STUDIES OF THE EFFECTS OF VARIOUS LITHOGENIC AND ANTILITHOGENIC DIETARY SUPPLEMENTS ON CALCIUM OXALATE KIDNEY STONE RISK FACTORS IN SOUTH AFRICAN BLACK AND WHITE SUBJECTS

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“Now faith is the substance of things hoped for, the evidence of things not seen”

The Bible

Hebrews 11: 1

TO MOM

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CONFERENCE PROCEEDINGS

Publications

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- Bungane NB, Rodgers AL, Allie-Hamdulay S, Lewandowski S. Investigation of the effect of oral glucose, sorbitol and xylitol loads on the urinary excretion of calcium, oxalate and phosphate in South African black and white subjects. *Euro Urol Meetings* 2007; **2**(1): 31.

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- Rodgers AL, Bungane NB, Allie-Hamdulay S, Lewandowski S, Webber D. Calciuria, oxaluria and phosphaturia after ingestion of glucose, sorbitol and xylitol in two population groups with different stone-risk profiles. *Urol Res*, September 2008.

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ABSTRACT

In South Africa, the incidence of urolithiasis in the white population occurs to the same extent as in other western societies. However, in the black population, this disease is extremely rare. Differences in the gastrointestinal and renal handling of several lithogenic and antilithogenic agents in the two ethnic groups have been reported, but the rarity of kidney stone disease in the black group remains a riddle. The studies described in this thesis were undertaken to address aspects of this phenomenon.

Carbohydrate and oxalate-containing dietary agents were identified as being of interest since surveys have paradoxically demonstrated that the consumption of these potentially lithogenic substances is significantly higher in the black group. The carbohydrates selected for study were glucose, sorbitol and xylitol, while the oxalate-containing agents were rhubarb, spinach and an aqueous solution of sodium oxalate itself. Finally, taurine, which has been shown to reduce urinary glycolate and oxalate in animal models, was also selected for investigation in the two population groups.

In all studies, similar protocols were adopted. These were approved by the Research and Ethics Committee of the University of Cape Town. Subjects were recruited from the black and white student cohorts of the university. All gave their informed consent. In general, 24 h urine samples were collected before and after ingestion of the particular test substance while following a standardized diet. In some protocols, urine samples were also collected at various time intervals during the 24 h period. Urines were analysed by routine biochemical techniques. In addition, some specialized analytes were determined in certain studies. Urinary sorbitol was analysed by high performance anion exchange chromatography (HPAEC) and by fluorescent assay. In the studies involving the oxalate-rich challenges, N-acetyl-β-glucosaminidase (NAG), a urinary marker enzyme of renal injury was measured by a colorimetric assay. Cellular oxidative stress was also evaluated in these particular studies by measuring 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative damage to DNA, using an enzyme-linked immunosorbent assay (ELISA). In the study involving taurine, urinary glycolate was measured using gas chromatography tandem mass spectrometry (GC/MS). Several crystallization experiments were performed on the urine samples. These included determination of the calcium oxalate metastable limit using a Coulter Counter, ^{14}C-oxalate deposition kinetics

using a scintillation counter, calcium oxalate aggregation inhibition by a spectrophotometric technique, particle volume-size distributions using a Coulter Counter and the Bonn Risk Index (BRI) by an automated titration method. In addition, crystals formed in these experiments were examined by scanning electron microscopy.

In the carbohydrate studies, glucose caused a decrease in urinary phosphate excretion in black subjects. Sorbitol induced an increase in urinary oxalate and a decrease in urinary phosphate excretion in white subjects. The latter observation was also noted in black subjects. Finally, xylitol provoked an increase in the urinary excretion of oxalate in black subjects. The decreases in phosphaturia are attributed to reduced renal filtered load of phosphate consequent to entry of phosphate into cells after glucose or sorbitol ingestion. On the other hand, it is proposed that the elevation of oxaluria following sorbitol and xylitol occurs via metabolic pathways which are governed by certain enzymes that convert the two sugars into oxalate. The observed difference in the urinary excretion of this parameter is ascribed to the difference in the activity of the enzyme aldolase which was determined in the two groups.

In the studies involving dietary oxalate challenges, rhubarb did not alter significantly the urinary excretion of oxalate in both groups. However, spinach and sodium oxalate provoked an increase in oxaluria in black subjects but did not concomitantly change either NAG or 8-OHdG excretion. The implication of these results is that the increases in oxaluria were not injurious suggesting resistance in this group to calcium oxalate stone formation.

In the study involving the taurine challenge, there were no changes in either group with respect to urinary oxalate and glycolate excretion. Interferences by other ingredients in the source of taurine, Red Bull Energy Drink, used in this study, may have limited the inhibitory effect previously observed in animal models.

The studies described in this thesis have demonstrated that subjects from South Africa's black and white population groups respond differently to some lithogenic and antilithogenic dietary challenges. The different handling mechanisms that are likely to be involved in these different responses may contribute towards understanding why stone disease occurs in the one group but not the other.

LIST OF ABBREVIATIONS

8-OHdG	8-hydroxydeoxyguanosine
ANOVA	analysis of variance
BRI	Bonn risk index
BSTFA	Bis(trimethylsilyl)trifuoroacetamide
CaOx	calcium oxalate
CaP	calcium phosphate
COM	calcium oxalate monohydrate
COD	calcium oxalate dihydrate
DNA	deoxyribonucleic acid
GC/MS	gas chromatography tandem mass spectrometry
GAO	glycolic acid oxidase
Glu	glucose
GO	glycolate oxidase
HPAEC	high performance anion exchange chromatography
HPLC	high performance liquid chromatography
LDH	lactate dehydrogenase
MSL	metastable limit
NAG	N-acetyl-β-glucosaminidase
Na_2Ox	sodium oxalate
p	p-value
P	phosphate
PAD	pulsed amperometric detection
RS	relative supersaturation
SE	standard error
SEM	scanning electron microscope
Sor	sorbitol
XRD	x-ray powder diffraction
Xyl	xylitol

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Chapter One

Introduction

1.1 Urolithiasis

Urolithiasis is a common clinical disorder which occurs in both industrialized and developing countries. Increased incidence of kidney stones in the industrialized world is associated with affluent standards of living and is strongly associated with race or ethnicity and region of residence (Parmar 2004). In general, a range of risk factors have been identified for kidney stone formation. These include life style, positive family history of stones, nutrition and nutrition deficiencies and associated diseases and these will be discussed.

Approximately 80% of kidney stones contain calcium, and the majority of them are composed primarily of calcium oxalate (60%) (Heilberg *et al.* 2006). The other common constituents of kidney stones are calcium phosphate (10%), magnesium ammonium phosphate (struvite) (5-10%), uric acid (5-10%) and cystine (1%) (Morton *et al.* 2002). Kidney stones occur in different shapes and sizes and are formed in the kidneys or anywhere along the urinary tract.

1.2 Renal Function and Stone Formation

1.2.1 Overview of the Kidney

The kidney is one of the vital organs in the body which plays a twofold pivotal role, namely in the elimination of waste products which are either ingested or produced by metabolism (e.g. urea, creatinine, uric acid, drugs) and in the maintenance of body homeostasis. The kidneys are located on the posterior wall of the abdomen and each is made up of about 1 million nephrons, which control and regulate the processes involved in urine formation in the kidney (Guyton and Hall 1996). The number of nephrons is fixed and therefore new nephrons cannot be regenerated by the kidney once damaged. Figure 1 depicts a schematic overview of the nephron and the parts which are involved in urine formation.

Each nephron is composed of various segments which effectively and exclusively function in the production of urine. The glomerulus is the initial segment where urine formation begins. A large amount of fluid (from blood) is filtered in this component into the Bowman's capsule and then into the proximal tubule. Partial- and complete-reabsorption back into the blood of electrolytes (e.g sodium ions and chloride ions)

and nutritional substances such as amino acids and glucose, respectively, occurs in this region (Guyton and Hall 1996). From the proximal tubule, the fluid flows into the Loop of Henle and then enters the distal tubule. The final urine is processed in the collecting duct and is passed into the ureter which in turn propels the urine toward the bladder where it is stored until it is excreted (Guyton and Hall 1996).

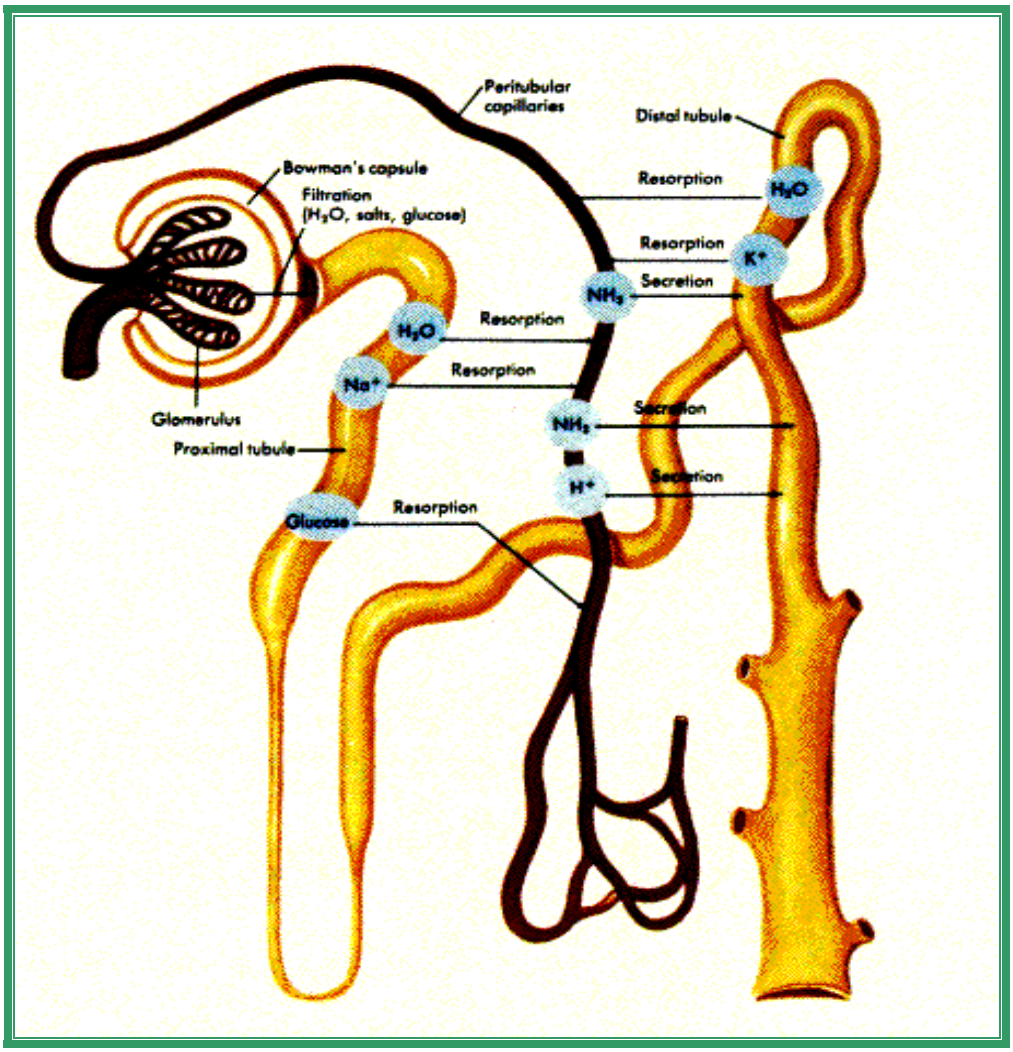

Figure 1: *Schematic representation of the nephron*
(http://www.home.byway.net/rjnoonan/humans_in_space/fluid.html)

1.2.2 Mechanisms of stone formation

Crystal formation in the kidneys can result in the formation of a kidney stone. The precipitation of these crystals in the urinary tract when the urine is supersaturated, i.e. when the concentration of salts is higher than what can be retained in solution, ultimately results in the formation of a stone. An imbalance between factors that promote crystallization and those that inhibit crystal formation and growth usually precedes stone formation (Coe *et al.* 2005). The interaction of Ca and/or Ox with other urinary ions and macromolecules that are present in different quantities, may give rise to the crystallization process. It is believed that urine from healthy controls and stone formers is always supersaturated with CaOx and that everyone forms CaOx crystals in urine from time to time (Ryall 1997). In the kidney, the supersaturation of calcium oxalate (CaOx) usually occurs in the collecting ducts of the nephron, whereas calcium phosphate (CaP) precipitation occurs rather in the earlier parts, such as the Loop of Henle or distal tubules. As the precipitated CaP is propelled through the tubules, it dissolves as the pH is lower in the collecting ducts and it can then act as a nucleation site for CaOx crystals at this region (Mandel 1996, Højgaard and Tiselius 1999a). A brief description of the crystallization steps involved in stone formation, namely, nucleation, growth, aggregation and retention follows.

Crystal Nucleation

Nucleation is defined as the formation of new crystal nuclei in a supersaturated solution capable of spontaneous growth into large crystals of a more stable solid phase (Finlayson 1978, Brown and Purich 1992). This transformation is influenced by supersaturation. Supersaturation is essential, although not a prerequisite, for stone formation as it lays the foundation for crystal nucleation to begin. The crystallization process is dependent on the supersaturation and as the supersaturation ratio increases, the nucleation rate also increases (El-Shall *et al.* 2004). Crystals can form if the relative supersaturation is greater than 1 but they dissolve if it is less than 1 (Finlayson 1978).

The increase in the concentration of calcium and oxalate ions in an aqueous CaOx solution can occur to such an extent that the relative supersaturation escalates to values greater than 10, in which the solution becomes unstable and crystals form (Hess and Kok 1996). It is postulated that the nucleation process is initiated when

complexes of stone salt ions in a solution combine to form tiny clusters, which gradually increase in size over time and with a concomitant addition of new clusters (Boskey 1981). Finlayson (1978) posited that the conversion of these clusters further into stable nuclei depends on changes in free energy. There are two types of nucleation, namely homogeneous and heterogeneous nucleation. Homogeneous nucleation is the process whereby nucleation occurs in a pure urine solution and the formation of crystals is spontaneous (Finlayson 1978).

In a case whereby the concentration of calcium and oxalate ions is augmented beyond the relative supersaturation value in a solution containing calcium, oxalate and CaOx crystals, secondary nucleation occurs on the on the surface of pre-existing CaOx crystals (Hess and Kok 1996). This mechanism, however, is very rare as urine in most cases is not concentrated enough. Nucleation can therefore occur via another process called heterogenous nucleation, i.e. nucleation in an impure urine solution resulting in facilitated crystal formation (Lonsdale 1968). In this process crystalline particles of foreign substances which act as nucleating substrates could facilitate the formation of calcium oxalate monohydrate (COM) crystals.

Crystal Growth

Subsequent to nucleation, crystal growth process occurs, a prerequisite for the formation of particles in renal tubules and thus stone formation. Crystals can either grow or aggregate individually to form stones or they can concomitantly grow and aggregate to produce larger particles which ultimately lead to stone formation (Fleisch 1978). New crystal components are incorporated into a crystal nucleus thereby reducing the overall free energy of atoms or molecules as they bond to each other (Hess and Kok 1996). The process whereby the newly formed crystal in the urine travels through the solution and ultimately sticks to the crystal to facilitate the growth of the crystal is termed bulk diffusion (Hess and Kok 1996). The rate at which crystal components are directly incorporated into the solid crystal lattice is believed to be dependent on supersaturation, which must be above 1 (Kavanagh 2006). The crystal growth process is also governed by other crucial factors like pH, size and shape of the molecule (Kok *et al.* 1990a).

Crystal Aggregation

The most determinant process in the formation kidney stones is called crystal aggregation which can be defined in simple terms as the sticking together of crystals in solution thereby forming larger particles (Hess and Kok 1996). This process is controlled by aggregating forces that promote crystal aggregation (i.e. van der Waals, viscous binding, solid bridges), disaggregating forces that disrupt crystal aggregation (i.e. electrostatic repulsion/Zeta potential), as well as shear forces which play a dual role (Finlayson 1978, Robertson *et al.* 1981, Hess 1991). Two types of mechanisms which govern the crystal aggregation process are primary and secondary aggregation (Sohnel and Grases 1995). On the one hand, primary aggregation occurs through the attachment of crystals into another pre-existing crystal to form an aggregate. Secondary aggregation, on the other hand proceeds via the collision of free particles which are suspended in solution. However, the structure of the aggregate which is formed as a result is usually irregular and of poor order compared to that of the aggregate formed via primary aggregation (Hess and Kok 1996). The crystal aggregation process is very fast compared to crystal growth as it produces larger particles within seconds. In contrast, the latter produces particles of insignificant size at a much slower rate (Robertson and Nordin 1982, Kok and Papapoulos 1993). However, crystal aggregation is also dependent on supersaturation, as it is the case with crystal growth (Hounslow *et al.* 2001).

Clinical studies have shown that recurrent CaOx stone formers excrete urine with larger crystal aggregates than their healthy controls (Robertson *et al.* 1969, Hallson and Rose 1976). This is believed to be due to the reduced ability of the urine of stone formers to inhibit crystal aggregation. Evidence suggests that citrate plays a major role in the inhibition of crystal aggregation and the correlation between hypocitraturia and renal stone formation has been reported (Kok *et al.* 1990a). In addition, it has been reported that crystal growth and aggregation can be affected by urinary macromolecules such as Tamm Horsfall protein, albumin, bikunin, osteopontin, inter-α-trypsin and prothrombin fragment 1, which may promote or inhibit these two crystallization processes (Worcester and Beshensky 1995, Marengo *et al.* 1998, Atmani and Khan 1999, Wesson *et al.* 2000).

Crystal Retention

The retention of crystals in the kidney is required prior to their conversion into a stone. It is conceded that crystals mechanistically adhere to renal epithelial cells of the kidney through crystal-cell interactions (Kok and Khan 1994, Mandel 1996). The composition of the renal tubular epithelial cell surface, which makes the surface either adherent or non-adherent, is regarded as an important determining factor for crystal retention. Evidence suggests that CaOx urolithiasis is initiated by hyperoxaluria (i.e. high urinary oxalate levels) which induces crystal formation and then deposition in the epithelial cells of the kidney (Khan 1996). It is postulated that the bound crystals on the renal epithelial cells induce injury, partly by reactive oxygen species (Aihara *et al.* 2003). An increased urinary excretion of γ-glutamyl transpidase, N-acetyl-β-glucosaminidase and β-galactosidase, has been observed in hyperoxaluric patients, indicating damage to the epithelial cells (Baggio *et al.* 1983, Khan *et al.* 1989). It is therefore hypothesized that crystal binding to the epithelium lining of the renal tubules may be triggered by renal injury. In some cases, with individual variability, the primary constituent of crystal aggregates is COM and these are often found adhered to the papillary tip of the epithelial cells. Calcium oxalate dihydrate (COD) crystals are less common and are in most cases found in hypercalciuric patients. Hyperoxaluric patients on the other hand often have predominantly COM crystals in their urines.

Another factor which facilitates crystal retention is the inability of larger crystals to pass through the tubular lumen, which then get trapped as result. The morphologies of COM and COD crystals are different and thus are dragged with urine at a different pace. When crystals flow at a slower rate and close to the tubule wall they tend to form crystal-cell interactions, which promote crystal attachment (Khan 2006). Figure 2 outlines the factors involved in the process of stone formation.

