPENDIX G

ASSESSMENT OF RENAL TUBULAR ACIDOSIS

The assessment of distal renal tubular acidosis (dRTA) is only carried out if indicated as described below:

Test 1:

The test followed by the Johannesburg stone clinic uses furosemide instead of the nausea and vomiting production associated with the original acid load test described by Wrong and Davies (1959)[1]. This was later modified by Walsch et al (2007)[2] using furosemide plus fludrocortisone (FF-test). Because this modification was questioned by recent animal studies, Bech et al (2017)[3] reconfirmed the accuracy and validity of the FF-test for the diagnosis of dRTA.

Test 2:

Another alternative for the rapid diagnosis of dRTA is under investigation at the Johannesburg stone clinic. The technique is to obtain arterial blood for blood gas estimation and thereafter to assess the changes that are relevant. However, there are three caveats to consider before taking this test as valid:

a) Proximal tubular acidosis must be excluded.
b) The patient must be at rest and not hyperventilating when the arterial puncture is made. Resting periods of pre and immediately after obtaining the arterial blood, respiratory rates are noted and, in the face of hyperventilation, the results would be non-valid. Patient choices therefore are also important.
c) As the technique is still under investigation the results will determine the validity or otherwise. If successful a formal research study will be performed.

[1] Wrong O, Davies HEF. The excretion of acid in renal disease. QJM: An International Journal of Medicine, Volume 28, Issue 2, April 1959, Pages 259–313. https://doi.org/10.1093/oxfordjournals.qjmed.a066844
[2] Walsh SB, Shirley DG, Wrong OM, Unwin RJ. Urinary acidification assessed by simultaneous furosemide and fludrocortisone treatment: an alternative to ammonium chloride. Kidney Int. 2007 Jun;71(12):1310-6. Epub 2007 Apr 4. DOI: https://doi.org/10.1038/sj.ki.5002220
[3] Bech AP et al. Use of the Furosemide Fludrocortisone Test to Clinically Assess Distal Tubular Acidification. American Journal of Kidney Diseases, Volume 70, Issue 4, 589 – 591 doi: https://doi.org/10.1053/j.ajkd.2017.05.009

APPENDIX H

ROUTINE 24-HOUR URINE TESTS

Two 24-hour urine microscopy and culture collections (one on Sunday (specimen I) and one on the following Monday (specimen II) to assess the "weekend" effect). Electrolyte : creatinine ratios are not accurate enough and 24-hour collections are essential.[1]

- **Collection bottles:**
  - Neither 1L or 2L plastic bottles are permitted for urine collection for obvious reasons i.e. multiple specimen bottles increase the risk of contamination.
  - Patient to label the bottles clearly.
  - Bottles with samples must be refrigerated at all times and for travel it must be kept in an adequate cool bag which can accommodate a 4L bottle and 3 to 5 free ice gel blocks. This includes the specimen taken by the patient on the Tuesday morning to the central stone clinic laboratory. Note: Distant laboratories cannot be used as delay in specimen handling is not acceptable (e.g. contamination notes).

- **Urine acidity or basicity levels:**
  - pH must be read on a sophisticated pH meter as soon after sample delivery as possible. Urine dipstick methods are not permitted.
  - Notes on pH levels:
    - A pH of ≤5.3 rules out type 1 distal renal tubular acidosis but pH of ≥ 5.8 suggests high food protein acid load.
    - A pH of ≥6.4 is unusual on a normal 24-hour urine.
    - A pH of 7.6 is suggestive of either complete type 1 distal renal tubular acidosis or a patient on a carbonic anhydrase inhibitor.
    - A pH of ≤7.0 is highly suggestive of a CaP (brushite) stone. Again, to remember, brushite calculi are associated with a marked increase of fibrosis and a higher rate of developing significant future renal dysfunction.

  The laboratory measurement of oxalate, urine pH should be performed with a urine pH of ≤3 (some suggest 1.5) using either hydrochloric acid or concentrated nitric acid.

- **Low urine volume**
  - Renal stone formers should pass at least 2.25 to 2.5 (up to 3) litres of urine per day.

[1] Reynolds TM. ACP Best Practice No 181: Chemical pathology clinical investigation and management of nephrolithiasis. J Clin Pathol. 2005 Feb;58(2):134-40. doi: 10.1136/jcp.2004.019588 https://jcp.bmj.com/content/jclinpath/58/2/134.full.pdf

- Highly concentrated urines are more common in hot areas, However, the commonest stones found in these regions is not CaOx but rather uric acid.
- It has been noted that recent climate changes may be linked to an increase in stone formation in children [20] and adults [21]

- **An important stand-alone point on the discussion with the patient**
  - It is mandatory to emphasize that when performing 24-hour urine tests for mineral and other measurements of stone promotors and inhibitors, NO advice on fluid intake adequacy is to be discussed with the patient. The reason for this is the essential knowledge of assessing whether the "correct" volume of fluid, as assessed by a 24-hour urinary volume, is being ingested.

