The effects of calcium, magnesium and citrate health supplements on urinary risk factors for calcium oxalate kidney stone formation

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SUMMARY

This thesis was undertaken to investigate the effects of various health supplements on the risk of calcium oxalate (CaOx) kidney stone formation. Four separate studies comprised the entire project.

In the first study, a new approach was implemented to investigate the individual, additive and synergistic effects of calcium, magnesium and citrate health supplements on the urinary risk factors for CaOx kidney stone formation in eight healthy males. While previous studies have examined the effects of individual preparations, few (if any) have addressed the issue of chemical interactions or synergism between different agents. The experimental design which was employed was a "Complete Latin Square Design" in which each subject was allocated to a randomized sequence of seven supplemental protocols in addition to the normal diet. These protocols comprised of calcium, magnesium and citrate supplements and combinations thereof. Supplementation lasted for 1 week. 24-hour urines were collected at baseline and during the final day of each supplemental protocol. The urine composition, relative supersaturation (RS), Tiselius risk index, metastable limit (MSL) and particle volume-size distributions were measured for each urine sample. The results showed that the activity of citrate-containing protocols in reducing several physicochemical risk factors for CaOx stone formation was superior to those containing calcium or magnesium alone and also that it behaves this way irrespective of whether the latter two components are present or not. The study also identified three interactions, viz. magnesium enhances citrate's ability to lower RS brushite (favourable synergistic interaction) and attenuates its ability to raise urinary pH (unfavourable synergistic interaction) and finally, calcium enhances citrate's ability to lower RS uric acid (additive effect).

In the second study, the potential prophylactic and therapeutic properties of a South African product (CitroSoda: sodium citrate bicarbonate tartrate), previously untested in the management of CaOx urolithiasis, was investigated. 30 healthy males (MC), 30 male CaOx stone formers (MSF), 30 healthy females (FC) and 30 female CaOx stone formers (FSF) participated in the study. The experimental design which was implemented was a placebo controlled, randomized, "within-patient" design. All the subjects were required to provide two 24h urine collections prior to the commencement of the trial which were used as baseline samples. Twenty subjects in each group ingested CitroSoda and ten subjects

in each group ingested a blinded placebo. Supplementation lasted for 7 days. In addition to the baseline 24h urine samples, subjects provided 24h collections on day 7 (final day of supplementation) and on day 10 (3 days after supplementation was suspended). The urine composition, relative supersaturation (RS), Tiselius risk index, metastable limit (MSL), particle volume-size distribution and [^{14}C]-oxalate deposition were measured for each urine sample. Scanning electron microscopy was also performed on selected samples. The results showed that CitroSoda favourably altered four biochemical risk factors in all four groups: pH and citrate excretion increased and the RS of uric acid and the RS of CaOx decreased. No unfavourable changes occurred in any of the risk factors. These results demonstrated that CitroSoda has the potential to be an effective preparation for the prophylactic and therapeutic management of CaOx urolithiasis.

Since the question of whether there is a stone forming risk associated with the ingestion of calcium supplements with meals or between meals remains unanswered, the third study was undertaken under strictly controlled conditions, to investigate urinary oxalate excretion as a function of the time at which a calcium supplement is ingested relative to an oxalate-rich meal. 20 healthy males participated in the study in which six different protocols were investigated over a six-hour time period. Subjects provided a fasting overnight urine sample on the morning of trial and thereafter ingested a standardized oxalate-rich meal. In the first protocol no calcium supplement was administered; in the 2nd protocol the calcium supplement was given with the meal and in the 3rd, 4th, 5th and 6th protocol, the supplement was given 1hr, 2hrs, 3hrs and 4hrs respectively after the meal. Subjects were required to provide urine samples at hourly intervals throughout the protocol, after which the urine composition and ion activity product of calcium oxalate (AP(CaOx) index) for each sample was calculated. The study demonstrated that calcium supplements taken 1 hour after an oxalate-rich meal produced the lowest total oxalate excretion over the 6-hour period following the ingestion of the oxalate-rich meal, relative to other protocols. The urinary calcium excretion was fairly steady in this protocol whereas for the other protocols, a dramatic surge was observed at some stage during the 6 hour test period. The AP(CaOx) index values supported the choice of this protocol as the optimum one because it displayed a level trend which was relatively lower (or equal) to that of the other protocols.

In the final study, a speciation program (JESS) which is not well-known in urolithiasis research, was firstly used to compare RS outputs with those of the more familiar EQUIL2.

Secondly, it was used to interpret the effects of the CitroSoda preparation on RS CaOx, RS brushite and RS uric acid observed in the study described above. Good correlation was found between the RS outputs of the two programs. However, because of JESS's superior capability for determining mixed ligand speciation, it was possible to demonstrate convincingly that the increase in urinary pH after administration of CitroSoda is the all-important urinary effect which caused the observed decrease in RS CaOx. This important result provides motivation for proposing that the therapeutic strategy of choice which should be adopted in the management of idiopathic CaOx urolithiasis is to attempt to raise the urinary pH using appropriate medication.

Thus, the results of the four studies presented in this thesis have yielded important findings in the context of CaOx urolithiasis research and in the context of the clinical management of this disease.

ABBREVIATIONS

CaOx	Calcium oxalate
COD	Calcium oxalate dihydrate
COM	Calcium oxalate monohydrate
FC	Female control
FSF	Female stone former
JESS	Joint Expert Speciation System
K-Cit	Potassium citrate
K-Mg-Cit	Potassium magnesium citrate
MC	Male control
MSF	Male stone former
MSL	Metastable limit
Na-Cit	Sodium citrate bicarbonate tartrate
Na-K-Cit	Sodium potassium citrate
RS	Relative supersaturation
SE	Standard error

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Chapter 1

General Introduction

1.1 KIDNEY STONES AND THEIR COMPOSITION

Urolithiasis is one of the most painful disorders to afflict human beings. The disease can be traced back approximately 6690 years corresponding to the age of a bladder stone removed from an Egyptian mummy (Rodgers 1991). It is evident from the literature that urolithiasis is a multi-factorial disease dependent on a wide range of epidemiological variables such as climate, geography, race, occupation, diet, drinking water as well as clinical conditions.

Most stones form in the urinary tract and contain calcium in combination with either oxalate or phosphate (Herring 1962, Prien 1963, Gibson 1974, Westbury 1974). Since about 70% of renal stones are composed predominantly of calcium oxalate (CaOx) (Marangella *et al.* 1999) it is important to try to inhibit crystallization of this salt. The formation of calcium oxalate stones is believed to occur in urine that is supersaturated with respect to CaOx and/or low content of crystallization inhibitors. As a consequence, urinary inhibitors of stone formation have long been of interest to stone researchers. Other stone types are uric acid (10-20%), calcium phosphate (approximately 8%) struvite (5-10%) and cystine (1-2%) (Ramello *et al.* 2000).

1.2 EPIDEMIOLOGY

1.2.1 Geography and Climate

As the atmospheric temperature increases, the incidence of stone disease has been shown to increase among people who do not drink adequate amounts of fluid (Blacklock 1982). This trend is particularly obvious in the South Eastern United States (South Eastern Stone Belt), (Blacklock 1982), Australia (Blacklock 1982) and Saudi Arabia (Robertson *et al.* 1994). A possible explanation is that as perspiration and dehydration increase, urinary output decreases resulting in an increase in the concentration of various stone-forming salts and their relative supersaturations.

Parry and Lister (1975) reported an increase in urinary calcium excretion in troops after arrival in a desert area. A similar trend in the excretion of urinary calcium was observed by Robertson *et al.* (1974) in the summer months in the United Kingdom. These authors suggest that prolonged exposure of the skin to UV radiation activates the conversion of 7-dehydrocholesterol to vitamin D_3 which together with its hydroxylated derivative, 25-

1-25-dihydroxyvitamin D_3, acts on the intestine and bone to increase plasma Ca^{2+} and PO_4^{3-} levels resulting in an increase in intestinal calcium absorption and as a result causes a potential increase in stone formation (Broadus *et al.* 1984).

1.2.2 Occupation

Many investigators have found an increase in the incidence of stone formation amongst certain occupational groups (Mates 1969, Blacklock 1969, Ferrie *et al.* 1984, Borghi *et al.* 1993). The prevalence of stone formation was found to be higher in people with sedentary work (Mates 1969, Blacklock 1969, Borghi *et al.* 1993). For example, a higher incidence of stone formation has been reported amongst aviation pilots and truck drivers (Borghi *et al.* 1993, Zheng *et al.* 2002). Mates (1969) also found that the incidence of stone disease in rural areas amongst farmers and forestry workers is negligible and attributed this observation to their active lifestyle.

1.2.3 Race, Gender and Age

Certain race groups have lower stone incidence rates than others (Bateson 1977, Rodgers 1991). A well known example occurs in South Africa where stone disease is extremely rare in the black population but occurs in approximately 15% of the white population (Wise *et al.* 1961, Modlin 1967, Meyers 1994, Whalley *et al.* 1998). This is surprising as studies of the urine composition of these two groups revealed that blacks have lower urinary citrate compared to whites (Modlin 1967, Whalley *et al.* 1998), which would have increased their risk of stone formation. A recent study proposed that a possible explanation for the difference in stone incidence could be due to the different renal handling mechanisms of calcium in the two race groups (Rodgers *et al.* 2002). Recent studies have focused on two inhibitory urinary proteins, Tamm-Horsfall mucoprotein (THM) (Craig *et al.* 1999) and urinary prothrombin fragment 1 (UPTF1) (Webber *et al.* 2002). These studies have suggested that the composition of urine in the black population enhances the inhibitory activity of the respective urinary protein. A lower prevalence of stones has also been observed in other race groups like Eskimos, American blacks and Aborigines (Bateson 1977, Rodgers 1991). In 1994, Soucie *et al.* examined the lifetime prevalence of kidney stones amongst blacks and whites in the United States and found that the prevalence amongst whites was twice that amongst blacks (Soucie *et al.* 1994).

Many studies have shown that urolithiasis is age-dependent (Ljunghall *et al.* 1977, Robertson *et al.* 1984a, Hesse *et al.* 1986) and that it is most prevalent between the ages of 30 and 60 (Hesse *et al.* 1986). This can be attributed to the excretion of certain urinary components which are influenced by age-dependent metabolism (Hesse *et al.* 1986). Evidence also indicates that twice as many males (7-15%) are afflicted by this disease than females (3-6%) (Scott *et al.* 1977, Blacklock 1982, Robertson *et al.* 1983, Hesse *et al.* 1986). Hesse and his co-workers (1986) observed that in females there is an increase in calcium and phosphate excretion and a decrease in pH in the 40-60 year age-group, and as the subject gets older there is a steady decrease in magnesium excretion in subjects over 30 years old. They also found that the urinary parameters in males are not as age-dependent as with females. There is evidence that oestrogen protects women against urolithiasis by lowering the urinary saturation of stone-forming salts (Heller et al. 2002) and that in men testosterone may increase the risk of stone formation by increasing urinary oxalate excretion (Lee *et al.* 1996).

1.2.4 Economics

The prevalence of stone disease in industrialized countries ranges from 1 to 10% (Ramello *et al.* 2000). This can be attributed to the increase in food consumption as a country becomes industrialized and societies become more affluent. Generally, the population starts consuming more animal proteins (Robertson *et al.* 1979, Robertson *et al.* 1982) which results in an increased risk of stone formation.

Treatment and management of stone disease are economic issues (Robertson 1999a, Strohmaier 1999, Tiselius 2000). Over the past 3 decades there has been a considerable improvement in the techniques used for stone removal from open surgery to non-invasive or slightly-invasive techniques (Tiselius 2003). These procedures are very costly, and are unable to solve the problem of stone recurrence. There is thus a strong need for preventative treatment. Indeed, Parks and Coe (1996) investigated the economic benefits of a metabolic risk-evaluation in 1100 patients and found that a medical stone preventative programme resulted in an annual average saving of US$ 2158 per patient. It is difficult to compare this figure to that obtained from other studies done in other countries because the cost of stone removal and prevention varies considerably from one country to the next and so does the choice of treatment. However, there is no doubt that it places a huge burden on the economy of a country and therefore the financial benefit of

reducing the recurrence of stones outweighs the cost of patient evaluation and medical therapy.

1.2.5 Genetic factors

Idiopathic calcium oxalate stone formation is a polygenic disorder (Goodman *et al.* 1995).

1.3 URINARY RISK FACTORS

It has long been recognized that urolithiasis is the combined result of a number of individual determinant factors which are called biochemical risk factors (Finlayson 1974a, Robertson *et al.* 1978, Robertson *et al.* 1981a, Hess *et al.* 1996). These factors can be considered as those determining the degree of supersaturation of urine, and those which affect the tendency of the crystals to enlarge and aggregate together once nucleation has occurred. The factors which affect 24-hour urine most include urinary pH, volume and the excretion rates (renal output) of calcium, citrate, magnesium, oxalate and uric acid. Of the seven risk factors mentioned, low urine volume and increased excretion of urinary oxalate are considered to be the most important risk factors for urine to become highly supersaturated (Hess *et al.* 1996). Each of these risk factors is discussed below.

1.3.1 Urinary pH

There appears to be a dual role of pH in calcium oxalate urolithiasis. On the one hand the solubility of calcium oxalate increases with decreasing pH (Tiselius 1981), while on the other hand inhibitory activity towards calcium oxalate crystallization increases with increasing pH (Pak 1994, Hesse *et al.* 1997). An explanation for the latter observation is that at a higher pH, more phosphate and citrate ions are dissociated which in turn increases the complexation of calcium, thereby reducing the urinary saturation of CaOx (Pak 1994). Therefore patients with CaOx urolithiasis would benefit from therapeutic alkalanization but this could cause an increase in the risk of calcium phosphate (brushite) formation (Tiselius *et al.* 1981). Tiselius (1981) has suggested that in patients with hypercalciuria (high concentration of calcium in the urine), therapeutic alkalanization should be combined with calcium reducing measures to avoid brushite stone formation.

On the other hand, an acidic pH increases the risk of uric acid stones (Finlayson *et al.* 1974b, Tiselius 2003).

1.3.2 Urine Volume

A high fluid intake has for a long time been the recommended treatment for kidney stones (Pak *et al.* 1980, Consensus Conference 1988, Borghi *et al.* 1996, Borghi *et al.* 1999a). Pak *et al.* (1980) investigated the effect of diluting urine, by implementing a high water intake, on the crystallization of calcium salts and they found that this regimen could inhibit the formation of kidney stones by reducing the saturation of calcium salts. They expressed concern that diluting the urine resulted in dilution of the inhibitors which could decrease the effect of the reduced saturation of the calcium salts but concluded that increasing the urine volume from 1 to 2 litres per day increased the CaOx metastable limit (MSL) suggesting that diluting the urine could have a protective effect on CaOx crystallization.

In a randomized study Borghi *et al.* (1996) also showed the clinical significance of a high fluid intake on kidney stone formation. They found that by increasing fluid intake to achieve a urinary volume of 2 litres per day, both the urinary calcium and urinary oxalate concentrations decreased significantly, leading to a reduction in the stone recurrence rate from 27% to 12 %. Borghi *et al.* (1999a) investigated the effect of increasing the urine volume on the CaOx supersaturation and the effect of macromolecules on CaOx crystallization. They found that the increase in water intake reduced the CaOx saturation, but did not substantially alter the CaOx MSL and that the dilution of the urine did not change the ability of macromecules to inhibit CaOx crystallization.

Fluids other than water can also be consumed to achieve a higher urinary volume, e.g. apple juice (Vahlensieck 1986), herbal teas (Hesse *et al.* 1993), lemon juice (Seltzer *et al.* 1996) and cranberry juice (McHarg and Rodgers 2003), without posing a risk for urolithiasis. Beverages that should be avoided are those with a high oxalate content, e.g. cola (Rodgers 1999) and hot chocolate (Hesse *et al.* 1993).

From these studies it can be concluded that a low urine volume is a crucial risk factor of kidney stone formation as it can result in an increase in the saturation of stone-forming salts.

1.3.3 Urinary Calcium

It is well-established that hypercalciuria is a risk factor for recurrent stone formation (Ettinger 1979, Coe *et al.* 1982, Vahlensieck 1986, Goldfarb 1994, Borghi *et al.* 1996, Borghi et al. 1999b) since the calcium ion is common in both CaOx and calcium phosphate stones. Indeed, a significantly higher incidence of hypercalciuria has been recorded in stone formers compared to normal controls (Flocks 1939, Robertson *et al.* 1978, Coe *et al.* 1992, Pak 1998, Heilberg 2000, Curhan *et al.* 2001). However, some studies were unable to confirm this finding (Tiselius *et al.* 1978, Ryall and Marshall 1983, Ryall *et al.* 1984).

It has been reported that patients with idiopathic hypercalciuria present with either absorptive (Broadus et al. 1984, Coe and Favus 1986) or renal hypercalciuria (Coe and Favus 1986). Patients presenting with the former condition absorb calcium far more efficiently from their food than healthy subjects, leading to elevated levels of calcium in their blood which in turn is excreted in the urine after which the serum calcium normalizes. According to some investigators (Coe *et al.* 1973, Pak *et al.* 1975, Broadus *et al.* 1981), patients presenting with the latter condition have increased levels of parathyroid hormone. However, not all investigators agree with this observation (Burckhardt *et al.* 1981, Coe *et al.* 1982, Lilienfeld *et al.* 1982, Lien *et al.* 1983, Olmer *et al.* 1983). Studies have demonstrated that a low calcium diet reduces urinary calcium excretion significantly and increases urinary oxalate excretion significantly (Marshall *et al.* 1972, Barilla *et al.* 1978, Bataille *et al.* 1983).

Hypercalciuria is not only dependent on calcium intake; it can be caused by increased levels of sodium (Muldowney *et al.* 1982, Robertson 1987, Hesse *et al.* 1993, Sakhaee *et al.* 1993, Martini *et al.* 1998), as calcium and sodium have similar renal tubular handling mechanisms (Agus *et al.* 1981), and can also be caused by increased protein intake (Robertson 1979, Robertson 1987, Breslau et al. 1988, Trinchieri et al. 1991, Curhan 1993). It was also found that stone formers with hypercalciuria are twice as sensitive to sodium intake as normal stone formers (Brutis *et al.* 1994). Therefore a decrease in dietary sodium and protein is recommended for CaOx stone formers since this regimen decreases the calcium excretion and a concomitant decrease in oxalate and uric acid excretion is observed (Hesse 1999).

1.3.4 Urinary Oxalate

Urinary oxalate is known to have a far greater effect on the degree of supersaturation of urine with respect to calcium oxalate than calcium (Finlayson 1974a, Ito *et al.* 1992, Robertson *et al.* 1981a, Robertson *et al.* 1981b). Although various studies have shown that CaOx stone formers present a higher mean oxalate excretion than healthy controls (Robertson 1978, Goldfarb 1988, Schwille *et al.* 1989, Wilson *et al.* 1989), other studies were unable to corroborate this finding (Robertson 1968, Galosy *et al.* 1980, Ryall and Marshall 1983). This could be due to the difficulty experienced when measuring urinary oxalate accurately prior to 1984, as it was found that ascorbate converts to oxalate spontaneously at alkaline pH which could interfere with oxalate measurements (Mazzachi *et al.* 1984, Robertson and Scurr 1984b). The accurate determination of oxalate has only become possible in recent years (Robertson 1999b).

In 1999, Robertson (1999b) performed an *in vitro* experiment and found that only a small amount of sodium oxalate was required to initiate CaOx crystallization, whereas it was almost impossible to initiate crystallization by adding calcium chloride. This confirmed previous observations that although both calcium and oxalate are required for the formation of calcium oxalate stones, oxalate seems to play a far greater role than calcium [Ito *et al.* 1992, Robertson *et al.* 1981a]. As a result, it is more important to reduce the amount of oxalate excreted in the urine in order to prevent the recurrence of these stones.

Various mechanisms have been identified as the causes of hyperoxaluria (increased urinary oxalate excretion), viz. an increased dietary intake of oxalate (Robertson 1999b, Merhoff et al. 2001), increased intestinal absorption of oxalate from food, increased endogenous production of oxalate (Robertson 1999b, Merhoff et al. 2001) and a deficiency in oxalate-degrading bacteria in the gut (Allison et al. 1986, Robertson 1999b).
Since more than 80% of urinary oxalate is derived from endogenous production (Menon and Mahle 1982, Sutton and Walker 1994) it is very difficult to alter the amount of oxalate excreted. However, more recent studies refute this figure and this might alter the overall picture (Liebman & Chai 1997, Holmes *et al.* 2001).

1.3.5 Urinary Citrate

Numerous studies over the past twenty years have demonstrated the prevalence of hypocitraturia (low urinary citrate excretion) in CaOx kidney stone sufferers, thereby providing convincing evidence in support of it being a critical risk factor in this disease (Rudman *et al.* 1982, Nicar *et al.* 1983, Hosking *et al.* 1985, Laminski *et al.* 1990, Cupisti *et al.* 1992).

In vitro experiments during this same time period have demonstrated that citrate is an effective inhibitor of CaOx crystal nucleation (Hallson *et al.* 1983, Nicar *et al.* 1987, Schwille *et al.* 1999), growth (Ryall *et al.* 1981, Bek-Jensen *et al.* 1996) and aggregation (Ryall *et al.* 1981, Kok *et al.* 1986, Kok *et al.* 1987, Tiselius *et al.* 1993). Thus, citrate is able to retard all three of the recognised crystallization mechanisms of stone-forming salts. It achieves this by complexing calcium and reducing ionic calcium concentration. As such, the formation of CaOx and other salts such as calcium phosphate is inhibited (Meyer *et al.* 1975). Thus, the role of citrate in preventing stone formation may be ascribed to its ability to function as an inhibitor as well as form soluble complexes with calcium.

1.3.6 Urinary Magnesium

Several decades ago it was established that magnesium can decrease the crystallization of calcium oxalate by complexing with oxalate ions to form soluble magnesium oxalate, thus reducing the amount of free oxalate ions in the urine (Desmars *et al.* 1973, Hallson *et al.* 1982). This is advantageous since, as mentioned earlier, oxalate plays a far greater role in calcium oxalate stone formation than calcium. In vitro studies have shown that magnesium decreases nucleation and growth of calcium oxalate crystals (Li *et al.* 1985, Kohri *et al.* 1988). Magnesium has also been found to lower the urinary saturation of CaOx (Lindberg *et al.* 1990a) and increase urinary citrate (Lindberg *et al.* 1990b).

There is conflicting evidence regarding the relative levels of urinary magnesium in stone formers and controls. In some studies, urinary magnesium in stone formers was found to be lower than that of controls (Faragalla and Gershoff 1963, Trinchieri *et al.* 1991, Trinchieri *et al.* 1992) whereas in other studies it was found to be equal to or higher (Johansson *et al.* 1980, Drach 1988).

1.3.7 Urinary Uric Acid

Hyperuricosuria (a high uric acid excretion in the urine) is another known risk factor for the nucleation, growth and aggregation of CaOx crystals (Robertson et al. 1978, Coe 1983, Griffith et al. 1986). Stones composed of uric acid can be dissolved by increasing the urinary pH to approximately 6.5 (Hesse *et al.* 1997). Approximately one third of patients with CaOx stones have raised urinary uric acid excretion (Coe 1978). The latter author suggested that there are two possible mechanisms for the increased excretion of

uric acid, viz. overproduction of uric acid and a high intake of dietary protein (rich in purine). He found that only 30% of the cases were due to the former, whereas the latter accounted for 70% of the cases. This observation therefore warrants the dietary restriction of foods rich in purine e.g. fish, chicken and organ meat. (Coe 1978, Scheinman 2000).

Evidence suggests that Allopurinol therapy is effective in reducing stone recurrence in CaOx stone formers by blocking uric acid production (Ettinger 1991) and by reducing purine absorption (Pak *et al.* 1978).

1.3.8 Relative Supersaturation and Risk Index

The supersaturation of urine is a thermodynamic concept which is most often expressed as the relative supersaturation (RS). RS, which can easily be computed using special software called EQUIL 2, is the ratio between the activity product of the stone-forming ions in urine and the corresponding solubility product obtained from artificial solutions (Werness *et al.* 1985). It gives an estimate of the driving forces favouring crystallization of common urinary salts, e.g. CaOx, brushite and uric acid. This is a valuable tool in urolithiasis research. The Tiselius risk index (Tiselius 1991) is also widely used as an indicator of the risk of stone formation.

1.4 DIET AND SUPPLEMENTS

Since diet affects urine composition, certain foodstuffs and supplements may be lithogenic while others may be anti-lithogenic. Among these are calcium, citrate and magnesium.

1.4.1 Dietary Calcium

Several studies have linked dietary calcium intake to urinary calcium excretion (Marshall *et al.* 1972, Bataille *et al.* 1983, Borghi *et al.* 1999b). In the past it was believed that a high dietary calcium intake increases the risk of kidney stone formation (Bleich *et al.* 1979, Robertson *et al.* 1987, Breslau 1994). This hypothesis was based largely on the finding that the majority of recurrent stone formers have hypercalciuria (Pak 1988). Consequently, patients with calcium-containing stones have routinely been recommended to decrease their calcium intake. However, studies have questioned this advice due to the potential risk of hyperoxaluria and bone loss (Bataille *et al.* 1983,

Messa et al. 1991, Curhan *et al.* 1993). In the latter study, Curhan and his co-workers examined the relationship between dietary calcium intake and the risk of developing kidney stones in a cohort study of 45,619 healthy men. They found an inverse relationship between dietary calcium intake and kidney stone formation. This observation was later confirmed in women (Curhan *et al.* 1997). An explanation for this could be that dietary calcium restriction reduces the binding of calcium to oxalate in the gut allowing more oxalate to be absorbed and excreted in the urine (Curhan *et al.* 1993).

In 1997, Messa *et al.* evaluated the effect of dietary calcium restriction on the relative supersaturation of calcium oxalate in the urine of stone-forming patients using a computer methodology which takes into account the main soluble complex species of oxalate. They found that dietary calcium restriction increases the relative supersaturation of calcium oxalate in the urine of stone formers, mainly because of an increase in oxalate excretion.

Studies in hypercalciuric patients have shown that dietary calcium restriction may lead to an imbalance of calcium and bone loss thereby increasing the risk of osteoporosis (Coe *et al.*1982) and can be associated with a 10 percent higher probability of stone formation (Curhan *et al.* 1993).

The above-mentioned studies therefore demonstrate convincingly that restriction of dietary calcium poses significant doubt on its efficacy as a therapeutic protocol in the management of CaOx urolithiasis.

1.4.2 Supplemental Calcium

There are conflicting results on this subject in the literature. Curhan *et al.* (1993, 1997) studied the relationship between supplemental calcium and the risk of kidney stone formation in two cohort studies comprising 45,619 healthy men and 91,731 healthy females respectively and showed that supplemental calcium had no significant effect on the risk of stone formation in men (Curhan *et al.*1993) but increased the risk of stone formation in women (Curhan *et al.* 1997). It is noted that Curhan does not offer an explanation for the different results between men and women. Curhan *et al.* (1993, 1997) also compared the relationship between dietary and supplemental calcium in the same studies and showed that supplemental calcium did not have the same protective effect on

kidney stone formation as dietary calcium. The authors suggest that a possible explanation for the increased risk of stone formation could be the timing of ingestion of the supplements (Curhan *et al.* 1993, Curhan *et al.* 1997). In the latter study the authors claim that if supplemental calcium is not ingested at the time of oxalate ingestion (i.e. at the time of the meal), it may provide very little or no protection from oxalate absorption while its own absorption may be greater. Both of these effects may lead to a greater risk of stone formation.

Several other studies have investigated stone forming risk associated with calcium supplements. While most of these have found that the risk does not increase with supplementation (Levine *et al.* 1994, Sakhaee *et al.* 1994, Caudarella *et al.* 2001, Williams *et al.* 2001, Domrongkitchaiporn *et al.* 2002), none have addressed the question of the timing at which the supplement is ingested i.e. with meals or between meals. This aspect is fully described in Chapter 4.

1.4.3 Supplemental Citrate

The role of citrate and citrate-containing preparations in urolithiasis has been extensively described by Pak in three excellent reviews (Pak 1987, Pak 1991, Pak 1994). Compelling evidence in support of the use of such compounds in the management of CaOx urolithiasis is based on a mass of data derived from three sources: in vitro crystallization experiments, measurement of urinary citrate in normal and CaOx stone-forming subjects and, finally, empirical determinations of CaOx urinary stone risk factors and stone recurrence rates in patients following administration of various citrate-containing preparations themselves.

Several different kinds of citrate-containing compounds have been used in clinical trials to investigate their efficacy in the treatment and management of urolithiasis. Among these are potassium citrate (K-Cit) (Pak *et al.* 1985. Pak *et al.* 1986a, Abdulhadi *et al.* 1993, Barcelo *et al.* 1993, Whalley *et al.* 1996), sodium potassium citrate (Na-K-Cit) (Schwille *et al.* 1985, Schwille *et al.* 1987, Rumenapf *et al.* 1987, Berg *et al.* 1990, Hofbauer *et al.* 1994, Ogawa 1994), calcium citrate (Sakhaee *et al.* 1994, Levine *et al.* 1994), calcium-sodium citrate (Schwille *et al.* 1997) and potassium-magnesium citrate (K-Mg-Cit) (Pak *et al.* 1992a, Ettinger *et al.* 1997). A combination of calcium lactate and Na-K-Cit has also been administered (Ito *et al.* 1992). Historically, sodium citrate has been used in the

management of uric acid urolithiasis (Sakhaee *et al.* 1983, Preminger *et al.* 1988) but has not been considered as an efficacious drug for the treatment of calcium urolithiasis because of reports that it may increase the risk of urinary crystallization of calcium oxalate and calcium phosphate *in uric acid stone patients* (Sakhaee *et al.* 1983, Preminger *et al.* 1988, Sakhaee *et al.* 1982, Pak *et al.* 1986b).

It is of interest that K-Cit appears to be the alkaline citrate preparation of choice by North American researchers (Pak *et al.* 1985. Pak *et al.* 1986a, Abdulhadi *et al.* 1993, Barcelo *et al.* 1993) while Na-K-Cit is the choice of investigators in Europe (Schwille *et al.* 1985, Schwille *et al.* 1987, Rumenapf *et al.* 1987, Berg *et al.* 1990, Hofbauer *et al.* 1994). Indeed, Ogawa has drawn attention to this difference of treatment modality (Ogawa 1994).

Based on an extensive review of the literature, it is worth noting that there is no significant difference in the respective efficacies of K-Cit and K-Na-Cit. Both preparations have been found to consistently increase urinary pH (K-Cit: Sakhaee *et al.* 1982, Sakhaee *et al.* 1983, Pak *et al.* 1985, Pak *et al.* 1986a, Pak *et al.* 1986b, Barcelo *et al.* 1993; K-Na-Cit: Schwille *et al.* 1985, Schwille *et al.* 1987, Rumenapf *et al.* 1987, Ogawa 1994) and increase urinary citrate (K-Cit: Sakhaee *et al.* 1982, Sakhaee *et al.* 1983, Pak *et al.* 1985. Pak *et al.* 1986a, Pak *et al.* 1986b, Preminger *et al.* 1988, Abdulhadi *et al.* 1993, Barcelo *et al.* 1993, Whalley *et al.* 1996; K-Na-Cit: Schwille *et al.* 1985, Schwille *et al.* 1987, Rumenapf *et al.* 1987, Berg *et al.* 1990, Ogawa 1994). However, trials have not been quite as convincing regarding their effects on urinary calcium. Nevertheless, both preparations have been reported as having lowered this parameter (K-Cit: Whalley *et al.* 1996, Sakhaee *et al.* 1983, Sakhaee *et al.* 1982; K-Na-Cit: Schwille *et al.* 1987, Rumenapf *et al.* 1987, Berg *et al.* 1990). Although urinary oxalate has not been reported as having been affected by either preparation, some trials have demonstrated a reduction in the relative supersaturation of CaOx following therapy (K-Cit: Sakhaee *et al.* 1982, Sakhaee *et al.* 1983, Pak et al. 1985, Pak et al. 1986a, Preminger *et al.* 1988; K-Na-Cit: Berg *et al.* 1990). Thus far, there has not been a study which has directly compared the effects of these two therapies, as there have been for K-Cit and sodium citrate (Sakhaee *et al.* 1983, Preminger *et al.* 1988).

It is worth noting that the concerns which have been reported about sodium citrate administration have arisen following the treatment of uric acid urolithiasis. A study investigating its efficacy in the management of *calcium oxalate* stone formation is described in Chapter 3 of this thesis.