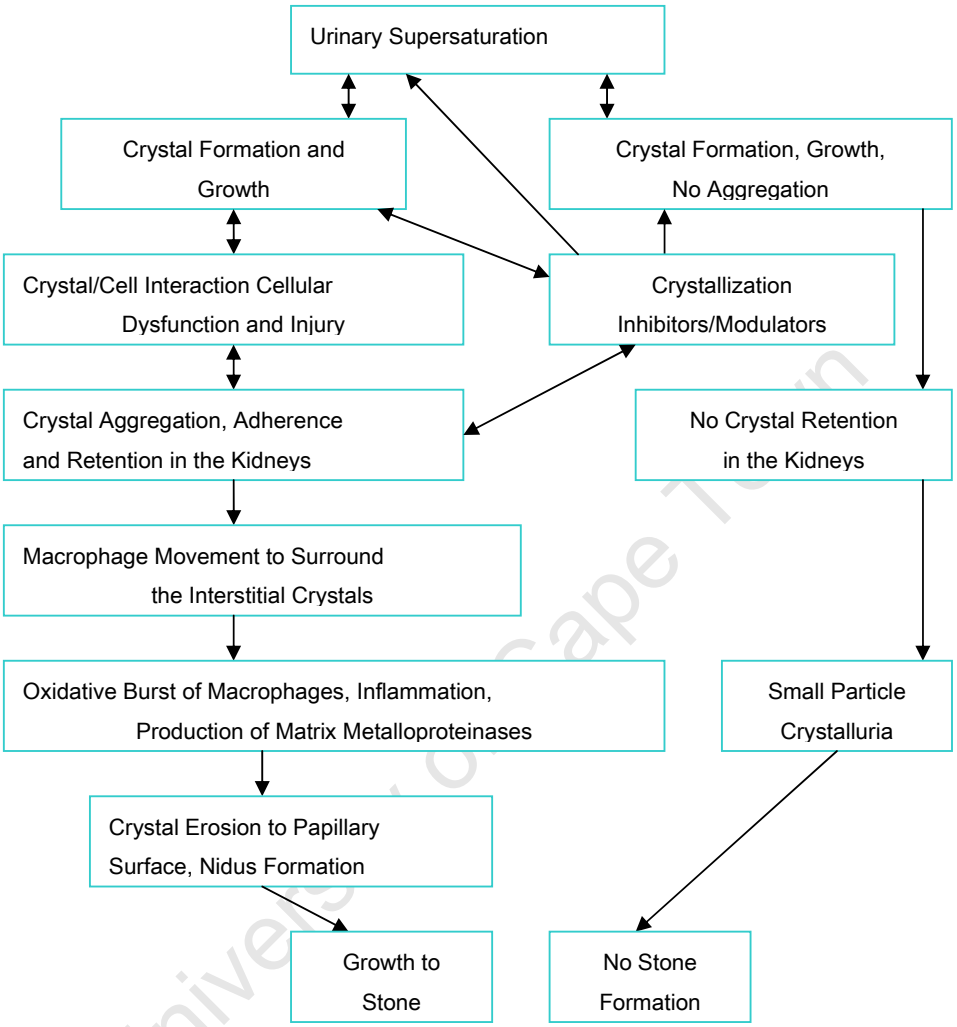

Figure 2: *Schematic outline of the processes of kidney stone formation* (Khan 2006).

1.3 Epidemiological factors

1.3.1 Extrinsic Risk Factors

Socio-economic conditions

The prevalence of urolithiasis has been persistently and progressively increasing in the last century in both developing and industrialized countries thereby inducing a concomitant elevation of costs of diagnosis and treatment of kidney stones. A direct proportionality between affluence and stone formation has emerged over the years. It has been observed that the higher the socio-economic class, the higher the prevalence of idiopathic nephrolithiasis (Robertson *et al.* 1980a). In 1993, it was estimated in the United States that the annual urolithiasis treatment cost is $1.83 billion (Clark *et al.* 1995). This is primarily due to the fact that the rate of stone incidence is rising.

Kidney stones usually recur and a recurrence rate of as high as 50% in 10 years has been documented (Trinchieri *et al.* 1999). Not much has been done to evaluate protection against stone recurrence. This impacts the economy unfavourably hence dietary measures have been taken in order to combat the formation of stones and thereby reducing the economic impact of this disease on health systems.

Occupation

Research has repeatedly indicated that people who have sedentary occupations are at a much higher risk of forming stones (Lonsdale 1968, Blacklock 1969, Ferrie and Scott 1984, Borghi *et al.* 1993). A study by Sutor and colleagues (1974) confirmed a higher incidence of stone formation amongst the professional and managerial groups compared to manual workers. Astronauts have also been studied as they spend more time in the microgravity environment and have been noted to have a higher incidence of calcium oxalate and uric acid stone formation (Whitson *et al.* 1993). This is primarily due to the increase in the urinary excretion of calcium, a decrease in urinary citrate excretion, a decrease in urinary pH and a lower urine volume.

Climate and Season

Seasonal and climatic variations have been implicated in the incidence and recurrence of kidney stone disease. Research has indicated a prevalence of stone disease in warm and hot climates particularly during spring and summer (Robertson *et al.* 1975). Pierce and Bloom (1945) observed a high stone incidence amongst troops located in desert areas during war. It is postulated that more concentrated urine, as a result of dehydration, and increased production of vitamin D, subsequent to prolonged exposure to sunlight, are important risk factors governing the formation of stones in hot regions. It is hypothesized that skin exposure to excessive sunlight triggers the production of 25-hydroxycholecalciferol which is then converted in the kidneys to 1,25 dihydroxy-vitamin D. Subsequently, an increased intestinal absorption of calcium occurs, which then stimulates a high urinary excretion of calcium and thereby leading to stone propensity (Berlin *et al.* 1986).

1.3.2 Intrinsic Risk Factors

Race / Ethnicity

One of the important factors which influence the risk of stone incidence has been associated with race. Research has shown that idiopathic stone disease occurs more frequently in white Caucasians than in blacks (Ramello *et al.* 2000). Similary, results of a survey conducted in the United States revealed a high and low prevalence of kidney stones in whites and blacks, respectively and an intermediate prevalence was found in people of Asian and Hispanic ethnic origin (Soucie *et al.* 1994, Hiatt *et al.* 1982). Moreover, Maloney (2005) reported a higher incidence of hypercalciuria in white stone formers in the United States. In South Africa approximately 15% of the white population has a high incidence of calcium oxalate stone formation compared to less than 1% of the black population which has shown immunity towards this disease (Wise and Kark 1961, Modlin 1967, Whalley *et al.* 1998). These intriguing findings have resulted in further investigations of the two population groups and a study by Rodgers *et al.* (2002) showed that the difference in the renal handling of calcium could be one of the factors governing the difference in stone incidence between the two groups. More studies on this aspect will be discussed later.

Age and Gender

A positive correlation between age and urolithiasis has been reported in various studies (Robertson *et al.* 1980b, Daudon *et al.* 2004). It has been postulated that the peak age for the development of CaOx stones is between 50 and 60 years whilst UA stones tend to occur in an older population over the age of 60 years (Baker *et al.* 1993). The rate of kidney stone formation in women is about half that in men, with two peaks, the first among women aged 35 years and the second among those aged 55 years (Morton *et al.* 2002).

The prevalence of kidney stone disease has been rising over the years in both males and females with the incidence in the former being two to three times higher than in the latter. The reason for this male predominance is obscure. Presently, CaOx and uric acid (UA) stones are more frequent in males than in females, whereas calcium phosphate (CaP) and struvite stones are more prevalent in females (Daudon *et al.* 2004, Gault and Chafe 2000, Robertson *et al.* 1980b). A study by Fan and co-workers (1999) has shown that the higher prevalence of nephrolithiasis in males can be ascribed to the effect of sex hormones on some lithogenic risk factors wherein androgens appeared to increase, and estrogens to decrease, urinary oxalate excretion and kidney calcium oxalate deposition. It has been suggested that the female sex hormone oestrogen may play an important role in protection against stone formation by affecting calcium metabolism. An increased risk of stone formation has been observed to increase after menopause (Heller *et al.* 2002).

Genetics / Family History

Studies on genetics have postulated that kidney stone disease is a polygenic defect (Mc Geown 1960; Resnick *et al.* 1968; Goodman *et al.* 1995). It has been reported that in most cases a family history of kidney stones is more common and frequent in patients with kidney stones than in non-stone formers (Lavan *et al.* 1971). In addition, in a previous study, Curhan and co-workers (1997a) observed that the likelihood of a family history of kidney stones was three times more in men who had developed a stone than in other men.

1.4 Pathophysiological Aspects

The development of renal calculi usually involves urine composition factors. It has long been documented that the 24 hour urine studies have revealed that urinary volume and urinary pH as well as the excretion of oxalate, calcium, uric acid, magnesium and citrate, in no particular order, are risk factors which influence crystal generation (Robertson *et al.* 1978, Hess *et al.* 1996). The ease of crystallization depends on the degree of supersaturation, the presence of preformed particles (promoters) and the level of crystallization inhibitor, with the latter hindering crystal nucleation and/or growth (Grases *et al.* 2006). The following section discusses the urinary risk factors and their effects on stone formation.

Urine Volume

It is widely known that a suitable fluid intake is the key inhibitor of kidney stone formation as it can reduce supersaturation (Curhan *et al.* 1993, Borghi *et al.* 1996, Curhan 1998a). Recommendations for urinary output vary, but there is a broad consensus that it should exceed two liters per day, while some even encourage urinary outputs in excess of three liters per day (Sakhaee *et al.*1980, Menon and Resnick, 2002). To confirm this, Borghi *et al.* (1996) found, in a retrospective study lasting 5 years that the recurrence of stones was evident in patients who maintained a urine volume of about 2.6 liters per day compared to those who maintained about 1.2 liters per day.

Urinary pH

The pH of the urine plays an important role in the process of kidney stone formation. The influence of urinary pH on the crystallization of CaOx and CaP, in particular, has been studied. On the one hand, it has been shown that CaP stone formation occurs at higher pH (above 7.0). This is caused by the dissociation of phosphate ions at the higher pH which, in the presence of high urinary calcium excretion promotes complexation of phosphate with calcium (Tiselius 1978, Pak 1994, Højgaard *et al.* 1999). However, the converse of this is the concomitant favourable reduction of CaOx stone formation, as there will be less calcium ions to bind with oxalate. CaOx crystallization is known to occur at pH below 6.25 (Ahlstrand *et al.* 1984).

On the other hand, at lower pH (below 5.5-5.7), the risk of uric acid stone formation may be enhanced (Sperling 1964, Tiselius 2003). A low urinary pH alone is not entirely responsible for uric acid lithiasis, since it has been shown that healthy subjects have relatively low urinary pH values and thus other factors need to be considered (Frank *et al.* 1963).

Urinary Calcium

Calcium is the most abundant element present in most stones with CaOx being the most common compound (Holmes *et al.* 2001, Hall 2002, Moyad 2003). It is generally perceived that the supersaturation of urine with calcium, termed hypercalciuria, is one of the most common and important risk factors for kidney stones (Asplin *et al.* 2000). Parmar (2004) defines hypercalciuria as the excretion of urinary calcium exceeding 200 mg in a 24 hour collection or an excess of 4 mg calcium/kg/24 h. There are different types of hypercalciuria (Bhandari and Menon 2003, Parmar 2004), namely:

- *Idiopathic hypercalciuria:* Increased urinary calcium excretion in the absence of elevated serum calcium levels.
- *Absorptive hypercalciuria:* Enhanced renal filtered load of calcium as a consequence of its elevated absorption from the intestine.
- *Renal hypercalciuria:* Primary leak of calcium due to impaired renal tubular absorption.
- *Resorptive hypercalciuria:* Increased bone demineralization and enhanced intestinal absorption of calcium.

It is estimated that about 30 to 50 % of patients with kidney stones have idiopathic hypercalciuria (Bushinsky 2002). Studies have linked idiopathic hypercalciuria (IH) with intestinal hyperabsorption of calcium (Hennemann *et al.* 1958) and there is also supporting evidence that most patients with IH present abnormally high serum levels of 1,25-dihydroxyvitamin D_3 (Hess and Jaeger 1992). Recommendations of restricting dietary calcium intake in the case of absorptive hypercalciuria as a therapeutic measure against CaOx stone formation have been implemented (Breslau 1994), even though no clinical data has been reported in support of this hypothesis. More recently, this practise has been shown to increase the risk of CaOx stone formation (Hall 2002).

Urinary Oxalate

It has been demonstrated that the saturation of urine with CaOx rises significantly with small increases in oxalate concentration and this has led to the hypothesis that calcium binds with oxalate in the intestinal lumen, which results in its excretion in the stool and thereby abating oxalate absorption, subsequently increasing the risk of CaOx crystallization (Coe *et al.* 1992, Curhan and Curhan 1994, Krishnamurthy *et al.* 2003, Hatch and Freel 2003). The amount of oxalate available for absorption in the intestine and its excretion via the urinary tract can be limited by its complexation with calcium (Sutton *et al.* 1994, Nishiura *et al.* 2002, Seiner *et al.* 2003, Hatch and Freel 2003).

In CaOx stone formers the molar ratio of urinary calcium to oxalate is 5 : 1, therefore based on this fact, it is recommended that oxalate intake must be restricted as small changes in oxalate concentration have much larger effects on calcium oxalate crystallization than calcium concentration (Heilberg 2000). Theoretically, the excessive excretion of oxalate in the urine could result from firstly, increased absorption from the gut, secondly, increased renal clearance or thirdly, increased rate of synthesis (Williams and Smith 1968). Research has shown that healthy individuals excrete less urinary oxalate compared to stone formers (Goldfarb 1988, Schwille *et al.* 1989, Wilson *et al.* 1989). On one hand, most patients with urinary calculi appear to excrete normal quantities of oxalate, but a small proportion excretes moderately increased amounts i.e. 50-100 mg per day. On the other hand, patients with the rare genetic disorder of primary hyperoxaluria excrete more than 100 mg per day. By contrast, only 10-40 mg per day of oxalate is excreted by healthy adults on a normal diet (Zarembski and Hodgkinson 1969).

Another equally important factor which influences the absorption and subsequently the urinary excretion of oxalate is the deficiency of *Oxalobacter formigenes* (*O. formigenes*), an intestinal bacterium which plays a crucial role in degrading dietary oxalate (Allison *et al.* 1986, Troxel *et al.* 2003). Research has shown that *O. formigenes* is acquired in early childhood by children and is present in faeces of 70-80% of adults (Sidhu *et al.* 1997, Sidhu *et al.* 1999, Kwak *et al.* 2001, Kumar *et al.* 2002, Kwak *et al.* 2003). However, patients with enteric hyperoxaluria and calcium oxalate stone formers have been reported to have a lower colonization rate of *O. formigenes* than non-stone formers (Kleinschmidt *et al.* 1993, Sidhu *et al.* 1998, Troxel

et al. 2003, Kumar *et al.* 2004). In order to find further evidence for the role of *O. formigenes* in oxalate homeostasis in humans, a decrease in oxalate excretion has been observed in two adult human subjects after co-administration of *O. formigenes* with an oxalate supplement (Duncan *et al.* 2002).

Urinary Uric Acid

High urinary uric acid is well-known as one of the primary risk factors of kidney stone formation. The main dietary source of uric acid is animal protein (purines). It is believed that an excessive dietary intake of purines and an excessive production of uric acid result in a condition known as hyperuricosuria (i.e. increased urinary excretion of uric acid). The risk factors deemed to be crucial in uric acid nephrolithiasis are acidic urine pH, low urine volume and hyperuricosuria (of genetic or environmental origin) (Riese and Sakhaee 1992). Excessive sweating and dehydration result in a low urinary volume which is usually accompanied by an unduly low urine pH which speeds up the uric acid stone formation (Sakhaee 2007). It has been postulated that a low urinary pH (below 5.5), rather than high urinary excretion of uric acid is the principal determinant of uric acid supersaturation (Coe *et al.* 1980). The association of hyperuricosuria with calcium oxalate stone formation has been well documented (Gutman and Yu 1968, Coe 1978).

It has been indicated that the development of acidic urine in patients with idiopathic uric acid nephrolithiasis may be a result of overproduction of endogenous acid and defective urinary excretion of ammonium (Sakhaee *et al.* 2002, Cameron *et al.* 2006). The latter results in decreased urinary citrate excretion in these uric acid stone formers. Recent findings have also noted a positive correlation between uric acid nephrolithiasis and insulin resistance, with the former being the result of low urinary pH in patients who experience the latter condition (Abate *et al.* 2004, Maalouf *et al.* 2004).

The recommendations for preventing uric acid stone disease involve alkalinization of urine using citrate or bicarbonate to maintain a pH of 6.0-6.5 and a higher daily intake of large quantities of fluids (Morton *et al.* 2002). It has also been suggested that treatment with Allopurinol may be beneficial in patients with hyperuricosuria as it blocks uric acid production thereby causing a reduction in the heterogeneous nucleation of CaOx (Ettinger 1991).

Urinary Citrate

One of the most crucial inhibitors of stone formation is citrate. In the urine, citrate binds with free calcium ion thereby forming a soluble complex and inhibiting the precipitation of calcium oxalate and calcium phosphate (Meyer and Smith 1975, Kok *et al.* 1987). Nucleation, growth and aggregation of pre-formed calcium oxalate and calcium phosphate crystals are also retarded by citrate to hinder the development of stones (Ryall *et al.* 1981, Nicar *et al.* 1987, Kok *et al.* 1987, Tiselius *et al.* 1993, Parks *et al.* 1996, Schwille *et al.* 1999). Hypocitraturia (i.e. low urinary citrate excretion) has been found to differ in male and female controls. It is defined as urinary excretion of citrate less than 200 mg/day in men and less than 400 mg/day in women (Hodgkinson 1962). In both stone formers and non-stone formers hypocitraturia results from increased reabsorption of citrate in the renal proximal tubules of the kidney (Pajor 1999, Hering-Smith *et al.* 2000). It has been reported that about 19-63% of patients with CaOx nephrolithiasis have hypocitraturia (Pak 1991).

Hypocitraturia is correctable and alkali therapy has been prescribed for the treatment of this defect. However, the efficacy of alkali therapy on its own in treating hypercalciuria related hypocitraturia has been deemed insufficient hence the addition of a thiazide diuretic to the therapeutic regimen is necessary (Menon *et al.* 1998).

Urinary Magnesium

Hypomagnesuria (decreased urinary magnesium excretion) is one of the primary causes of calcium oxalate crystallization and crystal growth. It has been reported that addition of magnesium to a sodium oxalate load or to meals containing oxalate reduces the urinary excretion of oxalate (Barilla *et al.* 1978, Berg *et al.* 1990a). This has often been attributed to the complexation of magnesium with oxalate ions thus forming a soluble magnesium oxalate salt and thereby reducing the uncomplexed oxalate ions in the urine. A correlation between the two inhibitors of crystallization, magnesium and citrate has been observed as most patients with hypomagnesuria also have hypocitraturia (Preminger *et al.* 1989).

Studies have noted a difference in urinary magnesium excretion between adults and children in western regions. It was found that the former excrete lower urinary magnesium than the latter, which explains the low incidence of stone formation in children compared to adults (Churchill *et al.* 1980, Miyak *et al.* 1998, Schwille *et al.*

1999). Similarly, it has generally been conceded that stone formers exhibit lower levels of urinary magnesium than controls (Trinchieri *et al.* 1991, Deshmukh and Khan 2006). However, contrary to this observation, some studies found the levels to be equal if not higher (Johansson *et al.* 1980, Drach 1988).

1.5 Diet

1.5.1 Reputed dietary and/or supplemental stone promoters

Calcium

Contrary to popular belief, the high incidence of CaOx nephrolithiasis is not a consequence of increased dietary calcium consumption (Curhan 1993, Curhan 1997b, Bushinsky 2002). Studies have shown that calcium restriction is no longer advisable for stone formers, with or without hypercalciuria, as a low-calcium diet leads to a greater oxalate absorption in the gastrointestinal (GI) tract, and thereby results in enhanced urinary oxalate absorption and excretion (Ettinger 1976, Hesse *et al.* 1981, Bastille *et al.* 1983, Martini *et al.* 1993, Heilberg *et al.* 1996). In fact, Curhan and colleagues (1993, 1997b) also investigated the effect of dietary calcium on kidney stone formation in both men and women and they observed that high calcium intake does lower intestinal binding of oxalate by calcium and prevents urinary crystallization. A study on male smokers showed no increase in risk of stone formation after dietary calcium intake (Hirvonen *et al.* 1999). Additionally, to find further proof on these findings, a study of normal men showed that simultaneous ingestion of a sufficient amount of calcium with oxalate-containing foods can help to prevent a marked hyperoxaluria (Hess *et al.* 1998).