Following is the proposed patient information sheet and collection instructions for a 24-hour urine specimen:

PATIENT INFORMATION SHEET FOR 24-hour URINE TEST

How to do a 24-hour urine test with 0-1% error

- When not to measure a 24-hour collection:
  - In-hospital patients (always erratic unless an indwelling catheter)
  - In females during menstruation
  - In unreliable subjects
  - In young children
  - In patients with possible or definite UTI
- Importance of instructions:
  - Using correct materials (no metal or glass)
  - Store in fridge (why? Ph control and prevention of contamination)
  - Inability to comply – tests will show and process will have to be repeated before any treatment decisions can be made
- We can accurately assess the validity of a complete or incomplete 24-hour sample. If incomplete, patient will have to repeat the sample and his/her medical aid will be informed thereof.
- Urines for stone assessment are collected in 2 separate bottles - routinely on a Sunday (bottle 1) and a Monday (bottle 2). If urine is lost during this collection the process must be repeated the following weekend. The receptionist must be informed of this in order to move the next (assessment) appointment forward.
- All patients are charged R50 per bottle refundable at the next (assessment) appointment.

Collection instructions for a 24-hour urine specimen

- If awake at e.g. 7am, set alarm for 7am and get up immediately, go directly to toilet and empty bladder down toilet. Flush away and this will now be the “start-time”.
- Then every time urine is passed it must be collected into a plastic jug and poured directly into the collecting bottle.
- This goes on all day and all the coming night if you wake up to pass urine.
- Set alarm for 7am in the morning and immediately get up, pass urine into the jug and pour it into the collection bottle. This last urine represents the “stop-time” i.e. the 24-hour collection is complete.
- If there is bowel action, first collect the urine into the jug before allowing the bowels to work.
- If urine is lost down the toilet or if you do not have the bottle with you for collecting then the process must be stopped, urine already collected must be poured down the toilet, the bottle rinsed with tap water and the process started again the next morning. Your doctor will be able to tell from the results if the sample is a “true” sample or not and will act accordingly.
- Label clearly with date and name. If samples are for stone assessment the bottles must also be clearly marked “Sunday – bottle 1” and “Monday – bottle 2”.
- Once collection is complete the bottle must be taken to the laboratory as soon as possible for the tests to be carried out.
- No preservative is used and be careful to avoid any contamination with soaps, sprays, etc.

APPENDIX I

SUGGESTED STANDARD PRINTED FORMAT FOR WORK-UP DATA (FOR SA LABORATORY STAFF)

A Urine tests – Notes:

1. No collections to be undertaken if patients either have an associated UTI or during menstruation.
2. Laboratory staff to fill in:

	Yes	No
• Has the patient fasted	☐	☐
• Was the 24-hour collection complete (i.e. any urine "lost" e.g. down the toilet?)	☐	☐
• Did the 2 bottles arrive in a suitable cooler bag?	☐	☐

3. All other electrolytes and citrate to be measured on unchanged specimens
4. If specimen II tests positive on dipstick for nitrate and has excess leucocytes do <u>not</u> measure citrate. Please report as such so that a repeat collection can be arranged.
5. If urine microscopy needs to be done do so on specimen II. Put a slot in to be ticked if microscopy is needed.

B Instructions to laboratory staff:

- Please do not insert any result as e.g. mmol/L or as amount : creatinine
- Please do insert all result as quantity / 24 hour (including microalbumin).
- Please store adequate (10 ml) of urine from each specimen (I and II) (frozen).
- Please use an accurate urine pH meter only to measure pH.
- Please add either hydrochloric acid (HCL) or Nitric acid (HNO3) to aliquots for measurement of Ca and Ox. Get pH to 3 or even down to 1.5 (best).
- Please add sodium hydroxide to aliquot to measure urate. Get pH to ≥7.8.

C Urine Chart

Table I.1: Suggested printed format for work-up data

Measurement (24-hour)	Specimen I (Sunday collection urine)	Specimen II (Normal day collection urine)
24-hour urine pH		
Volume		
Creatinine		
Urea		
Calcium		
Na		
K		
Mg		
PO4		
Albumin *		
Citrate **		
Acidify	Acidify this urine	Acidify this urine
pH		
Calcium		
Oxalate		
Alkalize	No alkalisation of this urine	Add alkali to this
pH	No measurement	
Uric acid	No measurement	

* If urinary tract infection (dipstick positive for nitrate) is present, omit 24-hour microalbumin and repeat test later.