1.4.4 Dietary Magnesium

It has been suggested that dietary magnesium is beneficial, because it binds intestinal oxalate and in so doing forms soluble magnesium oxalate, reducing the excretion of oxalate (Desmars *et al.* 1973, Barilla *et al.* 1978, Berg *et al.* 1986). Since the magnesium oxalate complex formed is more soluble than CaOx a reduction in the saturation of CaOx is observed following ingestion of dietary magnesium (Lindberg *et al.* 1990a). It has also been found that magnesium inhibits calcium phosphate crystal growth (Bisaz *et al.* 1978, Pak *et al.* 1992a).

1.4.5 Supplemental Magnesium

There are conflicting results in the literature regarding the use of magnesium supplementation in the treatment of urolithiasis. The effect of oral administration of magnesium oxide on CaOx crystallization in urine was tested in a short-term study on four patients where the it was found that after magnesium therapy urinary pH increased in all four patients, urinary calcium excretion increased in two patients and urinary oxalate excretion decreased in only one patient (Fetner *et al.* 1978). From this study, no objective evidence for the beneficial effects of magnesium oxide in the treatment of recurrent calcium urolithiasis could be found. On the other hand, other studies have reported prophylactic effects (Moore and Bunce 1964, Prien and Gershoff 1974, Johansson *et al.* 1980, Parivar *et al.* 1996). More recently, a study by Zimmerman et al. (2003), investigated the influence of magnesium supplements on oxalate absorption in healthy subjects. They found a significant increase in magnesium excretion and a significant decrease in oxalate excretion and concluded that magnesium supplementation can be used as effective therapy for recurrent calcium oxalate stone formers.

1.5 OBJECTIVES OF THIS THESIS

Consideration of the various studies described in this chapter clearly demonstrates that calcium, magnesium and citrate supplements play a role in determining urine composition and hence affect the urinary risk factors for CaOx stone formation. An aspect which has not been previously investigated is the interaction that these supplements might experience when given in combination and their effects on urinary risk factors. This provides motivation for objective 1 given below.

Regarding conservative treatment, it is of some considerable interest that although a host of citrate-containing preparations have been tested in the management of CaOx urolithiasis, sodium citrate preparations have been disregarded because of their unfavourable affects in the management of *uric acid* stone formation. Objective 2 was undertaken to address this question.

Review of the literature reveals that the timing of ingestion of a calcium supplement relative to a meal (and the oxalate-content of the meal itself) is crucial for determining the extent of oxalate binding in the gut and the ultimate oxalate excretion in the urine. Such data are not available. Thus, a carefully controlled study to address this issue is warranted and is addressed in objective 3.

Finally, the calculation of the relative supersaturation of urinary salts using the computer program EQUIL2 is extremely well-established and widely used. Indeed, its value has been demonstrated in many studies. Nevertheless, the program has limitations and shortcomings. JESS is another speciation program which has greater capabilities than EQUIL2 but it has not been applied in the field of urolithiasis research. Objectives 4 and 5 aimed to explore differences between the two programs and to use JESS's superior capability to interpret the effects observed in the study defined in objective 2 above.

The objectives of this thesis are thus:

1. to investigate the individual, additive and synergistic effects of calcium, magnesium and citrate health supplements on the urinary risk factors for calcium oxalate kidney stone formation
2. to investigate the potential prophylactic and therapeutic properties of a sodium citrate preparation in the management of calcium oxalate kidney stone formation
3. to determine urinary oxalate excretion as a function of the time at which a calcium supplement is ingested relative to an oxalate-rich meal
4. to compare the outputs of the two speciation programs, EQUIL2 and JESS
5. to use JESS to interpret effects observed in the study involving the sodium citrate preparation.

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Chapter 2

Investigation of the individual, additive and synergistic effects of calcium, magnesium and citrate health supplements on the urinary risk factors for calcium oxalate kidney stone formation

2.1 INTRODUCTION

In Chapter 1 the role of urine composition in determining CaOx stone formation was described in considerable detail. Among the key urinary components which influence the chemical risk of crystallization are calcium, magnesium and citrate. As also described in Chapter 1, several studies have investigated whether stone disease might be managed by manipulating the individual urinary levels of these components by oral administration of supplements which contain them. Notwithstanding the individual effects of these supplements, it is recognized that there may be *interactions* between them. Indeed, Curhan has made mention of dietary interactions among nutrients that could affect bioavailability, gastrointestinal absorption and urinary saturation (Curhan *et al.* 1997). It appears that previous studies have not addressed this aspect and that identification of such interactions might be important in assessing the overall effects of such supplements on stone formation risk factors.

Investigation of the individual and interactive effects of calcium, magnesium and citrate health supplements is described in this chapter.

2.2 SUBJECTS AND METHODS

2.2.1 Study Design

Eight healthy white male subjects (from the Cape Town area) in the age group 22-30 years participated in the study. They were matched with respect to body mass index (BMI). The subjects' normal domestic food intake was assessed using 4x24hr dietary records. To determine nutrient content, all the data obtained from the dietary questionnaire were analyzed using food composition tables (Langenhoven *et al.* 1991). The subjects were instructed not to take any medication or supplements (other than those prescribed in the protocol) for one week before and throughout the study. Seven supplemental protocols were investigated in addition to the free diet (Table 2.1). These protocols comprised of calcium (administered as calcium carbonate, Solgar, U.S.A), magnesium (administered as magnesium oxide, Solgar, U.S.A) and citrate (administered as sodium citrate bicarbonate tartrate, Abbott Laboratories*, S.A) and combinations thereof. Each subject was required to ingest the supplement at mealtimes. The experimental design implemented in this study was a "Complete Latin Square Design" (Table 2.1) in which each subject was allocated at random to a randomized sequence of all seven supplemental protocols and the normal diet (free diet) in the form of a 2^3

factorial. Daily doses were 800 mg of calcium (calcium carbonate), 4604 mg citrate (sodium citrate bicarbonate tartrate) and 300 mg magnesium (magnesium oxide), given individually or in combination. Supplementation lasted for 7 days. 24-hour urines were collected at baseline and during the final day of each supplemental protocol. There was a 7-day wash-out period between each protocol. Subjects remained on their free diets throughout the study.

Table 2.1: Complete Latin Square Cross-Over Design applied to supplemental protocols.

1	2	3	4	5	6	7	8
A	B	C	D	E	F	G	H
B	C	D	E	F	G	H	A
H	A	B	C	D	E	F	G
D	E	F	G	H	A	B	C
G	H	A	B	C	D	E	F
F	G	H	A	B	C	D	E
C	D	E	F	G	H	A	B
E	F	G	H	A	B	C	D

KEY
SUBJECTS: 1 – 8
PROTOCOLS (A – H):
A – Normal Diet
B – Calcium
C – Magnesium
D – Citrate
E – Calcium + Magnesium
F – Calcium + Citrate
G – Magnesium + Citrate
H – Calcium + Magnesium + Citrate

2.2.2 *Urine Analysis*

There were no urine samples which tested positive for the presence of blood and/or infection (Combur 10 test strip, Boehringer Mannheim, Germany). After recording the volume and pH (pH meter), aliquots were filtered through a 0.74µm filter to remove cellular debris and proteinaceous material. The samples were then analyzed for calcium, potassium, magnesium and sodium (Fernandez *et al.* 1971, Trudeau *et al.* 1967 and Willis *et al.* 1961) using a Varian 1275 Model flame atomic absorption spectrometer. Oxalate was determined using oxalate decarboxylase (Chiriboga *et al.* 1963), while citrate was determined by conversion to oxaloacetate using citrate lyase (Gruber *et al.* 1966). Inorganic phosphorous was determined using ammonium molybdate (Dryer *et al.* 1963), creatinine using picric acid (Rock *et al.* 1986), uric acid using uricase (Fossati *et al.* 1980) and chloride using an ion selective chloride electrode.

Relative supersaturation (RS) values of calcium oxalate, brushite and uric acid were computed using the EQUIL program (Werness *et al.* 1985). The Tiselius risk index

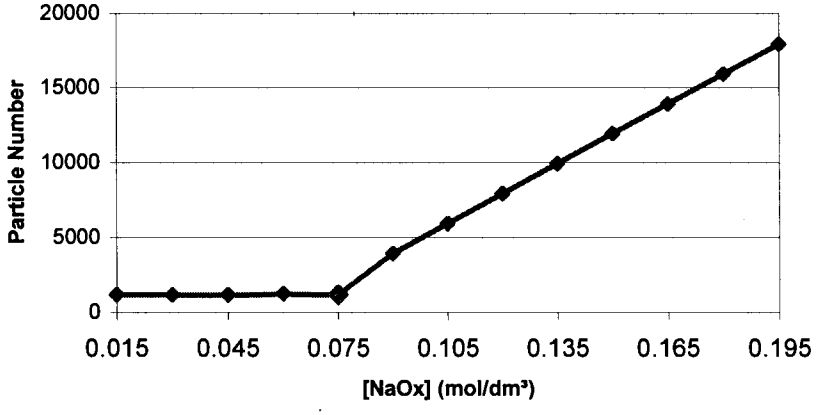
(Tiselius 1982) was also calculated for each urine sample. Data were statistically analyzed for individual and interactive effects by the method of analysis of variance (ANOVA) and the results were considered statistically significant if $p<0.05$. Since the means for the different variables in Tables 2.3-2.5 and those in Tables 2.6-2.8 are based on the same number of observations they have the same standard errors.

2.2.3 Crystallization Experiments

The ability of each urine sample to resist spontaneous nucleation, and thus calcium oxalate crystallization, was measured by spiking 10ml aliquots of filtered urine with 100µl of increasing concentrations of sodium oxalate and incubating at 37°Cin a shaking water bath for 30 minutes. A Coulter Multisizer (Multisizer 1, Coulter Electronic LTD, England, serial no. 030288) was then used to measure the number of particles in the urine aliquot at the different spiking concentrations. The minimum sodium oxalate (NaOx) concentration which induced crystallization was regarded as a measure of the CaOx metastable limit (MSL) (Ryall *et al.* 1985). This can be determined by plotting particle number vs NaOx concentration as shown in Figure 2.1. Once the MSL was determined, the urine was spiked with 1ml NaOx/100ml urine using a concentration of 15 mmol/dm^3 above the previously determined MSL. The urine was then incubated at 37°C in a shaking water bath (100rpm) for 120min and a Coulter Multisizer (140µm orifice, 2.8-90.0µm particle size range) was used to measure the particle volume-size distribution after 120min (Ryall *et al.* 1985). The total volume of particles precipitated during the incubation period and the mean particle size were determined.

Figure 2.1 demonstrates a typical graph for a CaOx MSL determination, where the concentration of NaOx corresponding to the grey point indicated on the graph corresponds to the MSL (final NaOx concentration in urine = 0.75 mmol/dm³).

Fig. 2.1 Typical Metastable Limit Plot



2.3 RESULTS

2.3.1 Dietary Analysis

The average nutrient intakes (including their ranges) derived from the dietary questionnaires are given in Table 2.2. The raw data is presented in Appendix CD: Chapter2/Data obtained from dietary questionnaires for each subject.xls. Also listed for comparative purposes are values reported in a previous study from our laboratory (Lewandowski *et al.* 2001).

2.3.2 Urine Composition and Physicochemical Risk Factors

Urine data for citrate-containing protocols (i.e. protocols D, F, G, H – Table 2.1) are given in Table 2.3. Collectively, these protocols favourably altered three recognized urinary risk factors significantly. Citrate excretion increased from 2.46 to 3.82 mmol/24hr ($p=0.0002$), calcium excretion decreased from 3.68 to 2.86 mmol/24hr ($p = 0.0091$), and the relative supersaturation (RS) of CaOx decreased from 2.36 to 1.43 ($p = 0.0012$). It is noted that sodium excretion increased significantly and that both oxalate and uric acid excretion showed an increasing trend, which tended weakly towards significance. No other significant urinary changes were observed.

Urine data for calcium-containing protocols (i.e. protocols B, E, F, H – Table 2.1) are given in Table 2.4. Collectively, these protocols significantly altered three urinary risk factors. Calcium excretion increased from 2.84 to 3.70 ($p = 0.0066$), phosphate excretion decreased from 30.70 to 23.09 ($p = 0.0054$) and pH increased from 6.96 to 7.14 ($p=0.0178$).

Urine data for magnesium-containing protocols (i.e. protocols C, E, G, H – Table 2.1) are given in Table 2.5. Collectively, these protocols did not alter any urinary risk factors significantly.

The raw data for the urine composition and physicochemical parameters for each subject are presented in Appendix CD: Chapter 2/Urine chemistry results and physicochemical parameters for each subject.doc.

Table 2.2: Comparison of mean nutrient intake of subjects in the present study to that reported in another study for subjects from the Cape Town area

Variables	Mean Present study	Range Present study	Range Previous study*
Total Protein (g/day)	103	74.1-146	82.7-156.5
Total Fat (g/day)	114	58.4-180.9	71.9-196.4
Total Carbohydrate(g/day)	285	142.4-399.8	204-685
Fibre (g/day)	21.6	13.1-30.4	15.7-69.3
Added Sugar (g/day)	69.2	19.1-113.2	48.2-120
Oxalate (mg/day)	134.4	38.6-318.6	75.9-753
Ca (mg/day)	861.0	376-1151	683-1955
Mg (mg/day)	405.5	262-558	298-959
Phosphate (mg/day)	1617	954-2139	1198-3200
K (mg/day)	3418	2053-4625	2652-6972
Na (mg/day)	6262	3482-9486	2109-5879
Zn (mg/day)	12.7	7.95-17.4	8.86-22.56
Vitamin A (RE/day)	1207	365-4587	653-2633
Vitamin B6 (mg/day)	2.40	1.23-4.52	1.74-6.41
Vitamin C (mg/day)	93.9	26-172	55-538
Vitamin D (µg/day)	2.89	0.76-6.64	1.22-8.88
BMI	24.1	22.1-26.1	

*Lewandowski *et al.* 2001

Table 2.3: Effect of citrate-containing protocols on urine composition and physicochemical properties

Variables	Citrate Absent (Protocols A,B,C,E) Mean ± SE	Citrate Present (Protocols D,F,G,H) Mean ± SE	P - Value
pH	6.83 ± 0.05	7.40 ± 0.05	<0.0001
Volume (ml/24hr)	1694 ± 95	1921 ± 95	0.0989
Citrate Excretion (mmol/24hr)	2.46 ± 0.24	3.82 ± 0.24	0.0002
Oxalate Excretion (mmol/24hr)	0.17 ± 0.02	0.23 ± 0.02	0.0396
Calcium Excretion (mmol/24hr)	3.68 ± 0.21	2.86 ± 0.21	0.0091
Magnesium Excretion (mmol/24hr)	5.45 ± 0.34	5.05 ± 0.34	0.4131
Sodium Excretion (mmol/24hr)	196.5 ± 19.2	321.0 ± 19.2	< 0.0001
Potassium Excretion (mmol/24hr)	67.94 ± 5.03	57.42 ± 5.03	0.1467
Creatinine Excretion (mmol/24hr)	15.33 ± 0.43	15.64 ± 0.43	0.6129
Phosphate Excretion (mmol/24hr)	27.84 ± 1.84	25.96 ± 1.84	0.4726
Chloride Excretion (mmol/24hr)	137.0 ± 7.0	136.0 ± 7.0	0.9200
Uric Acid Excretion (mmol/24hr)	3.54 ± 0.168	3.96 ± 0.168	0.0847
RS Calcium Oxalate	2.36 ± 0.19	1.43 ± 0.19	0.0012
RS Brushite	1.57 ± 0.12	0.96 ± 0.12	0.0009
RS Uric Acid	0.58 ± 0.07	0.11 ± 0.07	<0.0001
MSL (mol/dm^3)	0.05 ± 0.07	0.07 ± 0.01	> 0.0500
Tiselius risk index	174 ± 16	172 ± 16	> 0.0500

Table 2.4: Effect of calcium-containing protocols on urine composition and physicochemical properties

Variables	Calcium Absent (Protocols A,C,D,G) Mean ± SE	Calcium Present (Protocols B,E,F,H) Mean ± SE	P - Value
pH	6.96 ± 0.05	7.14 ± 0.05	0.0178
Volume (ml/24hr)	1705 ± 95	1910 ± 95	0.1350
Citrate Excretion (mmol/24hr)	3.15 ± 0.24	3.13 ± 0.24	0.9681
Oxalate Excretion (mmol/24hr)	0.20 ± 0.02	0.20 ± 0.02	0.8779
Calcium Excretion (mmol/24hr)	2.84 ± 0.21	3.70 ± 0.21	0.0066
Magnesium Excretion (mmol/24hr)	4.92 ± 0.34	5.59 ± 0.34	0.1727
Sodium Excretion (mmol/24hr)	258.5 ± 19.2	333.9 ± 19.2	0.9964
Potassium Excretion (mmol/24hr)	65.47 ± 5.03	59.89 ± 5.03	0.4373
Uric Acid Excretion (mmol/24hr)	3.80 ± 0.168	3.70 ± 0.168	0.6756
Creatinine Excretion (mmol/24hr)	15.50 ± 0.43	15.46 ± 0.43	0.9478
Phosphate Excretion (mmol/24hr)	30.70 ± 1.84	23.09 ± 1.84	0.0054
Chloride Excretion (mmol/24hr)	129.4 ± 7.0	143.4 ± 7.0	0.1648
RS Calcium Oxalate	1.74 ± 0.19	2.04 ± 0.19	0.2681
RS Brushite	1.21 ± 0.12	1.32 ± 0.12	0.5242
RS Uric Acid	0.50 ± 0.0)	0.19 ± 0.07	0.0022
MSL (mol/dm^3)	0.07 ± 0.01	0.05 ± 0.01	> 0.0500
Tiselius risk index	155 ± 16	191 ± 16	> 0.0500

Table 2.5: Effect of magnesium-containing protocols on urine composition and physicochemical properties

Variables	Magnesium Absent (Protocols (A,B,D,F) Mean ± SE	Magnesium Present (Protocols C,E,G,H) Mean ± SE	P - Value
pH	7.01 ± 0.05	7.09 ± 0.05	0.2489
Volume (ml/24hr)	1767 ± 95	1848 ± 95	0.5503
Citrate Excretion (mmol/24hr)	3.16 ± 0.24	3.12 ± (0.24	0.8940
Oxalate Excretion (mmol/24hr)	0.20 ± 0.02	0.20 ± 0.02	0.3384
Calcium Excretion (mmol/24hr)	3.09 ± 0.21	3.45 ± 0.21	0.2349
Magnesium Excretion (mmol/24hr)	4.71 ±0.34	5.80 ± 0.34	0.0289
Sodium Excretion (mmol/24hr)	258.1 ± 19.2	259.4 ± 19.2	0.9961
Potassium Excretion (mmol/24hr)	58.68 ± 5.03	66.68 ± 5.03	0.2672
Uric Acid Excretion (mmol/24hr)	3.72 ± 0.17	3.79 ± 0.17	0.7694
Creatinine Excretion (mmol/24hr)	15.42 ± 0.43	15.54 ± 0.43	0.8445
Phosphate Excretion (mmol/24hr)	26.40 ± 1.84	27.40 ± 1.84	0.7017
Chloride Excretion (mmol/24hr)	141.9 ± 7.0	132.0 ± 7.0	0.3232
RS Calcium Oxalate	1.88 ± 0.19	1.90 ± 0.19	0.9407
RS Brushite	1.16 ± 0.12	1.37 ± 0.12	0.2291
RS Uric Acid	0.39 ± 0.07	0.30 ± 0.07	0.3494
MSL (mol/dm^3)	0.06 ± 0.01	0.06 ± 0.01	> 0.0500
Tiselius risk index	170 ± 16	176 ± 16	> 0.0500

Three different chemical interactions were identified (Tables 2.6, 2.7, 2.8). Firstly, magnesium and citrate interacted with each other to influence the RS of brushite (Table 2.6). Secondly, these two components influenced pH (Table 2.7). Finally, calcium and citrate interacted to influence the RS of uric acid (Table 2.8).

Table 2.6: Influence of the interaction between magnesium and citrate on the RS of Brushite

			PAIRWISE P - VALUES		
Magnesium	**Citrate**	**Mean ± SE**	**P + A**	**A + P**	**A + A**
Present	Present	0.89 ± 0.17	0.0003	0.5483	0.1027
Present	Absent	1.85 ± 0.17		0.0015	0.0247
Absent	Present	1.03 ± 0.17			0.2939
Absent	Absent	1.29 ± 0.17			

P = Present

A = Absent

Table 2.7: Influence of the interaction between magnesium and citrate on pH

			PAIRWISE P – VALUES		
Magnesium	**Citrate**	**Mean ± SE**	**P + A**	**A + P**	**A + A**
Present	Present	7.35 ± 0.07	0.0001	0.2904	0.0001
Present	Absent	6.83 ± 0.07		0.0001	0.0094
Absent	Present	7.46 ± 0.07			0.0001
Absent	Absent	6.55 ± 0.07			

P = Present

A = Absent

Table 2.8: Influence of the interaction between calcium and citrate on the RS of Uric Acid

			PAIRWISE P - VALUES		
Calcium	**Citrate**	**Mean ± SE**	**P + A**	**A + P**	**A + A**
Present	Present	0.09 ± 0.09	0.1637	0.8258	0.0001
Present	Absent	0.28 ± 0.09		0.2783	0.0001
Absent	Present	0.12 ± 0.09			0.0001
Absent	Absent	0.87 ± 0.09			

P = Present

A = Absent

2.3.3 *Particle Volume-Size Distributions*

Figure 2.2 shows the mean particle volume-size distribution in urine at 120min after administration of a NaOx load for each protocol, where the mean particle size precipitated is represented by the mode of this curve and the total volume of particles precipitated is represented by the total area under the curve. Individual plots for each subject are presented in the Appendix CD: Chapter 2/Particle volume-size distributions for each subject.doc.

Fig. 2.2 Mean Particle Volume - Size Distribution

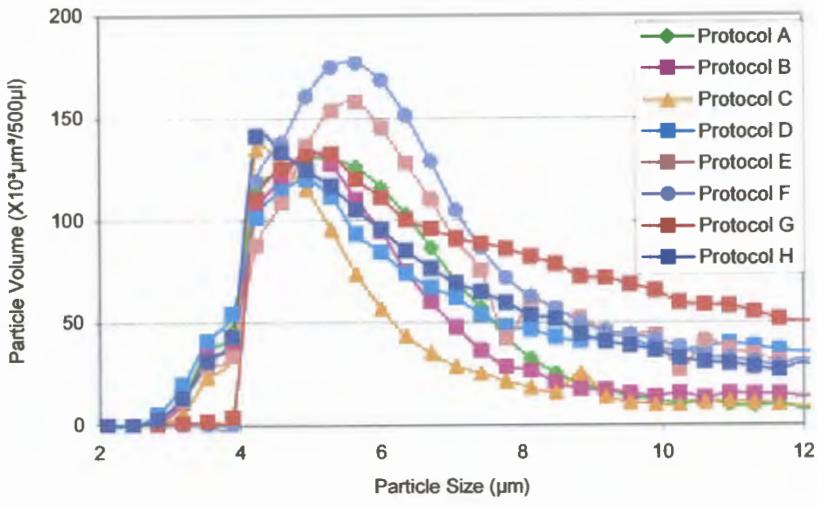
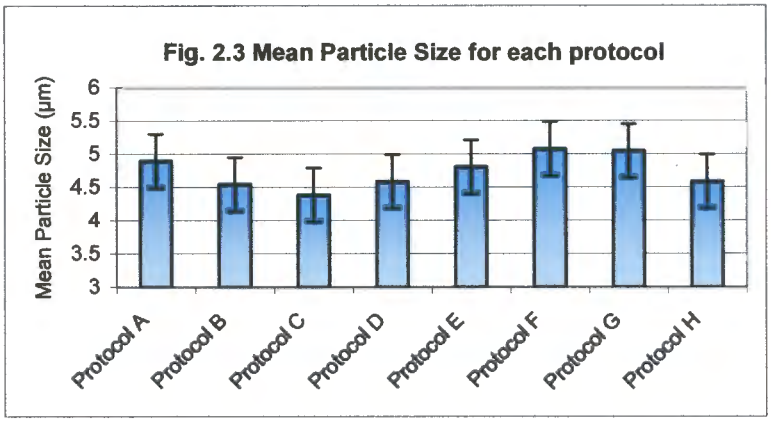
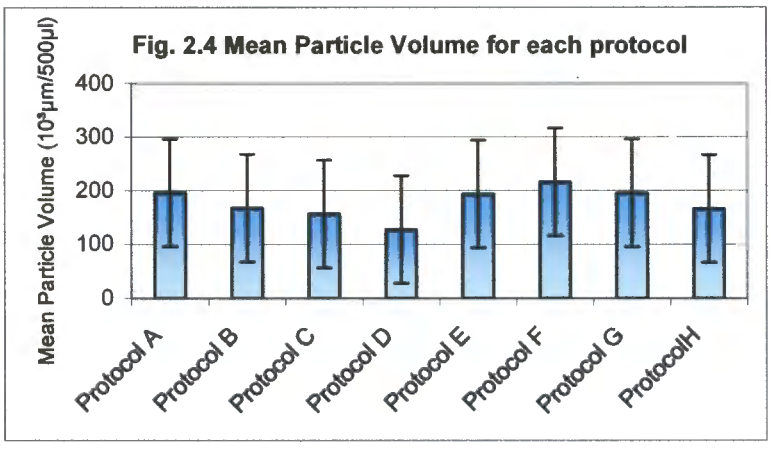


Figure 2.3 shows the mean particle sizes for each protocol corresponding to the particle size distributions above. The mean particle size values are reflected in Table 2.9. Most of the protocols showed a decreasing trend in the mean particle size with respect to Protocol A except for Protocols F and G which showed the opposite trend. There is no significant difference between the mean particle sizes for each of the protocols.

Figure 2.4 shows the mean particle volume, obtained from Figure 2.2, for each protocol. The mean particle volume values are reflected in Table 2.10. All the protocols showed a decreasing trend in its mean particle volume except for Protocol F which showed the opposite trend. There is no significant difference between the mean particle volumes for each protocol.

Figure 2.5 is a plot of the mean total volume, also obtained from Figure 2.2, for each protocol. These values are reflected in Table 2.11. The mean total particle volume data was only decreased by Protocols B and C, all the other protocols increased this parameter. Once again, there were no significant differences.





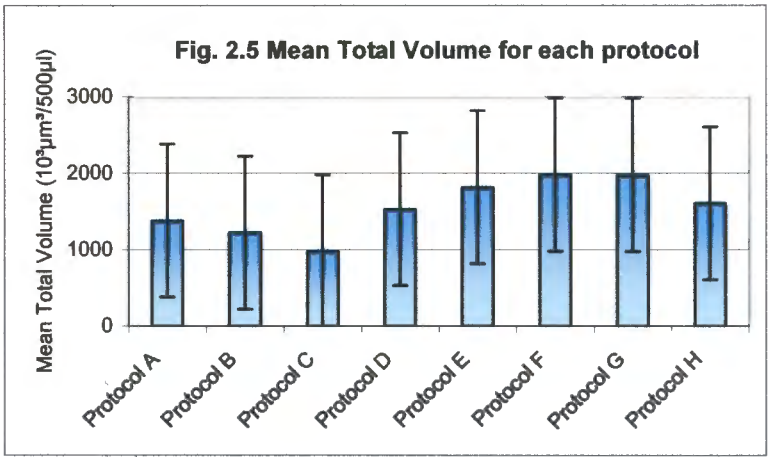


Table 2.9: Mean Particle Sizes (from Fig. 2.2) for each of the protocols

Diet	Mean Particle Size ± SE (µm)	% Decrease wrt Protocol A	% Increase wrt Protocol A	P-Value wrt Protocol A
A	4.90 ± 0.40			
B	4.55 ± 0.40	7.1		0.4101
C	4.39 ± 0.40	10.4		0.3218
D	4.59 ± 0.40	6.3		0.3193
E	4.81 ± 0.40	1.8		0.8533
F	5.08 ± 0.40		3.7	0.6872
G	5.05 ± 0.40		3.1	0.9994
H	4.59 ± 0.40	6.3		0.4366

Table 2.10: Mean Particle Volumes (from Fig. 2.2) for each of the protocols

Diet	Mean Particle Volume ± SE ($x10^3 µm^3/500µl$)	% Decrease wrt Protocol A	% Increase wrt Protocol A	P-Value wrt Protocol A
A	196.2 ± 100			
B	167.6 ± 100	14.6		0.5128
C	156.8 ± 100	20.1		0.5923
D	127.8 ± 100	34.9		0.0597
E	193.8 ± 100	1.2		0.9675
F	216.0 ± 100		10.1	0.6729
G	195.6 ± 100	0.3		0.7943
H	166.4 ± 100	15.2		0.4363

Table 2.11: Mean Total Particle Volumes (from Fig. 2.2) for each of the protocols

Diet	Mean Particle Volume ± SE ($x10^3 µm^3/500µl$)	% Decrease wrt Protocol A	% Increase wrt Protocol A	P-Value wrt Protocol A
A	1378.4 ± 1000			
B	1220.9 ± 1000	11.4		0.6755
C	977.8 ± 1000	19.9		0.4328
D	1529.0 ± 1000		10.9	0.8335
E	1815.7 ± 1000		31.7	0.4548
F	1979.0 ± 1000		43.6	0.2611
G	1974.2 ± 1000		43.2	0.4500
H	1606.6 ± 1000		16.6	0.5218

2.4 DISCUSSION

This study has addressed for the first time chemical interactions between calcium, magnesium and citrate-containing supplements. Although all of these components have been implicated individually in the past in influencing the risk of CaOx stone formation, the present study has revealed that citrate-containing protocols favourably altered three well-established risk factors (citrate excretion, calcium excretion and RS CaOx) while calcium favourably altered only two (phosphate excretion and pH). Magnesium-containing protocols did not alter any. Moreover, citrate was the common component in the three favourable chemical interactions which were identified. As such, this study has demonstrated that citrate-containing protocols are significantly more effective than magnesium and calcium-containing protocols in reducing the urinary risk factors for calcium oxalate stone formation. Of particular importance is the observation that citrate is able to behave in this way irrespective of whether or not it is chemically associated in the supplement with calcium and/or magnesium and this lends confidence to its use as a prophylactic and therapeutic agent.

The observation of an increase in citrate excretion and a decrease in calcium excretion following administration of citrate-containing protocols confirms the results of several other workers (Whalley *et al.* 1996, Hofbauer 1994, Sakhaee *et al.* 1983). The significantly raised urinary sodium in the present study is a consequence of the sodium salt administration. Potassium (Martini *et al.* 1998, Lemann *et al.* 1991) or phosphate (Breslau *et al.* 1998) over-consumption can also lead to lower urinary calcium excretion. Since the values for the ingestion of these nutrients in the present study fall within the respective ranges observed in our previous study (Table 2.2), it is unlikely that either is a confounding factor.

Of some considerable interest in the present study was the increasing trend of oxalate excretion following administration of citrate-containing protocols, albeit that this increase was not statistically significant. This trend might have occurred as a result of citrate binding calcium in the gut (Pak 1994) as reflected by the observation that calcium excretion was indeed reduced after citrate supplementation. This depletion of calcium

could result in greater absorption of oxalate (Bataille *et al.* 1983, Barilla *et al.* 1978, Langenhoven *et al.* 1991) leading to increased oxalate excretion (Earnest *et al.* 1974). Thus, the results indicate that there may be two antagonistic effects of citrate: it lowers urinary calcium on the one hand, but tends to increase urinary oxalate on the other. An obvious question concerns the relative importance of these effects. Consideration of RS CaOx might provide the answer. As reported above, RS CaOx was significantly lower after administration of citrate-containing protocols, thereby suggesting that lowering of urinary calcium was the more important factor. However, this argument is simplistic as it ignores the interactions of citrate which have been identified in this study (Tables 2.6, 2.7, 2.8) and which are likely to have played a determinant role in lowering RS CaOx.

It is important to comment on the effect of supplemental calcium in the context of the risk of stone formation since concerns have been expressed that its administration in the management of osteoporosis might increase such risk (Domrongkitchaiporn *et al.* 2000). Three significant effects were observed. Firstly calcium excretion increased in agreement with Levine *et al.* 1994 and Schwille *et al.* 1997. However, this is not regarded as clinically important as the mean value was within the normal range; nor did RS CaOx or the Tiselius risk index increase. Secondly, phosphate excretion decreased. While it is not possible to comment on the mechanism by which this occurs, the decrease in phosphate excretion lowers the risk of brushite formation and apatite at high pH. Thirdly, pH increased after calcium supplementation. This can have a favourable influence on the activity of inhibitors (Hesse *et al.* 1997), as discussed later. Thus, the results should allay the previously mentioned concerns about calcium supplementation. However, it should be emphasized that in the present study, the calcium-containing protocols (as well as the other protocols) were taken with meals, whereas some concerns have arisen as a consequence of between-meal supplementation (Curhan *et al.* 1993, Curhan *et al.* 1997). In certain circumstances the oxalate excretion might have decreased following calcium supplementation. A possible reason why this did not occur is that the supplement was ingested with breakfast which did not contain significant oxalate.