There have been contradicting findings with regard to the association of calcium supplements with the risk of stone formation. Two studies by Curhan and colleagues have shown supplementation with calcium induced a nonsignificant risk of stone formation in men (Curhan *et al.* 1993) and a significant increase in risk in women (20%) (Curhan *et al.* 1997b). Their explanation for the conflicting observations was the fact that the calcium supplement was taken with breakfast or without meals by most subjects, further from the time of their intake of dietary oxalate. The consequence of this is the elevation of absorption and urinary excretion of calcium, with a loss in opportunity for the intestinal binding of calcium to oxalate. This

highlights, therefore, the importance of the timing of ingestion of supplemental calcium for optimum results. Another study has reported an inverse correlation between the urinary oxalate-to-creatinine ratio and calcium intake in healthy men and women subjects subsequent to the elimination of calcium supplements after frequent consumption (Lemann *et al.* 1996).

However, by contrast, supplementation with calcium citrate in normal women revealed a nonsignificant increase in calcium oxalate saturation after three months (Sakhaee *et al.* 1994). Likewise, Levine *et al.* (1994) observed no significant changes in calcium oxalate saturation in female stone formers after six months of calcium citrate supplementation. More studies are therefore required to elucidate the association between calcium supplements and risk of kidney stones.

Oxalate

It is well known that an increased intake of dietary oxalate leads to hyperoxaluria, a key risk factor for calcium oxalate kidney stone formation. Urinary oxalate is derived not only from diet but also from endogenous synthesis. There seems to be uncertainty as to how much urinary oxalate comes from the diet. Some researchers have reported that diet contributes about 10 to 20 % (Williams and Smith 1983) while others have recently argued that it contributes 40-50 % (Holmes *et al.* 2001). The remainder and more substantial part of urinary oxalate is derived endogenously.

It has been estimated that a Western diet contains an oxalate content of 50 to 100 mg/day, whereas vegetarian diets have more than 150 mg/day (Hesse and Siener 1997, Holmes and Kennedy 2000). High oxalate levels are found in foodstuffs such as spinach, rhubarb, beets, nuts, tea, wheatbran, cocoa and strawberries. Ingested dietary oxalate is believed to be absorbed both actively and passively anywhere along the gastrointestinal tract (Prenen *et al.* 1984) and most researches have posited that 3-8 % of oxalate is absorbed from food (Finch *et al.* 1981, Brinkley *et al.* 1990). Furthermore, studies have revealed that stone formers possess a metabolic disorder in relation to gastrointestinal absorption of oxalate. It has been shown that stone formers absorb approximately 50 % more oxalate than non-stone formers (Hesse *et al.* 1999).

Berg *et al.* (1990b) studied the response to an oral load of ^{14}C-labeled oxalic acid in recurrent idiopathic calcium stone patients and normal subjects. Their results showed an increased calcium absorption (>15 %) in 40 % of the patients compared to <10 % in 68 % and ≤15 % in 32 % of normal subjects. A few plausible mechanisms have been proposed and they include, less available unbound calcium to bind to oxalate due to increased calcium binding to free fatty acids and a deficiency of oxalate-degrading bacteria in the gut or increased gut permeability consequential to augmented delivery of bile salts (Sutton and Walker 1994). This clearly indicates that an elevated consumption of oxalate-rich foods is not always a predisposing factor to hyperoxaluria in some CaOx stone formers. As mentioned earlier, it is imperative to avoid calcium restriction as it may result in increased intestinal absorption of oxalate and thus an elevated urinary oxalate excretion.

Evidence for the toxicity of oxalate to renal epithelial cells has been provided (Khan and Thamilselvan 2000, Koul *et al.* 2003, Jonassen *et al.* 2005). It is hypothesized that an increased urinary excretion of oxalate induces renal tubular injury, which is manifested by an elevated excretion of marker enzymes including N-acetyl-β-glucosaminidase in the urine (Huang *et al.* 2003). This topic will be dealt with later in much detail in Chapter Four of this thesis.

Animal Protein

The relation between the intake of animal protein and the high incidence of calcium oxalate kidney stone formation in industrialized countries has been examined. Curhan *et al.* (1993) found a 33 % risk increase in men who consumed ≥77 g/day of animal protein compared to those who consumed ≤50 g/day. Studies have indicated that increased dietary protein consumption triggers an unfavourable increase in the urinary excretion of calcium, oxalate and uric acid and lowers citrate excretion (Coe et al 1976, Robertson *et al.* 1979, Urivetzky 1987, Trinchieri *et al.* 1991, Holmes *et al.* 1993, Giannini *et al.* 1999, Nguyen *et al.* 2001). Hypercalciuria has been ascribed to an increase in the production of endogenous acids during metabolism. Consequently, bone resorption increases followed by the increase in the filtered load of calcium, hyperoxaluria (due to the high oxalate synthesis), hyperuricosuria (due to purine overconsumption) and hypocitraturia (due to high tubular resorption of citrate) (Hodgkinson 1962, Breslau *et al.* 1988, Barzel and Massey 1998).

Kok and co-workers (1990b), in a quest to examine the chemical composition of urine of healthy subjects after consumption of four different diets, with a high protein diet being one of the diets, found a significant increase in calcium and uric acid excretions and a decrease in the excretion of citrate. These results are in agreement with the findings of the aforementioned studies. In addition, COM crystallization kinetics, which these authors also measured, showed that the high protein diet resulted in the decreased inhibition of COM crystal aggregation. They attributed this effect to the reduced levels of urinary citrate, a well known inhibitor of CaOx stone formation. In light of these and other findings, the efficacy of a diet containing normal calcium, low animal protein and low sodium in the prevention and treatment of kidney stones has therefore been established and thus such a diet is highly recommended by Borghi *et al.* (2002).

Sodium

Many investigators have emphasized the importance of limiting the intake of sodium chloride (NaCl) as high amounts overtly elevate excretion of calcium (Muldowney *et al.* 1982, Kok *et al.* 1990b, Massey and Whitting 1996, Blackwood *et al.* 1999). This effect has been ascribed to the inhibition of sodium and calcium resorption.

Lemann *et al.* (1979) showed that every increase of 100mmol of sodium in the diet significantly increases urinary calcium by 25 mg. Yamakawa *et al.* (1996) investigated the effect of a simultaneous intake of NaCl and sodium oxalate (Na_2Ox) and of NaCl alone. Their results showed a significant decrease in the oxalate-to-creatinine ratio after a simultaneous intake of NaCl and Na_2Ox compared to the intake of Na_2Ox alone. On the other hand, both the simultaneous intake of NaCl and Na_2Ox and Na_2Ox alone induced no increase in the urinary calcium-to-creatinine ratio. However, intake of NaCl alone induced a remarkable increase in the calcium-to-creatinine ratio and a decrease in the oxalate-to-creatinine ratio. They deduced from their results that sodium chloride detemines oxalate absorption and increases the risk of stone formation.

A positive correlation between a high NaCl intake and bone loss has also been established. In fact, a previous study revealed that an intake of ≥16 g per day in calcium stone formers poses a risk of low bone mineral density (Martini *et al.* 2000).

Refined Carbohydrate

It is conceded that an excessive consumption of high carbohydrate diets, especially those containing refined carbohydrates, induces unfavourable changes in urine composition which may increase the risk of stone development (Hodgkinson and Heaton 1965, Lemann *et al.* 1969, Thom *et al.* 1978, Rao *et al.* 1982). Evidence suggests that consumption of refined carbohydrates brings about more pronounced and rapid changes in the urinary excretion of calcium and its absorption (MacLeod and Blacklock 1979). Sucrose, for instance, when consumed in large quantities is considerably lithogenic as it induces an elevation of the urinary calcium excretion which may increase the risk of renal stone formation (Lemann *et al.* 1969). The magnitude of this effect is more prominent in stone formers than in controls. Rao and co-workers (1982) postulated that insulin and glucagon, the hormones involved in carbohydrate metabolism, may be responsible for the differences in the response to a carbohydrate load in the two groups. The effect of plasma insulin has been elucidated by its role in the renal tubular electrolyte transport. It brings about changes in the urinary electrolyte composition as calcium reabsorption is reduced predominantly in the proximal tubules (DeFronzo *et al.* 1976).

Nguyen *et al.* (1986) administered a 75 g glucose load to healthy subjects, which provoked an increase not only in urinary calcium but also in oxalate excretion. Similarly, in a different study where stone formers and healthy controls ingested a 100 g glucose load, an increase in the excretion of the aforementioned urinary parameters was observed in both groups and the rise in the relative supersaturation with respect to calcium oxalate and uric acid was noted in the patient group (Gluszek 1988). In addition, a comparative study to investigate the effect of a glucose or fructose challenge in normal subjects was undertaken. On one hand, a concomitant augmentation of calcium and oxalate excretion in the urine was observed after the glucose challenge. On the other hand, fructose caused an increase in the urinary excretion of calcium and in contrast, a decrease in the urinary excretion of oxalate (Nguyen *et al.* 1989). However, a recent study examined the association between fructose intake and stone risk in three distinct cohorts comprising 93 730 older women, 101 824 younger women and 45 984 men and revealed a positive correlation (Taylor and Curhan 2008). Moreover, compelling evidence suggests that fructose augments the production of uric acid and thus leads to uric acid stone formation (Choi *et al.* 2005).

Of interest in the context of the present thesis is that consumption of carbohydrates has been shown to be higher in the South African black population than in the white population (Vorster and Nell 2001). This aspect will be fully explored in Chapter Two.

1.5.2 Reputed dietary and/or supplemental stone inhibitors

Citrate

The protective effect of potassium citrate (Pak and Fuller 1986, Abdulhadi *et al.* 1993, Barcelo *et al.* 1993) and sodium potassium citrate (Schwille *et al.* 1985, Berg *et al.* 1990, Hofbauer *et al.* 1994) supplementation in the management and treatment of urolithiasis has been of wide interest. The two citrate-containing products are associated with the formation of complexes with calcium thereby reducing the concentration of free Ca^{2+} and hence decreasing supersaturation of CaOx and CaP (Pak *et al.* 1982). They also increase the urinary pH, which concomitantly induces an increase in the dissociation of uric acid and reduces undissociated uric acid levels (Heilberg and Schor 2006). Calcium citrate (Sakhaee *et al.* 1994), calcium sodium citrate (Schwille *et al.* 1997) and potassium-magnesium citrate (Ettinger *et al.* 1997) have been rendered therapeutic in urolithiasis.

Magnesium

The efficacy of dietary magnesium in the inhibition of crystallization with respect to CaOx and CaP has been reported. It does so by complexing oxalate to form magnesium oxalate, thereby reducing urinary oxalate excretion (Barilla *et al.* 1978, Berg *et al.* 1986). A study conducted in healthy men whose diet was assessed every four years using a food frequency questionnaire over 14 years showed a reduced risk of stone formation after a dietary intake of magnesium (Taylor *et al.* 2004).

Controversy over the effect of magnesium supplementation on the treatment of kidney stones has been of interest. The administration of magnesium hydroxide in a randomized trial did not have a beneficial effect in preventing the recurrence of calcium oxalate urolithiasis (Ettinger *et al.* 1988). This is likely due to the fact that the magnesium supplement was not taken with meals and participants were on a low oxalate diet which may have resulted in reduced intestinal binding of oxalate by magnesium. Similarly, magnesium oxide has been proven ineffective in the treatment of recurrent stones (Fetner *et al.* 1978). On a positive note, magnesium

supplements have been endorsed in recent years as they have shown their effectiveness in increasing urinary magnesium excretion thus inhibiting oxalate absorption and excretion (Zimmerman *et al.* 2005). General therapeutic measures which have proved effective for a low urinary excretion of magnesium include magnesium citrate (Lindberg *et al.* 1990, Pak 1990) and intake of magnesium- and bicarbonate-rich mineral water (Karagülle *et al.* 2007).

1.5.3 Beverages

A question that is frequently asked is which fluids are recommended and which ones are prohibited. Water seems to the best beverage as it contains insignificant amounts of solutes and no caffeine. A previous study undertaken by Curhan and associates (1998b) in non-stone forming women demonstrated that of the 17 beverages that were studied, women who drank one daily 8 ounce glass of grapefruit had a 44% increased risk of forming stones. In contrast, women who consumed a similar amount of wine, coffee or tea had an 8% to 10% decreased stone risk.

The protective effect of coffee against stone formation has been supported by Goldfarb *et al.* (2005). However, notwithstanding this observation, a positive relationship between caffeine intake and urinary calcium excretion has been observed (Massey and Sutton 2004). Therefore moderate consumption of caffeinated beverages is highly recommended. In addition, apple juice has been found to have an adverse effect in stone formation (Curhan *et al.* 1996) and it is advised that excess consumption must be avoided. Consumption of lemonade, however, has a beneficial effect as it contains citrate, a well-known inhibitor of stone formation (Seltzer *et al.* 1996, Kang *et al.* 2007, Penniston *et al.* 2007). Studies have shown that taurine, which is one of the major ingredients in the well-known Red Bull energy drink, reduces the urinary excretion of both glycolate and oxalate in rats (Talwar *et al.* 1985). A study investigating the efficacy of taurine in lowering glycolate urinary excretion is described in Chapter Five of this thesis.

1. 6 Urolithiasis in South Africa

In South Africa, 12-15% of the white population is susceptible to CaOx stone formation but this disorder is virtually non-existent in the black population, affecting less than 1% of this race group, as mentioned earlier (Meyers *et al.* 1994, Whalley *et*

al. 1998). Studies have shown that South African blacks have lower urinary calcium (Muskat *et al.* 1951, Modlin 1967, Whalley *et al.* 1998) and relatively higher urinary oxalate levels than whites (in women only) (Whalley *et al.* 1998). In addition, studies on urine composition of these two ethnic groups have also shown that blacks have a lower urinary citrate compared to whites (Modlin 1967, Whalley *et al.* 1998). Some of these results are surprising and counterintuitive; none of them provides a compelling explanation for the rarity of urolithiasis in this population group.

In an attempt to elucidate the differences in stone frequency in the two race groups, several studies have been undertaken by the kidney stone research group at the University of Cape Town. These have identified different inhibitory roles for different urinary proteins (Craig *et al.* 1999, Webber *et al.* 2002, Rodgers *et al.* 2006). However, more importantly in the context of the present thesis, intriguing results have emerged from dietary studies. Among these, it has been demonstrated that South African black and white male subjects display different renal responses to various lithogenic and anti-lithogenic dietary challenges (Lewandowski *et al.* 2001, Rodgers and Lewandowski 2002, Lewandowski and Rodgers 2004). In addition, Lewandowski and co-workers (2005) found that despite a higher consumption of dietary oxalate in blacks compared to their white compatriots, urinary oxalate levels were equal to or lower in the former group. These findings prompted the authors to suggest that blacks have a superior renal handling mechanism for oxalate. As mentioned previously in this Chapter, dietary oxalate may lead to cellular injury. This aspect will be fully explored in Chapter Four.

1.7 Objectives

Based on the aforementioned studies and the ongoing interest of the Kidney Stone Research Laboratory at the University of Cape Town in the different renal handling mechanisms of black and white South Africans in response to different dietary and supplementary challenges, the following general objectives were defined for the present thesis:

- to investigate in healthy South African black and white subjects their response to the ingestion of refined carbohydrates

- to investigate the effect of the ingestion of dietary oxalate challenges on renal cell injury in the two race groups

More specific aims and objectives will be described in the chapters which follow.

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Chapter Two

Investigation of the effect of glucose, sorbitol and xylitol ingestion on the excretion of the key urinary risk factors in black and white South African subjects

2.1 Introduction

As discussed in the previous Chapter, numerous studies over the years have commonly identified a high-carbohydrate diet as one of the causes of increased excretion of urinary risk factors which result in CaOx kidney stone formation (Hodgkinson and Heaton 1965, Lemann *et al.* 1969, Thom *et al.* 1978, Rao *et al.* 1982). Evaluation of the effect of intake of sugars such as glucose (Glu) or sucrose (Suc) in CaOx stone formers and normal subjects, indicated a marked increase in urinary calcium excretion in the former (Lemann 1969). Nguyen and coworkers (1994) observed an increase in urinary calcium and oxalate excretion in healthy subjects after ingestion of dark chocolate containing Suc. Whilst a rise in plasma insulin with hypercalciuria following a carbohydrate load has been reported in idiopathic calcium stone formers (Rao *et al.* 1982), a comparative study showed no hypercalciuretic effect in spite of the high plasma insulin levels in noninsulin-dependent diabetics and controls (Garg *et al.* 1990). The latter study was contrary to the suggestions by DeFronzo *et al.* (1975) that high plasma insulin levels are a prelude to higher urinary calcium excretion.

The association of carbohydrate ingestion and kidney stone formation prompted investigators to study another class of carbohydrates known as polyols such as lactitol (Lac), mannitol (Man), sorbitol (Sor), xylitol (Xyl) and maltitol (Mal). Also known as sugar alcohols, polyols are low digestible carbohydrates which are commonly used in the food industry as sweeteners. Chemically, the aldehyde or ketone group found in other common sugars like Glu is replaced by a hydroxyl group in polyols which would account, partly, to their metabolic differences. Structural differences in the polyols themselves are worthy of note, for example, Xyl has a shorter chain than Sor and even though they are both monosaccharides their metabolism could be different. With the exception of erythrol (Ery), polyols are produced by hydrogenation of glucose, lactose, maltose, palatinose, xylose and partially hydrolysed starch derivatives (Oku and Nakamura 2002). What sparked interest in studying these is that, unlike other carbohydrates, they are slowly and partially absorbed (ranging from 0% for Lac to about 80% for Sor) (Patil *et al.* 1987, Langkilde *et al.* 1994) from the small intestine and are metabolized in an insulin-independent manner and thus have little or no effect on plasma glucose, which makes them more suitable for diebetics (de Kalbermatten *et al.* 1980).

A number of clinical and experimental studies on polyols conducted in humans and animals have been well documented. Hypercalciuria has been observed in rats after administration of high dietary concentration of certain polyols and this has been attributed to the likelihood of increased calcium absorption (Hämäläinen *et al.* 1985, Amman *et al.* 1988, Brommage *et al.* 1993). Several human studies have demonstrated that polyols have little or no adverse effects on the urinary risk parameters for CaOx kidney stone formation even at elevated rates of consumption (Förster *et al.* 1981, Francis *et al.* 1986, Egger *et al.* 1989). For example, in contrast to the observation of hypercalciuria in rats, when healthy male and female subjects were given a 40-100 g oral dose of Xyl per day over a period of 18 days, no increase in plasma or urinary calcium was observed (Förster *et al.* 1981). Results of a study conducted by Mäkinen and colleagues (1981) showed that intake of 50 g per day of Xyl for 2 years by a group of 41 male and female subjects produced no differences in urinary calcium in comparison to the control groups which were administered fructose or sucrose. Similarly, no changes in the urinary excretion of calcium were observed after ingestion of 20-40 g of Lac per day for one month by 12 subjects (Egger *et al.* 1989). On the other hand, 10 g of Sor resulted in the reduction of the fractional absorption of radiolabeled calcium in 10 women (Francis *et al.* 1986).

Furthermore, in a study undertaken by Nguyen *et al.* (1993) to compare the effects of a 20 g oral load of four polyols-Sor, Xyl, Lyc and Mal- with that of Glu in 10 healthy subjects over a 5-day period, Xyl was the only polyol that overtly increased the urinary excretion of calcium as well as that of oxalate and phosphate.