** NB: If dipstick is positive for white blood count and nitrate then no citrate to be done but repeat 24-hour collection for citrate only.

APPENDIX J

AUTHORISATIONS

Appendix J.1

Publications

Table J.1: Publication authorisations

Article / Publication [Reference Number]	Name of publication	Journal / Book	Year of public-ation	Permission / Authorisation granted	Page
1 [92]	Nephron	**J**	**1990**	**e-mail received 21 March 2018 04:53 AM**	**212**
2 [90]	British Journal of Urology	**J**	**1998**	**Open access guidelines extract**	**213**
3 [180]	British Journal of Urology	**J**	**1991**	**e-mail received 23 November 2017 05:40 AM**	**214**
4 [229]	British Journal of Urology	**J**	**1999**	**e-mail received 01 November 2017 11:34 PM**	**215**
5 [117]	Journal of Urology	**J**	**2002**	**Open access guidelines extract**	**216**
6 [185]	British Journal of Urology	**J**	**1996**	**e-mail received 07 November 2017 01:14 AM**	**219**
7 [210]	British Journal of Urology	**J**	**1996**	**e-mail received 16 November 2017 12:47 AM**	**220**

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March 21, 2018 EBR/mes

Professor Anthony M. Meyers,
Division of Nephrology, Department of Medicine,
Metabolic Stone Clinic
Johannesburg,
South Africa

AUTHORIZATION LETTER

Dear Professor Meyers,

I am pleased to inform you that permission is granted hereby to reprint your article

Prevalence of Hypocitraturia and Hypopyrophosphaturia in Recurrent Calcium Stone Formers: As Isolated Defects or Associated with Other Metabolic Abnormalities
Laminski N.A. · Meyers A.M. · Sonnekus M.I. · Smyth A.E.
Nephron 1990;56:379–386 (DOI:10.1159/000186179)

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Yours sincerely,
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S. Glücklich

Silvia Glücklich (Mrs)
Rights Manager
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Appendix J.2

Clarification on the role of the candidate (Prof AM Meyers) in the publications presented in Chapter 6

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1st October 2019

To whom it may concern

Re: Contribution of Prof Anthony Meyer to the 7 publications submitted in his MD thesis

The research ideas and protocols used in the publications were predominantly from Prof Meyers (about 80%). Maria Martins wrote papers 5 and 6 and I wrote papers 1 to 4 and no 7 but Prof Meyers always proofread and edited them. He also helped in the laboratory on many occasions. Prof Meyers got Dr Kumar Grover from Adelaide to work with us in our laboratory and Prof Meyers was often in the laboratory with him. Prof Meyers also went to Cape Town to help Alan Rodgers do the x-ray diffraction crystallography study and did all the scanning electron microscopy work.

Thus his input to the papers was about 70% except for the cystine paper where his input was about 90%.

Yours sincerely

Dr Natalie Whalley

Dept. of Molecular Medicine and Haematology

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Appendix J.3

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Moraes MF	e-mail dated 20 March 2018 11:20 AM	2	225
Pretorius SS	e-mail dated 15 May 2018 11:55 AM	2	226
Rodgers AL	e-mail dated 24 February 2020 11:35 AM	5	227
Shar TG	Emigrated - not traceable	2	-
Smyth AE	Not traceable	1	-
Sonnekus MI	Deceased	1, 3	-
Whalley (Laminski) NA	Letter dated 25 October 2017 - e-mail 25 October 2017 10:51 AM	1, 2, 3, 4, 5, 6, 7	228

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Cc: nkfsa@mweb.co.za
Subject: Re: Kidney stine research

Dear Natalie
So nice to hear from you! Hope you and Prof Meyers are well. Of course he can use the work.

I am still in touch with SS Pretorius, her email address is sandra.pretorius@icloud.com

I do not know the others.

Kind regards
Robin

Robin Dolman
PhD Dietetics
Program Leader: Dietetics
School of Physiology, Nutrition and Consumer Science Faculty of Health Sciences North West University Potchefstroom 2520 South Africa

Tel: +27 - 18-299 4059
Fax: +27 - 18-299 2464
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Many thanks and kind regards
Natalie Whalley nee Laminski.

Maria da C Sales Martins

13 Pinkhill Park

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26 March 2018

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Your sincerely,

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To: Natalie Whalley
Subject: Re: Permission request from Prof Anthony Meyers

Dearest Nathalie

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I enclose my CV. I highlighted our paper in yellow.

And I hope Prof. Meyers will be happy.