The finding that magnesium protocols did not alter urinary risk factors agrees with the findings of Fetner *et al.* who conducted a study to test the effect of oral administration of magnesium oxide on the crystallization of calcium oxalate and brushite in the urine of 4 recurrent stone formers and found no beneficial effects of magnesium oxide in the treatment of his patients (Fetner *et al.* 1978, References Chapter 1).

In this study the CaOx MSL did not change significantly after any of the protocols. A favourable decreasing trend in the mean particle size was obtained for all of the protocols except for Protocols F and G, and an increasing trend in the total particle volume was observed for all of the protocols except for Protocols B and C. The latter observation can be explained by the limitations of the Coulter Multisizer which simply measures the total volume of particles in a particular suspension e.g. urine, irrespective of what the particles are composed of. For example, when crystals (which often are irregular in shape) aggregate to form a "particle" the Coulter Multisizer measures the crystals plus the air or fluid in the pores which are created by packing or the aggregation of the crystals, thereby resulting in an overestimation of the volume for the particles (Ryall *et al.* 1995). Attention is drawn to the fact that the above-mentioned changes were not statistically significant.

In the present study it is hypothesized that two different types of chemical interactions are feasible, viz. synergism and addition. A synergistic interaction occurs when the effect of the combination of the components is significantly greater than the sum of the effects due to each component individually (positive synergism) or when the effect of the combination is significantly lower than the effect of the weakest component (negative synergism). An additive interaction occurs when the effect of the combination is greater than that of each of the individual components (but is less than their sum) or is equal to the sum of their effects. In those cases where the effect of the combination lies between the stronger and weaker components, negative synergism is proposed. A proposed model illustrating these definitions is given in Table 2.12. In this model, two supplemental components A and B interact to influence urinary parameter Z. Suppose component A increases parameter Z by a value x and component B increases parameter Z by a value y, where y>x.

Table 2.12: A proposed model illustrating the different types of chemical interactions

Magnitude of combination (A and B)	Effect
Greater than (x + y)	Positive Synergism
Less than x	Negative Synergism
Greater than x, but less than y	Negative Synergism
(x + y)	Additive Effect
Less than (x + y), but greater than y	Additive Effect

As indicated in the results, this study has identified three interactions. Firstly, citrate and magnesium interacted with each other to influence the RS of brushite. Table 2.6 shows

that the presence of citrate lowers the value of this parameter from 1.29 to 1.03 ($p > 0.0500$) while magnesium causes a significant *increase* to 1.85 ($p = 0.0247$). The combination of citrate and magnesium yields an even lower value (than citrate) of 0.89 ($p > 0.0500$).

Thus, the effect of citrate alone was to change the RS value by 1.29 - 1.03 = + 0.26 while the change achieved by magnesium was in the opposite direction i.e. to a *higher* value (effect = 1.29 – 1.85 = - 0.56). The sum of the effects of the individual components is thus 0.26 – 0.56 = - 0.30. The actual effect achieved by the combination was 1.29 – 0.89 = +0.40. Thus, although the changes are not significantly different, there is a suggestion of positive synergism here, since magnesium alone *increased* the RS, citrate alone *decreased* the RS and the combination *decreased* the value by an amount that was greater than that achieved by citrate alone. The table also shows that citrate alone produces a significantly lower value than magnesium alone ($p = 0.0015$) and that the effect of the combination is significantly lower than that produced by magnesium alone ($p = 0.0003$). This latter comparison demonstrates that citrate plays the dominant role in lowering the RS of brushite.

The reduction in the RS brushite is an impressive result when viewed in the light of the higher phosphate ingestion of the unrestricted diet relative to that of the typical Cape Town diet (Table 2.2). The lowering of RS brushite is favourable since it has been reported that this compound might provide the heterogeneous nucleus for CaOx stone formation (Pak *et al.* 1973).

The second interaction again involved citrate and magnesium, which combined to influence pH. Table 2.7 shows that pH increases significantly from 6.55 to 7.46 in the presence of citrate alone ($p = 0.0001$). Similarly, in the presence of magnesium alone the pH increases significantly from 6.55 to 6.83 ($p = 0.0094$). The sum of the two effects is therefore (7.46 – 6.55) + (6.83 – 6.55) i.e. 0.91 + 0.28 = 1.19. In the presence of both magnesium and citrate, the pH increases significantly from 6.55 to 7.35 ($p = 0.0001$). Since the magnitude of this combined effect (0.80) lies between the magnitudes of the individual effects of magnesium (0.28) and citrate (0.91), a negative synergistic interaction on pH by citrate and magnesium is indicated. Comparison of the value for the combination (7.35) with that obtained in the absence of citrate (6.83) $p = 0.0001$, and with that obtained in the absence of magnesium (7.46) $p = 0.2904$, demonstrates that citrate

has a significantly stronger effect than magnesium in raising the pH. The same result emerges by comparing the value obtained with citrate alone (7.46) with magnesium alone (6.83) p = 0.0001.

The implication of the increase in urinary pH is worthy of comment. Previous studies have revealed that there appears to be a dual role of pH in calcium oxalate urolithiasis. On the one hand the solubility of CaOx increases with decreasing pH (Tiselius 1981), while on the other hand (as mentioned earlier) inhibitory activity towards calcium oxalate crystallization increases with increasing pH (Hesse *et al.* 1997). The increase in pH observed in the present study would therefore enhance inhibition by citrate which, in any event, was elevated by administration of citrate–containing supplements. Although magnesium attenuates the increase in pH achieved by citrate alone, its effect is nonetheless more beneficial than the effect when magnesium is administered on its own.

The third chemical interaction occurred between citrate and calcium which combined to influence the RS of uric acid. Table 2.8 shows that in the presence of citrate alone, the RS of uric acid decreases significantly from 0.87 to 0.12 (p = 0.0001). The magnitude of the citrate effect is thus 0.87 – 0.12 = 0.75. Similarly, in the presence of calcium alone, the RS decreases from 0.87 to 0.28 (p = 0.0001). The magnitude of the calcium effect is thus 0.87 – 0.28 = 0.59. In the presence of both citrate and calcium, the RS decreases significantly to 0.09 (p = 0.0001). The magnitude of this effect is 0.87 – 0.09 = 0.78 which is greater than that of either component alone, thereby suggesting an additive effect. Comparison of the value obtained for the combination (0.09) with that obtained in the absence of calcium but presence of citrate (0.28) and in the absence of citrate but in the presence of calcium (0.12) shows that neither of these two components play a dominant role in achieving the additive effect (p = 0.1637 and p = 0.8258 respectively). The decrease in RS uric acid by the combination of calcium and citrate is regarded as favourable since uric acid has been reported as playing a role in CaOx stone formation (Ryall *et al.* 1990).

Since no significant changes were observed in the particle sizes (Figure 2.3) and particle volumes (Figures 2.4 and 2.5) after ingestion of the different supplements, it can be concluded that the above-mentioned interactions cannot be attributed to the change in size of the particles formed.

Finally, attention is drawn to the fact that the citrate product used in the present study (CitroSoda, Abbott Laboratories*, S.A) is a sodium salt and that previous studies have not administered citrate in this form. The favourable individual and interactive results achieved by this salt are therefore noteworthy and warrant further investigation. This aspect is addressed in Chapter 3 of the present thesis.

In conclusion, the present study has demonstrated the application of a new approach towards assessing efficacy of stone reducing preparations. In this approach, individual, additive and synergistic interactions between all the components commonly found in such preparations were independently and collectively evaluated. The results showed that the activity of citrate-containing protocols in reducing several physicochemical risk factors for calcium oxalate stone formation was superior to those containing calcium or magnesium and that the latter two components interacted with each other to enhance the effect of citrate in reducing the RS of uric acid and the RS of brushite respectively. Thus, clinicians can be reassured that citrate in combination with either of these components will have favourable effects.

* This product is now owned by Adcock Ingram Limited, S.A.

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Chapter 3

Investigation of the prophylactic and therapeutic properties of sodium citrate bicarbonate tartrate in the management of calcium oxalate kidney stone formation

3.1 INTRODUCTION

In the previous chapter a study was described where sodium citrate bicarbonate tartrate (CitroSoda, Abbott Laboratories*, South Africa) was administered to 8 healthy male volunteers to examine its synergistic effects with two other health supplements. Several favourable changes in the urinary risk factors for CaOx urolithiasis were recorded and attributed to CitroSoda. These results, as well as the absence of any previous rigorous investigation involving a sodium citrate preparation in the treatment of CaOx stones, prompted the expansion of the size and scope of the earlier study (described in Chapter 2) and to focus specifically on the effects of CitroSoda.

The role of citrate and citrate-containing preparations in urolithiasis has been extensively described in Chapter 1 of this thesis.

Numerous studies over the past twenty years have demonstrated the prevalence of hypocitraturia in CaOx kidney stone sufferers, thereby providing convincing evidence in support of it being a critical risk factor in this disease (Rudman et al. 1982, Nicar et al. 1983, Hosking *et al.* 1985, Laminski *et al.* 1990, Cupisti *et al.* 1992). Thus, there is a sound rationale and strong motivation for the synthesis and testing of citrate-containing compounds which might induce favourable changes in the risk factors associated with CaOx urolithiasis.

Historically, sodium citrate has been used in the management of uric acid urolithiasis (Sakhaee *et al.* 1983, Preminger *et al.* 1988) but has not been considered as an efficacious drug for the treatment of calcium urolithiasis because of reports that it may increase the risk of urinary crystallization of CaOx and calcium phosphate *in uric acid stone patients* (Sakhaee *et al.* 1983, Preminger *et al.* 1988, Sakhaee *et al.* 1982, Pak *et al.* 1986). Thus a rigorous study investigating its efficacy in the management of CaOx oxalate stone formation is warranted. Such a study is described in this chapter.

3.2 SUBJECTS AND METHODS

3.2.1 Study Design

In this study, four groups of subjects were investigated viz. 30 healthy white males with no history of kidney stone disease (MC), 30 healthy white females with no history of kidney stone disease (FC), 30 white male calcium oxalate stone-formers (MSF) and 30 white female calcium oxalate stone-formers (FSF). All of the stone-formers (age group 30-60 years) had passed a CaOx stone during the preceding 6 months and were recruited from private urologists. The healthy subjects (age group 22-30 years) were recruited from the student cohort of the University of Cape Town. The experimental design used in this study was a placebo controlled, randomized, "within-patient" design. All of the subjects were required to provide two 24h urine collections prior to the commencement of the trial. These were used as baseline samples. Since subjects remained on their free unrestricted diets, each of them was required to complete a 24h-recall dietary questionnaire at various times throughout the trial to identify potential confounding factors. The raw data is presented in the Appendix CD: Chapter3/"Mean data obtained from dietary questionnaires.doc". Twenty subjects in each group took 4 sachets CitroSoda (CitroSoda, Abbott Laboratories*, S.A) per day (equivalent to a total of 2452mg sodium citrate, 6864mg sodium bicarbonate, 2808mg citric acid and 3432mg tartaric acid). This preparation will henceforth be referred to as Na-Cit. It does not contain any sugar, sweetener or colourant. Ten subjects in each group were given a blinded placebo (glucose powder: Pinnacle Pharmaceuticals, South Africa). Subjects were required to ingest Na-Cit (or placebo) at mealtimes (2 sachets at breakfast and 2 sachets at lunchtime). Supplementation lasted for 7 days. In addition to the baseline 24h urine samples, subjects provided 24h collections on day 7 (final day of supplementation) and on day 10 (3 days after supplementation was suspended).

3.2.2 Urine Analysis

Urines were pre-treated and analyzed as previously described (Chapter 2, page 31).

3.2.3 Crystallization Experiments

Crystallization experiments were conducted in each urine as described in chapter 2 (page 32). These involved the determination of each sample's CaOx MSL (Ryall *et al.* 1985) followed by measurement of the particle size distribution of the samples, 120 min after crystallization of CaOx had been initiated (Ryall *et al.* 1995). These particle size

distribution curves are presented in the results section. Data were statistically analyzed using ANOVA. Attention is drawn to the fact that the statistical analysis was performed on the *total volume* (i.e. integrated area under the distribution curve) and on the *mean particle size* (represented by the mode of the distribution curve).

3.2.4 Crystal Deposition Experiments

In order to determine the kinetics of CaOx crystallization as opposed to the kinetics of (possible) *particle* formation, deposition experiments involving radioactive ^{14}C-oxalate (Ryall *et al.* 1995) were conducted on the baseline and day 7 samples. The procedure for these experiments is described below.

Urine samples were filtered and 15ml aliquots were incubated in a shaking waterbath at 37°C for 10 min. 3.125μCi/100ml of ^{14}C-oxalate (NEN, Boston, USA) was added to each 15ml aliquot. CaOx crystallization was induced in each aliquot by the addition of a NaOx load in the same manner as for the crystallization experiments as described in paragraph 3.2.3 above. At 30 min intervals, 2.5 ml samples were filtered through a 0.22μm Millipore filter into 250 μl of concentrated hydrochloric acid, which quenched the reaction, using a disposable syringe and filter holder. 100μl aliquots of the acidic solution were then added to 10ml of scintillation fluid (Zinsser Analytic, Frankfurt) and counted in a liquid scintillation counter (1900CA Tri-Carb Liquid Scintillation Analyser, Packard Instruments Co, Inc., Meriden CT, USA) for 10 minutes. This step was performed in duplicate and the mean values at 120 minutes were statistically analyzed using ANOVA.

3.2.5 Scanning electron microscopy

Crystallization was induced by the addition of NaOx in the same way as for the crystallization experiments. At the end of the 120 minute incubation period, 2ml aliquots of the urine specimens were passed through 0.22μm Millipore filters. The filters were air-dried, mounted on aluminium stubs and coated with gold and palladium once. The stubs were examined using a scanning electron microscope (Leica S440 Scanning Electron Microscope, Leica Cambridge, England). An accelerating voltage of 15kV was used at a working distance of10-15mm and a probe current of 30pA.

3.3 RESULTS

3.3.1 Male Controls

Urine Composition

Statistically significant favourable changes occurred in **5** key risk factors after 7 days of Na-Cit ingestion (Table 3.1): pH (6.40 ± 0.07 versus 7.35 ± 0.09, $p < 0.0001$) and citrate excretion (2.95 ± 0.21 versus 3.68 ± 0.28, $p = 0.0363$) increased while Ca excretion (3.33 ± 0.23 versus 2.40 ± 0.31, $p = 0.0166$) and the RS values of CaOx (2.04 ± 0.18 versus 1.34 ± 0.26, $p = 0.0286$) and uric acid (0.97 ± 0.16 versus 0.18 ± 0.23, $p = 0.0058$) decreased. Increased urinary citrate excretion was still apparent at day 10 (2.95 ± 0.21 versus 3.71 ± 0.28, $p = 0.0307$) i.e. 3 days after suspension of Na-Cit, thereby indicating a carry-over effect. There were no unfavourable changes. No significant changes occurred after taking placebo (Table 3.2). The raw data for the urine composition and physicochemical parameters for each subject is presented in the Appendix CD: Chapter 3/Data for male controls/"Urine excretions and physicochemical data for each subject.xls".

Table 3.1: Male Controls: Average urine composition and physicochemical parameters after Na-Cit administration

Variables	*Baseline*	*Day 7*	*P-Values Baseline vs 7 days*	*Day 10*	*P-Values Baseline vs 10 days*
pH	6.40 ± 0.07	7.35 ± 0.09	<0.0001*	6.60 ± 0.09	0.0682
Volume (ml/24h)	1477 ± 78.4	1497 ± 105.4	0.8783	1475 ± 105.5	0.9904
Citrate (mmol/24h)	2.95 ± 0.21	3.68 ± 0.28	0.0363*	3.71 ± 0.28	0.0307*
Oxalate (mmol/24h)	0.16 ± 0.01	0.14 ± 0.01	0.1949	0.15 ± 0.01	0.7354
Calcium (mmol/24h)	3.33 ± 0.23	2.40 ± 0.31	0.0166*	2.94 ± 0.31	0.3066
Magnesium (mmol/24h)	4.96 ± 0.27	4.42 ± 0.36	0.2281	2.64 ± 0.36	0.3251
Sodium (mmol/24h)	222.7 ± 18.6	253.9 ± 25.0	0.3171	236.6 ± 25.0	0.6557
Potassium (mmol/24h)	47.24 ± 5.47	49.06 ± 7.36	0.8422	72.74 ± 7.36	0.7673
Urate (mmol/24h)	3.49 ± 0.16	3.48 ± 0.22	0.9566	3.58 ± 0.22	0.7397
Creatinine (mmol/24h)	16.60 ± 0.67	15.82 ± 0.90	0.4901	16.26 ± 0.90	0.7659
Phosphate (mmol/24h)	29.39 ± 1.51	27.98 ± 2.03	0.5798	29.40 ± 2.03	0.9960
Chloride (mmol/24h)	155.0 ± 10.3	134.0 ± 13.8	0.3853	161.8 ± 13.8	0.6927
Tiselius risk Index	112 ± 26	93 ± 36	0.6661	119 ± 38	0.8799
RS Brushite	1.36 ± 0.11	1.33 ± 0.16	0.8651	1.44 ± 0.17	0.7022
RS Uric Acid	0.97 ± 0.16	0.18 ± 0.23	0.0058*	0.51 ± 0.24	0.1113
RS CaOx	2.04 ± 0.18	1.34 ± 0.26	0.0286*	1.71 ± 0.27	0.3114
CaOx MSL (mol/dm^3)	0.07 ± 0.01	0.08 ± 0.01	0.4929	0.08 ± 0.01	0.3319

*Significant changes

Table 3.2: Male Controls: Average urine composition and physicochemical parameters after placebo administration

Variables	*Baseline*	*Day 7*	*P-Values Baseline vs 7 days*	*Day 10*	*P-Values Baseline vs 10 days*
pH	6.32 ± 0.092	6.15 ± 0.146	0.3285	6.30 ± 0.146	0.9083
Volume (ml/24h)	1496 ± 109	1483 ± 172	0.9510	1515 ± 172	0.9245
Citrate (mmol/24h)	2.78 ± 0.29	2.92 ± 0.46	0.7910	2.69 ± 0.46	0.8694
Oxalate (mmol/24h)	0.13 ± 0.01	0.15 ± 0.02	0.2449	0.13 ± 0.02	0.7925
Calcium (mmol/24h)	1.92 ± 0.32	1.55 ± 0.50	0.5369	1.76 ± 0.50	0.7877
Magnesium(mmol/24h)	3.98 ± 0.37	3.37 ± 0.59	0.3926	4.1 ± 0.59	0.8584
Sodium (mmol/24h)	227.8 ± 25.8	166.2 ± 40.8	0.2062	231.4 ± 40.8	0.9412
Potassium (mmol/24h)	56.83 ± 7.61	44.20 ± 12.02	0.3778	61.19 ± 12.02	0.7608
Urate (mmol/24h)	3.05 ± 0.23	2.94 ± 0.36	0.8077	3.43 ± 0.36	0.3709
Creatinine (mmol/24h)	14.10 ± 0.93	14.24 ± 1.47	0.9376	16.60 ± 1.47	0.1542
Phosphate (mmol/24h)	23.51 ± 2.10	29.34 ± 3.33	0.1425	30.80 ± 3.33	0.0673
Chloride (mmol/24h)	140.5 ± 14.3	167.7 ± 22.6	0.4678	167.7 ± 22.6	0.3124
Tiselius risk index	116 ± 36	96 ± 59	0.7663	63 ± 59	0.4428
RS Brushite	0.60 ± 0.16	0.40 ± 0.26	0.5053	0.66 ± 0.26	0.8583
RS Uric Acid	1.03 ± 0.23	1.69 ± 0.38	0.1365	1.29 ± 0.38	0.5492
RS CaOx	1.09 ± 0.26	1.18 ± 0.43	0.8541	1.25 ± 0.43	0.7425
CaOx MSL (mol/dm^3)	0.07 ± 0.01	0.07 ± 0.01	0.7757	0.06 ± 0.01	0.5460

CaOx MSL and Particle Size-Volume Distribution

No significant changes in the CaOx MSL were observed after Na-Cit (Table 3.1) and placebo administration (Table 3.2).

Similarly, no significant changes in the total particle volume and mean particle size were observed after 7 days of Na-Cit ingestion and 3 days after suspension of Na-Cit (Figure 3.1a). The same (statistically insignificant) effect on the total particle volume and mean particle size occurred after placebo ingestion for 7 days and 3 days after suspension thereof (Figure 3.1b). The raw data for the particle volume-size data are presented in the Appendix CD: Chapter 3/Data for male controls/"Particle volume-size data.xls".

Crystal Deposition Rate

A favourable decrease in the CaOx crystal deposition rate occurred after ingestion of Na-Cit (Figure 3.2a), $p = 0.0381$. This effect did not occur after the placebo protocol (Figure 3.2b). The raw data for the urine crystal deposition rates are presented in the Appendix CD: Chapter 3/Data for male controls/"Crystal deposition data.xls".

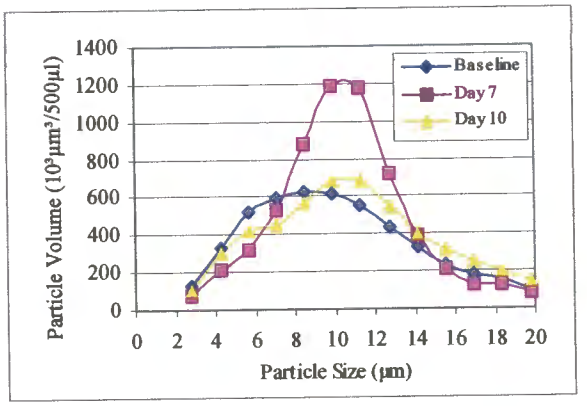


Figure 3.1a: Particle volume-particle size distribution after addition of oxalate. **Male controls'** urine before (Baseline) and after ingestion of CitroSoda (Day 7 & Day 10).

Baseline vs Day 7: % Increase in total volume = 27.48%, p>0.05. % Increase in mean particle size = 25.0%, p>0.05.
Baseline vs Day 10: % Increase in total volume = 8.67%, p>0.05. % Increase in mean particle size = 25.0%, p>0.05.

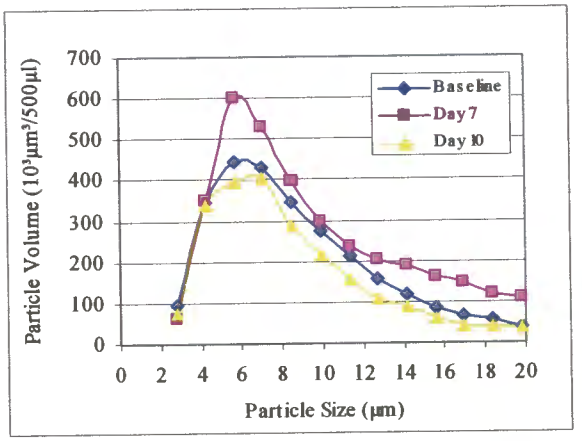


Figure 3.1b: Particle volume-particle size distribution after addition of oxalate. **Male controls'** urine before (Baseline) and after ingestion of Placebo (Day 7 & Day 10).

Baseline vs Day 7: % Increase in total volume = 27.34%, p>0.05. % Decrease in mean particle size = 1.7%, p>0.05.
Baseline vs Day 10: % Increase in total volume = 14.98%, p>0.05. No change in mean particle size.

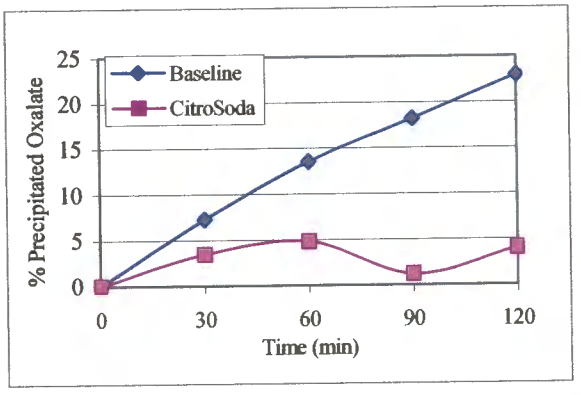


Figure 3.2a: Percentage precipitated [^{14}C]-oxalate after addition of oxalate. **Male controls'** urine before and after ingestion of CitroSoda.

% Decrease in precipitated oxalate after 120 min = 85.46%, p<0.05.

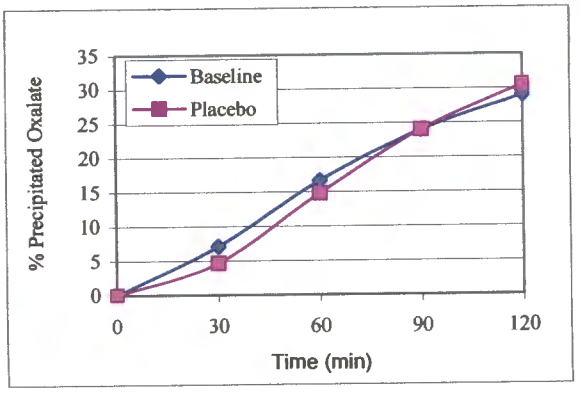


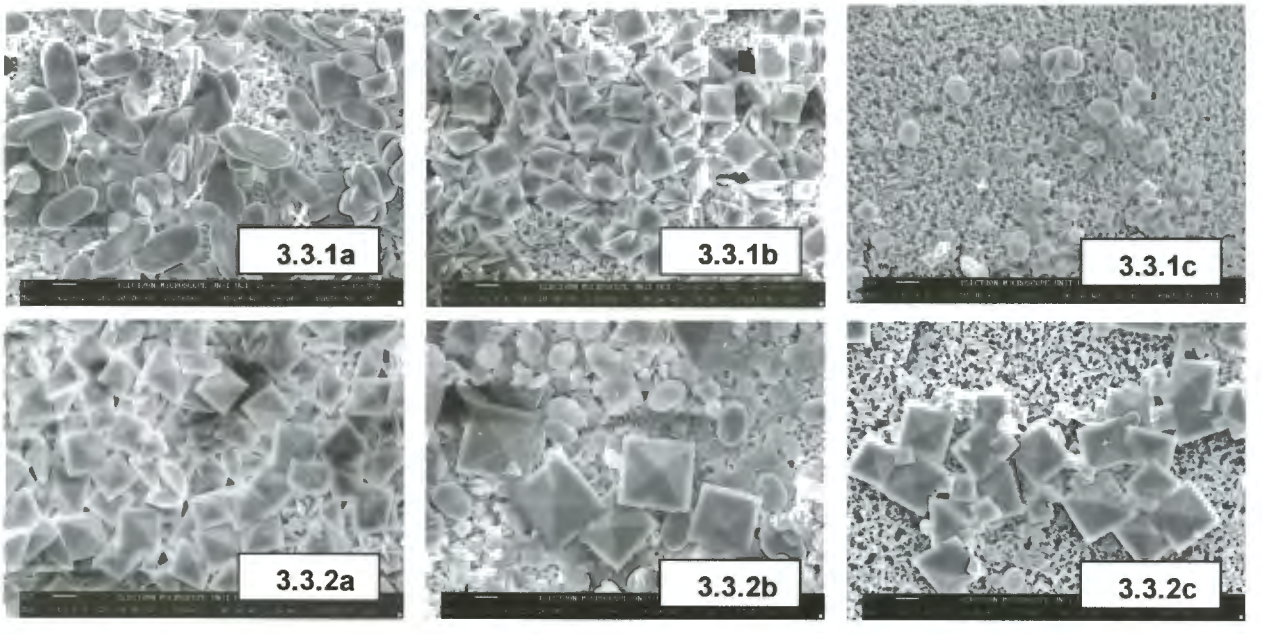
Figure 3.2b: Percentage precipitated [^{14}C]-oxalate after addition of oxalate. **Male controls'** urine before and after ingestion of Placebo.

% Increase in precipitated oxalate after 120 min = 17.17%, p>0.05.

Scanning electron microscopy

Figure 3.3 shows scanning electron micrographs of urine crystals, obtained after the induction of CaOx crystallization, from two male control subjects (Figures 3.3.1 and 3.3.2). In figure 3.3.1a (baseline), coffin-shaped calcium oxalate monohydrate (COM) crystals are predominant, whereas in figure 3.3.2a the micrograph recorded at baseline shows predominantly COD crystals. In general, the number and size of the crystals either remained constant or decreased in both subjects following Na-Cit ingestion (Figures 3.3.1b and 3.3.2b) and these effects carried over after ingestion was suspended (Figures 3.3.1c and 3.3.2c).

Figure 3.3: Male Controls: Scanning electron micrographs of CaOx crystals before and after administration of Na-Cit. Fig 3.3.1a, baseline sample at mag 7.62kX; Fig 3.3.1b, sample after 7 days of Na-Cit ingestion at mag 7.62kX; Fig 3.3.1c, sample 3 days after suspension of Na-Cit ingestion at mag 7.62kX; Fig 3.3.2a, baseline sample at mag 7.62kX; Fig 3.3.2b, sample after 7 days of Na-Cit ingestion at mag 7.62kX; Fig 3.3.2c, sample 3 days after suspension of Na-Cit ingestion at mag 7.62kX.



3.3.2 Male CaOx Stone Formers

Urine Composition

Statistically significant favourable changes occurred in **6** key risk factors after 7 days of Na-Cit ingestion (Table 3.3). The same risk factors were favourably changed in this group as were changed in the control group. However, in addition, oxalate excretion (0.18 ± 0.01 versus 0.12 ± 0.01, p<0.0001) was significantly decreased in the stone formers (Table 3.3). Since oxalate excretion is a crucial risk factor in CaOx stone formation, the decrease in this variable following Na-Cit ingestion is a highly significant clinical result. However, after placebo ingestion, urinary oxalate was also significantly decreased at day 7 (0.19 ± 0.01 versus 0.13 ± 0.01, p = 0.0004), (Table 3.4). After 10 days (i.e. 3 days after suspension of Na-Cit ingestion), urinary calcium (3.26 ± 0.22 versus 2.27 ± 0.31, p=0.0099) and oxalate were still significantly lower than at baseline (0.18 ± 0.01 versus 0.13 ± 0.01, p<0.0001), thereby resulting in the RS CaOx being significantly lower too (3.16 ± 0.18 versus 1.61 ± 0.27, p<0.0001), (Table 3.3). These results indicate that there is a carry-over effect associated with Na-Cit ingestion.

Statistically significant favourable changes occurred in **4** key risk factors after 7 days of placebo ingestion (Table 3.4). The raw data for the urine composition and physicochemical parameters for each subject is presented in Appendix CD: Chapter 3/Data for male stone formers/"Urine excretions and physicochemical data for each subject.xls".