In view of the reported differences between black and white subjects in terms of their renal handling of different lithogenic agents, the present study was undertaken to measure the response to the two polyols sorbitol (Sor) and xylitol (Xyl), as well as glucose (Glu) in subjects of South Africa's two population groups and to examine whether different handling mechanisms of the three sugars exist in the two groups.

The main objectives of this study are:

- to examine the effect of oral administration of Glu, Sor and Xyl on the excretion of the key urinary risk factors in black and white healthy male South African subjects.

- to assess the physico-chemical properties of the urines from the two groups before and after ingestion of the three sugars.

- to compare serum glucose excretions in the two groups post Glu, Sor and Xyl loading.

2.2 Subjects and Methods

Subjects

Ten black and ten white healthy male subjects (age range 18-30 years) from the student cohort of the University of Cape Town volunteered for the study. The protocol was submitted to the Ethics Committee of the University and approval was granted. Clinical examinations revealed normal renal function, no history of renal stone disease or diabetes mellitus. Subjects were not allowed to ingest any drugs or supplements which might affect calcium, oxalate or phosphorus metabolism.

Test Sugars

Glu (Tongaat Hulett Starch, Germiston, South Africa, 99% purity with composition [mol/l x 10^{-3}] Cit: 3.34, Ox: 0.01, Ca: 0.42, Mg: 0.06, Na: 7.41, K: 0.12, P: <0.2), Sor (Sigma Aldrich, Steinheim, Germany, 97% purity with composition [mol/l x 10^{-3}] Cit: 3.35, Ox: 0.01, Ca: 0.32, Mg: 0.16, Na: 5.23, K: 0.56, P: <0.2) and Xyl (Sigma Aldrich, Steinheim, Germany, 99% purity with composition [mol/l x 10^{-3}] Cit: 1.11, Ox: 0.01, Ca: 0.25, Mg: 0.04, Na: 7.41, K: 0.02, P: <0.2) were administered randomly to each subject.

Experimental Procedure

A double-blind design was adopted for the study and a 1 week washout period was observed between the protocols. Subjects were on a self-selected diet which controlled for calcium (1 g/day) and carbohydrate (300 g/day) intake for 7 days. Table 2.1 shows a diet sheet comprising foods and portions that subjects were instructed to choose from and consume daily during the 7-day period. On day 8, subjects consumed a strict standardized diet (Table 2.2) with the nutrient content as shown in Table 2.3 and collected urine for 24 h. During this period, subjects fasted overnight for 12 h and on day 9 they drank 250 ml of water (Caledon Natural Spring Water with composition (mg/l) of Ca: 6.1, Mg: 1.0, Na: 17.0, K: 5.0, Fe: 0.01, Cl: 23.0, SO_4: 1.7, bicarbonate: 32.0, pH: 6.4) in the morning (corresponding to time 0h), collected a baseline urine sample 1 h later and then ingested immediately a solution of Glu, Sor or Xyl (20 g in 250 ml water). Blood samples were drawn at the end of the baseline period and 30 min after ingestion of the sugar solutions by a professional nurse. Urine was collected hourly for the next 3 hours. The standardized diet was then resumed and urine was collected for the remaining 20 h

of the 24 h test period. Sera and urine samples were stored at 4°C until analysis. After each test, the full 24 h urine collection was reconstituted by volumetric addition of the various fractions.

Table 2.1: Self-selected standardized sheet depicting the variety of foods and portions consumed by subjects during 7 days before ingestion of each sugar

DAILY ALLOWANCE	*PORTION SIZE (1 PORTION EXAMPLES)*
3 milk portions	1 cup milk 1 cup yoghurt
Choose any 7 fat portions	1 tsp butter 1 tsp margarine 1 tsp sunflower, canola or olive oil 1 tsp mayonnaise ¼ of a avocado pear 2 tsp peanut butter
Choose any 3 vegetable portions	½ cup cooked vegetables 1 cup green fresh salad * see critical food list
Choose any 3 fruit portions	any medium-sized fruit ½ cup fruit juice * see critical food list
Choose any 14 starch portions	1 slice of bread ½ cup cooked porridge ½ cup cereal 1 weetbix 3 provitas 1 medium / 2 small potatoes ½ cup cooked rice, samp, pasta ½ cup legumes (eg lentils, beans, chickpeas, soya)
Choose any 7 protein portions	30g meat/chicken/fish = 1matchbox 45g cottage cheese = 1 heaped Tbsp 30g hard cheese = 1 matchbox 2 cheese wedges/4tsp cheese spread 1 egg
Choose any 3 sugar portions	1 tsp sugar 200ml pudding 50ml cool drink 2 blocks chocolate (not to be exceeded) 2 Jelly Beans

*Appendix CD: Chapter Two

Table 2.2: Constituents of the strict standardized diet ingested at day 8 and 9 and the time of consumption of each meal

MEAL	*DAY 8*	*DAY 9*
Breakfast: 8h00	50 g bread roll 8 g butter 25 g strawberry jam 60 g cornflakes 250 ml 2% low fat milk 250 ml coffee	50 g bread roll 8 g butter 25 g strawberry jam 60 g cornflakes 250 ml 2% low fat milk No coffee
Snack: 11h00	27 g muesli bar ½ cup fruit salad	27 g muesli bar ½ cup fruit salad
Lunch: 13h00	140 g soya mince* 120 g mixed vegetables 200 g pasta	140 g soya mince 120 g mixed vegetables 200 g pasta
Snack: 16h00	150 g apple 175 g low fat fruit yoghurt	150 g apple 175 g low fat fruit yoghurt
Supper: 18h00	4 slices of whole wheat bread 16 g butter 56 g cheddar cheese 50 g tomato	4 slices of whole wheat bread 16 g butter 56 g cheddar cheese 50 g tomato
Snack: 20h00	1 slice of whole wheat bread 20 g peanut butter	1 slice of whole wheat bread 20 g peanut butter

* soya mince for 7 portions: 1 onion, 1 heaped tsp garlic, 125 g button mushrooms, 1 tin peeled tomatoes, 1 cup soya mince (3 cups water), salt, pepper, paprika, cayenne pepper

Beverages (2.25 l/day):

250 ml coffee (4 g coffee powder, 4 g creamer, 10 g sugar in 250 ml water (Day 8 only))
700 ml Caledon water until 13h00
600 ml Caledon water until 19h00
700 ml Caledon water until 22h00

Table 2.3: Nutrient content of the standardized diet

VARIABLE	*AMOUNT*
Energy (kJ)	1434
Total protein (g)	109.5
Total fat (g)	112
Carbohydrate (g)	302
Calcium (mg)	1001
Magnesium (mg)	415
Phosphorus (mg)	1781
Citric acid (mg)	1509
Oxalic acid (mg)	66

Analytical Methods

The hourly urine fractions (0-3 h) as well as the reconstituted 24 h urines were tested in order to detect any unfavourable presence of blood and/or infection (Combur 10 test strip, Boehringer Mannheim, Germany). Samples which tested positive were discarded. The pH and volume of each urine sample were measured routinely. Urinary calcium, potassium, magnesium and sodium (Fernandez and Kahn 1971, Trudeau and Freier 1967 and Willis 1961) were determined using a Varian 1275 flame atomic absorption spectrometer, whereas uric acid was measured using uricase (Fossati *et al.* 1980) and chloride using an ion selective electrode. Oxalate was determined using oxalate by an enzymatic method involving oxalate decarboxylase (Chiriboga 1963), citrate using citrate lyase (Gruber and Mollering 1966), inorganic phosphorus using ammonium molybdate (Dryer and Routh 1963) and creatinine using picric acid (Rock *et al.* 1986).

Serum glucose was measured using an oxygen rate method employing a Beckman Oxygen electrode in which 10 µl of a blood sample was injected in a reaction cup containing a glucose oxidase solution. The concentration of glucose which is directly proportional to the peak rate at which oxygen is consumed when reacting with glucose to form gluconic acid, was then determined (Kadish *et al.* 1968).

The relative supersaturations (RS) of calcium oxalate (CaOx), brushite and uric acid were computed using the EQUIL 2 programme (Werness *et al.* 1985), whereas the Bonn Risk Index (BRI) was measured using a calcium electrode (Laube *et al.* 2004). All of these were assessed or measured in 24 h urine samples.

Crystallization Experiments

CaOx crystallization kinetics experiments were performed on 24 h urines filtered consecutively through a 0.75 μm pre-filter (Macherey-Nagel; GmBH and Co., Germany) and 0.45 μm nitrocellulose filter (Sartorius AG, Germany). These experiments were done in order to measure CaOx Metastable Limit, ^{14}C-oxalate deposition and crystal aggregation. In addition, the particle volume-size distribution was also measured to assess the particle formation kinetics. The description of these experiments follows

CaOx Metastable Limit

Filtered 10 ml aliquots of urine were spiked with increasing 100 μl concentrations of Na_2Ox every 2 min to induce CaOx crystallization and were incubated for 30 minutes at 37°C in a shaking water bath (Labcon Marketing Services, Johannesburg). The number of particles in the aliquots of urine was measured using a Coulter Counter Multisizer (Multisizer II, Coulter Electronic Ltd, England) (140 μm orifice, 2.8-90.0 μm particle size range). The minimum Na_2Ox concentration which was able to induce crystallization was used to determine the metastable limit (MSL) from a plot of particle number versus concentration of Na_2Ox (Ryall *et al.* 1985). An example of a graph for the determination of MSL is shown in Figure 2.1 in which the green dot corresponds to the MSL.

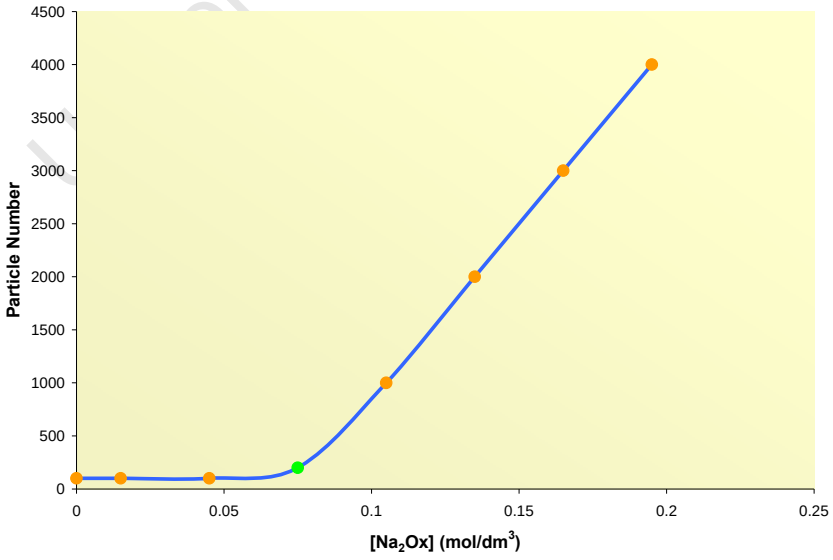


Figure 2.1: *Typical graph for the determination of MSL*

Crystal Deposition

30 ml aliquots of the urine samples were incubated at 30°C in a shaking water bath for approximately 10 min after being filtered. To each aliquot, 3.125 (Ci/100ml of radiolabeled oxalate (^{14}C-oxalate) (NEN, Boston, USA) was added, followed by spiking with 300 µl of the similar concentration of Na_2Ox used for the particle volume-size distribution experiment described shortly (page 55). Over 120 min, at 30 min intervals, the reaction was quenched by passing 2.5 ml of the aliquots into 250 µl of concentrated hydrochloric acid through a 0.22 µm Millipore filter. To each 10 ml of scintillation fluid (Zinsser Analytic, Frankfurt), 1000 µl of the acidified aliquots were then added and this step was performed in duplicate. The number of precipitated ^{14}C-oxalate crystals was then counted using a scintillation counter (1900CA Tri-Carb Liquid Scintillation Analyser, Packard Instruments Co, Inc., Meriden CT, USA). The percentage of precipitated ^{14}C-oxalate was determined from the following equation (Doyle *et al.* 1995):

$$\% \ ^{14}\text{C-oxalate precipitated} = 100 - 100x \ (\text{cpm}_{x\ \text{min}} / \text{cpm}_{0\ \text{min}})$$

[$\text{cpm}_{x\ \text{min}}$ = counts per minute at x min, $\text{cpm}_{0\ \text{min}}$ = counts per minute at 0 min]

CaOx Crystal Aggregation

I. Preparation of COM crystals

COM crystals were prepared prior to the aggregation experiment using a method by Pak *et al.* (1975). Calcium chloride (100 mM) and Na_2Ox (100 mM) were mixed by introducing equal volumes at a constant speed of 1.0 ml/min using a peristaltic pump (Gilson, France), with stirring, at room temperature. The mixture was stirred at 4°C for 1 week and then filtered using a 0.22 µm filter paper. Methanol was used to wash the crystals followed by drying at 37 °C for 1 h. The composition of the crystals was characterized by x-ray powder diffraction (XRD). X-ray diffraction patterns, as shown in Figure 2.2, were recorded using a Philips PW 1050/80 vertical goniometer in the 12-40 ^{o} 2θ range with CuKα radiation wavelength of 1.5418 Å produced at 40 kV and 25 mA. The d spacings and relative intensities were used to confirm the presence of COM crystals.

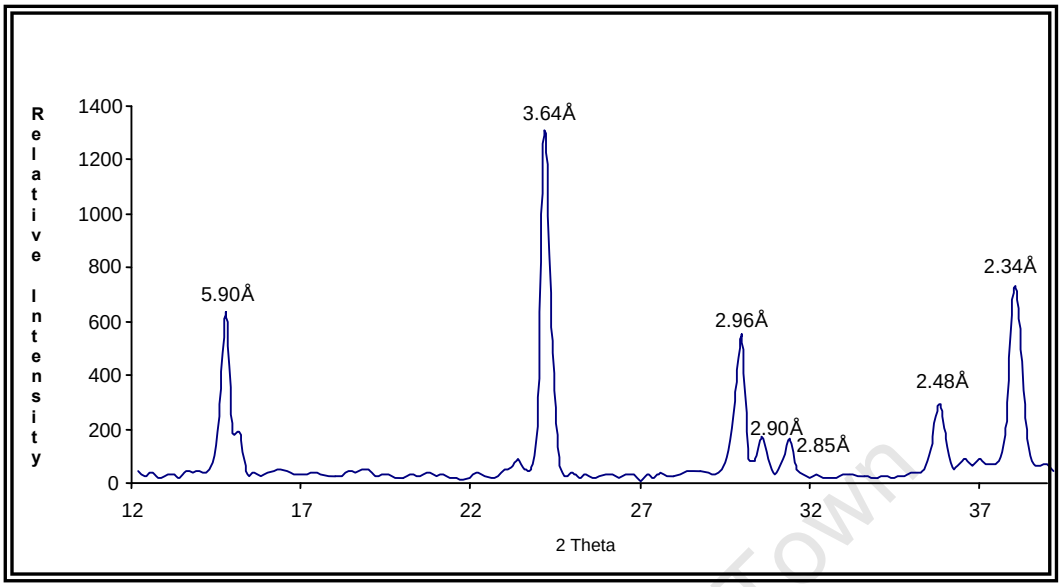


Figure 2.2: *XRD pattern of COM crystals*

II. Determination of inhibition of COM crystal aggregation

COM crystal slurry was prepared by mixing 0.8 mg of COM crystals with 1 ml of a buffer containing Tris-aminomethane (Tris).HCL (10 mM) and NaCl (90 mM) and the pH was adjusted to 7.2. The slurry was equilibrated while stirring at 1100 rpm overnight at 37°C. Filtered urine aliquots (400 µl) were then combined with the COM slurry (1600 µl) and the mixture was equilibrated at 37°C for 2 h with stirring. The control was 2000 µl of the slurry alone. The absorbance of was monitored at 620 nm using a spectrophotometer (Specord 40, United Scientific, South Africa) coupled with a stirrer for 15 min. Initially, slow stirring at 1100 rpm to induce crystal aggregation was applied until equilibrium was reached. Thereafter, the stirrer was stopped and the decreasing absorbance which indicated the aggregation rate of the COM crystals was then monitored. The rate of crystal aggregation was determined from the slope of the linear decrease in absorbance in the graph of absorbance vs time. The following equation was then used to determine percentage inhibition of aggregation (Hess *et al.* 1989):

$$\% \text{ Inhibition of aggregation} = 100\text{-}100(S_c/S_t)$$

[S_c = slope of the control sample, S_t = slope of the test sample]

Particle Volume-Size Distribution

The urine was spiked with 0.5 ml Na_2Ox/50 ml urine using a 15 mmol/dm^3 concentration above the previously determined MSL and was then incubated at 37°C in a shaking water bath for 120 min. The particle volume-size distribution was measured over 120 min using a Coulter Multisizer followed by the determination of the mean particle-size and the total volume of precipitated particles (Ryall *et al.* 1985).

Statistical Analysis

Data were analysed by one-way analysis of variance (ANOVA) at statistical significance of $p \leq 0.05$.

2.3 Results

Baseline

The mean 24 h urinary excretions of various parameters before the ingestion of Glu, Sor or Xyl are presented in Table 2.4. Blacks had significantly lower urinary calcium, magnesium, sodium, urate and phosphate. In addition, the RS of brushite was lower in blacks while the RS of uric acid was lower in whites. Moreover, the BRI was higher in the latter group. No further statistically significant differences were observed between the two groups.

GLUCOSE

24h Collections

Subsequent to the ingestion of Glu, no statistically significant changes were observed in the 24 h urine parameters in either race group, except for a significant increase in urine volume in blacks as shown in Table 2.5.

Fractional Collections (Non-Normalized Excretion)

At 0 h (Table 2.6) prior to the ingestion of Glu, the urinary citrate and potassium excretion were higher in blacks, with chloride approaching significance. Following the intake of Glu, at 1 h, the only significant difference was the decrease in urinary sulphate in whites, while the urine volume was approaching a significant increase in this group, as indicated in Table 2.7.

However, at 2 h a number of significant differences were observed (Table 2.8). Glu caused an increase in urine volume and a decrease in urinary potassium, creatinine and phosphate in blacks. On the other hand, it decreased the urinary excretion of sulphate in whites. Elevated urine volume levels were also observed in this group.

Glu provoked a significant increase in the urinary pH and a decrease in urinary potassium and phosphate, with creatinine approaching a significant decrease in blacks at 3 h (Table 2.9). The urine volume also increased significantly in this group. Similarly, in the white group the urinary pH increased as well as the urine volume. A rise in urinary citrate excretion was also noted and a fall in urinary sulphate levels. In addition, urate was approaching a significant increase in this group.