I of course allow Prof. Meyers to proceed as it best suits him

I miss you all

With my best personal regards to You and Prof. Myers

Maria Francisca Moraes-Fontes

Stone Clinic Publication:

Whalley NA, Moraes MF, Shar TG, Pretorius SS, Meyers AM. Lithogenic risk factors in the urine of black and white subjects. Br J Urol 1998 Dec;82(6):785-790.

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Dr Sandra Pretorius
Postdoctoral Fellow
Centre for Exercise Science and Sports Medicine
University of the Witwatersrand
Mobile: +27(0)82 908 6814
e-mail: sandra.pretorius@icloud.com
sandra.pretorius@wits.ac.za

National Kidney Foundation of South Africa

From: Allen Rodgers <allen.rodgers@uct.ac.za>
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Subject: RE: Co-author agreement

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With best wishes
Allen

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Senior Research Scholar
Department of Chemistry
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Table 3.1	8	Panel 1		232
Figure 3.3	41	Figure 1	e-mail dated 20/01/2020 12:01	233
Figure 3.4	29	Figure 5	Letter dated 03/02/2020	231
Figure 3.6	130	Figure 5	e-mail dated 21/01/2020 10:03	234

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3 February 2020

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Recurrent renal stone disease—advances in pathogenesis and clinical management

Author: Geoffrey Bihl,Anthony Meyers

Publication: The Lancet
Publisher: Elsevier
Date: 25 August 2001

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APPENDIX K

ETHICS CLEARANCE

Human Research Ethics Committee (Medical) 50 years 1966 – 2016

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Office email: hrec-medical.researchoffice@wits.ac.za
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May 10, 2018.

To whom it may concern in the Faculty of Health Sciences.

This letter deals with the ethics clearance of a study of renal calculi. Professor Anthony Meyers is preparing an MD thesis with the approved title "Past and future perceptions on the important role of a dedicated clinic for the prevention and management of recurrent calcium oxalate nephrolithiasis".

Some weeks ago I was approached by Professor Meyers for a copy of his ethics clearance; he no longer had it due to clearance of papers when he retired. Meyers' first paper for his MD was published in 1990 and the last for the MD in 2001. My experience is that the ethics clearance would have been issued between 1987 and 1988. All the publications deal with kidney stones in patients so only one clearance would have been needed. While the HREC (Medical) office staff found many of Professor Meyers' study clearances in the current database and University registry but not the one he was searching for. Nowadays after 2-3 years the registry copies are passed on to a commercial storage company off campus – there was no success from a search there which is often the case.

Before 2002 Wits ethics clearances had an unlimited life (the same was at the other HRECs at UCT, UP, US, UKZN and Medunsa) - a specified period (5 years at Wits) was only introduced at all the South African universities after the site inspections at the six universities above by the three members of the US Office of Human Research Protections. The lack of a specified period at all these universities was the only criticism of the inspecting team. Nowadays renewals of clearances help the ethics database searches. Another help nowadays is the inclusion in publications of the ethics clearance number.

I have known Professor Meyers since I was a medical student in the second half of the 1960s. Whenever he is about to submit an application he has phoned me to ensure he has included pertinent information. Bearing this in mind the current Chair, Dr. Clement Penny contacted Dr. N. Whalley, a co-author on half the papers to be submitted for the MD, she confirmed that there was an ethics clearance.

I therefore confirm that ethics clearance for a study of renal calculi was approved.

Signed.
Professor Peter Cleaton-Jones, HREC (Medical) Chair 1985-2017.

Noted. 14th May, 2018.
Dr. Clement Penny, HREC (Medical) Chair 2018-

APPENDIX L

ADDITIONAL READING MATERIAL

These references are classified into subject groups to add to what is already cited in the main text. This has been done to enhance the thesis relevance and to put forward as much data as possible. The groups are:

Group 1: General research on urolithiasis

Group 2: Stones in children

Group 3: Epidemiology – general

Group 4: Epidemiology – specific; including population differences

Group 5: Pathogenesis – basic

Group 6: Pathogenesis – special

Group 7: Pathogenesis – racial differences

Group 8: Work-up – standard and new facts

Group 9: Work-up – Special and cutting-edge tests

Group 10: Management – Primary causes

Group 11: Management – Secondary causes

Group 12: Complications

Group 13: Stone clinic – cost and effectiveness

ADDITIONAL READING REFERENCES:

GROUP 1: GENERAL UROLITHIASIS

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GROUP 2: STONES IN CHILDREN

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GROUP 4: EPIDEMIOLOGY – SPECIFIC; INCLUDING POPULATION DIFFERENCES

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GROUP 6: PATHOGENESIS – SPECIAL

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