Table 3.3: Male Stone Formers: Average urine composition and physicochemical parameters Na-Cit administration

Variables	*Baseline*	*Day 7*	*P-Values Baseline vs 7 days*	*Day 10*	*P-Values Baseline vs 10 days*
pH	6.07 ± 0.06	7.04 ± 0.09	<0.0001*	6.14 ± 0.09	0.5332
Volume (ml/24h)	1364 ± 76	1371 ± 106	0.9582	1266 ± 106	0.4526
Citrate (mmol/24h)	2.40 ± 0.20	3.10 ± 0.28	0.0453*	2.65 ± 0.28	0.4600
Oxalate (mmol/24h)	0.18 ± 0.01	0.12 ± 0.01	<0.0001*	0.13 ± 0.01	<0.0001*
Calcium (mmol/24h)	3.26 ± 0.22	2.16 ± 0.31	0.0046*	2.27 ± 0.31	0.0099*
Magnesium (mmol/24h)	2.60 ± 0.26	2.38 ± 0.36	0.6312	2.64 ± 0.36	0.9203
Sodium (mmol/24h)	188.8 ± 17.9	241.5 ± 25.0	0.0895	154.6 ± 25.0	0.2681
Potassium (mmol/24h)	61.19 ± 5.29	64.72 ± 7.36	0.6972	72.74 ± 7.36	0.2052
Urate (mmol/24h)	3.43 ± 0.16	3.86 ± 0.22	0.1091	3.40 ± 0.22	0.9185
Creatinine (mmol/24h)	15.51 ± 0.64	15.02 ± 0.90	0.6641	14.30 ± 0.90	0.2753
Phosphate (mmol/24h)	30.96 ± 1.46	28.72 ± 2.03	0.3731	28.25 ± 2.03	0.2825
Chloride (mmol/24h)	168.8 ± 9.9	156.4 ± 13.8	0.4655	146.5 ± 13.83	0.1927
Tiselius risk index	162 ± 26	114 ± 36	0.2791	108 ± 38	0.2327
RS Brushite	1.10 ± 0.11	1.04 ± 0.16	0.7697	0.90 ± 0.17	0.3240
RS Uric Acid	1.91 ± 0.16	0.32 ± 0.23	<0.0001*	1.80 ± 0.24	0.6792
RS CaOx	3.16 ± 0.18	1.15 ± 0.26	<0.0001*	1.61 ± 0.27	<0.0001*
CaOx MSL (mol/dm^3)	0.05 ± 0.01	0.08 ± 0.01	0.0013*	0.06 ± 0.01	0.2845

*Significant changes

Table 3.4: Male Stone Formers: Average urine composition and physicochemical parameters after placebo administration

Variables	*Baseline*	*Day 7*	*P-Values Baseline vs 7 days*	*Day 10*	*P-Values Baseline vs 10 days*
pH	6.02 ± 0.09	6.23 ± 013	0.1576	6.19 ± 0.13	0.2509
Volume (ml/24h)	1714 ± 109	1516 ± 149	0.2853	1422 ± 149	0.1170
Citrate (mmol/24h)	2.84 ± 0.29	2.86 ± 0.39	0.9647	2.61 ± 0.39	0.6373
Oxalate (mmol/24h)	0.19 ± 0.01	0.13 ± 0.01	0.0004*	0.16 ± 0.01	0.1011
Calcium (mmol/24h)	4.33 ± 0.32	3.93 ± 0.44	0.4536	4.12 ± 0.44	0.6916
Magnesium (mmol/24h)	3.00 ± 0.37	2.91 ± 0.51	0.8841	2.82 ± 0.51	0.7796
Sodium (mmol/24h)	209.0 ± 25.8	206.2 ± 35.3	0.9475	236.7 ± 35.3	0.5287
Potassium (mmol/24h)	45.86 ± 7.60	47.53 ± 10.40	0.8972	56.63 ± 10.40	0.4051
Urate (mmol/24h)	3.93 ± 0.23	3.64 ± 0.31	0.4439	3.77 ± 0.31	0.6702
Creatinine (mmol/24h)	17.33 ± 0.93	16.30 ± 1.27	0.5127	14.07 ± 1.27	0.0402*
Phosphate (mmol/24h)	31.58 ± 2.10	32.53 ± 2.89	0.7895	30.13 ± 2.88	0.6855
Chloride (mmol/24h)	274.6 ± 14.3	148.3 ± 19.6	0.8078	157.1 ± 19.56	0.9052
Tiselius risk index	275 ± 36	119 ± 51	0.0151*	305 ± 51	0.6314
RS Brushite	0.89 ± 0.16	1.52 ± 0.23	0.0239*	1.14 ± 0.23	0.3588
RS Uric Acid	2.46 ± 0.23	1.88 ± 0.33	0.1492	1.63 ± 0.33	0.0403*
RS CaOx	3.71 ± 0.26	2.35 ± 0.37	0.0032*	2.61 ± 0.37	0.0163*
CaOx MSL (mol/dm^3)	0.09 ± 0.01	0.09 ± 0.01	0.8422	0.08 ± 0.01	0.4451

*Significant changes

CaOx MSL and Particle Size-Volume Distribution

A significant increase in the CaOx MSL was observed after 7 days of Na-Cit ingestion (p=0.0013). No significant changes were observed 3 days after the suspension of Na-Cit (Table 3.3) and after placebo ingestion (Table 3.4).

Similarly, no significant changes in the total particle volume and mean particle size were observed after 7 days of Na-Cit ingestion and 3 days after suspension thereof (Figure 3.4a). The same (statistically insignificant) effect on the total particle volume and mean particle size occurred after 7 days of placebo ingestion, no change in the latter was observed 3 days after suspension of placebo (Figure 3.4b). The raw data for the particle volume-size data are presented in the Appendix CD: Chapter 3/Data for male stone formers/"Particle volume-size data.xls".

Crystal Deposition Rate

No significant changes were observed in the CaOx deposition rate after ingestion of Na-Cit (Figure 3.5a) and after ingestion of placebo (Figure 3.5b). The raw data for the urine crystal deposition rates are presented in the Appendix CD: Chapter 3/Data for male stone formers/"Crystal deposition data.xls".

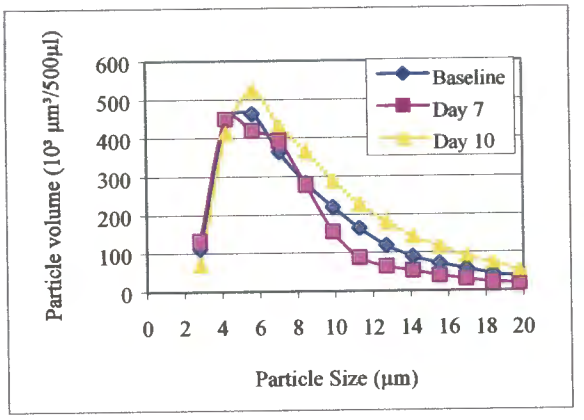


Figure 3.4a: Particle volume-particle size distribution after addition of oxalate. **Male stone formers'** urine before (Baseline) and after ingestion of CitroSoda (Day 7 & Day 10).

Baseline vs Day 7: % Decrease in total volume = 13.01%, p>0.05. % Decrease in mean particle size = 7.08%, p>0.05.
Baseline vs Day 10: % Increase in total volume = 21.95%, p>0.05. % Increase in mean particle size = 9.8%, p>0.05.

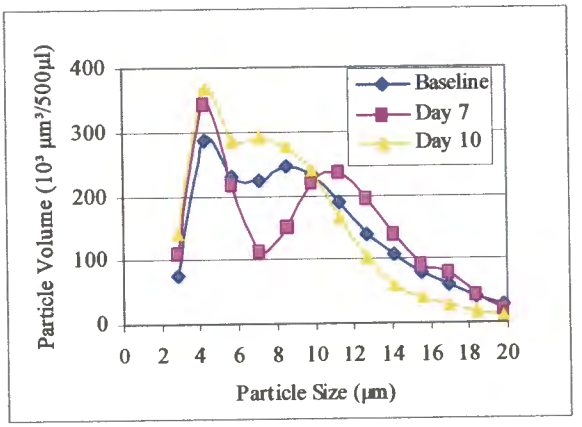


Figure 3.4b: Particle volume-particle size distribution after addition of oxalate. **Male stone formers'** urine before (Baseline) and after ingestion of Placebo (Day 7 & Day 10).

Baseline vs Day 7: % Increase in total volume = 1.03%, p>0.05. % Decrease in mean particle size = 2.2%, p>0.05.
Baseline vs Day 10: % Increase in total volume = 5.15%, p>0.05. No change in mean particle size.

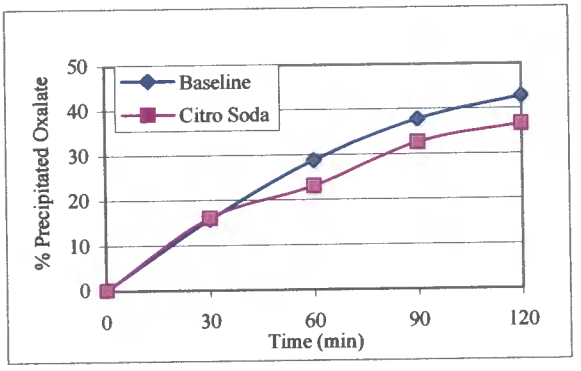


Figure 3.5a: Percentage precipitated [^{14}C]-oxalate. **Male stone formers'** urine before (Baseline) and after ingestion of CitroSoda.

% Decrease in precipitated oxalate after 120 min = 14.37%, p>0.05.

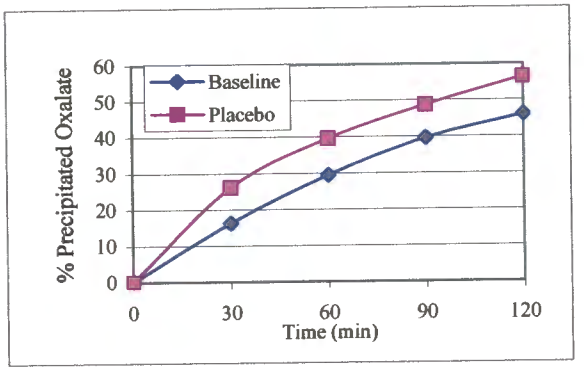


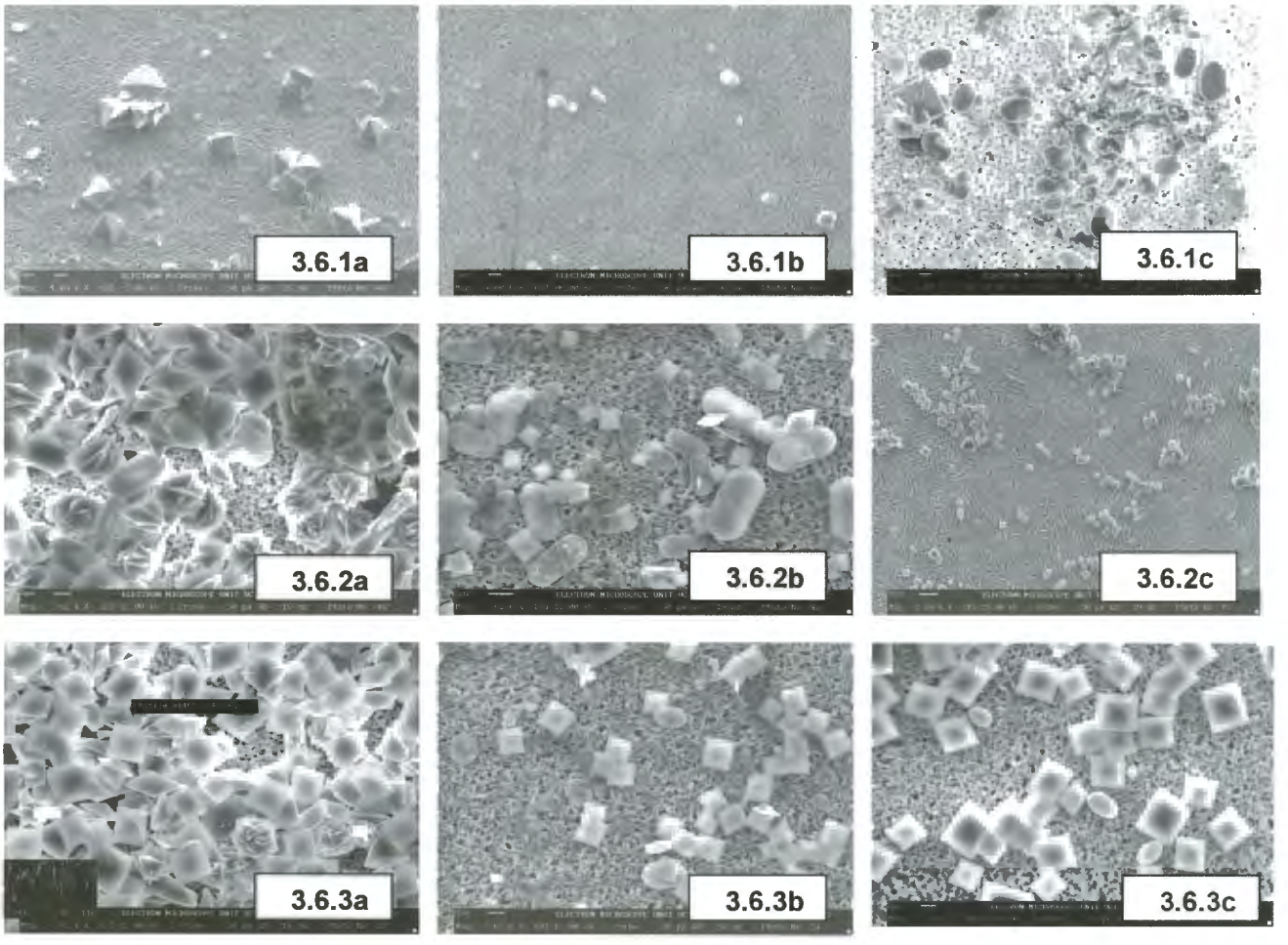
Figure 3.5b: Percentage precipitated [^{14}C]-oxalate. **Male stone formers'** urine before (Baseline) and after ingestion of Placebo.

% Increase in precipitated oxalate after 120 min = 22.72%, p>0.05.

Scanning electron microscopy

Figure 3.6 shows scanning electron micrographs of urine crystals, obtained after the induction of CaOx crystallization, from three male CaOx stone forming subjects (Figures 3.6.1, 3.6.2 and 3.6.3). The predominance of both single and aggregated COD crystals in figures 3.6.1a, 3.6.2a and 3.6.3a (baseline) is evident. Attention is drawn to the definite reduction in the number of crystals precipitated after 7 days of Na-Cit ingestion in all three subjects (figures 3.6.1b, 3.6.2b and 3.6.3b). There were no obvious consistent changes in crystal size.

Figure 3.6: Male Stone Formers: Scanning electron micrographs of CaOx crystals before and after administration of Na-Cit. Fig 3.6.1a, baseline sample at mag 4.00 KX; Fig 3.6.1b, sample after 7 days of Na-Cit ingestion at mag 4.00 KX; Fig 3.6.1c, sample 3 days after suspension of Na-Cit ingestion at mag 4.00 KX; Fig 3.6.2a, baseline sample at mag 7.62 KX; Fig 3.6.2b, sample after 7 days of Na-Cit ingestion at mag 7.62 KX; Fig 3.6.2c, sample 3 days after suspension of Na-Cit ingestion at mag 1.86 KX; Fig 3.6.3a, baseline sample at mag 7.62 KX; Fig 3.6.3b, sample after 7 days of Na-Cit ingestion at mag 7.62 KX; Fig 3.6.3c, sample 3 days after suspension of Na-Cit ingestion at mag 7.62 KX.



3.3.3 Female Controls

Urine Composition

Statistically significant favourable changes occurred in **6** key risk factors including a reduction in oxalate excretion (Table 3.5), after 7 days of Na-Cit ingestion: pH (6.42 ± 0.07 versus 7.50 ± 0.09, p<0.0001) and citrate excretion (2.91 ± 0.17 versus 3.90 ± 0.23, p = 0.0007) increased while oxalate excretion (0.20 ± 0.01 versus 0.15 ± 0.01, p<0.0001), Tiselius risk index (220.8 ± 17.8 versus 148.3 ± 26.0, p = 0.0234) and RS values for CaOx (2.22 ± 0.22 versus 0.61 ± 0.32, p<0.0001) and uric acid (0.78 ± 0.14 versus 0.06 ± 0.21, p = 0.0055) decreased. (Although urinary sodium increased significantly (101.2 ± 13.9 versus 252.3 ± 19.0, p<0.0001), there was not a concomitant increase in urinary calcium. Indeed, urinary calcium decreased (1.98 ± 0.17 versus 1.62 ± 0.23, p = 0.2068), albeit not significantly). After 10 days (i.e. 3 days after suspension of Na-Cit ingestion), urinary oxalate (0.20 ± 0.01 versus 0.16 ± 0.01, p = 0.0013) and RS CaOx (2.22 ± 0.22 versus 1.38 ± 0.32, p = 0.0305) was still significantly lower than at baseline, indicating a carry - over effect. There were no significant changes after placebo ingestion (Table 3.6). The raw data for the urine composition and physicochemical parameters for each subject is presented in the Appendix CD: Chapter 3/Data for female controls/"Urine excretions and physicochemical data for each subject.xls".

Table 3.5: Female Controls: Average urine composition and physicochemical parameters after Na-Cit administration

Variables	*Baseline*	*Day 7*	*P-Values Baseline vs 7 days*	*Day 10*	*P-Values Baseline vs 10 days*
pH	6.42 ± 0.07	7.5 ± 0.09	<0.0001*	6.55 ± 0.10	0.2971
Volume (ml/24h)	1674 ± 70	1896 ± 96	0.0659	1463 ± 100	0.0872
Citrate (mmol/24h)	2.91 ± 0.17	3.90 ± 0.23	0.0007*	3.06 ± 0.24	0.6144
Oxalate (mmol/24h)	0.20 ± 0.01	0.15 ± 0.01	<0.0001*	0.16 ± 0.01	0.0013*
Calcium (mmol/24h)	1.98 ± 0.17	1.62 ± 0.23	0.2068	1.99 ± 0.24	0.9704
Magnesium (mmol/24h)	2.37 ± 0.15	2.55 ± 0.21	0.4696	2.50 ± 0.22	0.6252
Sodium (mmol/24h)	101.2 ± 13.9	252.3 ± 19.0	<0.0001*	164.7 ± 19.7	0.0098*
Potassium (mmol/24h)	42.92 ± 3.40	32.90 ± 4.65	0.0848	38.18 ± 4.81	0.4234
Urate (mmol/24h)	2.75 ± 0.13	2.98 ± 0.18	0.2848	2.64 ± 0.18	0.6149
Creatinine (mmol/24h)	10.32 ± 0.31	10.31 ± 0.44	0.9905	10.46 ± 0.44	0.7899
Phosphate (mmol/24h)	19.36 ± 1.08	19.13 ± 1.53	0.9003	21.63 ± 1.53	0.2293
Chloride (mmol/24h)	111.1 ± 7.2	125.1 ± 10.2	0.2613	119.4 ± 10.2	0.5055
Tiselius risk index	221 ± 18	148 ± 26	0.0234*	162 ± 26	0.0632
RS Brushite	0.79 ± 0.12	0.52 ± 0.17	0.1924	0.73 ± 0.17	0.7463
RS Uric Acid	0.78 ± 0.14	0.06 ± 0.21	0.0055*	0.58 ± 0.21	0.4264
RS CaOx	2.22 ± 0.22	0.61 ± 0.32	<0.0001*	1.38 ± 0.32	0.0305*
CaOx MSL (mol/dm^3)	0.13 ± 0.01	0.17± 0.01	<0.0001*	0.14± 0.01	0.3011

*Significant changes

Table 3.6: Female Controls: Average urine composition and physicochemical parameters after placebo administration

Variables	*Baseline*	*Day 7*	*P-Values Control vs 7 days*	*Day 10*	*P-Values Control vs 10 days*
pH	6.33 ± 0.09	6.43 ± 0.13	0.5152	6.28 ± 0.13	0.7672
Volume (ml/24h)	1447 ± 96	1420 ± 136	0.8729	1429 ± 136	0.9155
Citrate (mmol/24h)	2.38 ± 0.23	2.74 ± 0.32	0.3673	2.63 ± 0.32	0.5293
Oxalate (mmol/24h)	0.16 ± 0.01	0.15 ± 0.02	0.5209	0.15 ± 0.02	0.5534
Calcium (mmol/24h)	2.99 ± 0.23	3.46 ± 0.32	0.2309	3.12 ± 0.32	0.7346
Magnesium (mmol/24h)	2.33 ± 0.21	2.53 ± 0.30	0.5867	2.39 ± 0.30	0.8728
Sodium (mmol/24h)	94.2 ± 19.0	82.0 ± 26.9	0.7108	67.6 ± 26.9	0.4210
Potassium (mmol/24h)	34.65 ± 4.65	42.18 ± 6.58	0.3520	40.72 ± 6.58	0.4528
Urate (mmol/24h)	3.12 ± 0.17	2.85 ± 0.24	0.3650	2.63 ± 0.24	0.1017
Creatinine (mmol/24h)	11.64 ± 0.43	11.70 ± 0.60	0.9352	11.41 ± 0.60	0.7553
Phosphate (mmol/24h)	22.02 ± 1.48	22.12 ± 2.09	0.9673	25.30 ± 2.09	0.2012
Chloride (mmol/24h)	122.1 ± 9.8	126.4 ± 13.9	0.8007	129.4 ± 13.9	0.6683
Tiselius risk index	215 ± 25	194 ± 36	0.6329	196 ± 36	0.6619
RS Brushite	1.23 ± 0.17	1.53 ± 0.24	0.3072	0.10 ± 0.24	0.4272
RS Uric Acid	1.31 ± 0.20	0.88 ± 0.29	0.2236	0.99 ± 0.29	0.3630
RS CaOx	3.30 ± 0.31	3.00 ± 0.43	0.5849	2.70 ± 0.43	0.2626
CaOx MSL (mol/dm^3)	0.11 ± 0.01	0.10 ± 0.01	0.3095	0.09 ± 0.01	0.0944

CaOx MSL and Particle Size-Volume Distribution

A significant increase in the CaOx MSL was observed after 7 days of Na-Cit ingestion ($p<0.0001$). No significant changes were observed 3 days after the suspension of Na-Cit and after placebo ingestion.

A significant decrease in the total particle volume which was deposited after Na-Cit ingestion for 7 days was observed (Figure 3.7a), $p = 0.0035$, while no significant change in the mean particle size was observed. No statistically significant changes in afore-mentioned variables were observed 3 days after suspension of Na-Cit (Figure 3.7a). No effect on the total particle volume and mean particle size occurred after 7 days of placebo ingestion and neither was any change observed 3 days after suspension of placebo (Figure 3.4b). The raw data for the particle volume-size data are presented in the Appendix CD: Chapter 3/Data for female controls/"Particle volume-size data.xls".

Crystal Deposition Rate

A favourable decrease in the CaOx deposition rate occurred after Na-Cit ingestion ($p = 0.0239$) (Figure 3.8a) but not after placebo (Figure 3.8b). The raw data for the urine crystal deposition rates are presented in the Appendix CD: Chapter 3/Data for female controls/"Crystal deposition data.xls".

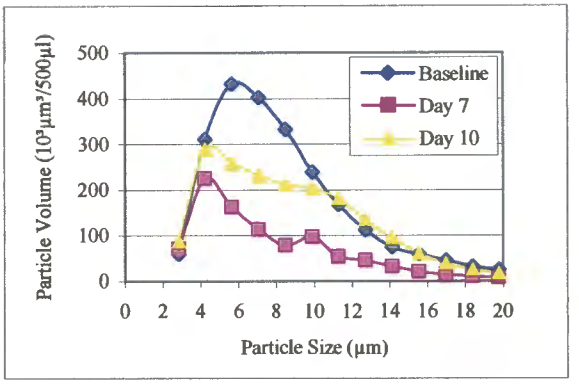


Figure 3.7a: Particle volume-particle size distribution after addition of oxalate. **Female controls'** urine before (Baseline) and after ingestion of CitroSoda (Day 7 & Day 10).
Baseline vs Day 7: % Decrease in total volume = 61.57%, $p<0.05$. % Decrease in mean particle size = 26.7%, $p>0.05$.
Baseline vs Day 10: % Decrease in total volume = 17.03%, $p>0.05$. % Decrease in mean particle size = 30.0%, $p>0.05$.

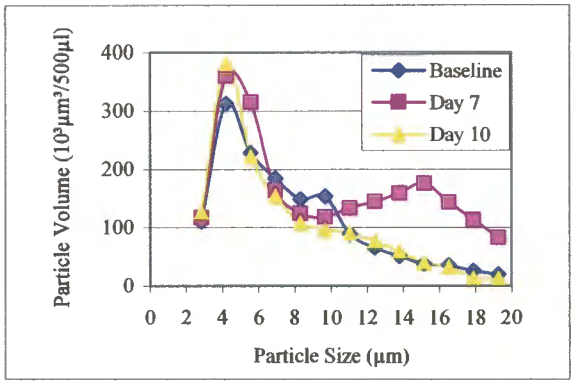


Figure 3.7b: Particle volume-particle size distribution after addition of oxalate. Female controls' urine before (Baseline) and after ingestion of Placebo (Day 7 & Day 10).
Baseline vs Day 7: % Increase in total volume = 47.95%, $p>0.05$. No change in mean particle size.
Baseline vs Day 10: % Decrease in total volume = 2.05%, $p>0.05$. % Decrease in mean particle size = 9.5%, $p>0.05$.

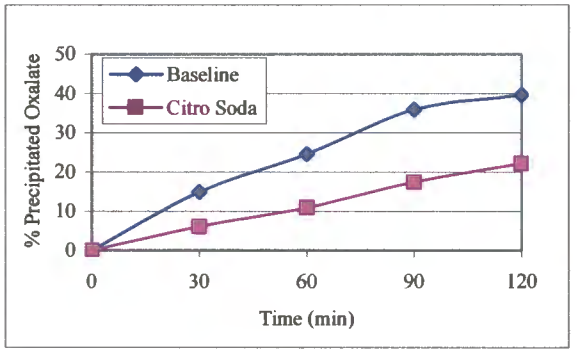


Figure 3.8a: Percentage precipitated [^{14}C]-oxalate. **Female controls'** urine before (Baseline) and after ingestion of CitroSoda.

% Decrease in precipitated oxalate after 120 min = 43.98%, $p<0.05$.

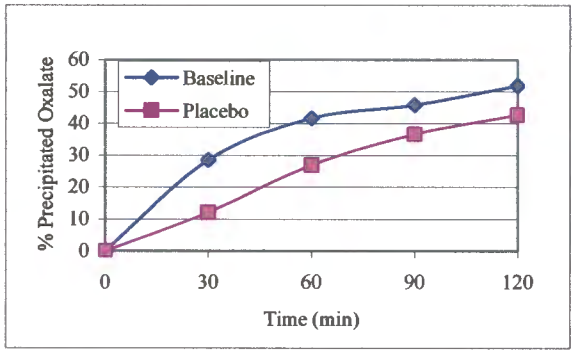


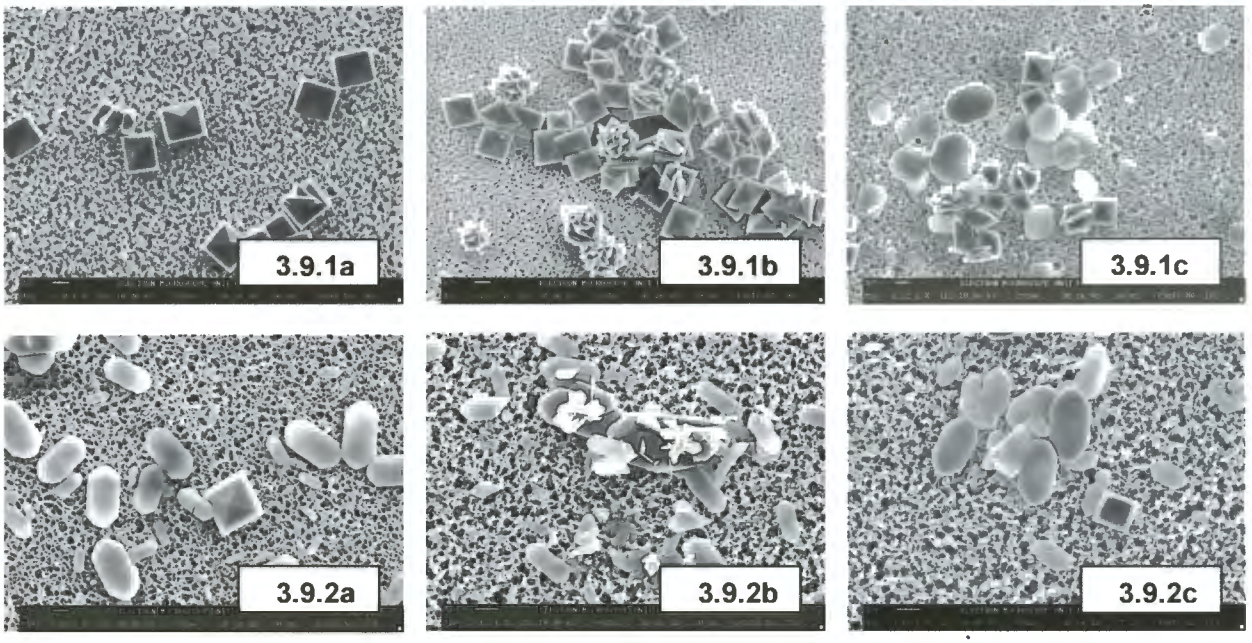
Figure 3.8b: Percentage precipitated [^{14}C]-oxalate. **Female controls'** urine before (Baseline) and after ingestion of Placebo.

% Decrease in precipitated oxalate after 120 min = 17.68%, $p>0.05$.

Scanning Electron Microscopy

Figure 3.9 shows scanning electron micrographs of urine crystals, obtained after the induction of CaOx crystallization, from two white female control subjects (Figures 3.9.1 and 3.9.2). Figure 3.9.1a (baseline) shows COD crystals while the urine from the second subject, Figure 3.9.2a (baseline), shows predominantly COM crystals. No obvious effect on the number and size of the crystals were observed after Na-Cit ingestion (Figures 3.9.1b and 3.9.2b) and after suspension thereof (Figures 3.9.1c and 3.9.2c).

Figure 3.9: Female Controls: Scanning electron micrographs of CaOx crystals before and after administration of Na-Cit. Fig 3.9.1a, baseline sample at mag 5.30 KX; Fig 3.9.1b, sample after 7 days of Na-Cit ingestion at mag 5.28 KX; Fig 3.9.1c, sample 3 days after suspension of Na-Cit ingestion at mag 6.32 KX; Fig 3.9.2a, baseline sample at mag 7.62 KX; Fig 3.9.2b, sample after 7 days of Na-Cit ingestion at mag 7.62 KX; Fig 3.9.2c, sample 3 days after suspension of Na-Cit ingestion at mag 7.62 KX.



3.3.4 Female CaOx Stone Formers

Urine Composition

Statistically significant favourable changes occurred in **7** key risk factors after 7 days of Na-Cit ingestion (Table 3.7). Of these, 6 corresponded with the risk factors, which were altered in the controls. After 10 days (i.e. 3 days after suspension of Na-Cit ingestion), urinary citrate was still significantly higher than at baseline (2.47± 0.17 versus 3.25 ± 0.26, p = 0.0131), while urinary oxalate (0.17 ± 0.01 versus 0.11 ± 0.01, p = 0.0002) and RS CaOx (3.29 ± 0.22 versus 1.94 ± 0.35, p = 0.0016) were still significantly lower than at baseline, indicating a favourable carry-over effect (Table 3.7). None of these changes occurred after placebo ingestion at 7 days. However, after 10 days (placebo protocol), there were statistically significant decreases in urinary calcium (3.72 ± 0.24 versus 2.48 ± 0.34, p = 0.0037), creatinine (10.30 ± 0.45 versus 8.41 ± 0.63, p = 0.0166), RS CaOx (4.91 ± 0.31 versus 3.53 ± 0.50, p = 0.0215) and chloride (134.8 ± 10.3 versus 103.4 ± 14.6, p = 0.0476). These unexpected changes may be due to the possibility that the 10 day collections in this group may have been incomplete, as suggested by the significantly lower 24h urinary excretion of creatinine relative to baseline (Table 3.8). The raw data for the urine composition and physicochemical parameters for each subject is presented in Appendix CD: Chapter 3/Data for female stone formers/"Urine excretions and physicochemical data for each subject.xls".