Table 2.4: Mean baseline 24 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.07 ± 0.08	6.22 ± 0.06	0.0865
Volume (ml/24h)	1924 ± 110	2060 ± 87.6	0.2698
Citrate (mmol/24h)	2.99 ± 0.23	3.04 ± 0.23	0.7885
Oxalate (mmol/24h)	0.27 ± 0.01	0.29 ± 0.01	0.1881
Calcium (mmol/24h)	2.60 ± 0.27	4.19 ± 0.24	*0.0001
Magnesium (mmol/24h)	1.79 ± 0.22	3.45 ± 0.18	*0.0001
Sodium (mmol/24h)	93.1 ± 12.0	148 ± 20.5	*0.0328
Potassium (mmol/24h)	46.0 ± 4.98	54.4 ± 4.82	0.1560
Urate (mmol/24h)	2.94 ± 0.15	3.51 ± 0.14	*0.0061
Creatinine (mmol/24h)	17.8 ± 3.84	15.5 ± 0.68	0.5237
Phosphate (mmol/24h)	18.6 ± 1.16	27.3 ± 1.68	*0.0001
Chloride (mmol/24h)	160 ± 8.25	164 ± 7.71	0.9577
Sulphate (mmol/24h)	17.4 ± 0.86	20.3 ± 1.28	0.0815
Tiselius risk index	198 ± 24.2	284 ± 19.8	0.6843
RS Brushite	0.32 ± 0.24	4.39 ± 1.67	*0.0002
RS CaOx	3.29 ± 1.25	4.39 ± 1.67	0.2119
RS Uric Acid	1.54 ± 1.24	0.83 ± 0.77	*0.0231
MSL (mol/dm^3)	0.15 ± 0.01	0.13 ± 0.01	0.1313
BRI (/l)	1.13 ± 0.40	3.87 ± 0.70	*0.0096

*statistically significant

Table 2.5: Mean 24 h urinary variables ± SE in black and white subjects after ingestion of Glu

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Glu*	*p*	*Baseline*	*Post-Glu*	*p*	*p Post-Glu*
pH	6.07±0.08	6.37±0.16	0.0628	6.22±0.06	6.41±0.07	0.0931	0.8093
Volume (ml/24h)	1924±110	2452±126	*0.0201	2060±87.6	2453±147	0.2850	0.9986
Citrate (mmol/24h)	2.99±0.23	2.92±0.51	0.9858	3.04±0.23	3.17±0.35	0.7675	0.6822
Oxalate (mmol/24h)	0.27±0.01	0.31±0.02	0.0907	0.29±0.01	0.32±0.03	0.9771	0.8157
Calcium (mmol/24h)	2.60±0.27	2.22±0.20	0.5020	4.19±0.24	3.57±0.46	0.1873	*0.0245
Magnesium (mmol/24h)	1.79±0.22	1.77±0.28	0.9292	3.45±0.18	2.45±1.20	0.0647	0.2813
Sodium (mmol/24h)	93.1±12.0	112±18.2	0.4815	148±20.5	155±41.9	0.3478	0.2551
Potassium (mmol/24h)	46.0±4.98	36.1±10.2	0.4462	54.4±4.82	36.6±8.77	0.2074	0.9624
Urate (mmol/24h)	2.94±0.15	2.59±0.25	0.2851	3.51±0.14	3.35±0.28	0.5881	0.0705
Creatinine (mmol/24h)	17.8±3.84	13.1±1.68	0.5287	15.5±0.68	15.5±1.40	0.9606	0.2991
Phosphate (mmol/24h)	18.6±1.16	18.5±3.83	0.9748	27.3±1.68	25.5±3.73	0.6274	0.2237
Chloride (mmol/24h)	160±8.25	153±11.8	0.5612	164±7.71	180±17.3	0.3397	0.2663
Sulphate (mmol/24h)	17.4±0.86	18.9±1.08	0.4202	20.3±1.28	17.1±2.24	0.2266	0.5450
Tiselius Risk Index	198±24.2	299±48.1	0.6368	284±19.8	254±27.4	0.7381	0.3697
RS Brushite	0.32±0.24	0.28±0.02	0.7374	4.39±1.67	0.65±0.04	0.0859	*0.0315
RS CaOx	3.29±1.25	2.65±0.38	0.3782	4.39±1.67	2.79±0.99	0.1084	0.8681
RS Uric Acid	1.54±1.24	0.52±0.03	0.1014	0.83±0.77	0.49±0.03	0.0843	0.8602
MSL (mol/dm³)	0.15±0.01	0.17±0.02	0.3650	0.13±0.01	0.13±0.02	0.9140	0.1198
BRI (/l)	1.13±0.40	0.56±0.21	0.2415	3.87±0.70	2.32±1.05	0.2523	0.1392

*statistically significant

Table 2.6: Mean baseline 0 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.10 ± 0.25	6.13 ± 0.22	0.9316
Volume (ml/h)	198 ± 49.8	101 ± 21.5	0.0641
Citrate (mmol/h)	0.34 ± 0.10	0.11 ± 0.02	*0.0133
Oxalate (mmol/h)	0.05 ± 0.01	0.04 ± 0.01	0.8771
Calcium (mmol/h)	0.16 ± 0.06	0.14 ± 0.03	0.6595
Magnesium (mmol/h)	0.15 ± 0.06	0.13 ± 0.02	0.6636
Sodium (mmol/h)	8.30 ± 2.93	5.50 ± 1.56	0.3756
Potassium (mmol/h)	6.31 ± 1.85	1.95 ± 0.58	*0.0202
Urate (mmol/h)	0.23 ± 0.08	0.09 ± 0.02	0.0667
Creatinine (mmol/h)	1.79 ± 0.58	0.85 ± 0.10	0.0778
Phosphate (mmol/h)	3.76 ± 1.63	1.07 ± 0.25	0.0704
Chloride (mmol/h)	18.3 ± 6.34	6.70 ± 1.62	†0.0555
Sulphate (mmol/h)	1.36 ± 0.55	0.71 ± 0.10	0.1631

*statistically significant; †approaching statistical significance

Table 2.7: Mean 1 h urinary variables ± SE in black and white subjects after ingestion of Glu

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Glu*	*p*	*Baseline*	*Post-Glu*	*p*	*p Post-Glu*
pH	6.10±0.25	6.05±0.19	0.8605	6.13±0.22	6.08±0.31	0.7011	0.9258
Volume (ml/h)	198±49.8	449±276	0.4663	101±21.5	164±37.8	†0.0546	0.3204
Citrate (mmol/h)	0.34±0.10	0.31±0.12	0.8375	0.11±0.02	0.12±0.02	0.9028	0.1630
Oxalate (mmol/h)	0.05±0.01	0.07±0.04	0.6367	0.04±0.01	0.03±0.01	0.2543	0.3114
Calcium (mmol/h)	0.16±0.06	0.28±0.19	0.6467	0.14±0.03	0.14±0.03	0.4661	0.4878
Magnesium (mmol/h)	0.15±0.06	0.24±0.17	0.7027	0.13±0.02	0.13±0.03	0.7651	0.5304
Sodium (mmol/h)	8.30±2.93	9.84±6.59	0.8551	5.50±1.56	4.46±1.31	0.3976	0.4334
Potassium (mmol/h)	6.31±1.85	8.26±6.06	0.6467	1.95±0.58	1.91±0.55	0.3424	0.3109
Urate (mmol/h)	0.23±0.08	0.47±0.34	0.5679	0.09±0.02	0.10±0.02	0.6275	0.2890
Creatinine (mmol/h)	1.79±0.58	2.66±1.68	0.6821	0.85±0.10	0.83±0.07	0.3381	0.2930
Phosphate (mmol/h)	3.76±1.63	4.41±3.47	0.8840	1.07±0.25	0.84±0.23	0.2284	0.3183
Chloride (mmol/h)	18.3±6.34	26.6±17.1	0.7024	6.70±1.62	7.00±1.28	0.9163	0.2692
Sulphate (mmol/h)	1.36±0.55	2.75±2.02	0.5758	0.71±0.10	0.58±0.08	*0.0173	0.2956

*statistically significant; †approaching statistical significance

Table 2.8: Mean 2 h urinary variables ± SE in black and white subjects after ingestion of Glu

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Glu*	*p*	*Baseline*	*Post-Glu*	*p*	*p Post-Glu*
pH	6.10±0.25	6.70±0.18	0.1091	6.13±0.22	6.42±0.25	0.3992	0.3919
Volume (ml/h)	198±49.8	328±26.6	*0.0286	101±21.5	291±38.4	*0.0004	0.4447
Citrate (mmol/h)	0.34±0.10	0.39±0.11	0.7307	0.11±0.02	0.19±0.04	0.0739	0.1099
Oxalate (mmol/h)	0.05±0.01	0.02±0.00	0.0988	0.04±0.01	0.03±0.01	0.2193	0.6189
Calcium (mmol/h)	0.16±0.06	0.07±0.02	0.1283	0.14±0.03	0.11±0.02	0.4506	0.1324
Magnesium (mmol/h)	0.15±0.06	0.06±0.01	0.1152	0.13±0.02	0.54±0.42	0.3438	0.2739
Sodium (mmol/h)	8.30±2.93	2.68±1.47	0.1007	5.50±1.56	4.14±1.19	0.4970	0.4510
Potassium (mmol/h)	6.31±1.85	1.48±0.45	*0.0112	1.95±0.58	2.05±0.54	0.8996	0.4250
Urate (mmol/h)	0.23±0.08	0.13±0.02	0.2134	0.09±0.02	0.13±0.02	0.1686	1.0000
Creatinine (mmol/h)	1.79±0.58	0.64±0.05	*0.0450	0.85±0.10	0.67±0.07	0.1670	0.7272
Phosphate (mmol/h)	3.76±1.63	0.35±0.07	*0.0314	1.07±0.25	0.55±0.14	0.0885	0.2161
Chloride (mmol/h)	18.3±6.34	6.60±0.93	0.0632	6.70±1.62	7.90±1.23	0.5629	0.4117
Sulphate (mmol/h)	1.36±0.55	0.48±0.20	0.1432	0.71±0.10	0.35±0.07	*0.0104	0.5571

*statistically significant

Table 2.9: Mean 3 h urinary variables ± SE in black and white subjects after ingestion of Glu

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Glu*	*p*	*Baseline*	*Post-Glu*	*p*	*p Post-Glu*
pH	6.10±0.25	6.89±0.14	*0.0128	6.13±0.22	6.72±0.13	*0.0343	0.4088
Volume (ml/h)	198±49.8	343±31.5	*0.0219	101±21.5	322±19.5	*0.0001	0.5638
Citrate (mmol/h)	0.34±0.10	0.61±0.17	0.2200	0.11±0.02	0.21±0.04	*0.0395	*0.0259
Oxalate (mmol/h)	0.05±0.01	0.02±0.00	0.1019	0.04±0.01	0.02±0.00	0.0609	0.8271
Calcium (mmol/h)	0.16±0.06	0.07±0.02	0.1181	0.14±0.03	0.13±0.03	0.9824	0.0807
Magnesium (mmol/h)	0.15±0.06	0.73±0.66	0.4555	0.13±0.02	0.53±0.41	0.3382	0.7883
Sodium (mmol/h)	8.30±2.93	4.48±2.84	0.3710	5.50±1.56	5.59±1.44	0.9659	0.7230
Potassium (mmol/h)	6.31±1.85	2.17±0.63	*0.0346	1.95±0.58	3.85±1.79	0.3271	0.4107
Urate (mmol/h)	0.23±0.08	0.14±0.02	0.2706	0.09±0.02	0.14±0.02	†0.0541	0.8550
Creatinine (mmol/h)	1.79±0.58	0.71±0.05	†0.0533	0.85±0.10	0.74±0.09	0.4378	0.7916
Phosphate (mmol/h)	3.76±1.63	0.43±0.09	*0.0354	1.07±0.25	0.65±0.14	0.1641	0.2194
Chloride (mmol/h)	18.3±6.34	8.11±1.24	0.0966	6.70±1.62	8.70±1.45	0.3691	0.7636
Sulphate (mmol/h)	1.36±0.55	0.37±0.08	0.0638	0.71±0.10	0.38±0.09	*0.0276	0.9553

*statistically significant; †approaching statistical significance

Fractional Collections (Excretion Normalized to Creatinine)

The concentrations of calcium, oxalate and phosphate were normalized by using creatinine as a reference standard, due to its constant excretion throughout the urine collection. The ratio of the urine parameters to creatinine provides a more accurate index which serves as a measure of the excretion of each parameter in urine samples collected randomly (Nordin 1959). Nguyen and coworkers (1993) expressed their urinary outputs as ratios.

The mean calcium, oxalate and phosphate to creatinine ratios at 0 h (baseline), 1 h, 2 h and 3 h for the two race groups before and after the intake of Glu are shown in Figures 2.3, 2.4 and 2.5, respectively. No changes in the urinary excretion of calcium (Ca), oxalate (Ox) and phosphate (P) were noted within the white group. However, phosphate excretion was significantly decreased in blacks at 2 h (p=0.0153) and 3 h (p=0.0269).

***Figure 2.3**: Mean hourly values for urinary calcium excretion [(mmol Ca per mmol Creatinine) x 10^2] for black and white subjects following the Glu challenge*

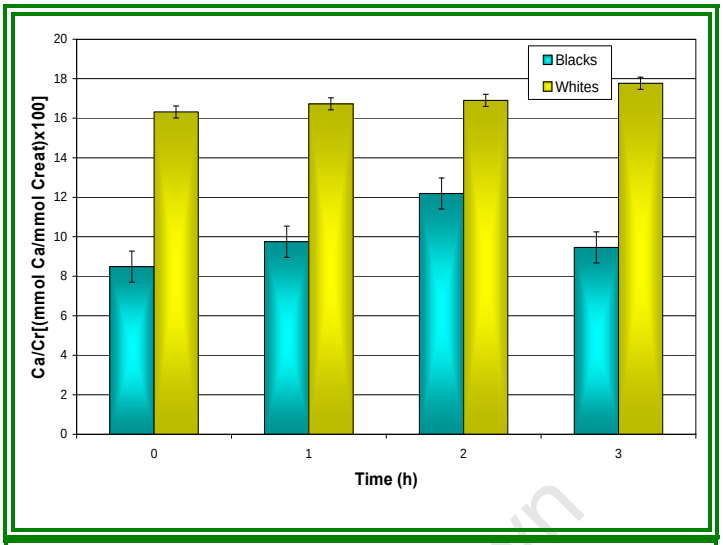


***Figure 2.4**: Mean hourly values for urinary oxalate excretion [(mmol Ox per mmol Creatinine) x 10^2] for black and white subjects following the Glu challenge*

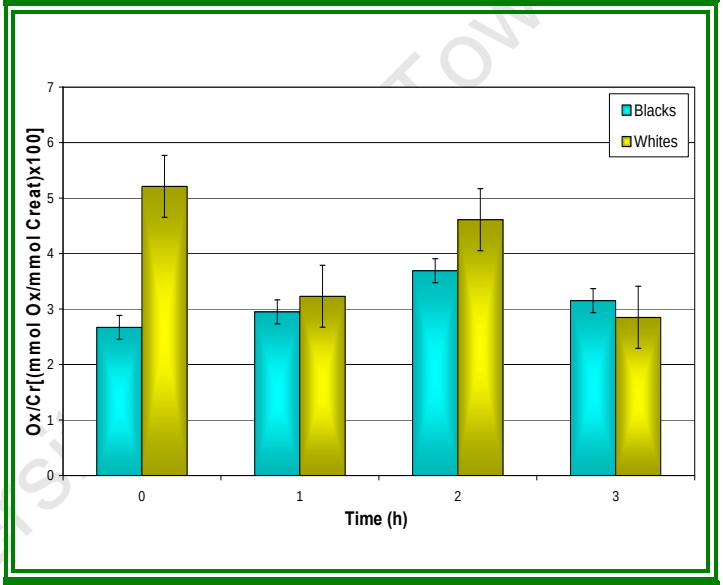
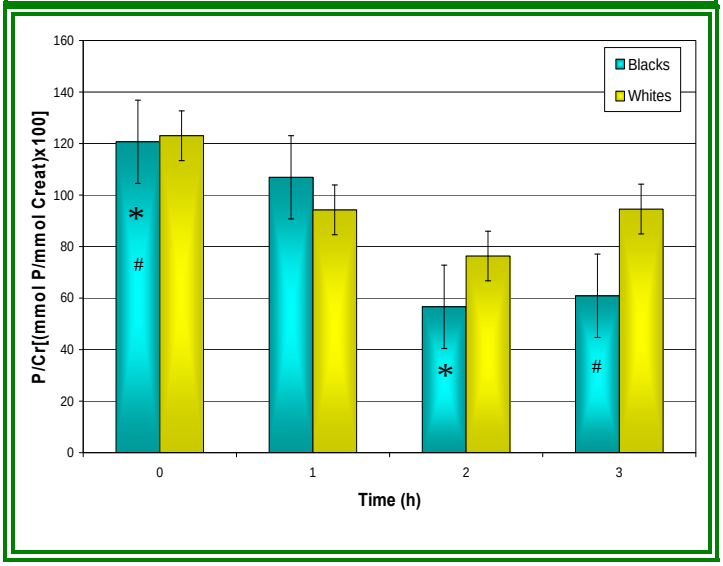


***Figure 2.5**: Mean hourly values for urinary phosphate excretion [(mmol P per mmol Creatinine) x 10^2] for black and white subjects following the Glu challenge*



Histograms labelled with the same symbol are significantly different ($p<0.05$)

Particle Formation Kinetics

Particle Volume-Size Distributions

The mean particle volume-size distribution at 120 min in urines of black and whites subjects before and after the ingestion of Glu is shown in Figure 2.6. The total area under the curve represents the total volume of precipitated particles, while the mode of the curve gives the mean size of the precipitated particles. The mean particle size values are given in Table 2.10. Glu induced a decrease in mean particle size in blacks but no change in whites. The mean particle volume was reduced in both groups.

Table 2.10: Mean particle size (µm) in urines of black and white subjects before and after ingestion of Glu

	Blacks	*Whites*
Baseline	4.37	4.37
Post Glu	2.91	4.37

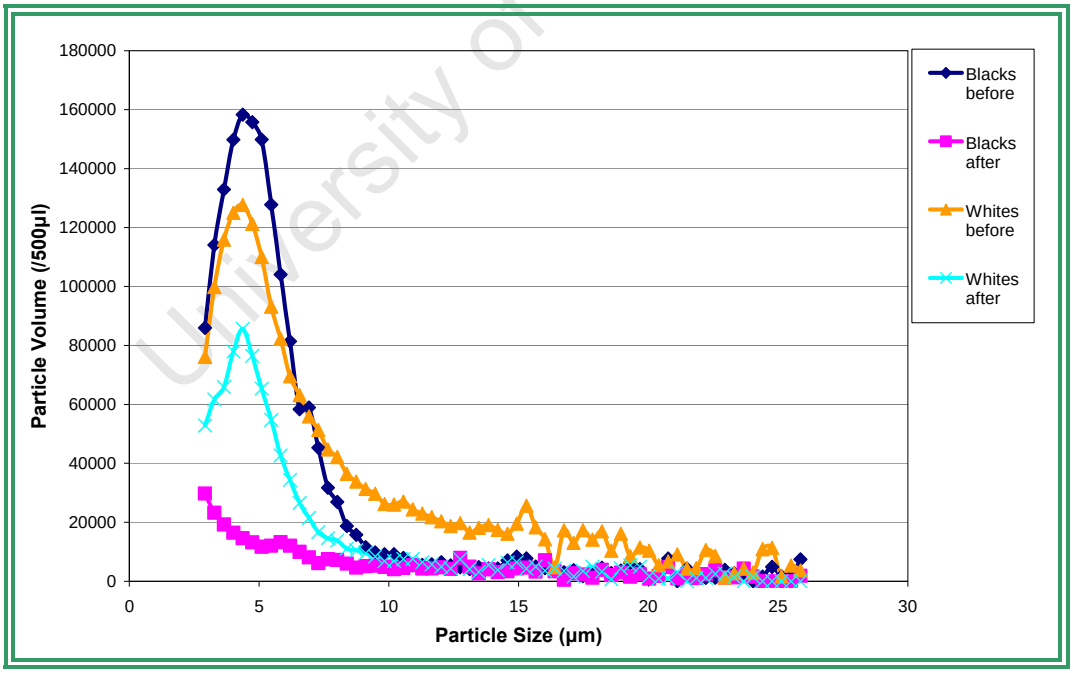


Figure 2.6: *Particle volume-size distribution in pre- and post-Glu ingestion in urines of black and white subjects*

Particle Number

The plots for the mean particle number versus time are shown in Figure 2.7. At 120 min the particle number decreased in whites post Glu load. Similarly, in blacks the Glu provoked a decrease in particle number but to a greater extent.

