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