Table 3.7: Female Stone Formers: Average urine composition and physicochemical parameters after Na-Cit administration

Variables	*Baseline*	*Day 7*	*P-Values Baseline vs 7 days*	*Day 10*	*P-Values Baseline vs 10 days*
pH	6.33 ± 0.07	7.16 ± 0.09	<0.0001*	6.16 ± 0.11	0.1919
Volume (ml/24h)	1304 ± 72	1323 ± 96	0.8747	1314 ± 112	0.9341
Citrate (mmol/24h)	2.47 ± 0.17	3.17 ± 0.23	0.0149*	3.25 ± 0.26	0.0131*
Oxalate (mmol/24h)	0.17 ± 0.01	0.14 ± 0.01	0.0387*	0.11 ± 0.01	0.0002*
Calcium (mmol/24h)	3.08 ± 0.17	2.60 ± 0.23	0.0952	3.69 ± 0.26	0.0553
Magnesium (mmol/24h)	2.18 ± 0.16	1.86 ± 0.21	0.2313	2.27 ± 0.24	0.7550
Sodium (mmol/24h)	131.7 ± 14.2	140.8 ± 19.0	0.7005	149.5 ± 22.1	0.4971
Potassium (mmol/24h)	40.32 ± 3.46	36.54 ± 4.66	0.5162	53.44 ± 5.39	0.0422*
Urate (mmol/24h)	2.90 ± 0.13	2.66 ± 0.17	0.2780	2.98 ± 0.20	0.7391
Creatinine (mmol/24h)	10.57 ± 0.32	9.85 ± 0.43	0.1765	9.89 ± 0.49	0.2487
Phosphate (mmol/24h)	20.55 ± 1.10	20.70 ± 1.48	0.9317	24.32 ± 1.71	0.0650
Chloride (mmol/24h)	122.1 ± 7.3	97.9 ± 9.8	0.0505	127.6 ± 11.4	0.6821
Tiselius risk index	283 ± 18	204 ± 26	0.0145*	180 ± 28	0.8310
RS Brushite	0.93 ± 0.12	1.40 ± 0.17	0.0266*	1.02 ± 0.19	0.6942
RS Uric Acid	1.70 ± 0.14	0.48 ± 0.21	<0.0001*	1.56 ± 0.24	0.6142
RS CaOx	3.29 ± 0.22	2.13 ± 0.31	0.0027*	1.94 ± 0.35	0.0016*
CaOx MSL (mol/dm^3)	0.08 ± 0.01	0.11 ± 0.01	0.0037*	0.07 ± 0.01	0.4603

*Significant changes

Table 3.8: Female Stone Formers: Average urine composition and physicochemical parameters after placebo administration

Variables	*Baseline*	*Day 7*	*P-Values Baseline vs 7 days*	*Day 10*	*P-Values Baseline vs 10 days*
pH	6.24 ± 0.10	6.33 ± 0.14	0.6062	6.27 ± 0.14	0.8825
Volume (ml/24h)	1076 ± 102	771 ± 144	0.0863	839 ± 144	0.1823
Citrate (mmol/24h)	1.63 ± 0.24	1.35 ± 0.34	0.4980	1.33 ± 0.34	0.4730
Oxalate (mmol/24h)	0.15 ± 0.01	0.13 ± 0.02	0.5322	0.12 ± 0.02	0.2752
Calcium (mmol/24h)	3.72 ± 0.24	3.13 ± 0.34	0.1582	2.48 ± 0.34	0.0037*
Magnesium (mmol/24h)	1.85 ± 0.22	1.54 ± 0.31	0.4292	1.59 ± 0.31	0.4963
Sodium (mmol/24h)	81.7 ± 20.1	64.5 ± 28.4	0.6221	78.0 ± 28.4	0.9157
Potassium (mmol/24h)	34.03 ± 4.90	34.51 ± 6.93	0.9549	24.02 ± 6.93	0.2411
Urate (mmol/24h)	2.91 ± 0.18	2.41 ± 0.26	0.1130	2.31 ± 0.26	0.0579
Creatinine (mmol/24h)	10.30 ± 0.45	9.40 ± 0.63	0.2487	8.41 ± 0.63	0.0166*
Phosphate (mmol/24h)	21.13 ± 1.56	17.87 ± 2.20	0.2277	17.23 ± 2.20	0.1505
Chloride (mmol/24h)	134.8 ± 10.3	108.2 ± 14.6	0.0352*	103.4 ± 14.6	0.0476*
Tiselius risk index	309 ± 25	299 ± 38	0.8310	249 ± 38	0.1997
RS Brushite	1.87 ± 0.17	2.23 ± 0.26	0.2475	1.46 ± 0.26	0.1900
RS Uric Acid	1.54 ± 0.20	1.86 ± 0.31	0.3904	2.12 ± 0.31	0.1237
RS CaOx	4.91 ± 0.31	5.11 ± 0.47	0.7239	3.53 ± 0.50	0.0215*
CaOx MSL (mol/dm^3)	0.07 ± 0.01	0.07 ± 0.01	0.9146	0.07 ± 0.01	0.8582

*Significant changes

CaOx MSL and Particle Size-Volume Distribution

A significant increase in the CaOx MSL was observed after 7 days of Na-Cit ingestion ($p<0.0001$). The change observed 3 days after the suspension of Na-Cit was not statistically significant. No change was observed after placebo ingestion.

No significant changes in the total particle volume and mean particle size were observed after 7 days of Na-Cit ingestion and 3 days after suspension of Na-Cit (Figure 3.10a).

The same (statistically insignificant) effect on the total particle volume and mean particle size occurred after placebo ingestion for 7 days and 3 days after suspension thereof (Figure 3.10b). The raw data for the particle volume-size data are presented in the Appendix CD: Chapter 3/Data for female stone formers/"Particle volume-size data.xls".

Crystal Deposition Rate

No statistically significant changes in the CaOx deposition rate occurred after Na-Cit and placebo ingestion (Figures 3.11a & 3.11b). The raw data for the crystal deposition rates are presented in the Appendix CD: Chapter 3/Data for female stone formers/"Crystal deposition data.xls".

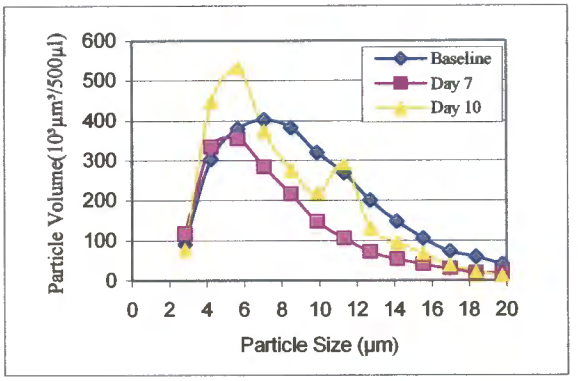


Figure 3.10a: Particle volume-particle size distribution after addition of oxalate. **Female stone formers'** urine before (Baseline) and after ingestion of CitroSoda (Day 7 & Day 10).

Baseline vs Day 7: % Decrease in total volume = 35.31%, p>0.05. % Decrease in mean particle size = 28.6%, p>0.05.
Baseline vs Day 10: % Increase in total volume = 9.44%, p>0.05. % Increase in mean particle size = 14.3%, p>0.05.

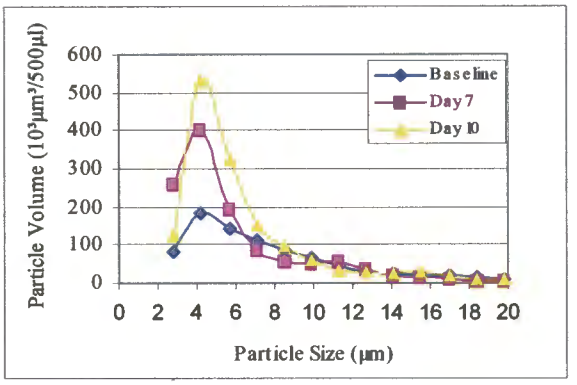


Figure 3.10b: Particle volume-particle size distribution after addition of oxalate. **Female stone formers'** urine before (Baseline) and after ingestion of Placebo (Day 7 & Day 10).

Baseline vs Day 7: % Increase in total volume = 41.67%, p>0.05. % Decrease in mean particle size = 7.1%, p>0.05.
Baseline vs Day 10: % Increase in total volume = 73.81%, p<0.05. % Decrease in mean particle size = 2.4%, p>0.05.

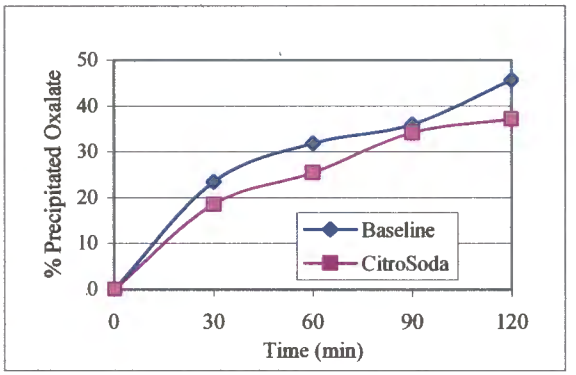


Figure 3.11a: Percentage precipitated [^{14}C]-oxalate. **Female stone formers'** urine before (Baseline) and after ingestion of CitroSoda.

% Decrease in precipitated oxalate after 120 min = 18.82%, p>0.05.

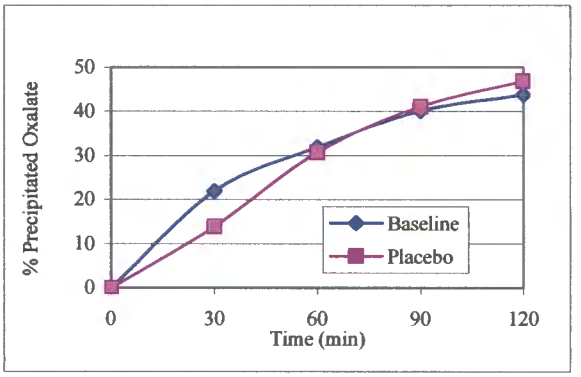


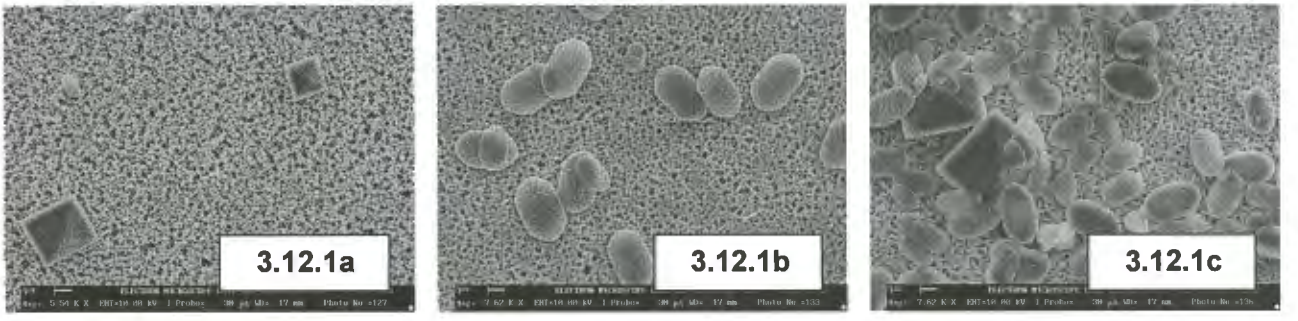
Figure 3.11b: Percentage precipitated [^{14}C]-oxalate. **Female stone formers'** urine before (Baseline) and after ingestion of Placebo.

% Increase in precipitated oxalate after 120 min = 6.59%, p>0.05.

Scanning electron microscopy

Figure 3.12 shows scanning electron micrographs of urine crystals, obtained after the induction of CaOx crystallization, from a white female stone forming subject (Figures 3.12.1). The micrograph of the baseline sample shows 2 single COD crystals (figure 3.12.1a). After 7 days of Na-Cit ingestion (Figure 3.12.1b) and 3 days after the suspension thereof (figure 3.12.1c), an increase in the overall number and size of crystals were observed.

Figure 3.12: Female Stone Formers: Scanning electron micrographs of CaOx crystals before and after administration of Na-Cit. Fig 3.12.1a, baseline sample at mag 5.54 KX; Fig 3.12.1b, sample after 7 days of Na-Cit ingestion at mag 7.62 KX; Fig 3.12.1c, sample 3 days after suspension of Na-Cit ingestion at mag 7.62KX.



3.4 DISCUSSION

The results of the present study have provided convincing physiological evidence to warrant the use of CitroSoda as a prophylactic and therapeutic agent in the conservative treatment and management of CaOx urolithiasis. The study has shown that this preparation unequivocally alters four biochemical risk factors in all of the groups which were studied, viz. pH, citrate excretion, RS CaOx and the RS of uric acid. Other physiological risk factors were favourably altered too. Of particular interest is oxalate excretion. It is noteworthy that this parameter decreased significantly in both FC and FSF following Na-Cit administration, and that the same effect was not achieved after placebo. On the other hand, no effect on MC was observed, but oxalate excretion decreased in MSF after CitroSoda *and* placebo. This latter observation is puzzling; a possible reason for this could be that this might be an example of the stone clinic effect (Hosking *et al.* 1983) in which the patients might have become more vigilant of their dietary oxalate intake once they had commenced the (placebo) protocol. As such, their urinary oxalate excretion decreased. It could be argued that the decrease in oxalate excretion observed in FC and FSF might have occurred for the same reason. However, this argument is easily

dismissed since the placebo protocol did not lower urinary oxalate in these 2 groups. Thus, the decrease in oxalate excretion in FC and FSF cannot be attributed to the stone clinic affect and must be correctly credited to CitroSoda. Decreases in oxalate excretion have not been reported for any of the citrate-containing preparations which are currently in wide use. Indeed, an increase in this parameter has been observed following K-Mg-Cit therapy (Ettinger *et al.* 1997). Thus, the lowering of urinary oxalate following Na-Cit administration represents a very important finding.

It is also worth noting that an increase in citrate excretion and a concomitant decrease in calcium excretion was observed following Na-Cit administration even though it is well known that sodium intake *increases* calcium excretion (Muldowney *et al.* 1982, Martini *et al.* 1998). It is also worth noting that bicarbonate decreases urinary calcium excretion (Siener *et al.* 2004). The increase in citrate excretion after citrate administration could be due to the excretion of absorbed Na-Cit which escaped metabolic degradation in vivo and directly appeared in the urine (Pak 1992). The increase in pH observed is as a result of the in vivo oxidation of citrate to bicarbonate, which results in a change in the acid-base balance of the urine (Pak 1992). The presence of bicarbonate in the Na-Cit preparation probably also attributed to the increase in urinary pH, which consequently led to an increase in citrate excretion (Keβler and Hesse 2000).

The present study has also provided physicochemical data which demonstrate the favourable effect of Na-Cit administration on CaOx urolithiasis. This point is illustrated by the RS values for CaOx and uric acid. The RS of CaOx was uniquely and favourably decreased in all of the groups studied except in MSF where the placebo also achieved this result (mainly due to the decrease in oxalate excretion following placebo administration discussed above). Despite this latter apparent anomaly, the decrease in RS of CaOx *in all of the groups* represents another important finding in favour of Na-Cit administration. The same is true for the observation of decreased RS values for uric acid. As will be pointed out later in the discussion, a previously reported concern about Na-Cit therapy has been the observed increase in the RS of sodium urate. Although this parameter was not directly measured in the present study, it is unlikely to have been affected since sodium excretion did not increase in 3 of the 4 groups. The only group in which sodium excretion increased was FC; and suggest that the possible risk of sodium urate precipitation would have been attenuated by the decrease in RS of uric acid. Another reported concern has been an increase in the RS of brushite (in uric acid stone formers) (Sakhaee *et al.* 1983). In the present study (involving healthy controls and CaOx

stone formers), this physicochemical risk factor did not increase in any of the groups following CitroSoda administration. To the contrary, a *decrease* was observed in FSF. The decrease in the Tiselius risk index in female controls and female stone formers is yet another favourable finding, even though the same effect did not occur in males.

A significant increase in the CaOx MSL was observed in all the groups after Na-Cit administration except for the male controls. Since an increase in the CaOx MSL is indicative of urine in which crystallization is relatively more difficult, this study has shown that urine exhibits enhanced inhibitory properties following Na-Cit administration.

Although there were no statistically significant changes (except in FC) in the total volume of deposited particles, the mean particle size and the CaOx deposition rates, some of the observed trends are interesting and worthy of speculative comment. The total volume of particles deposited and the mean particle size showed decreasing trends in all of the groups except MC (where increasing trends were observed) after Na-Cit administration. The (apparent) anomalous increase in particle volume in MC after Na-Cit administration was also achieved after placebo. As such, not much relevance is attached to this finding. CaOx crystal deposition rates showed decreasing trends in all of the groups albeit that the placebo also achieved a decrease in FC and FSF, but this latter effect (i.e. due to placebo) was weaker than that achieved by Na-Cit. Thus, it can be speculated that the volume-size distributions and the crystal formation kinetics tend to support the compelling urine data which demonstrated favourable therapeutic effects for Na-Cit.

The scanning electron micrographs were generally inconclusive. Nevertheless some noteworthy features were observed. The formation of COM or COD or mixtures thereof varied from one urine to another, suggesting that hydrate formation is a function of intrinsic urine composition. SEM observations in MC showed that crystal size and number either remained constant or decreased in urines after ingestion of Na-Cit. This is inconsistent with the size-volume distributions which showed an increasing trend. In MSF, SEM showed decreasing crystal numbers, tending to support the volume-size data which showed a decreasing trend in particle volume. In FC, no obvious effect on crystal size or volume was observed in the micrographs, while in FSF an increase in particle number and size was detected. This latter observation contradicts the decreasing trend which was speculated from the volume-size distributions. The apparent lack of consistency between SEM and volume-size data could be attributed to the relatively

small number of samples which were examined by the former technique. Attention is drawn to the fact that volume-size distributions and SEM were performed on urines which had been spiked with sodium oxalate. This provides a measure of how the urine (after ingestion of Na-Cit) supports CaOx crystallization. It would have been interesting to have measured the volume-size distribution curves and SEM on *raw urine* as well.

The results of the present study also provide evidence for carry-over effects: citrate excretion (MC), oxalate excretion (MSF, FC, FSF) and RS CaOx (MSF, FC, FSF) remained significantly altered, three days after suspension of Na-Cit administration. Moreover, negative side effects such as mild nausea, epigastric pain and abdominal distention, which have been reported for other citrate-containing preparations, did not occur in any of the subjects.

The 24h-recall dietary questionnaires revealed that there were no significant differences in the key nutrients of calcium and oxalate during the study period. Thus, urinary calcium and oxalate would not have been directly confounded by the subjects' unrestricted diet. Nevertheless, significant differences occurred in a few cases involving the nutrients magnesium, sodium, potassium, phosphate, citrate, vitamin A and vitamin C. These may have indirectly affected urinary excretions to a small extent thereby highlighting the need for a rigorous standard diet to have been administered.

It is of considerable interest in the context of this study to review the reasons for the apparent abandonment of Na-Cit as a potential therapeutic agent in the treatment of CaOx urolithiasis. Firstly, it is noted that in studies comparing the effects of K-Cit and Na-Cit, Sakhaee *et al.* 1983 and Preminger *et al.* 1988), refer to papers which have reported an increase in the risk of calcium stone formation following sodium *alkali* therapy. Attention is drawn to the fact that the alkalinizing agent in these studies was sodium bicarbonate and not sodium citrate *per se* (Parfitt *et al.* 1969, Robson *et al.* 1978). Secondly, there have indeed been very few studies on stone risk factors following Na-Cit administration (Sakhaee *et al.* 1982, Sakhaee *et al.* 1983, Preminger *et al.* 1988 and that, more importantly, these studies have involved very small patient groups (4, 5 and 6 subjects respectively). This raises serious doubts about the validity of the statistical analyses.

Thirdly, it appears as if the main concern about Na-Cit therapy is that it increases the urinary saturations of sodium urate (Sakhaee *et al.* 1982, Sakhaee *et al.* 1983, Preminger *et al.* 1988) and calcium phosphate (brushite) (Sakhaee *et al.* 1983). Although these parameters constitute urinary physicochemical risk factors, in vitro studies have demonstrated that the former effect could promote urate-induced crystallization of calcium oxalate (Pak *et al.* 1976, Pak *et al.* 1977) while the latter would increase the risk of brushite precipitation. However, it is intriguing that K-Cit therapy has caused the same two effects (Sakhaee *et al.* 1983), yet it has not been discredited in any way. Admittedly, the inhibitor activity against calcium oxalate precipitation (given by the formation product ratio) significantly increased with K-Cit while Na-Cit had no effect (Sakhaee *et al.* 1983), but this can be regarded as being indicative of *relative* risk rather than *absolute* risk.

Finally, it is noted that the concerns which have been reported about Na-Cit administration have arisen in the treatment of uric acid urolithiasis while there does not seem to be any reported concerns relating to the use of this preparation in calcium oxalate urolithiasis.

In conclusion, it can be stated that this study is unique for several reasons. Firstly, it is the first in-depth calcium oxalate stone risk investigation involving a sodium citrate preparation. Secondly, unlike many of the studies involving other citrate-containing preparations, the present study investigated *four* groups of subjects. The participation of males and females allowed certain gender-based similarities and differences to be identified, while the participation of healthy subjects allowed potential prophylactic effects of CitroSoda to be examined. Thirdly, the group sizes (30 subjects) used in this study were substantially larger than in many other studies, thereby lending credibility to the statistical analyses of this study. Fourthly, the results obtained from this study have dispelled the concerns that sodium citrate may increase the risk of stone formation in calcium oxalate stone formers. While such a concern may be true for uric acid stone formers, the present results have demonstrated convincingly that sodium citrate, in the form of CitroSoda, is an effective preparation for the prophylactic and therapeutic management of CaOx urolithiasis.

* This product is now owned by Adcock Ingram Limited, S.A.

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Chapter 4

Investigation of urinary oxalate excretion as a function of the time at which a calcium supplement is ingested relative to an oxalate-rich meal

4.1 INTRODUCTION

Several studies have addressed the question of stone forming risk associated with the administration of calcium supplements. Levine *et al.* (1994) and Sakhaee *et al.* (1994) tested the effects of calcium citrate on stone forming and normal females respectively. In both studies there were no significant increases in the relative supersaturation of CaOx. Since neither study indicated whether the supplement was taken with meals or between meals it is not possible to conclude whether these data (collected 3 years prior to publication of Curhan *et al.'s* paper) support the latter's hypothesis or not, viz. if supplemental calcium is not ingested with a meal it may provide little or no protection from oxalate absorption, which may lead to an increased risk of stone formation. Calcium carbonate supplements have also been investigated. Williams et al. (2001) administered this supplement to 8 male stone formers at breakfast and demonstrated a reduction in the calcium oxalate product when taking modest amounts of the supplement regularly thereby illustrating the value of taking the supplement with a meal. When taken after a meal, no significant effects on stone risk were observed in healthy and stone-forming males (Caudarella *et al.* 2001) and in postmenopausal women with osteoporosis (Domrongkitchaiporn *et al.* 2002). These latter studies support Curhan's hypothesis (Curhan *et al.* 1997). Other studies have focused on the inverse relationship between colonic calcium and oxalate in stone formers (Medonca *et al.* 2003). It is therefore apparent that while the timing of calcium supplementation relative to a meal is a potentially very important factor, its precise role has not been conclusively demonstrated. Indeed, a comprehensive investigation, under strictly controlled conditions, has not been previously reported. The study described in this chapter addresses this question.

4.2 METHODS

Twenty healthy white male subjects (from the student cohort of the University of Cape Town) in the age group 18-20 years participated in the study. The subjects were instructed to avoid calcium- and oxalate-rich foods for three days prior to the commencement of the study (Appendix CD: Chapter 4/"List of foods to avoid.doc"). They were also instructed not to take any medication or supplements (other than those prescribed in the protocol). Six different protocols were investigated. These are listed in Table 4.1. Subjects provided a fasting overnight urine sample on the morning of trial, which was discarded. Thereafter they ingested a standardized oxalate-rich meal (Table 4.2). The nutrient content of the standardized meal is given in Table 4.3. The whole experimental

protocol was conducted in a controlled environment where subjects were given explicit instructions regarding the protocol.

Table 4.1: Protocol used in study

Protocol	
1	09h00: Subject ingests standardized oxalate-rich meal 10h00: Subject provides a urine sample and drinks 200ml water 11h00: Subject provides a urine sample and drinks 200ml water 12h00: Subject provides a urine sample and drinks 200ml water 13h00: Subject provides a urine sample and drinks 200ml water 14h00: Subject provides a urine sample and drinks 200ml water 15h00 Subject provides a urine sample
2	09h00: Subject ingests standardized oxalate-rich meal and ingests calcium supplement 10h00: Subject provides a urine sample and drinks 200ml water 11h00: Subject provides a urine sample and drinks 200ml water 12h00: Subject provides a urine sample and drinks 200ml water 13h00: Subject provides a urine sample and drinks 200ml water 14h00: Subject provides a urine sample and drinks 200ml water 15h00 Subject provides a urine sample
3	09h00: Subject ingests standardized oxalate-rich meal 10h00: Subject provides a urine sample, drinks 200ml water and ingests calcium supplement 11h00: Subject provides a urine sample and drinks 200ml water 12h00: Subject provides a urine sample and drinks 200ml water 13h00: Subject provides a urine sample and drinks 200ml water 14h00: Subject provides a urine sample and drinks 200ml water 15h00 Subject provides a urine sample
4	09h00: Subject ingests standardized oxalate-rich meal 10h00: Subject provides a urine sample and drinks 200ml water 11h00: Subject provides a urine sample, drinks 200ml water and ingests calcium supplement 12h00: Subject provides a urine sample and drinks 200ml water 13h00: Subject provides a urine sample and drinks 200ml water 14h00: Subject provides a urine sample and drinks 200ml water 15h00 Subject provides a urine sample
5	09h00: Subject ingests standardized oxalate-rich meal 10h00: Subject provides a urine sample and drinks 200ml water 11h00: Subject provides a urine sample and drinks 200ml water 12h00: Subject provides a urine sample, drinks 200ml water and ingests calcium supplement 13h00: Subject provides a urine sample and drinks 200ml water 14h00: Subject provides a urine sample and drinks 200ml water 15h00 Subject provides a urine sample
6	09h00: Subject ingests standardized oxalate-rich meal 10h00: Subject provides a urine sample and drinks 200ml water 11h00: Subject provides a urine sample and drinks 200ml water 12h00: Subject provides a urine sample and drinks 200ml water 13h00: Subject provides a urine sample, drinks 200ml water and ingests calcium supplement 14h00: Subject provides a urine sample and drinks 200ml water 15h00 Subject provides a urine sample

Table 4.2: Standardized oxalate-rich meal ingested at the beginning of each protocol

Food	*Quantity per oxalate meal*
Spinach (cooked)	160g
Chicken (1 breast + 1 wing)	220-270g
Roll	40g
Coffee (with coffee creamer – zero calcium)	250ml
Mineral water (6.1mg/l calcium, 1.0mg/l magnesium)	200ml

* The preparation and source of the spinach was the same for the entire trial

Table 4.3: Composition of the standardized oxalate-rich meal

Variable	Composition
Energy (kJ)	3669
Total protein (g)	71.9
Plant protein (g)	7.9
Animal protein (g)	64.0
Total fat (g)	42.8
Carbohydrate (g)	45.0
Sodium (mg)	931
Potassium (mg)	1203
Phosphorous (mg)	611
Calcium (mg)	294
Magnesium (mg)	182
Vitamin C (mg)	8
Citric acid (mg)	124
Oxalic Acid (mg)	901

The dosage of calcium in the supplement was 1200mg (administered as calcium carbonate, SOLGAR, U.S.A). There was a 7-day wash-out period between protocols, during which the subjects were on their unrestricted home diet for 4 days and avoided calcium and oxalate rich foods for 3 days prior to the next protocol. Urines were pre-treated and analyzed as previously described (Chapter 2, page 31). The ion activity product of calcium oxalate, termed AP(CaOx) index, for each sample was calculated according to the following formula where hourly excretions (mmol/hr) of calcium, oxalate, magnesium and citrate were used and the volume was measured in litres (Ahlstrand *et al.* 1984):

$$AP(CaOx)\ index = 9.05 \times Ca^{0.71} \times Ox \times Mg^{-0.14} \times Cit^{-0.10} \times \dot{V}^{-1.2}.$$

Urinalysis data were statistically analyzed using a Split Plot Analysis (Repeated Measures Design) and the results were considered statistically significant if $p<0.05$. In this analysis, the data for the fasting urine sample (09h00) were discarded as previously mentioned. The baseline mean for each variable was computed from the 10h00 values for all protocols except protocol 2 as the latter is the only one in which the supplement

was taken prior to 10h00. All p-values are calculated relative to the baseline mean for that particular variable.

4.3 RESULTS

All raw data are presented in the Appendix CD (Chapter 4/"Urinary excretion data for each subject.xls").

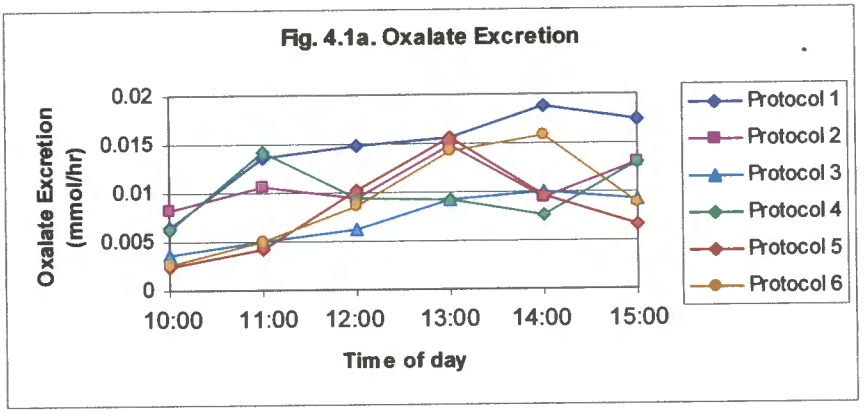
Peak oxalate excretions for each protocol are presented in Table 4.4a and oxalate excretions throughout the 6 hours of each protocol are given in Table 4.4b. Figures 4.1a and 4.1b present the data graphically. Baseline mean = 0.005 ± 0.001 mmol/hr. Figure 4.1c represents the *total* oxalate excretion for each protocol during the 6-hour period following ingestion of the oxalate-rich meal. These values were obtained by integration of the area under each graph in figure 4.1a.

Table 4.4a: Peak oxalate excretion for each protocol

Protocol	Time of day	Peak oxalate excretion ± SE
1	14:00	0.019 ± 0.003
2	13:00	0.015 ± 0.003
3	14:00	0.010 ± 0.003
4	11:00	0.014 ± 0.003
5	13:00	0.016 ± 0.003
6	14:00	0.016 ± 0.003

Table 4.4b: Mean Oxalate excretion (mmol/hr)

Protocol	Time of day	Mean ± SE	P – Value
1	10:00	0.006 ± 0.003	
1	11:00	0.014 ± 0.003	0.0045
1	12:00	0.015 ± 0.003	0.0014
1	13:00	0.015 ± 0.003	0.0006
1	14:00	0.019 ± 0.003	p < 0.0001
1	15:00	0.017 ± 0.003	0.0001
2	10:00	0.008 ± 0.003	
2	11:00	0.011 ± 0.003	0.0714
2	12:00	0.009 ± 0.003	0.1536
2	13:00	0.015 ± 0.003	0.0018
2	14:00	0.009 ± 0.003	0.1662
2	15:00	0.013 ± 0.003	0.0098
3	10:00	0.004 ± 0.003	
3	11:00	0.005 ± 0.003	0.9990
3	12:00	0.006 ± 0.003	0.7108
3	13:00	0.009 ± 0.003	0.1634
3	14:00	0.010 ± 0.003	0.0972
3	15:00	0.009 ± 0.003	0.1725
4	10:00	0.006 ± 0.003	
4	11:00	0.014 ± 0.003	0.0029
4	12:00	0.009 ± 0.003	0.1510
4	13:00	0.009 ± 0.003	0.1585
4	14:00	0.007 ± 0.003	0.4001
4	15:00	0.013 ± 0.003	0.0082
5	10:00	0.002 ± 0.003	
5	11:00	0.004 ± 0.003	0.7895
5	12:00	0.010 ± 0.003	0.0895
5	13:00	0.016 ± 0.003	0.0005
5	14:00	0.010 ± 0.003	0.1346
5	15:00	0.007 ± 0.003	0.5986
6	10:00	0.002 ± 0.003	
6	11:00	0.005 ± 0.003	0.9697
6	12:00	0.008 ± 0.003	0.2430
6	13:00	0.014 ± 0.003	0.0026
6	14:00	0.016 ± 0.003	0.0004
6	15:00	0.009 ± 0.003	0.2156



A 3-dimensional representation of the oxalate excretion is presented below (Figure 4.1b).