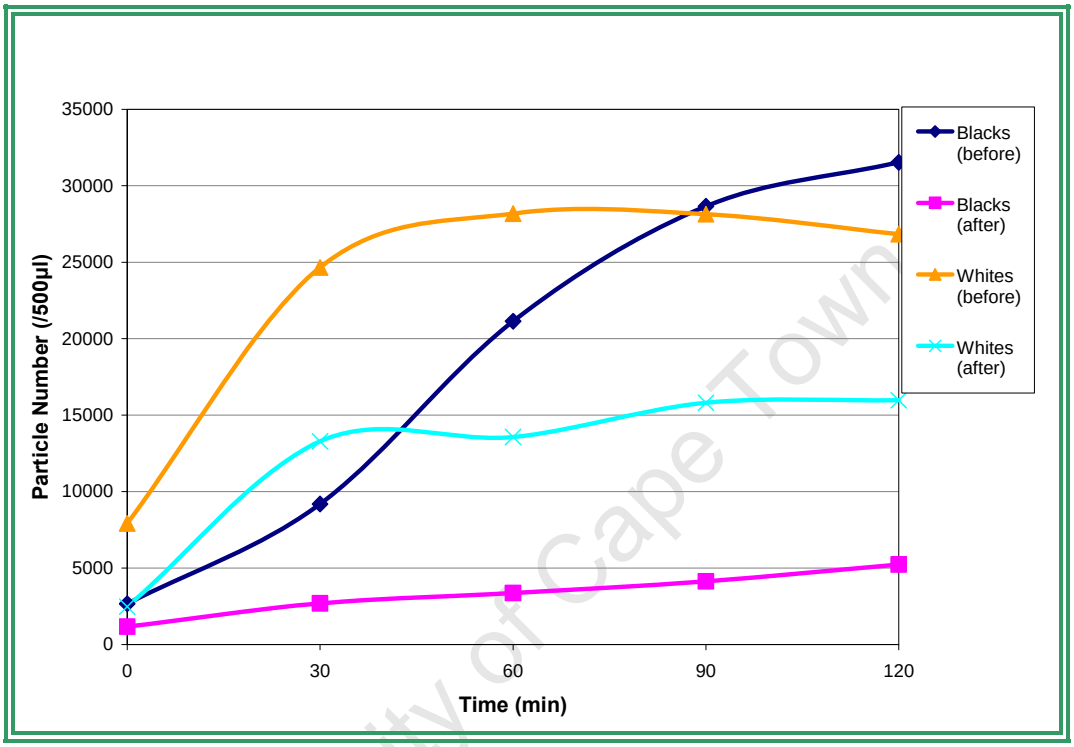


Figure 2.7: *Particle number in urines of blacks and whites before and after ingestion of Glu*

Crystallization Experiments

^{14}C-Oxalate Crystal Deposition

The rate of CaOx deposition is indicated by the gradient of each curve (top right-hand corner) for black and white subjects pre- and post-intake of Glu (Figure 2.8). A reduction in CaOx deposition rate was observed in the both groups after a Glu load.

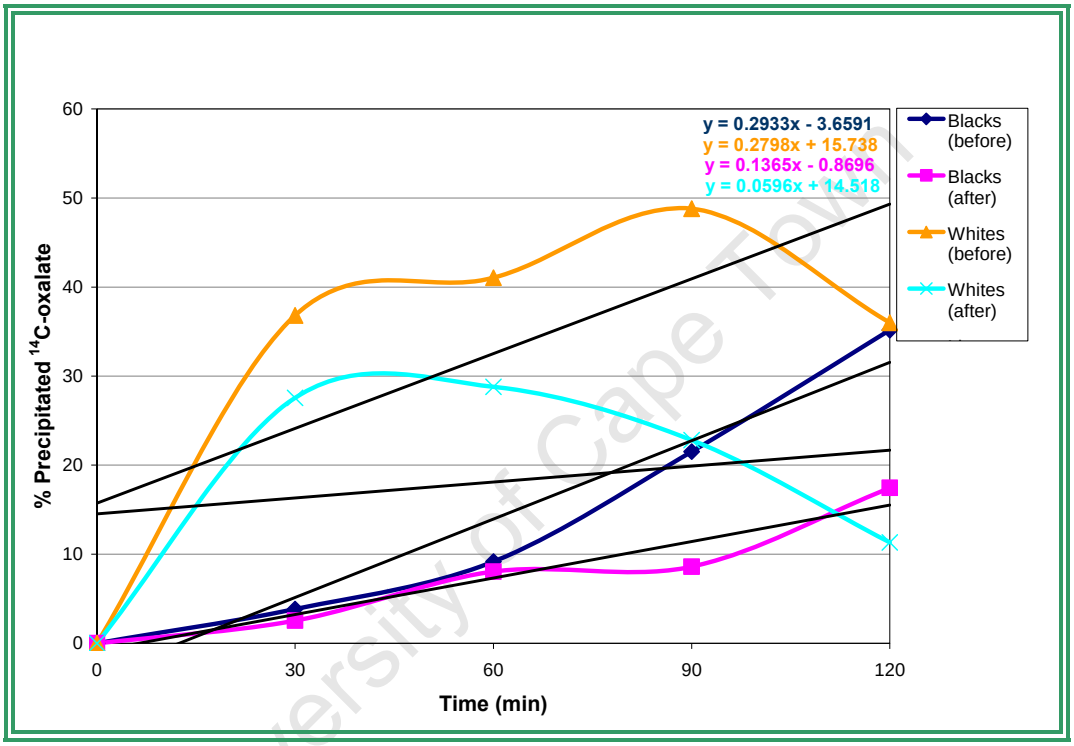


Figure 2.8: *Percentage precipitated ^{14}C-oxalate pre- and post-Glu ingestion in urines of black and white subjects*

Crystal Aggregation

The mean percentages for the inhibition of CaOx crystal aggregation in black and white subjects are given in Table 2.11, corresponding to the plots shown in Figure 2.9. Within each group no significant changes were noted after a Glu load. Similarly, inter-group comparisons revealed no changes both before and after Glu ingestion.

Table 2.11: Mean percentage inhibition of aggregation (SE) in black and white subjects before and after a Glu load

Variables	*Blacks*		*Whites*		*Blacks vs Whites*
	% Ia (SE)	*P*	*% Ia (SE)*	*P*	*P*
Baseline	91.1 (3.33)		62.4 (15.2)		0.0988
Post Glu	82.8 (4.16)	0.7293	71.6 (7.18)	0.8266	0.7905

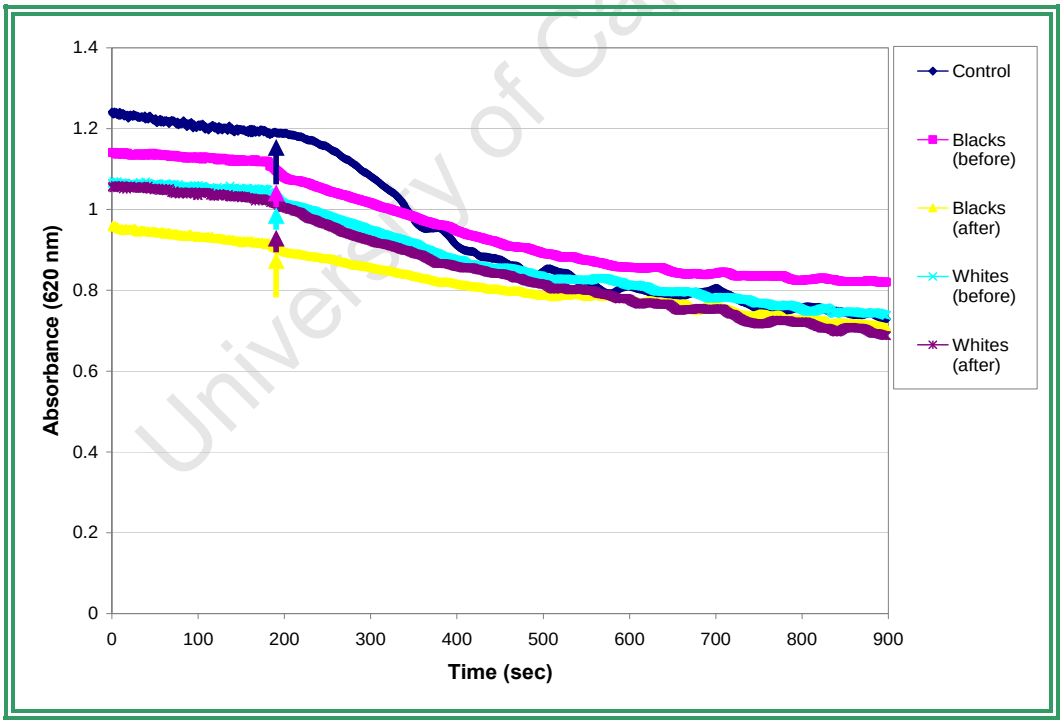


Figure 2.9: *Plot of absorbance versus time pre- and post-Glu load in urines of black and white subjects. The time at which stirring was stopped is indicated by the arrows.*

Summary of Urine Chemistry and Crystalluria Properties After Glu Load

	Blacks	Whites
24 h: BRI	↓	↑
Fractional (non-normalized)	↑ pH, ↓K, ↓Creat, ↓P	↑ Cit, ↑ pH
Fractional (normalized)	↓ P	↔
Particle size	↓	↔
Particle number	↓	↓
Deposition rate	↓	↓
Aggregation	↔	↔

SORBITOL

24h Collections

Table 2.12 shows the 24 h excretion of urinary parameters before and after a Sor load. In blacks, the urinary citrate excretion increased significantly. A decrease in urinary calcium, magnesium, urate, creatinine and phosphate excretion was noted in whites. The RS of brushite and BRI also decreased in this group. No other differences were observed.

Fractional Collections (Non-Normalized Excretion)

When comparing the two race groups at 0 h before Sor intake (Table 2.13), no significant differences were found between the two groups. At 1 h following the Sor ingestion (Table 2.14), phosphate decreased significantly in blacks but no changes were observed in whites. A significant decrease in urinary phosphate was further observed at 2 h in blacks (Table 2.15) and no other changes were observed in either group. At 3 h (Table 2.16), the only significant differences which were observed were an increase in the urinary pH and urine volume in blacks and whites, respectively, with citrate approaching a significant increase in the former.

Table 2.12: Mean 24 h urinary variables ± SE in black and white subjects after ingestion of Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post-Sor*
pH	6.07±0.08	6.21±0.11	0.2627	6.22±0.06	6.16±0.20	0.7271	0.8361
Volume (ml/24h)	1924±110	2008±139	0.6046	2060±87.6	1811±311	0.2872	0.5433
Citrate (mmol/24h)	2.99±0.23	4.27±0.32	*0.0035	3.04±0.23	2.36±0.55	0.2101	*0.0065
Oxalate (mmol/24h)	0.27±0.01	0.30±0.03	0.1613	0.29±0.01	0.24±0.04	0.0852	0.1510
Calcium (mmol/24h)	2.60±0.27	1.89±0.35	0.1635	4.19±0.24	2.45±0.50	*0.0020	0.3576
Magnesium (mmol/24h)	1.79±0.22	1.37±0.21	0.3403	3.45±0.18	2.25±0.46	*0.0069	0.0813
Sodium (mmol/24h)	93.1±12.0	76.5±19.5	0.4455	148±20.5	101±27.6	0.2728	0.4660
Potassium (mmol/24h)	46.0±4.98	32.3±7.64	0.2019	54.4±4.82	51.2±13.1	0.7784	0.2104
Urate (mmol/24h)	2.94±0.15	2.42±0.23	0.0780	3.51±0.14	2.34±0.32	*0.0006	0.8322
Creatinine (mmol/24h)	17.8±3.84	12.3±1.29	0.3792	15.5±0.68	11.5±1.96	*0.0190	0.7235
Phosphate (mmol/24h)	18.6±1.16	14.3±2.57	0.0971	27.3±1.68	17.6±4.70	*0.0208	0.5245
Chloride (mmol/24h)	160±8.25	149±15.2	0.3895	164±7.71	132±21.7	0.0888	0.5071
Sulphate (mmol/24h)	17.4±0.86	24.1±6.24	0.1107	20.3±1.28	15.3±3.01	0.0967	0.2620
Tiselius Risk Index	198±24.2	263±43.8	0.8855	284±19.8	268±40.4	0.9929	0.5508
RS Brushite	0.32±0.24	0.13±0.02	0.0713	4.39±1.67	0.39±0.04	*0.0148	*0.0259
RS CaOx	3.29±1.25	2.34±0.22	0.1559	4.39±1.67	3.73±0.86	0.5546	0.1561
RS Uric Acid	1.54±1.24	0.67±0.04	0.0976	0.83±0.77	1.25±0.29	0.3705	0.3728
MSL (mol/dm^3)	0.15±0.01	0.15±0.02	0.8765	0.13±0.01	0.15±0.02	0.3898	0.7979
BRI (/l)	1.13±0.40	0.98±0.35	0.7790	3.87±0.70	1.15±0.45	*0.0114	0.7698

*statistically significant

Table 2.13: Mean baseline 0 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	5.97 ± 0.17	6.17 ± 0.19	0.4475
Volume (ml/h)	112 ± 22.8	101 ± 32.2	0.7746
Citrate (mmol/h)	0.28 ± 0.07	0.20 ± 0.07	0.4606
Oxalate (mmol/h)	0.04 ± 0.01	0.02 ± 0.01	0.3599
Calcium (mmol/h)	0.20 ± 0.09	0.20 ± 0.09	0.9892
Magnesium (mmol/h)	0.12 ± 0.04	0.48 ± 0.24	0.1440
Sodium (mmol/h)	6.43 ± 1.81	15.2 ± 8.80	0.3191
Potassium (mmol/h)	3.98 ± 1.80	4.73 ± 2.19	0.7934
Urate (mmol/h)	0.11 ± 0.03	0.21 ± 0.09	0.3073
Creatinine (mmol/h)	0.94 ± 0.20	1.58 ± 0.65	0.3413
Phosphate (mmol/h)	0.97 ± 0.17	2.02 ± 1.29	0.4053
Chloride (mmol/h)	9.50 ± 1.88	13.6 ± 4.49	0.4000
Sulphate (mmol/h)	1.15 ± 0.33	1.88 ± 0.63	0.3115

Table 2.14: Mean 1 h urinary variables ± SE in black and white subjects after ingestion of Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post-Sor*
pH	5.97±0.17	5.87±0.24	0.7257	6.17±0.19	6.28±0.24	0.6399	0.2439
Volume (ml/h)	112±22.8	116±38.3	0.9392	101±32.2	75.1±19.3	0.5719	0.3583
Citrate (mmol/h)	0.28±0.07	0.23±0.06	0.6584	0.20±0.07	0.19±0.06	0.9225	0.6192
Oxalate (mmol/h)	0.04±0.01	0.02±0.01	0.1260	0.02±0.01	0.02±0.01	0.2714	0.7922
Calcium (mmol/h)	0.20±0.09	0.05±0.01	0.1591	0.20±0.09	0.11±0.02	0.2795	*0.0228
Magnesium (mmol/h)	0.12±0.04	0.05±0.02	0.1323	0.48±0.24	0.11±0.02	0.1440	*0.0450
Sodium (mmol/h)	6.43±1.81	2.90±1.04	0.1183	15.2±8.80	7.15±2.49	0.3153	0.1351
Potassium (mmol/h)	3.98±1.80	2.99±0.74	0.6352	4.73±2.19	3.34±0.60	0.623	0.7180
Urate (mmol/h)	0.11±0.03	0.08±0.02	0.1260	0.21±0.09	0.09±0.02	0.1936	0.5041
Creatinine (mmol/h)	0.94±0.20	0.66±0.08	0.1591	1.58±0.65	0.74±0.11	0.2568	0.0963
Phosphate (mmol/h)	0.97±0.17	0.45±0.08	*0.0097	2.02±1.29	0.43±0.09	0.3273	0.7511
Chloride (mmol/h)	9.50±1.88	6.24±1.28	0.1156	13.6±4.49	7.11±1.50	0.2268	0.3711
Sulphate (mmol/h)	1.15±0.33	0.51±0.10	0.0951	1.88±0.63	0.92±0.17	0.2141	†0.0552

*statistically significant; †approaching statistical significance

Table 2.15: Mean 2 h urinary variables ± SE in black and white subjects after ingestion of Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post-Sor*
pH	5.97±0.17	5.70±0.57	0.2168	6.17±0.19	6.39±0.22	0.4553	0.2889
Volume (ml/h)	112±22.8	130±24.9	0.6727	101±32.2	145±28.4	0.3162	0.4840
Citrate (mmol/h)	0.28±0.07	0.23±0.05	0.6246	0.20±0.07	0.17±0.07	0.7860	0.5765
Oxalate (mmol/h)	0.04±0.01	0.03±0.01	0.4244	0.02±0.01	0.02±0.00	0.9280	0.7759
Calcium (mmol/h)	0.20±0.09	0.09±0.03	0.3004	0.20±0.09	0.13±0.02	0.4571	0.4407
Magnesium (mmol/h)	0.12±0.04	0.07±0.02	0.2944	0.48±0.24	0.12±0.03	0.1607	0.2561
Sodium (mmol/h)	6.43±1.81	3.89±1.50	0.2935	15.2±8.80	5.32±1.70	0.2870	0.5159
Potassium (mmol/h)	3.98±1.80	2.76±0.60	0.5298	4.73±2.19	3.49±0.61	0.5956	0.4518
Urate (mmol/h)	0.11±0.03	0.08±0.01	0.3914	0.21±0.09	0.11±0.02	0.2963	0.2230
Creatinine (mmol/h)	0.94±0.20	0.67±0.06	0.2168	1.58±0.65	0.80±0.07	0.2524	0.2157
Phosphate (mmol/h)	0.97±0.17	0.33±0.06	*0.0022	2.02±1.29	0.36±0.10	0.2145	0.7411
Chloride (mmol/h)	9.50±1.88	13.5±6.72	0.5717	13.6±4.49	7.33±1.35	0.2036	0.4469
Sulphate (mmol/h)	1.15±0.33	0.58±0.09	0.2187	1.88±0.63	0.89±0.10	0.1461	*0.0434

*statistically significant

Table 2.16: Mean 3 h urinary variables ± SE in black and white subjects after ingestion of Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post-Sor*
pH	5.97±0.17	6.63±0.17	*0.0141	6.17±0.19	6.59±0.23	0.1775	0.9058
Volume (ml/h)	112±22.8	690±414	0.1806	101±32.2	273±39.4	*0.0038	0.3562
Citrate (mmol/h)	0.28±0.07	0.57±0.12	†0.0515	0.20±0.07	0.21±0.06	0.9269	*0.0214
Oxalate (mmol/h)	0.04±0.01	0.04±0.01	1.0000	0.02±0.01	0.03±0.01	0.3987	0.7112
Calcium (mmol/h)	0.20±0.09	0.11±0.03	0.4049	0.20±0.09	0.11±0.02	0.3741	0.9929
Magnesium (mmol/h)	0.12±0.04	0.05±0.02	0.1270	0.48±0.24	0.09±0.02	0.1246	0.1597
Sodium (mmol/h)	6.43±1.81	4.14±1.25	0.3112	15.2±8.80	5.05±1.27	0.2708	0.6179
Potassium (mmol/h)	3.98±1.80	2.87±0.67	0.5735	4.73±2.19	4.79±1.08	0.9800	0.1412
Urate (mmol/h)	0.11±0.03	0.16±0.02	0.2282	0.21±0.09	0.16±0.02	0.5612	0.8072
Creatinine (mmol/h)	0.94±0.20	0.69±0.05	0.2599	1.58±0.65	0.72±0.07	0.2105	0.7474
Phosphate (mmol/h)	0.97±0.17	0.55±0.13	0.0624	2.02±1.29	0.57±0.14	0.2770	0.9352
Chloride (mmol/h)	9.50±1.88	8.28±1.07	0.5786	13.6±4.49	9.33±0.94	0.3715	0.4745
Sulphate (mmol/h)	1.15±0.33	0.75±0.18	0.3034	1.88±0.63	1.28±0.38	0.4331	0.2108

*statistically significant; †approaching statistical significance

Fractional Collections (Excretion Normalized to Creatinine)

No significant changes were observed in the excretion of urinary calcium before and after Sor intake in both groups (Figure 2.10). However, oxalate was significantly increased within the white group at 3 h (p=0.0347) but there were no changes in blacks (Figure 2.11). A decrease in urinary phosphate was observed in blacks at 1 h (p=0.0181) and 2 h (p=0.0002), whereas in whites it was only observed at 2 h (p=0.0367) (Figure 2.12).