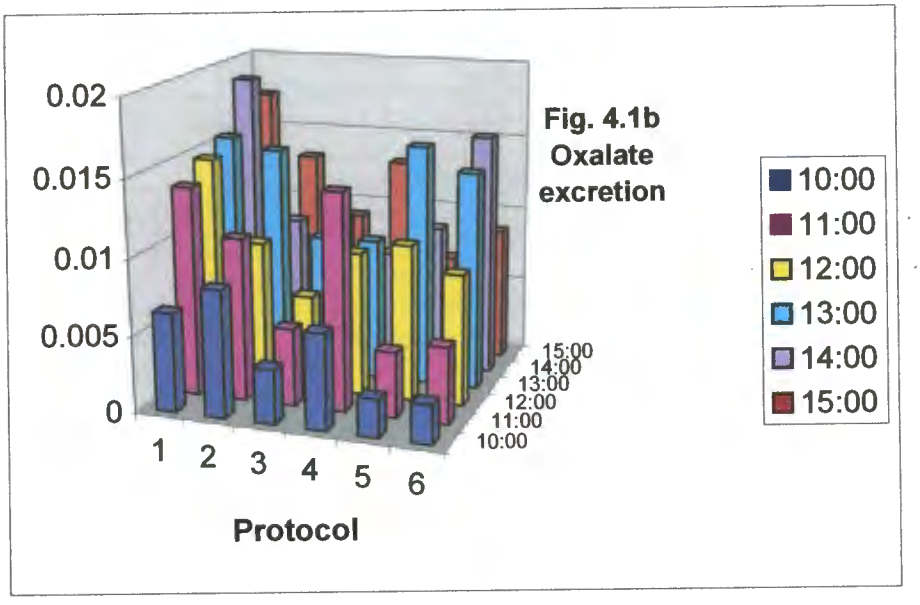
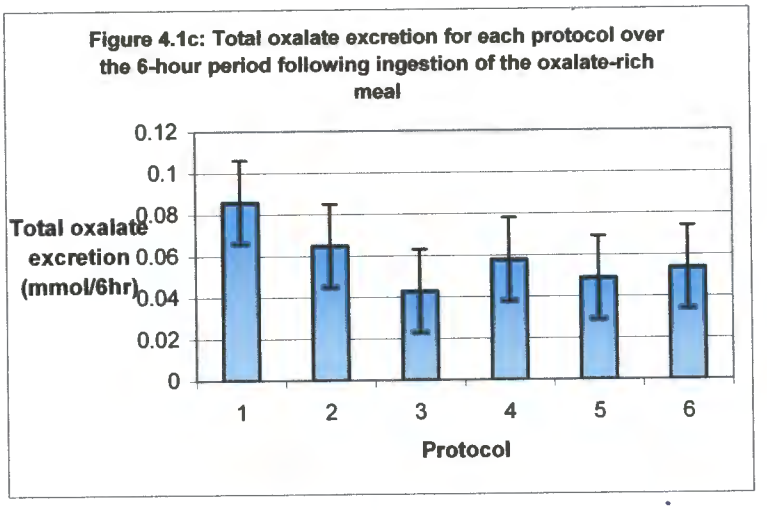


Fig. 4.1b Oxalate excretion

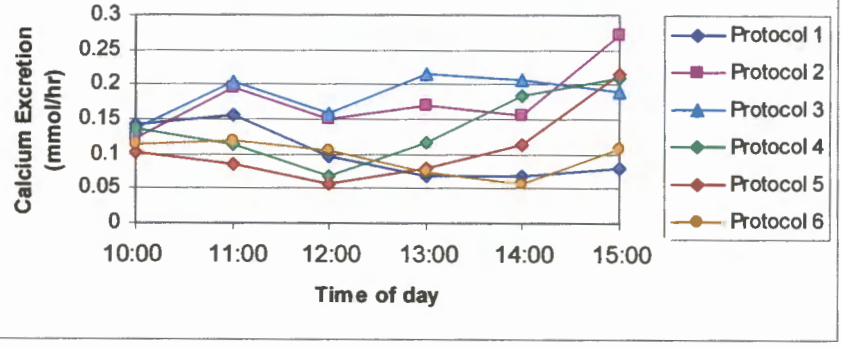


Calcium excretions throughout the 6 hours of each protocol are given in Table 4.5, while Figures 4.2 and 4.14 presents the data graphically. Baseline mean = 0.125 ± 0.013 mmol/hr.

Table 4.5: Mean Calcium excretion (mmol/hr)

Protocol	Time of day	Mean ± SE	P - Value
1	10:00	0.14 ± 0.03	
1	11:00	0.16 ± 0.03	0.2945
1	12:00	0.10 ± 0.03	0.3081
1	13:00	0.07 ± 0.03	0.0548
1	14:00	0.07 ± 0.03	0.0548
1	15:00	0.08 ± 0.02	0.1280
2	10:00	0.12 ± 0.03	
2	11:00	0.20 ± 0.02	0.0227
2	12:00	0.15 ± 0.02	0.4372
2	13:00	0.17 ± 0.02	0.1550
2	14:00	0.16 ± 0.02	0.3235
2	15:00	0.27 ± 0.02	p < 0.0001
3	10:00	0.14 ± 0.03	
3	11:00	0.20 ± 0.02	0.0098
3	12:00	0.16 ± 0.02	0.2516
3	13:00	0.21 ± 0.02	0.0033
3	14:00	0.21 ± 0.03	0.0059
3	15:00	0.19 ± 0.03	0.0308
4	10:00	0.14 ± 0.02	
4	11:00	0.11 ± 0.02	0.6938
4	12:00	0.07 ± 0.03	0.0480
4	13:00	0.12 ± 0.02	0.7190
4	14:00	0.18 ± 0.02	0.0497
4	15:00	0.21 ± 0.03	0.0053
5	10:00	0.10 ± 0.02	
5	11:00	0.09 ± 0.03	0.1827
5	12:00	0.06 ± 0.02	0.0198
5	13:00	0.08 ± 0.02	0.1280
5	14:00	0.11 ± 0.02	0.6444
5	15:00	0.21 ± 0.03	0.0032
6	10:00	0.11 ± 0.02	
6	11:00	0.12 ± 0.03	0.8334
6	12:00	0.10 ± 0.02	0.4849
6	13:00	0.07 ± 0.02	0.0851
6	14:00	0.06 ± 0.03	0.0213
6	15:00	0.11 ± 0.02	0.5393

Fig. 4.2 Calcium Excretion

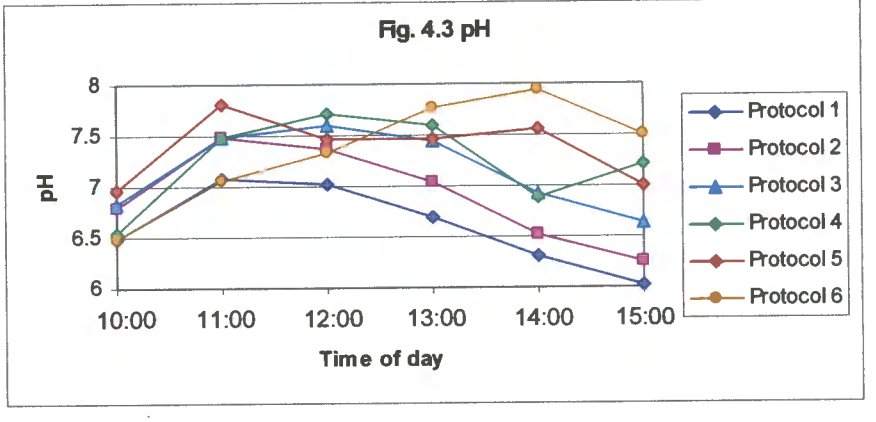


pH throughout the 6 hours of each protocol are given in Table 4.6, while Figure 4.3 presents the data graphically. Baseline mean = 6.66 ± 0.08.

Table 4.6: Mean pH

Protocol	Time of day	Mean ± SE	P - Value
1	10:00	6.48 ± 0.17	
1	11:00	7.07 ± 0.17	0.0367
1	12:00	7.02 ± 0.17	0.0631
1	13:00	6.69 ± 0.17	0.8602
1	14:00	6.31 ± 0.17	0.0775
1	15:00	6.02 ± 0.17	0.0015
2	10:00	6.78 ± 0.18	
2	11:00	7.48 ± 0.17	0.0001
2	12:00	7.37 ± 0.17	0.0005
2	13:00	7.04 ± 0.17	0.0571
2	14:00	6.52 ± 0.17	0.4835
2	15:00	6.25 ± 0.17	0.0450
3	10:00	6.82 ± 0.17	
3	11:00	7.49 ± 0.17	p< 0.0001
3	12:00	7.60 ± 0.17	p < 0.0001
3	13:00	7.44 ± 0.17	0.0001
3	14:00	6.92 ± 0.17	0.1776
3	15:00	6.64 ± 0.17	0.9533
4	10:00	6.54 ± 0.17	
4	11:00	7.48 ± 0.17	0.0001
4	12:00	7.72 ± 0.17	p < 0.0001
4	13:00	7.60 ± 0.17	p < 0.0001
4	14:00	6.89 ± 0.17	0.2383
4	15:00	7.21 ± 0.18	0.0052
5	10:00	6.96 ± 0.17	
5	11:00	7.80 ± 0.17	p < 0.0001
5	12:00	7.47 ± 0.17	0.0001
5	13:00	7.46 ± 0.17	0.0001
5	14:00	7.55 ± 0.17	p < 0.0001
5	15:00	7.00 ± 0.17	0.0787
6	10:00	6.48 ± 0.17	
6	11:00	7.06 ± 0.17	0.0420
6	12:00	7.33 ± 0.17	0.0007
6	13:00	7.77 ± 0.17	p < 0.0001
6	14:00	7.95 ± 0.17	p < 0.0001
6	15:00	7.50 ± 0.17	.p < 0.0001

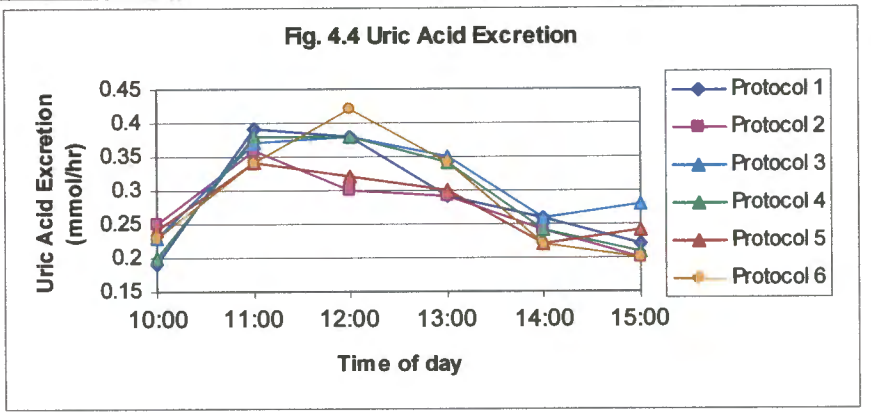
Fig. 4.3 pH



Uric acid excretions throughout the 6 hours of each protocol are given in Table 4.7, while Figure 4.4 presents the data graphically. Baseline mean = 0.22 ± 0.01 mmol/hr.

Table 4.7: Mean Uric Acid excretion (mmol/hr)

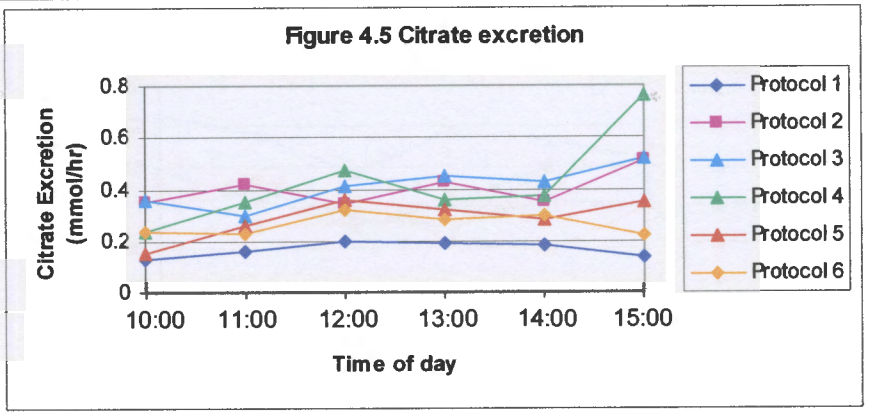
Protocol	Time of day	Mean ± SE	P – Value
1	10:00	0.19 ± 0.03	
1	11:00	0.39 ± 0.03	p < 0.0001
1	12:00	0.38 ± 0.03	p < 0.0001
1	13:00	0.29 ± 0.03	0.0240
1	14:00	0.26 ± 0.03	0.1373
1	15:00	0.22 ± 0.03	0.9280
2	10:00	0.25 ± 0.03	
2	11:00	0.36 ± 0.03	p < 0.0001
2	12:00	0.30 ± 0.03	0.0123
2	13:00	0.29 ± 0.03	0.0274
2	14:00	0.24 ± 0.03	0.5958
2	15:00	0.20 ± 0.03	0.5296
3	10:00	0.23 ± 0.03	
3	11:00	0.37 ± 0.03	p < 0.0001
3	12:00	0.38 ± 0.03	p < 0.0001
3	13:00	0.35 ± 0.03	0.0001
3	14:00	0.26 ± 0.03	0.1373
3	15:00	0.28 ± 0.03	0.0511
4	10:00	0.20 ± 0.03	
4	11:00	0.38 ± 0.03	p < 0.0001
4	12:00	0.38 ± 0.03	p < 0.0001
4	13:00	0.34 ± 0.03	0.0003
4	14:00	0.24 ± 0.03	0.3905
4	15:00	0.21 ± 0.03	0.9147
5	10:00	0.24 ± 0.03	
5	11:00	0.34 ± 0.03	0.0001
5	12:00	0.32 ± 0.03	0.0010
5	13:00	0.30 ± 0.03	0.0160
5	14:00	0.22 ± 0.03	0.9280
5	15:00	0.24 ± 0.03	0.4833
6	10:00	0.23 ± 0.03	
6	11:00	0.34 ± 0.03	0.0002
6	12:00	0.42 ± 0.03	p < 0.0001
6	13:00	0.34 ± 0.03	0.0003
6	14:00	0.22 ± 0.03	0.9766
6	15:00	0.20 ± 0.03	0.4696



Citrate excretions throughout the 6 hours of each protocol are given in Table 4.8, while Figure 4.5 presents the data graphically. Baseline mean = 0.22 ± 0.04 mmol/hr.

Table 4.8: Mean Citrate excretion (mmol/hr)

Protocol	Time of day	Mean ± SE	P - Value
1	10:00	0.13 ± 0.06	
1	11:00	0.16 ± 0.06	0.4687
1	12:00	0.20 ± 0.06	0.7405
1	13:00	0.19 ± 0.06	0.6397
1	14:00	0.18 ± 0.06	0.6019
1	15:00	0.14 ± 0.06	0.3163
2	10:00	0.35 ± 0.06	
2	11:00	0.42 ± 0.06	0.0365
2	12:00	0.34 ± 0.06	0.2004
2	13:00	0.43 ± 0.06	0.0269
2	14:00	0.35 ± 0.06	0.1649
2	15:00	0.51 ± 0.06	0.0027
3	10:00	0.36 ± 0.06	
3	11:00	0.30 ± 0.06	0.3743
3	12:00	0.41 ± 0.06	0.0292
3	13:00	0.45 ± 0.06	0.0092
3	14:00	0.43 ± 0.06	0.0148
3	15:00	0.52 ± 0.06	0.0008
4	10:00	0.24 ± 0.06	
4	11:00	0.35 ± 0.06	0.1288
4	12:00	0.47 ± 0.06	0.0042
4	13:00	0.36 ± 0.06	0.1160
4	14:00	0.37 ± 0.06	0.0936
4	15:00	0.76 ± 0.06	$p < 0.0001$
5	10:00	0.15 ± 0.06	
5	11:00	0.26 ± 0.06	0.7031
5	12:00	0.36 ± 0.06	0.1202
5	13:00	0.32 ± 0.06	0.2838
5	14:00	0.28 ± 0.06	0.5500
5	15:00	0.35 ± 0.06	0.1379
6	10:00	0.24 ± 0.06	
6	11:00	0.23 ± 0.06	0.9871
6	12:00	0.32 ± 0.06	0.2556
6	13:00	0.28 ± 0.06	0.5265
6	14:00	0.30 ± 0.06	0.3468
6	15:00	0.22 ± 0.06	0.9259

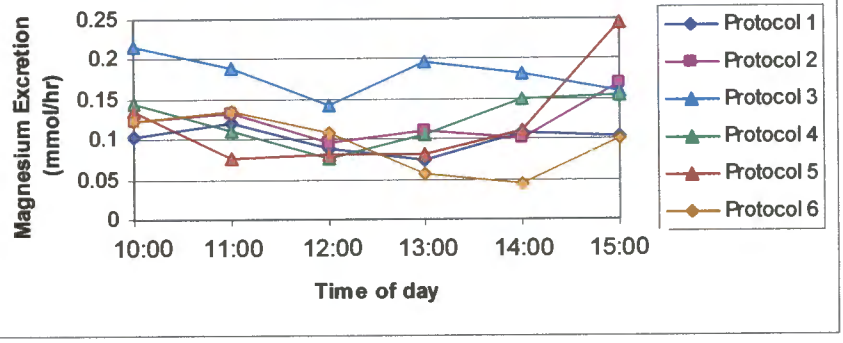


Magnesium excretions throughout the 6 hours of each protocol are given in Table 4.9, while Figure 4.6 presents the data graphically. Baseline mean = 0.144 ± 0.014 mmol/hr.

Table 4.9: Mean Magnesium excretion (mmol/hr)

Protocol	Time of day	Mean ± SE	P - Value
1	10:00	5.6 ± 1.7	
1	11:00	10.1 ± 1.7	0.3799
1	12:00	8.3 ± 1.7	0.9773
1	13:00	6.6 ± 1.7	0.4087
1	14:00	5.6 ± 1.7	0.1806
1	15:00	5.1 ± 1.7	0.1116
2	10:00	10.5 ± 1.8	
2	11:00	13.5 ± 1.7	0.0143
2	12:00	8.2 ± 1.7	0.9601
2	13:00	8.0 ± 1.7	0.8813
2	14:00	5.7 ± 1.7	0.2196
2	15:00	8.2 ± 1.7	0.9791
3	10:00	13.5 ± 1.7	
3	11:00	12.6 ± 1.7	0.0333
3	12:00	14.1 ± 1.7	0.0049
3	13:00	10.7 ± 1.7	0.2341
3	14:00	8.8 ± 1.7	0.7966
3	15:00	7.7 ± 1.7	0.7902
4	10:00	7.0 ± 1.7	
4	11:00	10.7 ± 1.7	0.2391
4	12:00	7.7 ± 1.7	0.7773
4	13:00	9.5 ± 1.7	0.5335
4	14:00	8.0 ± 1.7	0.8876
4	15:00	9.4 ± 1.8	0.5676
5	10:00	7.8 ± 1.7	
5	11:00	9.1 ± 1.7	0.6805
5	12:00	6.4 ± 1.7	0.3493
5	13:00	6.0 ± 1.7	0.2531
5	14:00	4.8 ± 1.7	0.0887
5	15:00	7.7 ± 1.7	0.7695
6	10:00	7.5 ± 1.7	
6	11:00	7.7 ± 1.7	0.7573
6	12:00	8.9 ± 1.7	0.7567
6	13:00	9.9 ± 1.7	0.4243
6	14:00	2.5 ± 1.7	0.0048
6	15:00	4.6 ± 1.7	0.0682

Fig. 4.6 Magnesium Excretion

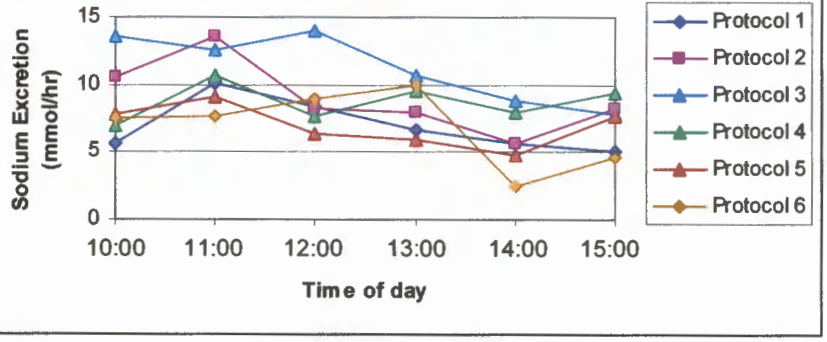


Sodium excretions throughout the 6 hours of each protocol are given in Table 4.10, while Figure 4.7 presents the data graphically. Baseline mean = 8.28 ± 0.85 mmol/hr.

Table 4.10: Mean Sodium excretion (mmol/hr)

Protocol	Time of day	Mean ± SE	P - Value
1	10:00	5.6 ± 1.7	
1	11:00	10.1 ± 1.7	0.3799
1	12:00	8.3 ± 1.7	0.9773
1	13:00	6.6 ± 1.7	0.4087
1	14:00	5.6 ± 1.7	0.1806
1	15:00	5.1 ± 1.7	0.1116
2	10:00	10.5 ± 1.8	
2	11:00	13.5 ± 1.7	0.0143
2	12:00	8.2 ± 1.7	0.9601
2	13:00	8.0 ± 1.7	0.8813
2	14:00	5.7 ± 1.7	0.2196
2	15:00	8.2 ± 1.7	0.9791
3	10:00	13.5 ± 1.7	
3	11:00	12.6 ± 1.7	0.0333
3	12:00	14.1 ± 1.7	0.0049
3	13:00	10.7 ± 1.7	0.2341
3	14:00	8.8 ± 1.7	0.7966
3	15:00	7.7 ± 1.7	0.7902
4	10:00	7.0 ± 1.7	
4	11:00	10.7 ± 1.7	0.2391
4	12:00	7.7 ± 1.7	0.7773
4	13:00	9.5 ± 1.7	0.5335
4	14:00	8.0 ± 1.7	0.8876
4	15:00	9.4 ± 1.8	0.5676
5	10:00	7.8 ± 1.7	
5	11:00	9.1 ± 1.7	0.6805
5	12:00	6.4 ± 1.7	0.3493
5	13:00	6.0 ± 1.7	0.2531
5	14:00	4.8 ± 1.7	0.0887
5	15:00	7.7 ± 1.7	0.7695
6	10:00	7.5 ± 1.7	
6	11:00	7.7 ± 1.7	0.7573
6	12:00	8.9 ± 1.7	0.7567
6	13:00	9.9 ± 1.7	0.4243
6	14:00	2.5 ± 1.7	0.0048
6	15:00	4.6 ± 1.7	0.0682

Fig. 4.7 Sodium Excretion

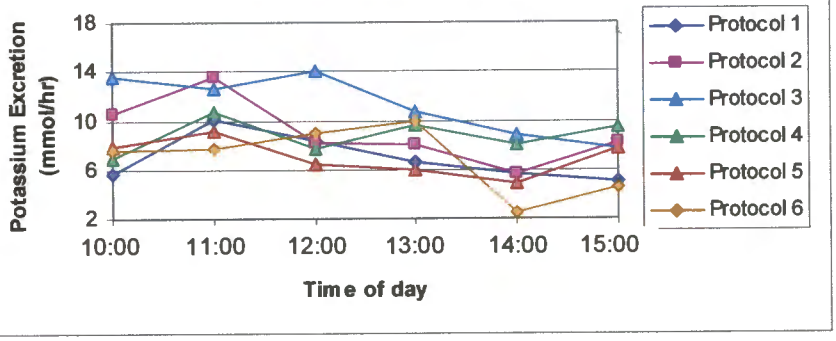


Potassium excretions throughout the 6 hours of each protocol are given in Table 4.11, while Figure 4.8 presents the data graphically. Baseline mean = 4.39 ± 0.35 mmol/hr.

Table 4.11: Mean Potassium excretion (mmol/hr)

Protocol	Time of day	Mean ± SE	P - Value
1	10:00	1.28 ± 0.67	
1	11:00	2.57 ± 0.67	0.0284
1	12:00	2.46 ± 0.65	0.0204
1	13:00	2.14 ± 0.65	0.0071
1	14:00	1.83 ± 0.65	0.0023
1	15:00	1.30 ± 0.65	0.0003
2	10:00	2.39 ± 0.69	
2	11:00	3.49 ± 0.65	0.2989
2	12:00	3.36 ± 0.65	0.2350
2	13:00	2.72 ± 0.65	0.0558
2	14:00	2.05 ± 0.65	0.0081
2	15:00	2.10 ± 0.65	0.0094
3	10:00	3.92 ± 0.67	
3	11:00	5.88 ± 0.65	0.0730
3	12:00	6.11 ± 0.65	0.0383
3	13:00	5.44 ± 0.65	0.2055
3	14:00	3.44 ± 0.67	0.2467
3	15:00	2.39 ± 0.67	0.0163
4	10:00	3.54 ± 0.65	
4	11:00	6.07 ± 0.65	0.0424
4	12:00	6.66 ± 0.67	0.0067
4	13:00	6.44 ± 0.65	0.0138
4	14:00	4.31 ± 0.65	0.9186
4	15:00	3.78 ± 0.69	0.4559
5	10:00	7.32 ± 0.65	
5	11:00	9.06 ± 0.67	$p < 0.0001$
5	12:00	8.84 ± 0.65	$p < 0.0001$
5	13:00	10.03 ± 0.65	$p < 0.0001$
5	14:00	7.00 ± 0.65	0.0019
5	15:00	5.76 ± 0.65	0.0976
6	10:00	5.89 ± 0.65	
6	11:00	7.69 ± 0.67	0.0001
6	12:00	9.49 ± 0.65	$p < 0.0001$
6	13:00	8.26 ± 0.65	$p < 0.0001$
6	14:00	7.95 ± 0.67	$p < 0.0001$
6	15:00	4.84 ± 0.65	0.5818

Fig. 4.8 Potassium Excretion

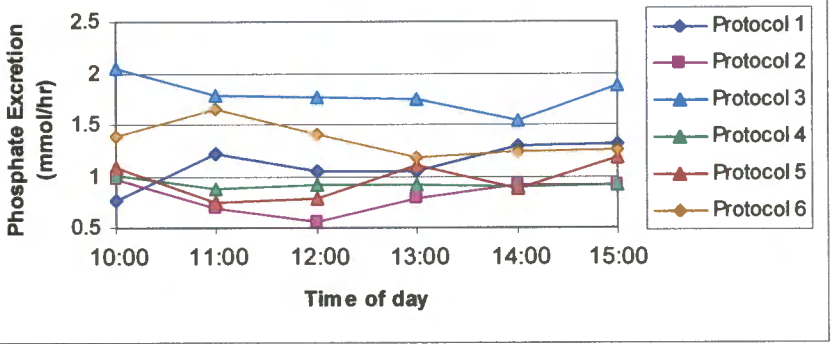


Phosphate excretions throughout the 6 hours of each protocol are given in Table 4.12, while Figure 4.9 presents the data graphically. Baseline mean = 1.25 ± 0.17 mmol/hr.

Table 4.12: Mean Phosphate excretion (mmol/hr)

Protocol	Time of day	Mean ± SE	P – Value
1	10:00	0.76 ± 0.14	
1	11:00	1.22 ± 0.14	0.9401
1	12:00	1.04 ± 0.14	0.5520
1	13:00	1.04 ± 0.14	0.5427
1	14:00	1.29 ± 0.14	0.9171
1	15:00	1.32 ± 0.14	0.8618
2	10:00	0.98 ± 0.15	
2	11:00	0.69 ± 0.14	0.1865
2	12:00	0.55 ± 0.14	0.0998
2	13:00	0.79 ± 0.14	0.2767
2	14:00	0.91 ± 0.14	0.4197
2	15:00	0.92 ± 0.14	0.4317
3	10:00	2.04 ± 0.14	
3	11:00	1.78 ± 0.14	0.1424
3	12:00	1.76 ± 0.14	0.1621
3	13:00	1.74 ± 0.14	0.1793
3	14:00	1.53 ± 0.14	0.4387
3	15:00	1.88 ± 0.14	0.0840
4	10:00	1.01 ± 0.14	
4	11:00	0.88 ± 0.14	0.2983
4	12:00	0.92 ± 0.14	0.3458
4	13:00	0.92 ± 0.14	0.3529
4	14:00	0.89 ± 0.14	0.3048
4	15:00	0.91 ± 0.14	0.3394
5	10:00	1.08 ± 0.14	
5	11:00	0.75 ± 0.14	0.1655
5	12:00	0.78 ± 0.14	0.1833
5	13:00	1.10 ± 0.14	0.6794
5	14:00	0.87 ± 0.14	0.2856
5	15:00	1.18 ± 0.14	0.8387
6	10:00	1.38 ± 0.14	
6	11:00	1.65 ± 0.14	0.8060
6	12:00	1.40 ± 0.14	0.6908
6	13:00	1.18 ± 0.14	0.8278
6	14:00	1.24 ± 0.14	0.9606
6	15:00	1.26 ± 0.14	0.9950

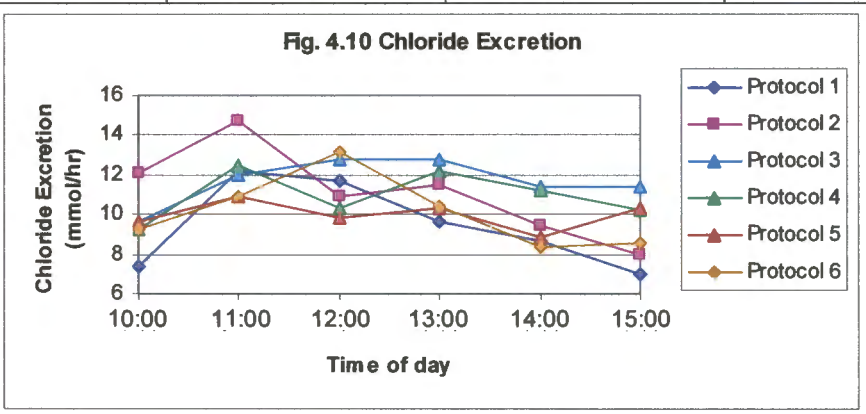
Fig. 4.9 Phosphate Excretion



Chloride excretions throughout the 6 hours of each protocol are given in Table 4.13, while Figure 4.10 presents the data graphically. Baseline mean = 9.04 ± 0.54 mmol/hr.

Table 4.13: Mean Chloride excretion (mmol/hr)

Protocol	Time of day	Mean ± SE	P – Value
1	10:00	7.4 ± 1.0	
1	11:00	12.1 ± 1.0	0.0145
1	12:00	11.7 ± 1.0	0.0367
1	13:00	9.6 ± 1.0	0.6674
1	14:00	8.6 ± 1.0	0.7451
1	15:00	7.0 ± 1.0	0.1058
2	10:00	12.1 ± 1.1	
2	11:00	14.7 ± 1.0	p < 0.0001
2	12:00	11.0 ± 1.0	0.1478
2	13:00	11.5 ± 1.0	0.0638
2	14:00	9.5 ± 1.0	0.7334
2	15:00	8.0 ± 1.0	0.4279
3	10:00	9.7 ± 1.0	
3	11:00	12.0 ± 1.0	0.0187
3	12:00	12.7 ± 1.0	0.0039
3	13:00	12.7 ± 1.0	0.0040
3	14:00	11.4 ± 1.0	0.0591
3	15:00	11.4 ± 1.0	0.0618
4	10:00	9.2 ± 1.0	
4	11:00	12.4 ± 1.0	0.0077
4	12:00	10.4 ± 1.0	0.2936
4	13:00	12.2 ± 1.0	0.0124
4	14:00	11.2 ± 1.0	0.0806
4	15:00	10.3 ± 1.0	0.3332
5	10:00	9.6 ± 1.0	
5	11:00	10.9 ± 1.0	0.1368
5	12:00	9.9 ± 1.0	0.5061
5	13:00	10.3 ± 1.0	0.3103
5	14:00	8.8 ± 1.0	0.8592
5	15:00	10.3 ± 1.0	0.3046
6	10:00	9.3 ± 1.0	
6	11:00	10.9 ± 1.0	0.1325
6	12:00	13.1 ± 1.0	0.0014
6	13:00	10.5 ± 1.0	0.0556
6	14:00	8.3 ± 1.0	0.5707
6	15:00	8.5 ± 1.0	0.6914

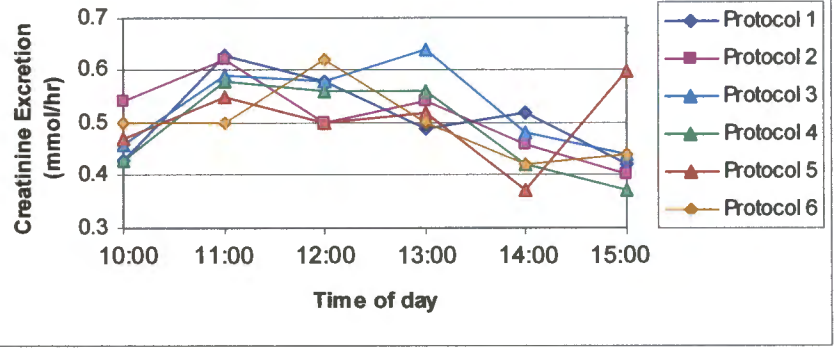


Creatinine excretions throughout the 6 hours of each protocol are given in Table 4.14, while Figure 4.11 presents the data graphically. Baseline mean = 9.04 ± 0.54 mmol/hr.