***Figure 2.10**: Mean hourly values for urinary calcium excretion [(mmol Ca per mmol Creatinine) x 10^2] for black and white subjects following the Sor challenge*

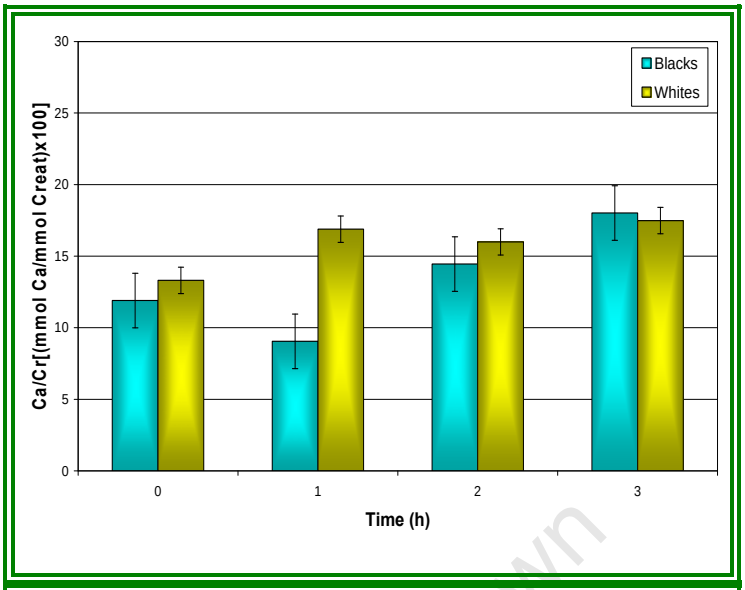


***Figure 2.11**: Mean hourly values for urinary oxalate excretion [(mmol Ox per mmol Creatinine) x 10^2] for black and white subjects following the Sor challenge*

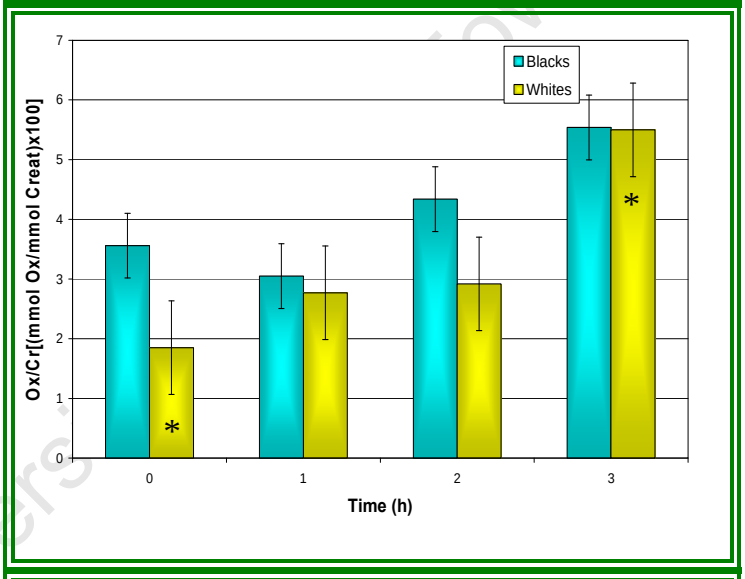
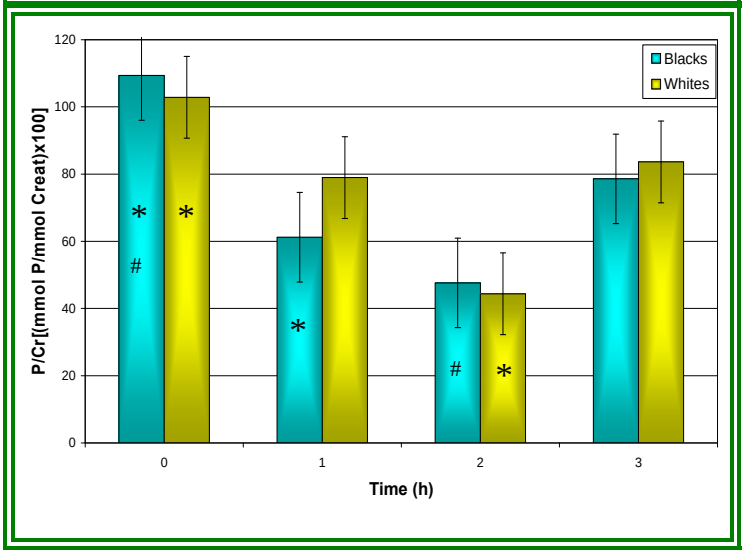


***Figure 2.12**: Mean hourly values for urinary phosphate excretion [(mmol P per mmol Creatinine) x 10^2] for black and white subjects following the Sor challenge*



Histograms labelled with the same symbol are significantly different ($p<0.05$)

Particle Formation Kinetics

Particle Volume-Size Distributions

The mean particle size values are given in Table 2.17. Sor provoked an increase in the mean particle size in blacks. Conversely, in whites the mean particle size decreased. The mean volume of precipitated particles decreased in both groups and was more pronounced in blacks. The mean particle volume-size distribution of urine from black and white subjects before and after Sor load is shown in Figure 2.13.

Table 2.17: Mean particle size (µm) in urines of black and white subjects before and after ingestion of Sor

	Blacks	*Whites*
Baseline	3.64	4.37
Post Sor	4.01	3.28

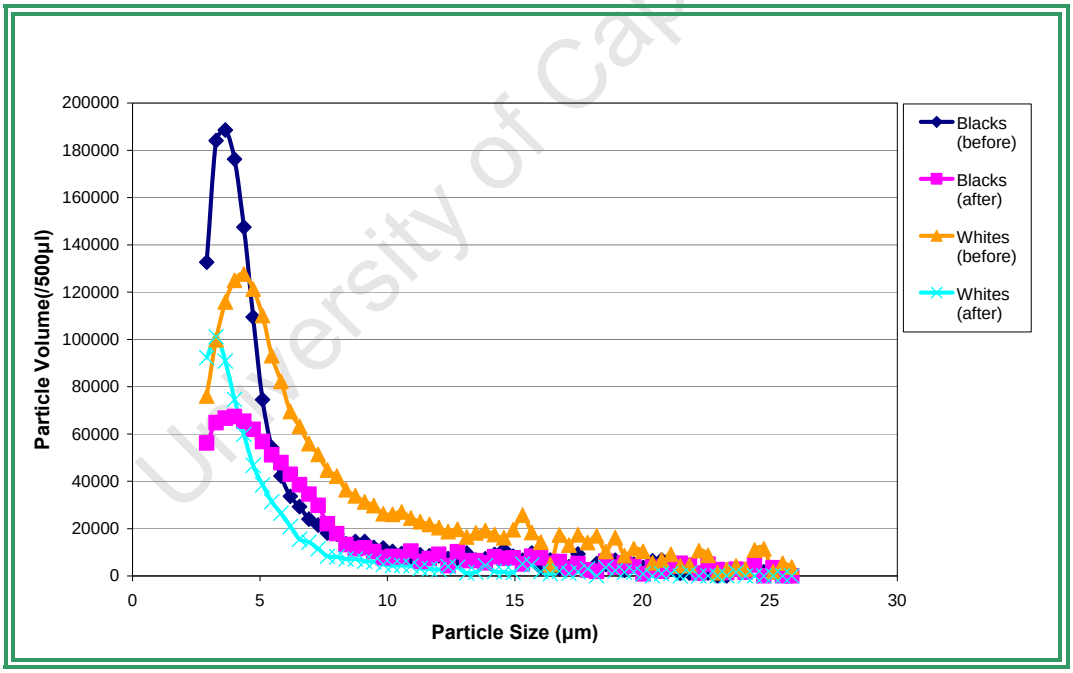


Figure 2.13: *Particle volume-size distribution in pre- and post-Sor ingestion in urines of black and white subjects*

Particle Number

The curves for particle number versus time are shown in Figure 2.14. At 120 min the mean particle number decreased in both groups (largely in the black group) post Sor load.

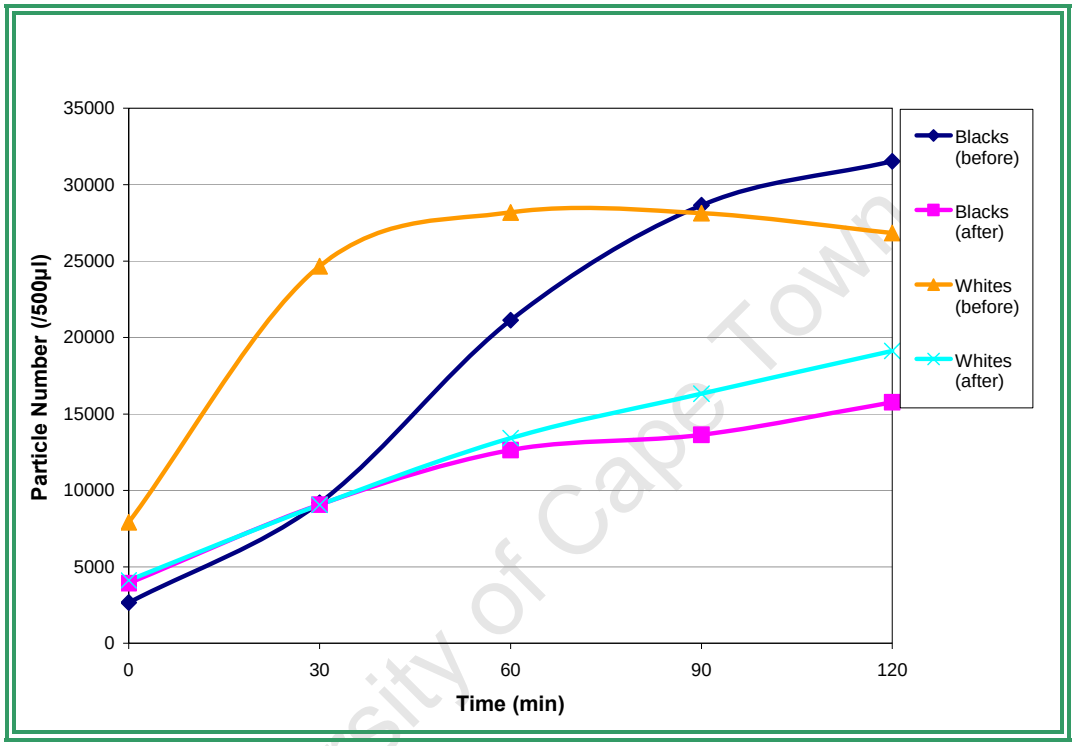


Figure 2.14: *Particle number in urines of blacks and whites before and after ingestion of Sor*

Crystallization Experiments

^{14}C-Oxalate Deposition

Sor induced a significant decrease in the rate of CaOx crystal deposition at 120 min in blacks (Figure 2.15). However, the rate of deposition did not change in whites.

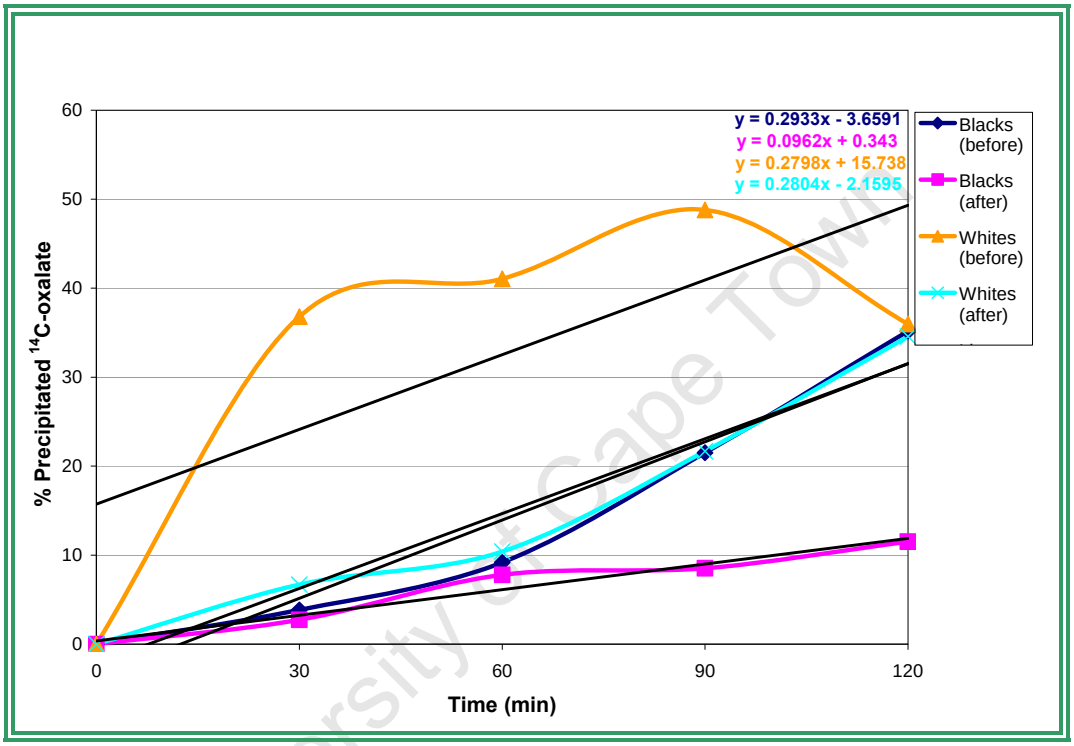


Figure 2.15: *Percentage precipitated ^{14}C-oxalate pre- and post-Sor ingestion in urines of black and white subjects*

Crystal Aggregation

The mean percentages for the inhibition of CaOx crystal aggregation in black and white subjects are given in Table 2.18, corresponding to the plots shown in Figure 2.16. Sor provoked a significant decrease in percentage inhibition of aggregation in blacks but no change was observed in whites. Inter-group comparisons revealed no changes both before and after Sor ingestion.

Table 2.18: Mean percentage inhibition of aggregation (SE) in black and white subjects before and after a Sor load

Variables	*Blacks*		*Whites*		*Blacks vs Whites*
	% Ia (SE)	*p*	*% Ia (SE)*	*p*	*p*
Baseline	85.1 (4.99)		61.3 (10.4)		0.0995
Post Sor	67.5 (3.82)	*0.0231	68.8 (6.99)	0.9776	0.9012

* statistically significant

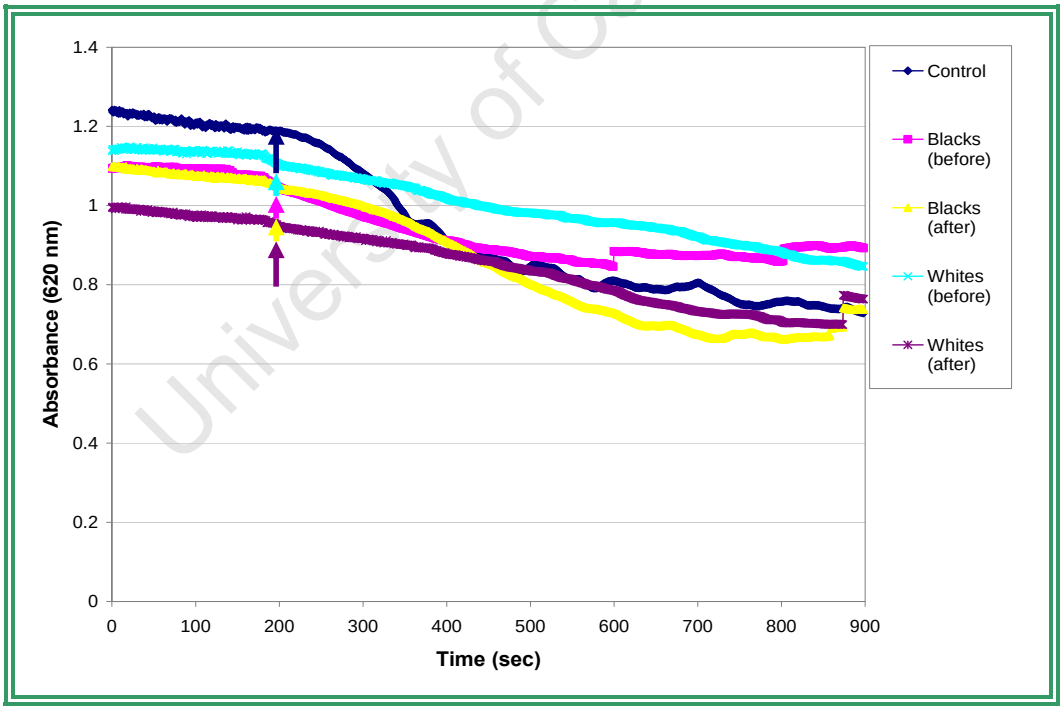


Figure 2.16: *Plot of absorbance versus time pre- and post-Sor load in urines of black and white subjects. The time at which stirring was stopped is indicated by the arrows.*

Summary of Urine Chemistry and Crystalluria Properties After Sor Load

	Blacks	Whites
24 h	↑ Cit, ↓ RS brushite	↓ Ca, ↓ Mg, ↓ Ur, ↓ Creat, ↓ P, ↓ RS brushite ↓ BRI
Fractional (non-normalized)	↓ P	↔
Fractional (normalized)	↓ P	↑ Ox, ↓ P
Particle size	↑	↓
Particle number	↓	↓
Deposition rate	↓	↔
Aggregation	↓	↔

XYLITOL

24h Collections

Xyl augmented the 24 h urinary excretion of oxalate and magnesium subsequent to its ingestion in blacks, as shown in Table 2.19. In the white group a significant decrease in the BRI was noted. No other significant changes were observed within the two groups.

Fractional Collections (Non-Normalized Excretion)

At 0 h, there were no differences in the two groups prior to a Xyl load (Table 2.20). After Xyl intake, at 1 h (Table 2.21), urinary potassium excretion increased in blacks. No additional changes in either group were observed. At 2 h (Table 2.22), the urinary sulphate excretion decreased and the urine volume increased in whites. The other variables within the two groups remained unchanged. The urinary pH increased in blacks 3 h post a Xyl load and the urine volume increased in both groups as shown in Table 2.23. No other changes were noticed.