Table 4.14: Mean Creatinine excretion (mmol/hr)

Protocol	Time of day	Mean ± SE	P – Value
1	10:00	0.43 ± 0.06	
1	11:00	0.63 ± 0.06	0.0069
1	12:00	0.58 ± 0.05	0.0482
1	13:00	0.49 ± 0.05	0.6646
1	14:00	0.52 ± 0.05	0.3143
1	15:00	0.42 ± 0.05	0.4742
2	10:00	0.54 ± 0.06	
2	11:00	0.62 ± 0.05	0.0097
2	12:00	0.50 ± 0.05	0.5005
2	13:00	0.54 ± 0.05	0.1613
2	14:00	0.46 ± 0.05	0.9170
2	15:00	0.40 ± 0.06	0.3260
3	10:00	0.46 ± 0.06	
3	11:00	0.59 ± 0.05	0.0328
3	12:00	0.58 ± 0.05	0.0399
3	13:00	0.64 ± 0.05	0.0045
3	14:00	0.48 ± 0.06	0.7149
3	15:00	0.44 ± 0.05	0.8231
4	10:00	0.43 ± 0.05	
4	11:00	0.58 ± 0.05	0.0580
4	12:00	0.56 ± 0.05	0.0980
4	13:00	0.56 ± 0.05	0.1155
4	14:00	0.42 ± 0.05	0.5811
4	15:00	0.37 ± 0.06	0.1340
5	10:00	0.47 ± 0.05	
5	11:00	0.55 ± 0.06	0.1271
5	12:00	0.50 ± 0.05	0.4969
5	13:00	0.52 ± 0.05	0.3143
5	14:00	0.37 ± 0.05	0.1475
5	15:00	0.60 ± 0.05	0.0219
6	10:00	0.50 ± 0.05	
6	11:00	0.50 ± 0.05	0.5502
6	12:00	0.62 ± 0.05	0.0091
6	13:00	0.50 ± 0.05	0.4465
6	14:00	0.42 ± 0.06	0.5652
6	15:00	0.44 ± 0.05	0.7599

Fig. 4.11 Creatinine Excretion

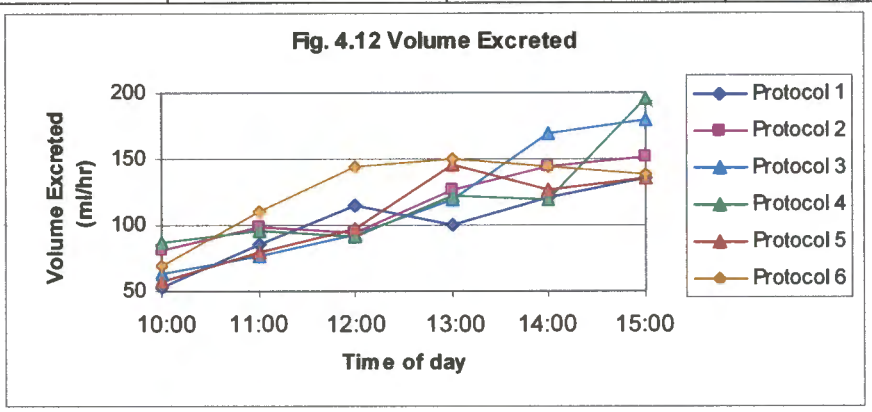


Urine volume excreted throughout the 6 hours of each protocol is given in Table 4.15, while Figure 4.12 presents the data graphically. Baseline mean = 66.3 ± 7.87 ml/hr.

Table 4.15: Mean Volume excreted (ml/hr)

Protocol	Time of day	Mean ± SE	P – Value
1	10:00	53.0 ± 17.1	
1	11:00	85.9 ± 17.1	0.3015
1	12:00	114.1 ± 16.6	0.0130
1	13:00	100.2 ± 16.6	0.0755
1	14:00	120.5 ± 16.6	0.0050
1	15:00	136.0 ± 16.6	0.0004
2	10:00	81.0 ± 17.6	
2	11:00	97.8 ± 16.6	0.1057
2	12:00	94.2 ± 16.6	0.1513
2	13:00	126.1 ± 16.6	0.0025
2	14:00	143.8 ± 16.6	0.0001
2	15:00	151.2 ± 16.6	p < 0.0001
3	10:00	63.4 ± 17.1	
3	11:00	76.4 ± 16.6	0.5941
3	12:00	92.4 ± 16.6	0.1696
3	13:00	119.0 ± 16.6	0.0064
3	14:00	168.6 ± 17.1	p < 0.0001
3	15:00	179.2 ± 16.6	p < 0.0001
4	10:00	87.4 ± 16.6	
4	11:00	96.1 ± 16.6	0.1180
4	12:00	91.4 ± 16.6	0.1872
4	13:00	121.4 ± 16.6	0.0044
4	14:00	119.2 ± 16.6	0.0061
4	15:00	194.9 ± 17.6	p < 0.0001
5	10:00	58.0 ± 16.6	
5	11:00	78.8 ± 17.1	0.5090
5	12:00	97.4 ± 16.6	0.1036
5	13:00	145.1 ± 16.6	0.0001
5	14:00	126.2 ± 16.6	0.0021
5	15:00	135.3 ± 16.6	0.0004
6	10:00	69.6 ± 16.6	
6	11:00	110.2 ± 17.1	0.0222
6	12:00	144.2 ± 16.6	0.0001
6	13:00	150.0 ± 16.6	p < 0.0001
6	14:00	143.8 ± 17.1	0.0001
6	15:00	137.9 ± 16.6	0.0003

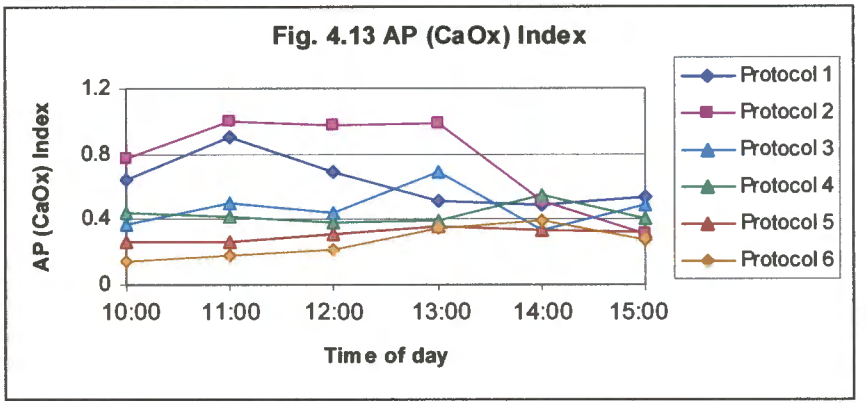
Fig. 4.12 Volume Excreted



AP(CaOx) Index throughout the 6 hours of each protocol are given in Table 4.16, while Figure 4.13 presents the data graphically. Baseline mean = 0.372 ± 0.055.

Table 4.16: Mean AP(CaOx) Index

Protocol	Time of day	Mean ± SE	P – Value
1	10:00	0.64 ± 0.10	
1	11:00	0.90 ± 0.10	0.0001
1	12:00	0.69 ± 0.10	0.0120
1	13:00	0.51 ± 0.10	0.2769
1	14:00	0.48 ± 0.10	0.3705
1	15:00	0.54 ± 0.10	0.1971
2	10:00	0.78 ± 0.10	
2	11:00	1.00 ± 0.10	p < 0.0001
2	12:00	0.98 ± 0.10	p < 0.0001
2	13:00	0.91 ± 0.10	0.0001
2	14:00	0.51 ± 0.10	0.3048
2	15:00	0.31 ± 0.10	0.6690
3	10:00	0.37 ± 0.10	
3	11:00	0.50 ± 0.10	0.3234
3	12:00	0.43 ± 0.10	0.6257
3	13:00	0.68 ± 0.10	0.0147
3	14:00	0.33 ± 0.10	0.7356
3	15:00	0.48 ± 0.10	0.3800
4	10:00	0.44 ± 0.10	
4	11:00	0.42 ± 0.10	0.7270
4	12:00	0.38 ± 0.10	0.9312
4	13:00	0.39 ± 0.10	0.8803
4	14:00	0.55 ± 0.10	0.1558
4	15:00	0.41 ± 0.11	0.7772
5	10:00	0.26 ± 0.10	
5	11:00	0.26 ± 0.10	0.3616
5	12:00	0.31 ± 0.10	0.6113
5	13:00	0.36 ± 0.10	0.9094
5	14:00	0.34 ± 0.10	0.7673
5	15:00	0.32 ± 0.10	0.6540
6	10:00	0.15 ± 0.10	
6	11:00	0.17 ± 0.11	0.1159
6	12:00	0.21 ± 0.10	0.2085
6	13:00	0.34 ± 0.11	0.8106
6	14:00	0.39 ± 0.11	0.9091
6	15:00	0.27 ± 0.10	0.4262



A 3-dimensional representation of the calcium excretion is given below (Figure 4.14).

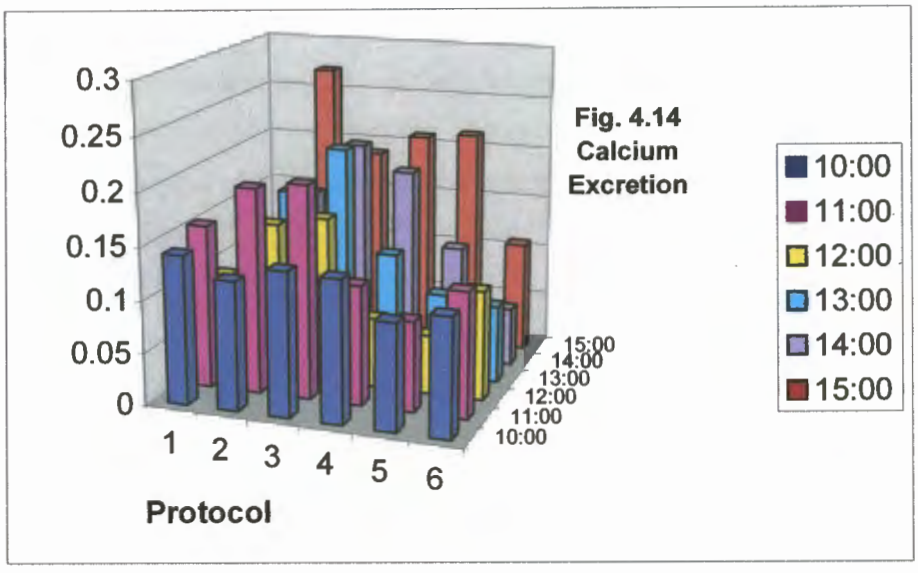


Fig. 4.14 Calcium Excretion

A general increasing trend in oxalate excretion is observed for each protocol (except protocol 4) after ingestion of the oxalate-rich meal (Table 4.4b and Figures 4.1a and 4.1b). Moreover, these data show that oxalate excretion was highest 4-5 hours after ingestion of the oxalate-rich meal irrespective of the time at which the calcium supplement was given (except for protocol 4), (Table 4.4a). The increase in oxalate excretion is significant at each time point throughout the collection period after ingestion of oxalate-rich meal for protocol 1 ($p<0.05$) (Table 4.4b, Figure 4.1a). Generally, all the protocols produced a lower oxalate excretion relative to protocol 1, i.e. all the protocols in which calcium was administered produced a lower oxalate excretion than the baseline protocol in which calcium was absent.

In protocols 2 and 3 the calcium excretion increased significantly after ingestion of the supplement. While an increase also occurred in protocol 5, it was not significant (Table 4.5 and Figures 4.2 and 4.14). In protocols 4 and 6 the calcium excretion decreased significantly after ingestion of the supplement.

Significant increases in pH were observed for each protocol immediately after ingestion of the oxalate-rich meal (Table 4.6 and Figure 4.3) followed by a decreasing trend a few hours after the meal.

A significant increase in uric acid excretion was observed for each protocol after ingesting the oxalate-rich meal i.e. between 10:00 and 11:00, $p<0.05$, (Table 4.7 and Figure 4.4). The uric acid excretion returned to baseline at the end of the collection period for all protocols.

An increasing trend in citrate excretion was observed for all the protocols between 10:00 and 11:00 except for protocols 3 and 6 where the opposite trend was observed (Table 4.8 and Figure 4.5). The excretion of this variable was lowest for protocol 1 throughout the collection period. In protocols 2, 3 and 4 the citrate excretion increased significantly relative to the baseline mean at 15:00 ($p<0.05$).

No obvious trends were observed for magnesium (Table 4.9 and Figure 4.6), phosphate (Table 4.12 and Figure 4.9), chloride (Table 4.13 and Figure 4.10) and creatinine excretion (Table 4.14 and Figure 4.11). A significant increase in sodium excretion (relative to the baseline mean) was observed 2hrs after ingestion of the calcium supplement in protocols 2 and 3 ($p<0.05$). No significant changes in this variable were observed in the other protocols (Table 4.10 and Figure 4.7).

A significant increase in potassium excretion ($p<0.05$) was observed after ingestion of the oxalate-rich meal in all the protocols except for protocol 2 ($p>0.05$), (Table 4.11 and Figure 4.8). Thereafter, a decrease in this variable was observed.

The volume excreted increased throughout the day for all the protocols (Table 4.15 and Figure 4.12).

The AP(CaOx) index increased significantly after ingestion of the oxalate-rich meal, in protocols 1 and 2, till 11:00 ($p<0.05$) and then decreased throughout the day (Table 4.16 and Figure 4.13). In protocol 3, very little change in this variable was observed for the first 3hrs after ingestion of the supplement this was followed by a significant increase ($p<0.05$), which was then followed by a decreasing trend. No significant changes were observed in protocols 4-6.

4.4 DISCUSSION

Spinach was chosen as the main component of the oxalate-rich meal as it is the only one of the food items tested by Brinkley *et al.* (1981) which is capable of causing hyperoxaluria. Although the amount of spinach that the subjects in the study ingested contained approximately 380mg of calcium, the latter is not very bioavailable from this source (Brinkley *et al.* 1981). Hence, there was no concern that this calcium would interfere with the study.

The observation in this study that oxalate excretion was highest 4-5hrs after a meal is in agreement with results reported by other workers (Tiselius *et al.* 1977, Ahlstrand *et al.* 1984). The present study has revealed that all protocols (irrespective of the time at which the calcium supplement was administered) produced a lower oxalate excretion relative to protocol 1 (Table 4.4b and Figures 4.1a and 4.1b), thereby demonstrating that ingesting a calcium supplement at any time relative to an oxalate-rich meal is better than ingesting no calcium supplement at all. Of all the protocols, protocol 3 is the only one in which the oxalate excretion did not increase significantly relative to the baseline at any time during the day. In all of the other protocols, a significant increase in oxalate excretion was observed at some time during the day, e.g. protocol 2 peaked at 13:00 ($p = 0.0018$), protocol 4 peaked at 11:00 ($p = 0.0029$), protocol 5 peaked at 13:00 ($p = 0.0005$) and protocol 6 peaked at 14:00 ($p = 0.0004$) relative to the baseline mean. Furthermore, figure 4.1c demonstrates that protocol 3 produced the lowest total excretion of oxalate over the 6-hour period following the ingestion of the oxalate-rich meal, relative to other protocols. As a result, subjects would have the lowest risk of a dangerous increase in urinary oxalate concentration following protocol 3. From this it can be concluded that the optimum time at which a calcium supplement should be taken in order to minimize excretion of oxalate is 1 hour after the oxalate challenge (protocol 3). It is surprising that the total oxalate excretion for protocol 2 was the second highest of all the protocols (Figure 4.1c).

The general trend that the calcium excretion decreases in protocol 1 (Table 4.5 and Figures 4.2 and 4.14) is due to the fact that no calcium supplement was administered in this protocol. This supports Brinkley et al.'s (1981) observation that the calcium in spinach (the oxalate-rich meal) is not bioavailable. The high oxalate excretion corresponding to this protocol could thus be attributed to absence of a calcium challenge.

The trends that were observed in this study regarding urinary calcium excretion (Table 4.5) agree with those of Brinkley *et al.* (1981) that this variable peaks between 4-6hrs after a calcium load. In protocol 2, the calcium excretion peaked 6 hrs after the calcium load ($p < 0.0001$), in protocol 3, the calcium excretion reached a peak 3-4 hrs after the calcium load ($p = 0.0033$), in protocol 4 the calcium excretion peaked 4 hrs after the calcium load ($p = 0.0053$) and in protocol 5 the calcium excretion peaked 3 hrs after the calcium load ($p = 0.0032$). Since urine samples were not collected after 15:00 for protocols 5 and 6, the calcium excretion peaks cannot be identified. It is noted that protocol 3 seems to have produced the best result for calcium excretion as no dramatic surge was observed for this protocol (Figure 4.2), i.e. the urinary calcium excretion was fairly steady in this protocol whereas for protocols 2, 4 and 5 a dramatic surge was observed at some stage during the day.

In agreement with previous studies that the alkalinizing aspect of vegetarian diets increase urinary pH (Rodgers *et al.* 2002, Siener and Hesse 2003), this variable increased significantly for at least 2 hours after ingestion of the oxalate-rich meal for all the protocols (Table 4.6 and Figure 4.3). It also agrees with the circadian rhythm of this variable where it reaches a maximum between 11:00 and 14:00 (Vahlensieck *et al.* 1982). In protocol 1 the urinary pH increases after ingesting the meal, which is expected as this variable is naturally lower after fasting and increases after the meal induced alkaline tide, and then decreases 3 hours after the meal and continues to decrease throughout the day. This decrease is observed for each of the other protocols but the administration of the calcium supplement after the meal (protocols 3, 4 and 5) seems to delay this observed decrease by 1-2hours. It can thus be concluded that supplemental calcium taken after an oxalate-rich meal is beneficial in keeping the urinary pH elevated. This can be regarded as an important finding as the inhibitory activity towards calcium oxalate crystallization increases with increasing pH (Kohri *et al.* 1991, Pak 1994, Hesse *et al.* 1997).

The increase in urinary uric acid excretion observed after the oxalate-rich meal (Table 4.7 and Figure 4.4) could be due to the animal protein in this meal as enough protein had to be given to the subjects to last them the whole day. This finding agrees with other authors who found that a lithogenic effect of animal protein intake includes an increased urinary excretion of uric acid (Breslau *et al.* 1988, Siener *et al.* 2002). The uric acid

excretion does not seem to be dependent on the calcium supplement as it returned to the baseline value for all of the protocols.

In protocols 2, 3 and 4 the citrate excretion increased significantly after administration of the calcium supplement but only remained significantly elevated in protocol 4 (Table 4.8 and Figure 4.5). No obvious trends were observed for magnesium, phosphate, chloride and creatinine excretion.

It is noted that the potassium excretion (Table 4.11 and Figure 4.8) followed a similar trend to that of urinary pH for all the protocols where it increased after ingestion of the oxalate-rich meal and then decreased throughout the day. This is expected since potassium is alkaline and would thus induce a concomitant increase in pH.

The urinary volume increased significantly throughout the day in all six protocols (Table 4.15 and Figure 4.12). This observation is consistent with the circadian rhythm of this variable where it reaches a maximum at midday (11:00-14:00) or early afternoon (14:00-17:00), (Vahlensieck *et al.* 1982).

It is noted that the AP(CaOx) values reach their peak immediately after ingestion of the oxalate-rich meal in protocol 1 and then decrease steadily(Table 4.16 and Figure 4.13). When the calcium supplement is given with the meal (protocol 2), the same initial increase in this variable is observed but is then maintained for a few hours, after which it decreases dramatically. The latter effect might correspond to the onset of oxalate binding by calcium. It is also noted that in protocols 3-6, the AP(CaOx) is lower than in protocol 1. This is surprising since the same values would have been expected during the early stages of the protocol. Protocols 3 and 4 follow similar trends, i.e. the AP(CaOx) index increases 3 hours after ingestion of the supplement and then decreases; the latter effect probably corresponds to the oxalate binding calcium. A possible reason for not being able to obtain more conclusive results for the AP(CaOx) indices could be that the hourly water intake was too high and as a result subjects were highly hydrated.

As discussed in chapter 1, urolithiasis has long been recognized as the combined result of a number of biochemical risk factors (Finlayson 1974, Robertson *et al.* 1978, Robertson *et al.* 1981, Hess *et al.* 1996) and as a result it should be possible to identify

which of the protocols in this study is able to produce the lowest risk of stone formation based on the results discussed above. The results of this study show the importance of taking a calcium supplement with or soon after an oxalate-rich meal (i.e. protocols 2 and 3). By investigating the administration of calcium supplements at various times with respect to an oxalate-rich meal, valuable information has been obtained regarding the inhibitory capacity of calcium supplements relative to an oxalate load. It is noted that protocol 3 seems to have produced the best result for both oxalate (Figures 4.1a and 4.1b) and calcium (Figures 4.2 and 4.14) excretion since the former produced the lowest excretion and the latter produced no dramatic increase in its excretion relative to other protocols. Furthermore, the AP(CaOx) index for this protocol also follows a favourable trend suggesting that this protocol might be the optimum one. However, further investigation of the relationship between the timing of administration of the calcium supplement with respect to the oxalate-rich meal in order to optimize the inhibitory role of the former is clearly warranted. Indeed, such a study is needed in CaOx stone formers in order to ascertain whether they respond differently to such a treatment compared to healthy individuals. Future studies in this area could be improved in several ways viz. a higher oxalate load could be used in comparison with the calcium supplement, the protocol could be extended for 2 hours further as the observation period for protocols 5 and 6 were very short, and a lower fluid intake could be recommended as the urine becomes very dilute with the high fluid intake that was used in the present study.

4.5 REFERENCES

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Chapter 5

Application of a powerful chemical speciation program (JESS) to explain the urinary relative supersaturation changes induced by administration of a citrate-containing preparation

5.1 INTRODUCTION

Chemical speciation is the term used to describe the concentration and form of every chemical species in a system (Williams 1998). This includes all gases, liquids and solids which may or may not be present at any given time in the actual system under investigation. A chemical species is any moiety that can exist on its own. Since the factors that govern crystallization processes are all dependent on the chemical species which are present, they can be studied by means of speciation programs which calculate the chemical speciation using extensive databases of equilibrium constants and solubility products. In this chapter the development of a theoretical urine model and its application to kidney stone disease will be described. Theoretical models are all based on the assumption that the system is at equilibrium. However, in modeling biological processes the system is often not at thermodynamic equilibrium and hence, the program predicts what the final result will be rather than the instantaneous situation. Thus, a major limitation of current models is that they do not accommodate kinetic factors. The theoretical model for a system consists of the total concentrations and phases of each component (an inorganic element or an organic compound) together with a database of all possible equilibria. Using appropriate software this information can be converted into the concentration of each chemical species i.e. the chemical speciation.

A well-known speciation program which has been extensively used in the field of kidney stone research to determine the relative supersaturation (RS)of various stone-forming salts found in urine is EQUIL2 (Werness *et al.* 1985). As its name suggests, it is an equilibrium model which calculates the RS of common stone forming species in urine (viz. calcium phosphate (brushite), CaOx and uric acid). The input data required by the program are the components listed in Table 5.1. RS values are of great interest in urolithiasis research and have exhaustively used as a measure of stone risk in multiple studies over the past 20 years (Pak *et al.* 1985, Pak and Fuller 1986, Berg and Tiselius 1989, Hesse *et al.* 1993, Ettinger *et al.* 1997, Borghi *et al.* 1999, Borghi *et al.* 2002, Siener and Hesse 2002, Siener *et al.* 2003a, Siener *et al.* 2003b).

Table 5.1: Input data required by EQUIL2

Components	
H^+	Citrate (mol/dm³)
Sodium (mol/dm³)	Oxalate (mol/dm³)
Potassium (mol/dm³)	Urate (mol/dm³)
Calcium (mol/dm³)	Chloride (mol/dm³)
Magnesium (mol/dm³)	Sulfate (mol/dm³)
NH_4 (mol/dm³)	CO_2 (mol/dm³)
PO_4 (mol/dm³)	Pyrophosphate (mol/dm³)

A speciation program which is not as well-known in urolithiasis research is JESS (Joint Expert Speciation System), (May and Murray 1991a). This program has a database of many thermodynamic constants and solubility products which can be used to model the chemical speciation under any specified conditions. A theoretical model needs to be constructed to mimic the real solution that is of interest. In other words, establishing a model requires the researcher to "estimate" realistic values for the chemical system in which speciation is to be calculated or to use experimentally determined values. For example, in order to calculate the speciation in urine after administration of CitroSoda (Chapter 3), the empirically determined concentrations shown in Table 5.2 and 5.3 would be used.

JESS has been widely used in medicine to model metal-ligand equilibria in human blood plasma (May PM and Murray K 1991a) and to model industrial processes under widely different conditions of temperature and pressure (May and Murray 1991a). It has also been used to predict the solubility of CaOx hydrates at different pH in artificial urine solutions (Streit et al. 1998).

There are various fundamental differences between EQUIL2 and JESS. These relate mainly to the constants and to the extent of their databases, their ability to determine the formation of different types of ligands and their scanning facilities. Features of JESS that make it superior to EQUIL2 are that it has a large on-line database of thermodynamic parameters which is much more comprehensive than those of other modeling packages, (e.g. it is able to perform speciation calculations from a primary database of over 12000 reactions in aqueous solution), it is able to estimate equilibrium constants that are not available in its database, it is able to calculate formation constants for mixed-ligand complexes and finally it is also able to take into account changes in ionic strength and

temperature (May PM and Murray K 1991b). The constants in JESS can also be updated whereas the constants used in EQUIL2 are fixed into the program. Different equilibria can be modelled using JESS, including redox, solubility, protonation, complex formation and adsorption. However, EQUIL2 is limited to protonation and complex formation. JESS also contains a scan feature which enables the researcher to scan variables *automatically* whereas EQUIL 2 does not. This feature allows the user to investigate the effect of changing any of the component concentrations or of changing pH in a systematic way. For example, if the effect of the citrate concentration in a urinary system is desired, one need only specify the maximum and minimum possible concentrations for this component, and the program will automatically calculate the speciation at different points within this range. In this way a graph of speciation versus component concentration is easily constructed.

There are a number of limitations associated with the process of chemical equilibrium modeling which should be borne in mind when attempting to model a real system. The first of these is the possible unreliability of the thermodynamic information used (Nordstrom DK and Ball JW 1984). The second limitation is that since equilibrium models are thermodynamic models (based on supersaturation) they do not take kinetic factors such as inhibitors (delays crystallization) and promoters (enhances crystallization) into consideration. Both JESS and EQUIL2 are thermodynamically based models and can be used to predict how far the urine is from equilibrium with respect to the solid phases found in kidney stones. Kinetic considerations, on the other hand give information about how fast crystallization occurs. Since the stone-forming process is a combination of these two factors it is important to recognize that ignoring the latter is a significant limitation of using equilibrium modeling in urolithiasis research.

5.2 OBJECTIVES

The objectives of the study described in this chapter were therefore defined as follows: (i) to compare the RS results obtained from the two programs in order to assess whether their output is similar, (ii) to determine whether JESS could be used to explain the effects on RS observed after the ingestion of Na-Cit (described in Chapter 3) and (iii) whether it could thus be utilized in the routine evaluation of urinary stone therapy.

5.3 METHODS

5.2.1 Comparison of the RS output for the two programs

The mean values of the various urinary components which were obtained from the CitroSoda study were utilized as the input data for both the EQUIL2 and JESS programs (Tables 5.2 and 5.3). Certain urinary components listed in Table 5.1, e.g. SO_4^{2-}, NH_4^+, CO_2, pyrophosphate and phosphate were not measured in this study and hence were not included in the RS calculations. For the JESS program the human body temperature, 37°C, was used while the ionic strength was calculated by the program itself. Logarithmic values of solubility products (log Ksp) which are stored in the databases of the two programs are given in Table 5.4. These were used to calculate the RS of brushite (Table 5.5), CaOx (Table 5.6) and uric acid (Table 5.7). As can be seen from Table 5.4, the database of JESS is far more extensive. Solubility products for the two programs cannot be compared directly as the stoichiometry of the components in each program are based on different formulae. For example, in order to calculate the Ksp of brushite using JESS the following formula is used: Ksp = $[Ca][H][PO_4][H2O]^2$, whereas to calculate the Ksp of brushite using EQUIL2 the following formula is used: Ksp = $[Ca][HPO_4][H2O]^2$. The convention used by JESS to express the name of the solid species is given using the JESS format in which the stoichiometry of each component is given in brackets and are separated by an underscore (Table 5.4).

Since the data in tables 5.2 and 5.3 are means of several urines, statistical analysis of the EQUIL2 and JESS outputs is redundant.

Table 5.2: Mean total urinary concentrations for males (obtained from the Na-Cit study) used as input data for the two programs

Component	Concentration x 10^3 for MC at Baseline	Concentration x 10^3 for MC at Day 7	Concentration x 10^3 for MSF at Baseline	Concentration x 10^3 for MSF at Day 7
pH	6.40	7.35	6.07	7.04
Na^+ (mol/dm^3)	150.8	169.6	138.0	176.0
K^+ (mol/dm^3)	32.0	32.8	44.9	47.2
Ca^{2+} (mol/dm^3)	2.26	1.60	2.39	1.58
Mg^{2+} (mol/dm^3)	3.36	2.95	1.90	1.74
Cl^- (mol/dm^3)	105.0	89.5	124.0	114.0
PO_4^{3-} (mol/dm^3)	19.9	18.7	22.7	20.9
Citrate (mol/dm^3)	1.99	2.46	1.76	2.26
Oxalate (mol/dm^3)	0.106	0.096	0.134	0.089
Uric acid (mol/dm^3)	2.36	2.32	2.51	2.52

Table 5.3: Mean total urinary concentrations for females (obtained from the Na-Cit study) used as input data for the two programs

Component	Concentration x 10^3 for FC at Baseline	Concentration x 10^3 for FC at Day 7	Concentration x 10^3 for FSF at Baseline	Concentration x 10^3 for FSF at Day 7
pH	6.42	7.50	6.33	7.16
Na^+ (mol/dm^3)	60.4	133.0	10.1	106.0
K^+ (mol/dm^3)	25.6	17.4	30.9	27.6
Ca^{2+} (mol/dm^3)	1.18	0.85	2.36	1.97
Mg^{2+} (mol/dm^3)	1.41	1.35	1.67	1.41
Cl^- (mol/dm^3)	66.4	66.0	93.7	74.0
PO_4^{3-} (mol/dm^3)	11.6	10.1	15.8	15.6
Citrate (mol/dm^3)	1.74	2.06	1.89	2.40
Oxalate (mol/dm^3)	0.122	0.078	0.131	0.107
Uric acid (mol/dm^3)	1.64	1.57	2.22	2.01

Table 5.4: Solubility products used to calculate the RS of the various species in the JESS and EQUIL2 programs

Solid Species		log (Ksp)	
JESS formula	EQUIL2 formula	JESS	EQUIL2
Ca+2_H+1_PO4-3_H2O(2) (Brushite)	$CaHPO_4(H2O)_2$	-17.233	-6.400
Ca+2_Oxalic-2 (Calcium Oxalate)	CaOx	-7.745	-5.210
H+1_Uric-1 (Uric Acid)	H_2U	-8.689	-3.583
Mg+2_OH-1(2)		16.540	-
Ca+2_H+1_PO4-3		-17.564	-
Ca+2(4)_H+1_PO4-3(3)		-43.449	-
Ca+2(5)_Cl-1_PO4-3(3)		-60.614	-
Ca+2(5)_OH-1_PO4-3		-39.075	-
Ca+2(3)_PO4-3(2)		-25.861	-
Ca+2_Oxalic-2_H2O(3)		-7.382	-
Ca+2_Oxalic-2_H2O		-7.642	-
Ca+2_Oxalic-2_H2O(2)		-7.250	-
Ca+2_H+1(4)_PO4-3(2)_H2O		-36.719	-
Ca+2(4)_H+1_PO4-3(3)_H2O(3)		-43.331	-
Ca+2_Uric-1(2)_H2O(6)		-8.608	-
Mg+2_H+1_PO4-3_H2O(3)		-16.230	-
Mg+2_Oxalic-2		-3.168	-
Mg+2(5)_OH-1_PO4-3(3)		-28.368	-

Note: The solubility products for the two programs cannot be compared directly as they are formulated differently.

5.2.2 Can JESS be used to explain the effects observed on RS after the ingestion of a sodium citrate preparation?

Using the urine data in Tables 5.2 and 5.3, JESS was used to determine the speciation of citrate, calcium and oxalate. Thereafter, the scan feature of the program (described in the Introduction) was implemented to investigate the speciation percentages as a function of varying pH and of varying oxalate, citrate and calcium concentrations.

5.4 RESULTS

5.3.1 Comparison of the RS output for the two programs

The output of the EQUIL2 program provides RS values while JESS gives "log SI". The term SI refers to "solubility index"; it is equivalent to "relative supersaturation". In order to facilitate comparison of the respective program's outputs, RS values from EQUIL2 have been expressed as "log RS" in Tables 5.5 (brushite), 5.6 (CaOx) and 5.7 (uric acid). Figures 5.1, 5.2 and 5.3 display the corresponding graphs for the two programs. (MC: male controls, MSF, male stone formers, FC: female controls, FSF: female stone formers).

Table 5.5: Comparison of JESS vs EQUIL2 for Brushite

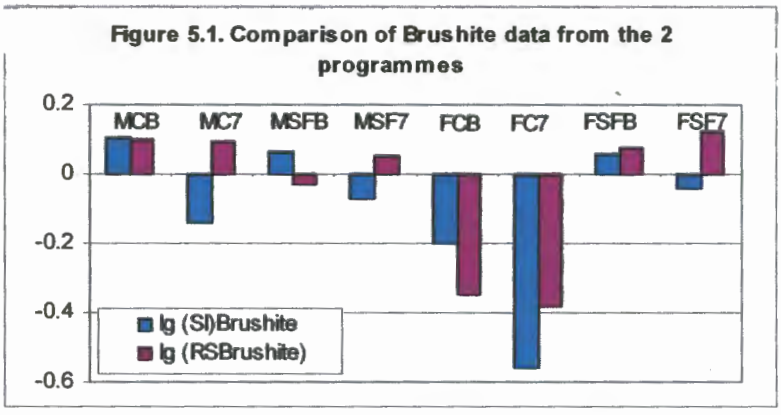
	JESS	EQUIL2	
	log (SI) Brushite	RS Brushite	log (RS Brushite)
MC Baseline (MCB)	0.108	1.261	0.101
MC Day 7 (MC7)	-0.142	1.248	0.096
MSF Baseline (MSFB)	0.067	0.938	-0.028
MSF Day 7 (MSF7)	-0.069	1.137	0.056
FC Baseline (FCB)	-0.199	0.447	-0.349
FC Day 7 (FC7)	-0.556	0.415	-0.382
FSF Baseline (FSFB)	0.058	1.191	0.076
FSF Day 7 (FSF7)	-0.042	1.333	0.125

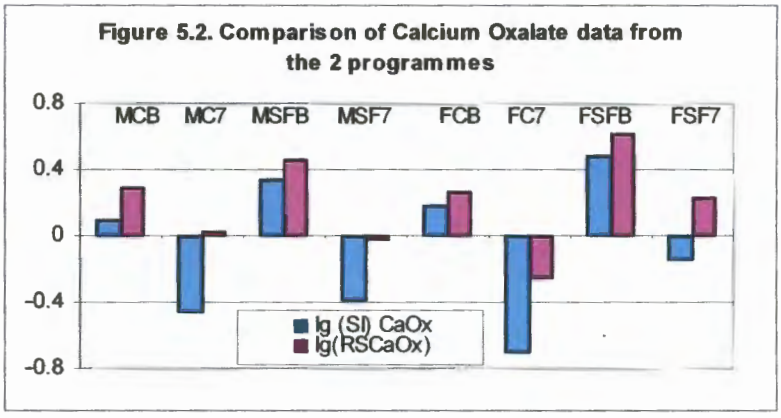
Table 5.6: Comparison of JESS vs EQUIL2 for Calcium Oxalate

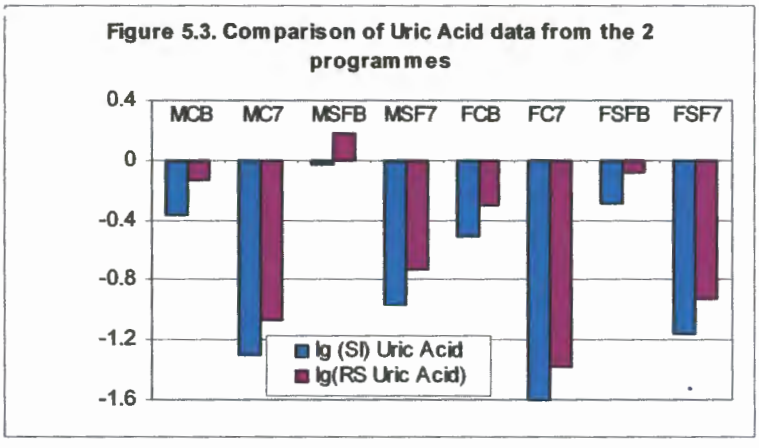
	JESS	EQUIL2	
	log (SI) CaOx	RS CaOx	log (RS CaOx)
MC Baseline (MCB)	0.092	1.980	0.297
MC Day 7 (MC7)	-0.463	1.055	0.023
MSF Baseline (MSFB)	0.339	2.881	0.460
MSF Day 7 (MSF7)	-0.390	0.942	-0.026
FC Baseline (FCB)	0.185	1.865	0.271
FC Day 7 (FC7)	-0.70	0.559	-0.253
FSF Baseline (FSFB)	0.486	4.200	0.623
FSF Day 7 (FSF7)	-0.146	1.722	0.236

Table 5.7: Comparison of JESS vs EQUIL2 for Uric Acid

	JESS	EQUIL2	
	log (SI) Uric Acid	RS Uric Acid	log (RS Uric Acid)
MC Baseline (MCB)	-0.362	0.727	-0.138
MC Day 7 (MC7)	-1.298	0.086	-1.065
MSF Baseline (MSFB)	-0.035	1.510	0.179
MSF Day 7 (MSF7)	-0.961	0.188	-0.727
FC Baseline (FCB)	-0.508	0.504	-0.297
FC Day 7 (FC7)	-1.601	0.042	-1.378
FSF Baseline (FSFB)	-0.291	0.828	-0.082
FSF Day 7 (FSF7)	-1.158	0.117	-0.930



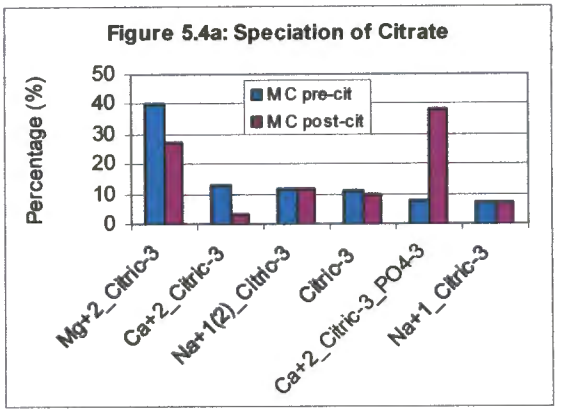


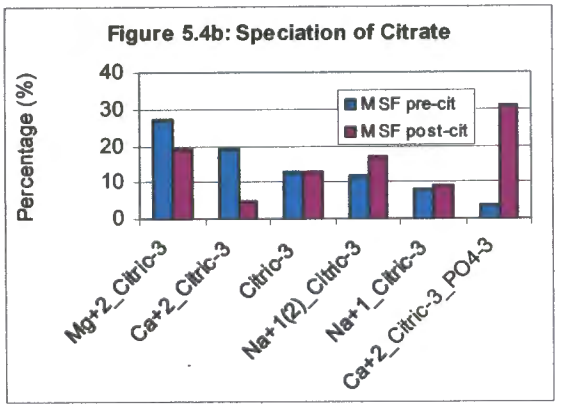


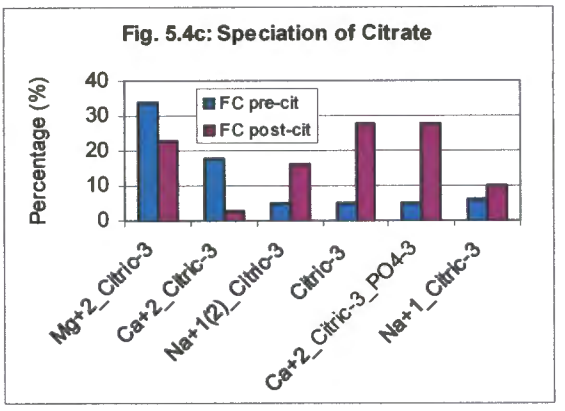
For each of the graphs the blue curve represents the results obtained from JESS and the pink curve represents the results obtained from EQUIL2. Very good agreement was obtained between the programs for the RS of brushite (Figure 5.1), CaOx (Figure 5.2) and uric acid (Figure 5.3).

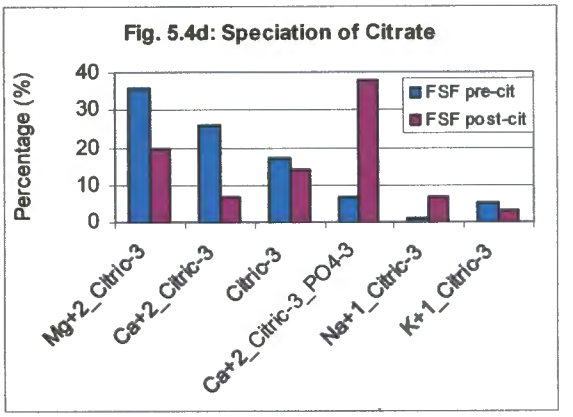
5.3.2 *Using JESS to explain the effects observed on RS after ingestion of a Na-Cit preparation*

Figures 5.4a-d depict the speciation of citrate in the urine of the MC, MSF, FC and FSF groups respectively while Figures 5.5a-d and Figures 5.6a-d depict the speciation of oxalate and calcium respectively. In these histograms the baseline samples will be referred to as "pre-cit" and the samples after 7 days of sodium citrate ingestion will be referred to as "post-cit".

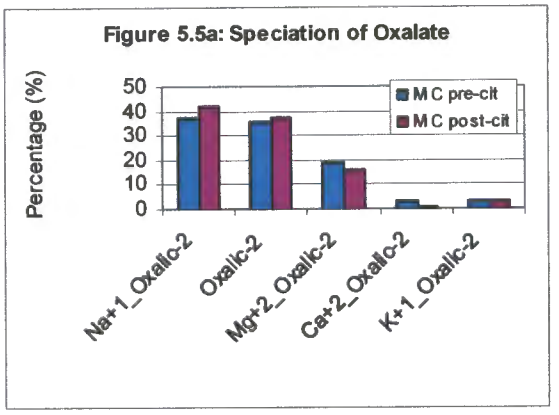


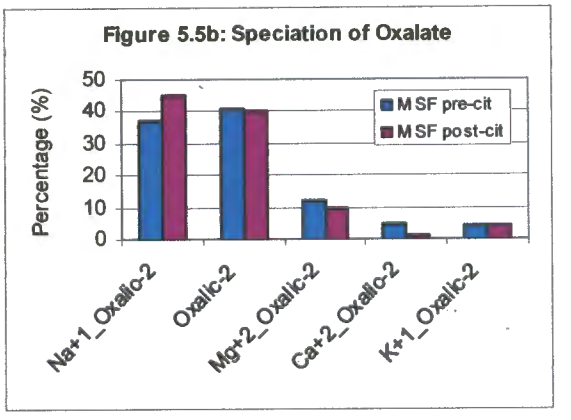


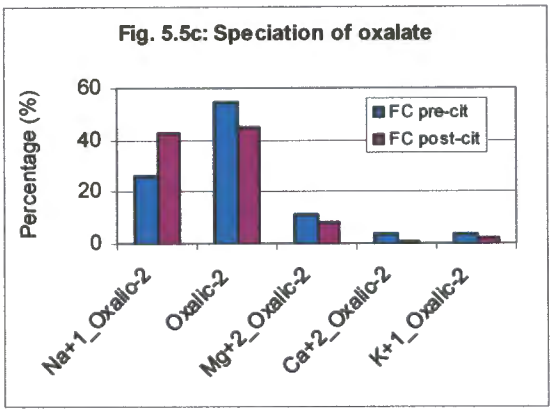


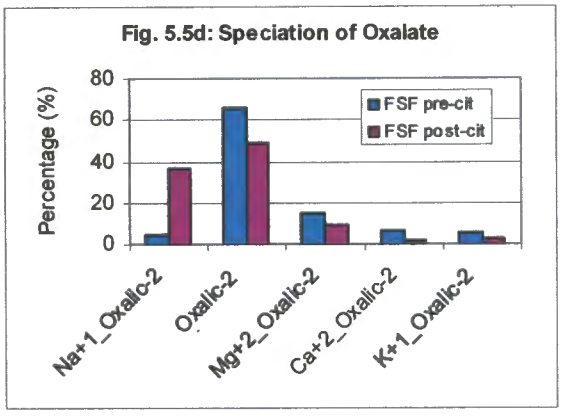


These histograms (Figures 5.4a-d) demonstrate a decrease in the percentage of Mg+2_Citric-3 and Ca+2_Citric-3 and an increase in the percentage Ca+2_Citric-3_PO4-3 in all four groups after the administration of CitroSoda.

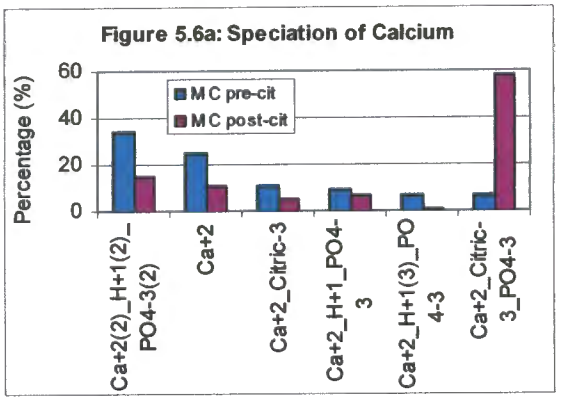


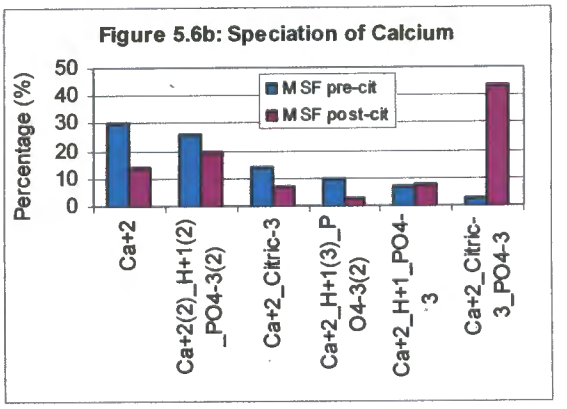


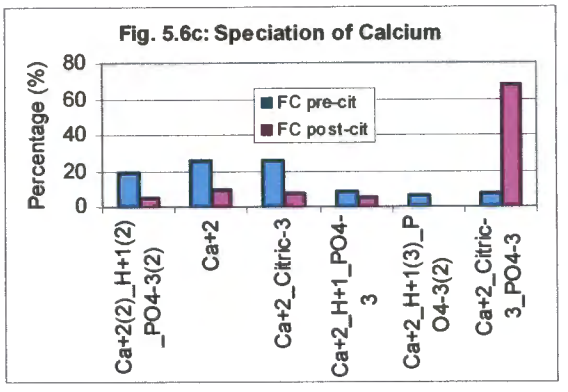


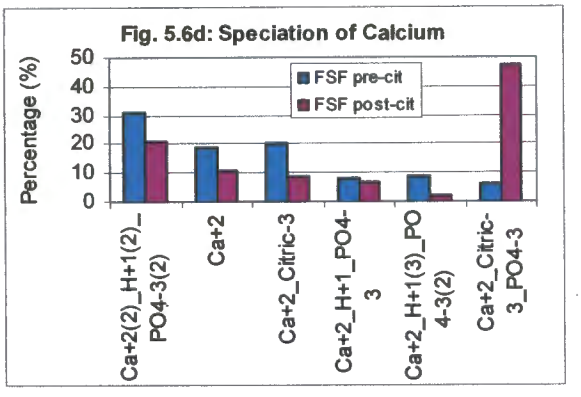


These histograms (Figures 5.5a-d) demonstrate that the percentage Na+1_Oxalic-2 increased in all four groups whereas the opposite trend was observed for Mg+2_Oxalic-2 and Ca_Oxalic-2. The percentage Oxalic-2 decreased in the FC and FSF groups (Figures 5.5c and 5.5d).









These histograms (Figures 5.6a-d) demonstrate that the percentage of Ca+2(2)_H+1(2)_PO4-3(2) and Ca+2 decreased and the percentage Ca+2_Citric-3_PO4-3 increased in all four groups after administration of CitroSoda.

Figures 5.7, 5.8, 5.9 and 5.10 depict the variation in the percentages of the various species as a function of varying concentrations of citrate, oxalate and calcium and varying values of pH respectively.

Since the speciation obtained for each of the calcium, citrate and oxalate components were very similar in the four groups it was feasible to use a single model to represent all of the groups. The MC group was arbitrarily selected for this purpose. Citrate concentration, calcium concentration, oxalate concentration and pH were scanned from below the lowest value obtained for each of the above-mentioned components in Tables 5.2 and 5.3 to the highest value.

Fig. 5.7: Scan of Citrate Concentration

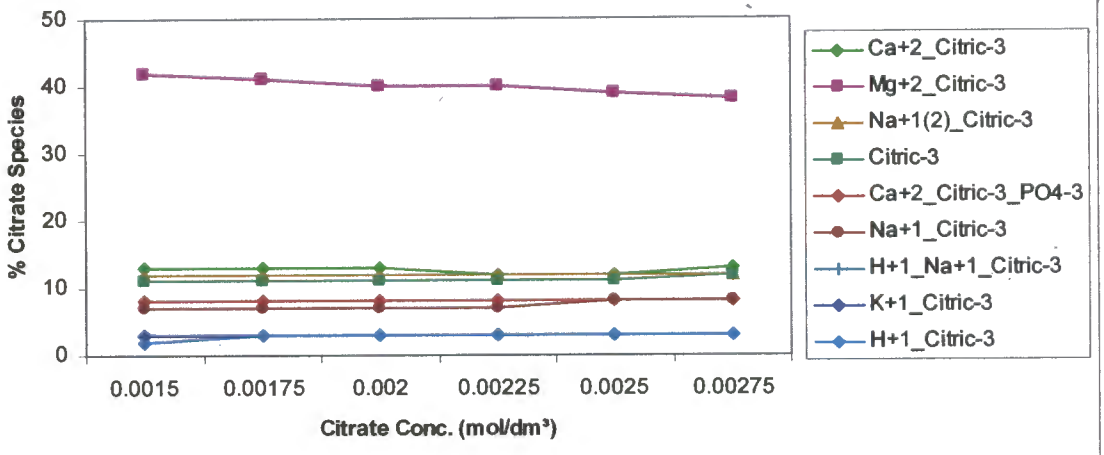


Fig. 5.8: Scan of Oxalate Concentration

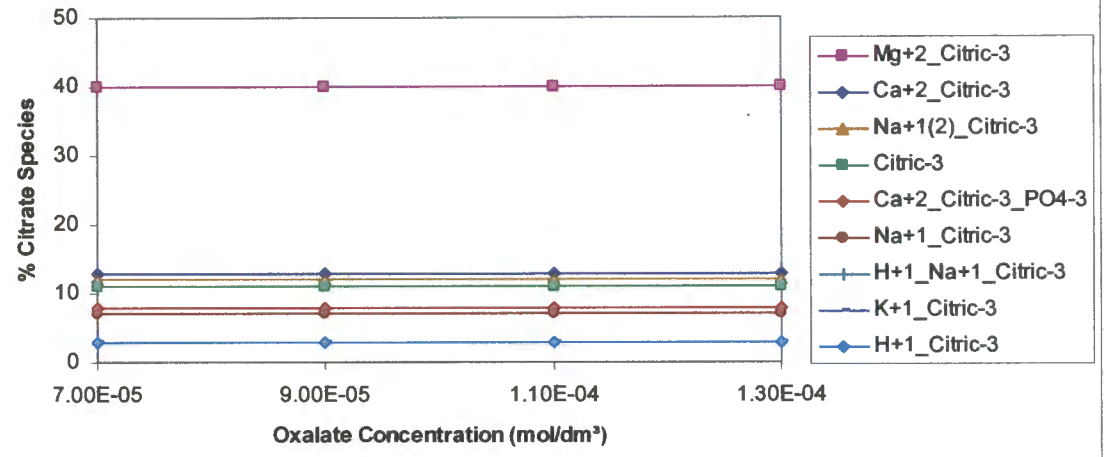
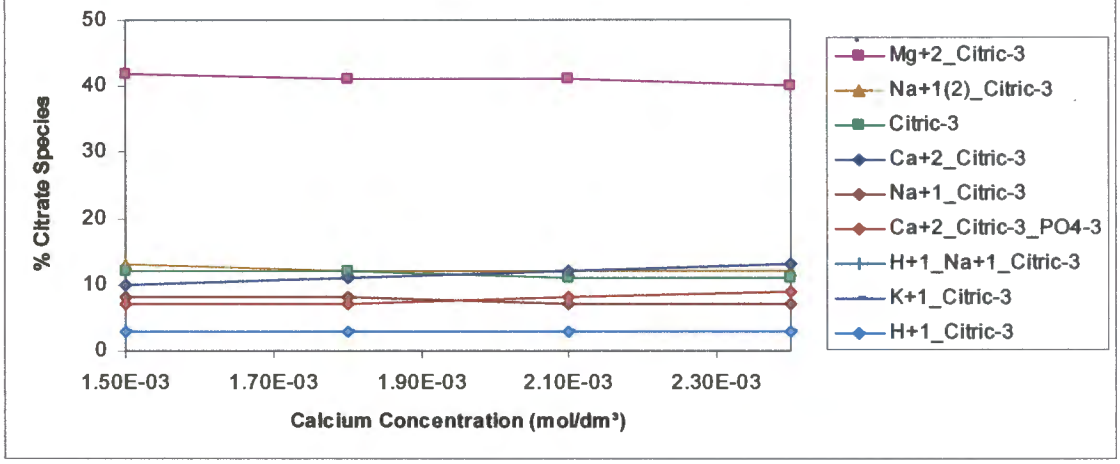
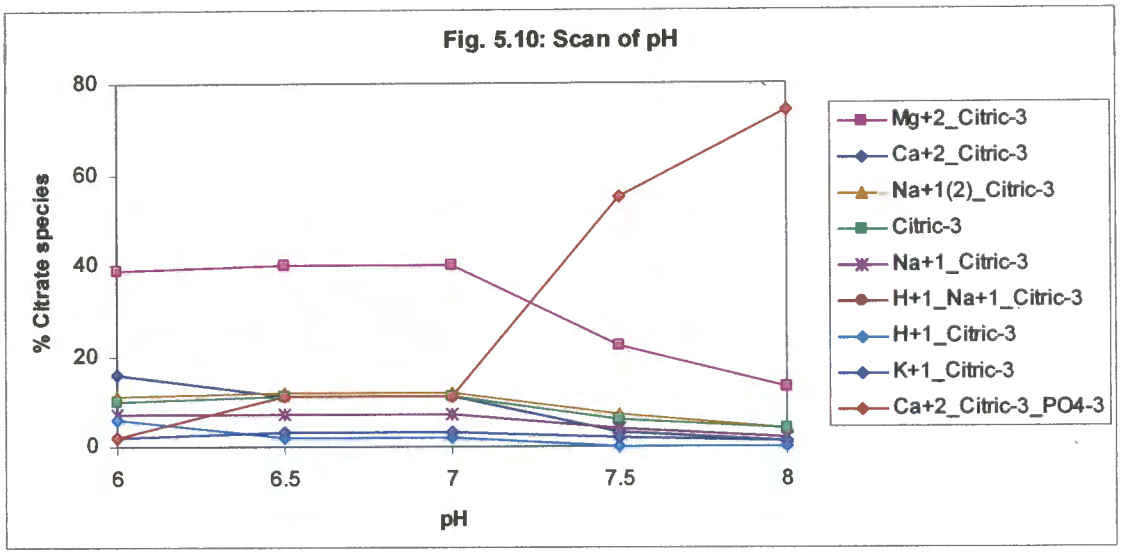


Fig. 5.9: Scan of Calcium Concentration





When scanning the citrate concentration (figure 5.7), hardly any changes in the species were observed except for the percentage formation of Mg+2_Citric-3 which decreased from 42% to 38% as the citrate concentration was increased. It is noted that this species is present at much higher levels than any other complex (also true in other scans).

When scanning the oxalate (figure 5.8) and calcium (figure 5.9) concentrations, no change in the percentage of the various species was observed. However, as mentioned above, the percentage of the magnesium complex is significantly higher than that of all the other complexes.

When the pH was scanned (figure 5.10) a decrease in the percentage formation of Mg+2_Citric-3 from 39% to 13% and an increase in the percentage formation of Ca+2_Citric-3_PO4-3 from 2% to 74% occurred.

5.5 DISCUSSION

In the first part of the study, good correlation was obtained between the two programs for their respective calculations of RS (Figures 5.1, 5.2 and 5.3). This lends confidence to both programs. However, identical results were not obtained. A possible reason for this could be that the constants in EQUIL2 are hard-wired into the program and therefore cannot be updated, whereas in JESS the database consists of more recent literature values for the solubility products of the species that were investigated. Another possible reason which limits the comparison of these two methods could be the lack of components in the EQUIL2 database. Since speciation simulations are dependent on the database and not on the program itself, JESS may therefore be regarded as being more reliable than EQUIL2. A second reason why JESS may be regarded as being more reliable is that it takes into account changes in ionic strength as well as the formation of mixed ligands and species whereas EQUIL2 does not. An obvious example is that of the Ca+2_Citric-3_PO4-3 species which has been calculated by JESS and has emerged in figures 5.4a-d as being of considerable significance.

In chapter 3, the RS CaOx, uric acid and brushite were calculated using the empirically determined urinary component concentrations in the four different groups as input data for the EQUIL2 program. A significant decrease in RS CaOx and RS uric acid was observed after administration of sodium citrate in all four groups. However, a significant increase in RS brushite was observed in the FSF group and not in the other three groups. Since RS is based on the chemical speciation in urine, it should be possible to explain these observations by considering the speciation in more detail. An attempt to do so is described in the paragraphs which follow.

Figures 5.5a-d (speciation of oxalate calculated using JESS) all show that there is a small decrease in the concentration of Ca+2_Oxalic-2 supporting the prediction by EQUIL2 of a decrease in RS CaOx. For this to have happened, either the free calcium and/or the free oxalate concentration must have decreased. The question that needs to be asked is, which of these changes occurred and why? For males (Figures 5.5 a-b) we note that there was no change in the free Oxalic-2 concentration while for females (Figures 5.5c-d) the free Oxalic-2 concentration decreased. Thus, the decrease in RS of females can possibly be accounted for by a decrease in the concentration of the free Oxalic-2. Notwithstanding this observation, inspection of figures 5.6a-d (speciation of calcium calculated using JESS) shows that there is a decrease in the Ca+2 concentration in all

four groups. Thus, the decrease in RS of all four groups can possibly be accounted for by a decrease in the concentration of the free Ca+2. The latter is itself explained by the utilization of free Ca+2 in the formation of Ca+2_Citric-3_PO4-3 in all four groups as demonstrated by the very large increase in the concentration (5-60%, Figures 5.6a-d) of this species. Thus, the decrease in RS CaOx predicted by EQUIL2 can be explained by a decrease in the free Ca+2 concentration in all four groups and (additionally) by a decrease in the free oxalate concentration in the females calculated by JESS.

It is interesting to note that figures 5.4a-d (speciation of citrate calculated using JESS) show a decrease in Mg+2_Citric-3 and Ca+2_Citric-3 concentrations and figures 5.5a-d shows an increase in the Na+1_Oxalic-2 concentration in all four groups. Although these observations are not of relevance to the current discussion it is important to note them because they may have relevance to other studies.

It is now of interest to examine whether the JESS calculations can explain the EQUIL2 output that RS brushite increased significantly in the FSF group but did not change in the other three groups. Interestingly, JESS predicts that there is little or no change in the concentration of brushite (Figures 5.6a-d) suggesting an apparent conflict in the programs with respect to the FSF group. However, a possible explanation for this difference could be the large increase in Ca+2_Citric-3_PO4-3, a species which is not taken into account in EQUIL2. Thus, in JESS, the formation of this species utilizes a significant quantity of Ca^{2+} and PO_4^{3-}, thereby leaving little of the latter ions for the formation of brushite. However, in the EQUIL2 calculation there would be more Ca^{2+} and PO_4^{3-} available for the formation of brushite.

What about uric acid? Can JESS be used to explain the changes in RS predicted by EQUIL2 for this compound? Since there were no observed changes in the speciation of uric acid using JESS, it is not possible to explain the changes in RS in terms of speciation of this species.

Having postulated above that the free Ca+2 concentration is probably the main urinary factor which was responsible for the decrease in RS CaOx, the question which now arises is what aspect of the Na-Cit formulation gives rise to this change? In comparing the empirically determined concentrations in the four different groups it is noted that pH ($p<0.05$), total urinary calcium (MC and MSF: $p<0.05$; FC and FSF: $p>0.05$), total urinary

oxalate (MC: $p>0.05$; MSF, FC and FSF: $p<0.05$) and total urinary citrate ($p<0.05$) changed (Chapter 3, Tables 3.1, 3.3, 3.5 and 3.7) after administration of sodium citrate. Which of these urinary changes account for the change in the free Ca+2 concentration predicted by JESS? A further question is whether these factors influence Ca+2 individually or in combination. In an attempt to answer these questions the effect of pH and calcium, oxalate and citrate concentrations were investigated using JESS. Figures 5.7-5.10 show the effect that changes in these component concentrations have on the percentage of each species. Figure 5.7 shows that Mg+2_Citric-3 accounts for 42% of the total citrate and it decreased marginally to 38% as the citrate concentration is increased. Changes observed in the other citrate species were even smaller. Similarly, little or no change in the percentage of the various species was observed after scanning the total oxalate (figure 5.8) and total calcium (figure 5.9) concentrations. Thus, the change in RS CaOx observed after administration of Na-Cit cannot be accounted for by changes in the total concentrations of these three components in the range studied (scanned). As noted previously, the change in RS CaOx must be due to a change in the free calcium and/or free oxalate concentrations. Figure 5.10 shows dramatic changes in the percentages of Mg+2_Citric-3 and Ca+2_Citric-3_PO4-3 species as the pH is increased. The former decreased from 39% to 13% while the latter increased from 2% to 74% as the pH was increased. This change occurs in the same pH range as that induced by Na-Cit (6.07-7.50). *Thus, it can be postulated that it is the change in pH after administration of sodium citrate which is responsible for the increase in the Ca+2_Citric-3_PO4-3 concentration which in turn lowers the free Ca+2 concentration resulting in a decrease in RS CaOx.*

This *theoretical* investigation has therefore demonstrated that by increasing the pH of urine, the RS of CaOx can be decreased. This confirms the results obtained by other *experimental* studies which have come to the conclusion that citrate preparations decrease RS CaOx (Sakhaee *et al.* 1983, Pak *et al.* 1985, Pak and Fuller 1986, Berg and Tiselius 1989, Pak 1991, Pak *et al.* 1992, Pak 1994, Ettinger *et al.* 1997). The study also provides for the first time, profound evidence that raising the urinary pH should be the therapeutic strategy of choice in the management of idiopathic CaOx urolithiasis.

This study has also shown that JESS could be utilized in the routine evaluation of urinary supersaturation and in the evaluation of the possible effectiveness of a given treatment in the prevention of CaOx stone formation. For example, in an attempt to ascertain whether

a particular diet or medical treatment is effective in reducing the supersaturation of CaOx, the pH and various other urinary parameters could be measured before and after administration of the treatment. The JESS program can then be used to evaluate the RS CaOx before and after its administration. If the urinary pH increases after its administration, the RS of CaOx should decrease.

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Chapter 6

Concluding Remarks

The results of the four studies described in this thesis have yielded important findings in the context of CaOx urolithiasis research and in the context of the clinical management of this disease. Of particular interest in the first study (chapter 2) was the identification of three different chemical interactions all involving citrate: firstly magnesium and citrate exerted a favourable synergistic effect on lowering RS brushite, secondly the same two components had an attenuating synergistic effect on raising urinary pH and thirdly, calcium and citrate exerted an additive effect on lowering RS uric acid. These findings may be of clinical significance in the treatment of brushite and uric acid stones while the effect of magnesium on citrate's action to raise urinary pH is important in the light of the JESS calculations, which demonstrated that raising urinary pH is a key treatment strategy.

An important finding in chapter 3 was the identification of a product (CitroSoda) which has hitherto not been investigated in terms of its effect on CaOx risk factors. The favourable performance of this preparation should be noted and a follow-up trial spanning several years and involving a large group of stone-formers should be undertaken to assess its true potential as a therapeutic agent for reducing stone recurrence rates.

As a result of the concerns associated with the ingestion of oxalate-containing foods and the concomitant ingestion of calcium health supplements, the findings in chapter 4 are of considerable significance. Clinicians now have compelling data which allow them to recommend to their patients with some confidence the optimum timing for ingesting calcium supplements relative to an oxalate-rich meal so that their risk of stone formation is minimized. However, patients should be cautioned that balance between dietary oxalate and calcium would be a more appropriate goal.

Finally, the value of the speciation program, JESS, as a research tool has been demonstrated. While EQUIL2 has been used successfully for many years, the application of an alternative, more powerful program should be an attractive proposition for stone researchers. Indeed, application of the JESS program to the urinalysis data acquired in Chapter 3 of this thesis has yielded the extremely important finding that an increase in urinary pH should be the therapeutic strategy of choice in the treatment of idiopathic CaOx urolithiasis.