Table 2.19: Mean baseline 24 h urinary variables ± SE in black and white subjects after ingestion of Xyl

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Xyl*	*p*	*Baseline*	*Post-Xyl*	*p*	*p Post-Xyl*
pH	6.07±0.08	6.17±0.13	0.4245	6.22±0.06	6.34±0.17	0.4176	0.4380
Volume (ml/24h)	1924±110	1982±74.0	0.6909	2060±87.6	1924±140	0.4610	0.7083
Citrate (mmol/24h)	2.99±0.23	2.87±0.36	0.8673	3.04±0.23	2.78±0.36	0.5953	0.8550
Oxalate (mmol/24h)	0.27±0.01	0.33±0.02	*0.0037	0.29±0.01	0.28±0.02	0.7337	0.0866
Calcium (mmol/24h)	2.60±0.27	2.18±0.33	0.4198	4.19±0.24	3.25±0.55	0.0867	0.1070
Magnesium (mmol/24h)	1.79±0.22	2.75±0.41	*0.0277	3.45±0.18	3.00±0.36	0.2598	0.6523
Sodium (mmol/24h)	93.1±12.0	87.1±23.5	0.8050	148±20.5	189±46.2	0.3779	0.0629
Potassium (mmol/24h)	46.0±4.98	51.7±8.82	0.4667	54.4±4.82	60.7±14.0	0.5899	0.5848
Urate (mmol/24h)	2.94±0.15	2.99±0.15	0.8183	3.51±0.14	3.19±0.16	0.2610	0.3719
Creatinine (mmol/24h)	17.8±3.84	14.5±0.88	0.6141	15.5±0.68	13.7±1.68	0.2529	0.6568
Phosphate (mmol/24h)	18.6±1.16	22.5±2.44	0.1061	27.3±1.68	29.1±4.71	0.5036	0.1639
Chloride (mmol/24h)	160±8.25	136±14.0	0.1066	164±7.71	151±9.16	0.8584	0.1704
Sulphate (mmol/24h)	17.4±0.86	18.7±1.97	0.5060	20.3±1.28	15.6±2.93	0.1090	0.3738
Tiselius Risk Index	198±24.2	243±32.5	0.6992	284±19.8	288±39.9	0.5830	0.9478
RS Brushite	0.32±0.24	0.37±0.03	0.7108	4.39±1.67	0.78±0.06	0.2817	*0.0251
RS CaOx	3.29±1.25	3.35±0.33	0.9264	4.39±1.67	3.22±0.40	0.2696	0.8667
RS Uric Acid	1.54±1.24	1.00±0.20	0.3369	0.83±0.77	0.94±0.19	0.7209	0.8924
MSL (mol/dm³)	0.15±0.01	0.15±0.01	0.9478	0.13±0.01	0.15±0.02	0.3388	0.9695
BRI (/l)	1.13±0.40	0.69±0.26	0.3849	3.87±0.70	1.08±0.41	*0.0089	0.4407

*statistically significant

Table 2.20: Mean baseline 0 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	5.90 ± 0.18	5.95 ± 0.18	0.8947
Volume (ml/h)	102 ± 22.8	113 ± 24.7	0.4155
Citrate (mmol/h)	0.16 ± 0.03	0.19 ± 0.05	0.3412
Oxalate (mmol/h)	0.02 ± 0.01	0.04 ±0.01	0.1906
Calcium (mmol/h)	0.06 ± 0.01	0.24 ± 0.09	0.0774
Magnesium (mmol/h)	0.09 ± 0.01	0.30 ± 0.11	0.0796
Sodium (mmol/h)	4.76 ± 2.10	13.9 ± 5.87	0.1605
Potassium (mmol/h)	2.46 ± 0.57	3.06 ± 0.55	0.4577
Urate (mmol/h)	0.10 ± 0.02	0.17 ± 0.04	0.2011
Creatinine (mmol/h)	0.87 ± 0.15	1.39 ± 0.41	0.2510
Phosphate (mmol/h)	1.07 ± 0.31	2.02 ± 0.69	0.2230
Chloride (mmol/h)	8.22 ± 1.90	10.7 ± 2.35	0.4305
Sulphate (mmol/h)	0.89 ± 0.15	1.42 ± 0.47	0.3015

Table 2.21: Mean 1 h urinary variables ± SE in black and white subjects after ingestion of Xyl

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Xyl*	*p*	*Baseline*	*Post-Xyl*	*p*	*p Post-Xyl*
pH	5.90±0.18	6.10±0.31	0.5650	5.95±0.18	6.04±0.23	0.9183	0.8777
Volume (ml/h)	102±22.8	86.3±33.6	0.7009	113±24.7	90.4±16.3	0.7906	0.9063
Citrate (mmol/h)	0.16±0.03	0.14±0.05	0.6706	0.19±0.05	0.14±0.03	0.2115	0.9655
Oxalate (mmol/h)	0.02±0.01	0.01±0.00	0.6289	0.04±0.01	0.02±0.00	0.4441	0.2289
Calcium (mmol/h)	0.06±0.01	0.07±0.02	0.8939	0.24±0.09	0.20±0.06	0.5293	0.0655
Magnesium (mmol/h)	0.09±0.01	0.11±0.03	0.5743	0.30±0.11	0.21±0.07	0.3317	0.2109
Sodium (mmol/h)	4.76±2.10	5.15±2.73	0.9102	13.9±5.87	10.6±2.89	0.4171	0.2024
Potassium (mmol/h)	2.46±0.57	3.08±0.75	*0.0025	3.06±0.55	3.80±0.72	0.6257	0.5050
Urate (mmol/h)	0.10±0.02	0.09±0.04	0.9045	0.17±0.04	0.16±0.03	0.3514	0.2031
Creatinine (mmol/h)	0.87±0.15	0.81±0.14	0.8609	1.39±0.41	0.93±0.14	0.4422	0.5561
Phosphate (mmol/h)	1.07±0.31	0.53±0.13	0.1739	2.02±0.69	1.01±0.28	0.2395	0.1812
Chloride (mmol/h)	8.22±1.90	7.43±2.58	0.8034	10.7±2.35	9.56±1.60	0.6489	0.4755
Sulphate (mmol/h)	0.89±0.15	0.63±0.17	0.2646	1.42±0.47	0.78±0.15	0.3273	0.5066

*statistically significant

Table 2.22: Mean 2 h urinary variables ± SE in black and white subjects after ingestion of Xyl

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Xyl*	*p*	*Baseline*	*Post-Xyl*	*p*	*p Post-Xyl*
pH	5.90±0.18	6.32±0.20	0.1372	5.95±0.18	6.19±0.19	0.3731	0.6413
Volume (ml/h)	102±22.8	169±25.9	0.0695	113±24.7	216±15.8	*0.0023	0.1306
Citrate (mmol/h)	0.16±0.03	0.28±0.06	0.1054	0.19±0.05	0.21±0.03	0.8270	0.2717
Oxalate (mmol/h)	0.02±0.01	0.02±0.00	0.9196	0.04±0.01	0.04±0.01	0.9846	0.0743
Calcium (mmol/h)	0.06±0.01	0.09±0.01	0.1644	0.24±0.09	0.16±0.03	0.3651	†0.0520
Magnesium (mmol/h)	0.09±0.01	0.13±0.03	0.3280	0.30±0.11	0.13±0.02	0.1262	0.9699
Sodium (mmol/h)	4.76±2.10	3.91±2.01	0.7751	13.9±5.87	5.85±1.74	0.1846	0.4749
Potassium (mmol/h)	2.46±0.57	2.46±0.48	1.0000	3.06±0.55	3.69±0.91	0.5731	0.2628
Urate (mmol/h)	0.10±0.02	0.17±0.04	0.2011	0.17±0.04	0.16±0.02	0.8907	0.8907
Creatinine (mmol/h)	0.87±0.15	0.92±0.20	0.8524	1.39±0.41	0.78±0.04	0.1404	0.4792
Phosphate (mmol/h)	1.07±0.31	0.49±0.07	0.0914	2.02±0.69	0.75±0.15	0.0730	0.1531
Chloride (mmol/h)	8.22±1.90	8.22±2.05	1.0000	10.7±2.35	9.50±1.26	0.6582	0.5942
Sulphate (mmol/h)	0.89±0.15	0.61±0.11	0.1526	1.42±0.47	0.40±0.09	*0.0382	0.1467

*statistically significant; †approaching statistical significance

Table 2.23: Mean 3 h urinary variables ± SE in black and white subjects after ingestion of Xyl

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Xyl*	*p*	*Baseline*	*Post-Xyl*	*p*	*p Post-Xyl*
pH	5.90±0.18	6.70±0.12	*0.0017	5.95±0.18	6.39±0.23	0.1469	0.2484
Volume (ml/h)	102±22.8	346±43.6	*0.0001	113±24.7	326±32.5	*0.0001	0.7199
Citrate (mmol/h)	0.16±0.03	0.30±0.06	0.0608	0.19±0.05	0.22±0.06	0.7953	0.3413
Oxalate (mmol/h)	0.02±0.01	0.04±0.01	0.1423	0.04±0.01	0.02±0.00	0.1242	0.8690
Calcium (mmol/h)	0.06±0.01	0.11±0.02	0.1205	0.24±0.09	0.14±0.03	0.2751	0.4668
Magnesium (mmol/h)	0.09±0.01	0.16±0.05	0.1724	0.30±0.11	0.13±0.02	0.1241	0.4783
Sodium (mmol/h)	4.76±2.10	2.29±0.69	0.2816	13.9±5.87	5.08±1.45	0.1427	0.1124
Potassium (mmol/h)	2.46±0.57	2.84±0.60	0.6527	3.06±0.55	3.65±0.60	0.4812	0.3502
Urate (mmol/h)	0.10±0.02	0.14±0.02	0.1501	0.17±0.04	0.18±0.02	0.7902	0.2698
Creatinine (mmol/h)	0.87±0.15	0.62±0.04	0.1261	1.39±0.41	0.69±0.08	0.0998	0.4961
Phosphate (mmol/h)	1.07±0.31	0.64±0.07	0.2059	2.02±0.69	0.92±0.17	0.1192	0.1590
Chloride (mmol/h)	8.22±1.90	7.22±1.30	0.6696	10.7±2.35	9.80±1.08	0.7332	0.1432
Sulphate (mmol/h)	0.89±0.15	0.59±0.10	0.1176	1.42±0.47	1.06±0.88	0.7322	0.6221

*statistically significant

Fractional Collections (Excretion Normalized to Creatinine)

The intake of Xyl did not have any significant effect on the urinary excretion of any of the three urinary parameters (Ca, Ox and P) within the white group (Figures 2.17-2.19). In blacks, on the other hand, Xyl induced an increase in the urinary oxalate excretion at 3 h (p=0.0024) (Figure 2.18). No further differences were observed.

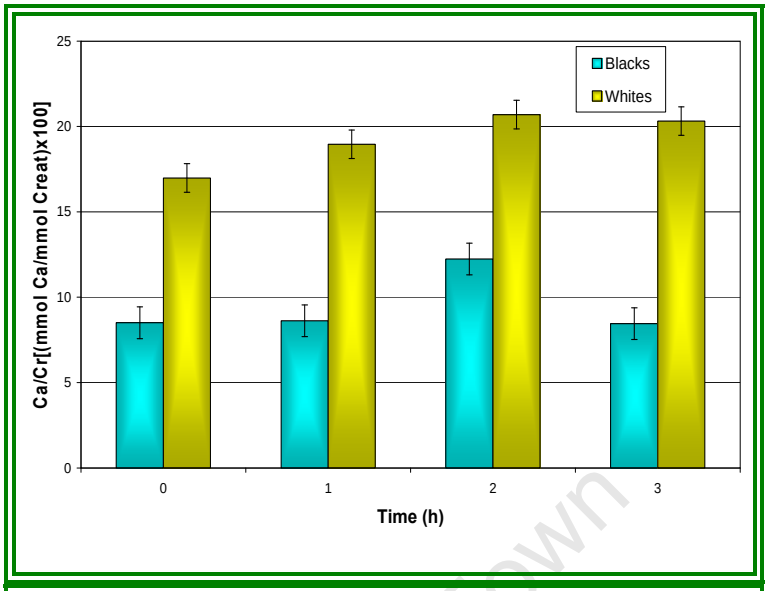


***Figure 2.17**: Mean hourly values for urinary calcium excretion [(mmol Ca per mmol Creatinine) x 10^2] for black and white subjects following the Xyl challenge*

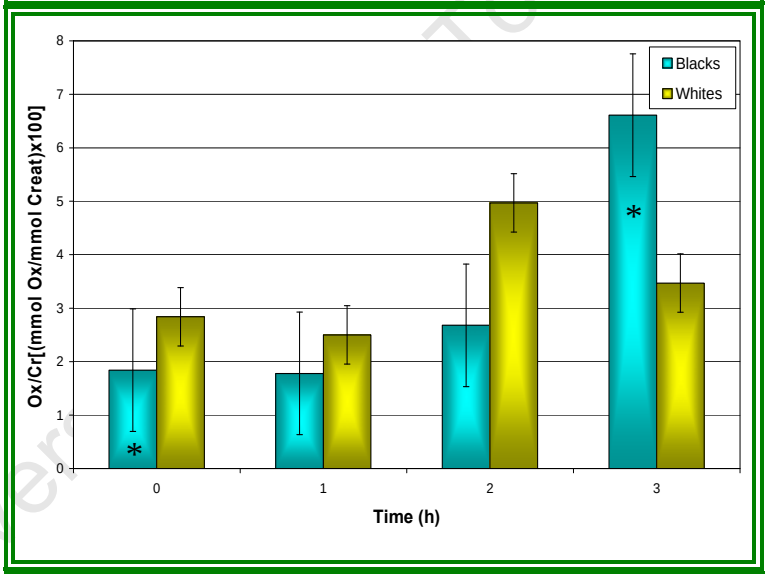


***Figure 2.18**: Mean hourly values for urinary oxalate excretion [(mmol Ox per mmol Creatinine) x 10^2] for black and white subjects following the Xyl challenge*

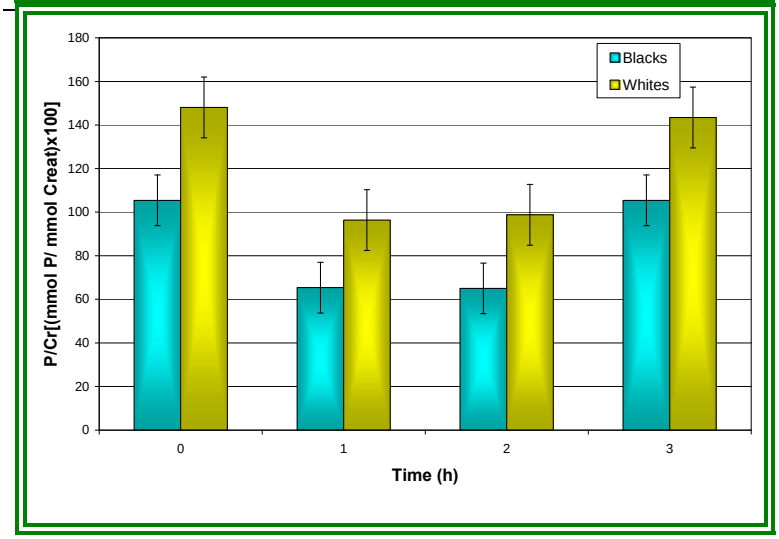


***Figure 2.19**: Mean hourly values for urinary phosphate excretion [(mmol P per mmol Creatinine) x 10^2] for black and white subjects following the Xyl challenge*

Histograms labelled with the same symbol are significantly different ($p<0.05$)

Particle Formation Kinetics

Particle Volume-Size Distributions

Subsequent to Xyl ingestion the mean particle size did not change in the black group but it decreased slightly in the white group (Table 2.24). The mean particle volume was decreased significantly in the former group and not in the latter (Figure 2.20).

Table 2.24: Mean particle size (µm) in urines of black and white subjects before and after ingestion of Xyl

	Blacks	*Whites*
Baseline	4.37	4.37
Post Xyl	4.37	4.01

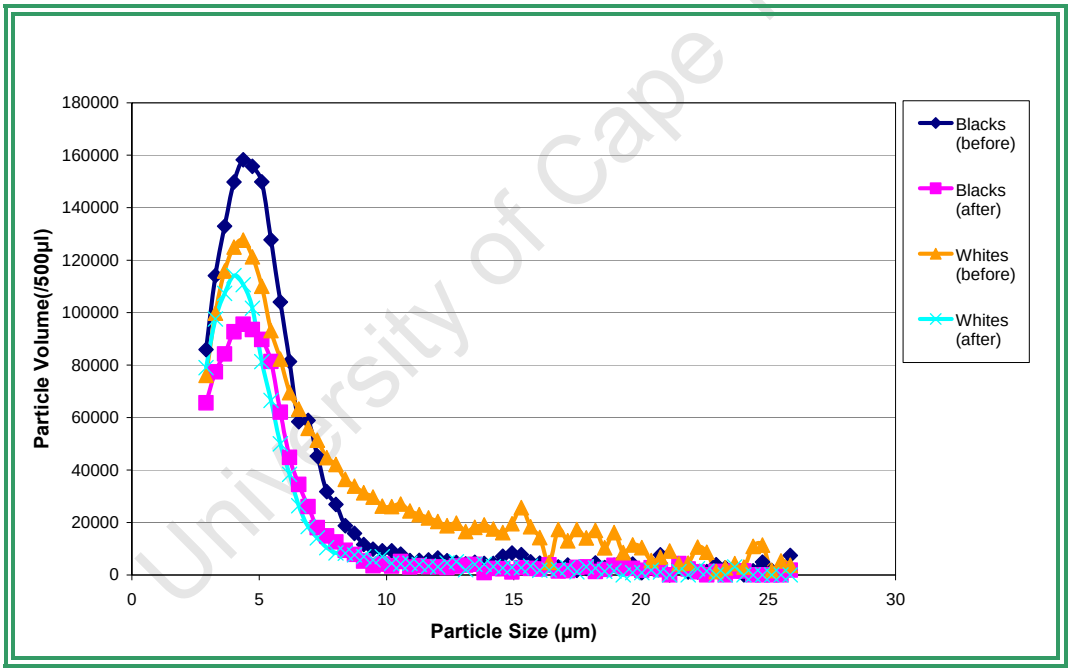


Figure 2.20: *Particle volume-size distribution in pre- and post-Xyl ingestion in urines of black and white subjects*

Particle Number

At 120 min the particle number decreased in both group subsequent to Xyl ingestion as shown in Figure 2.21. This occurred to a greater extent in black subjects.

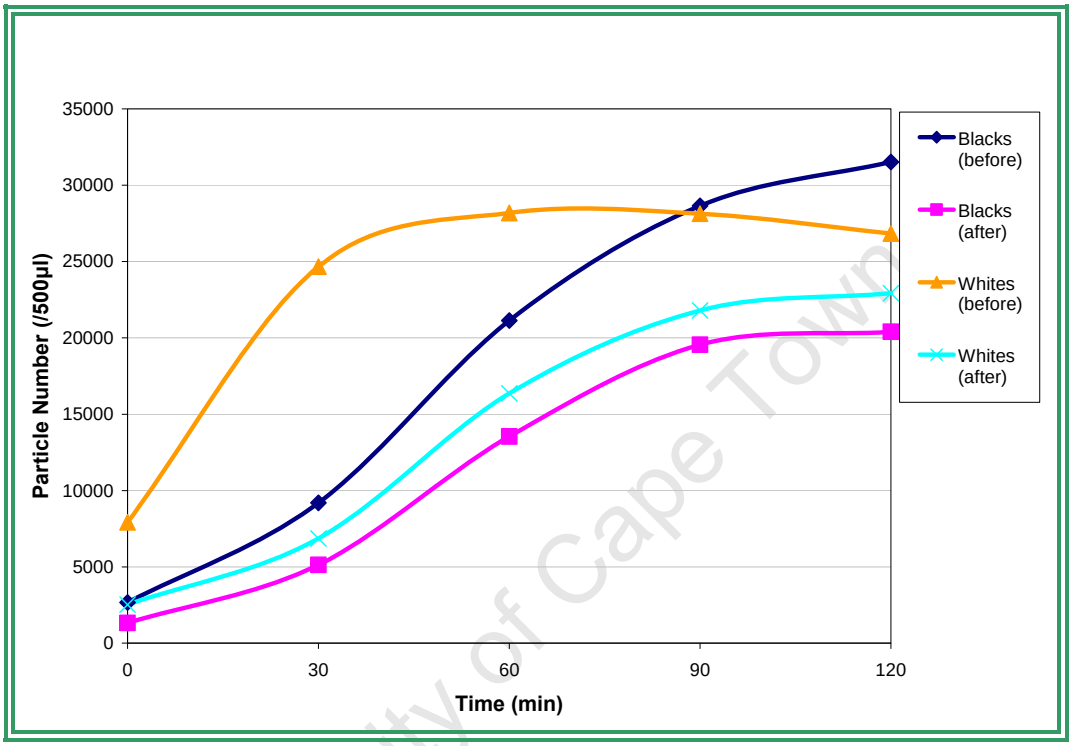


Figure 2.21: *Particle number in urines of blacks and whites before and after ingestion of Xyl*

Crystallization Experiments

^{14}C-Oxalate Deposition

A significant decrease in the rate of CaOx crystal deposition at 120 min was observed in whites following a Xyl load as shown in Figure 2.22, whereas no significant difference was noted in blacks.

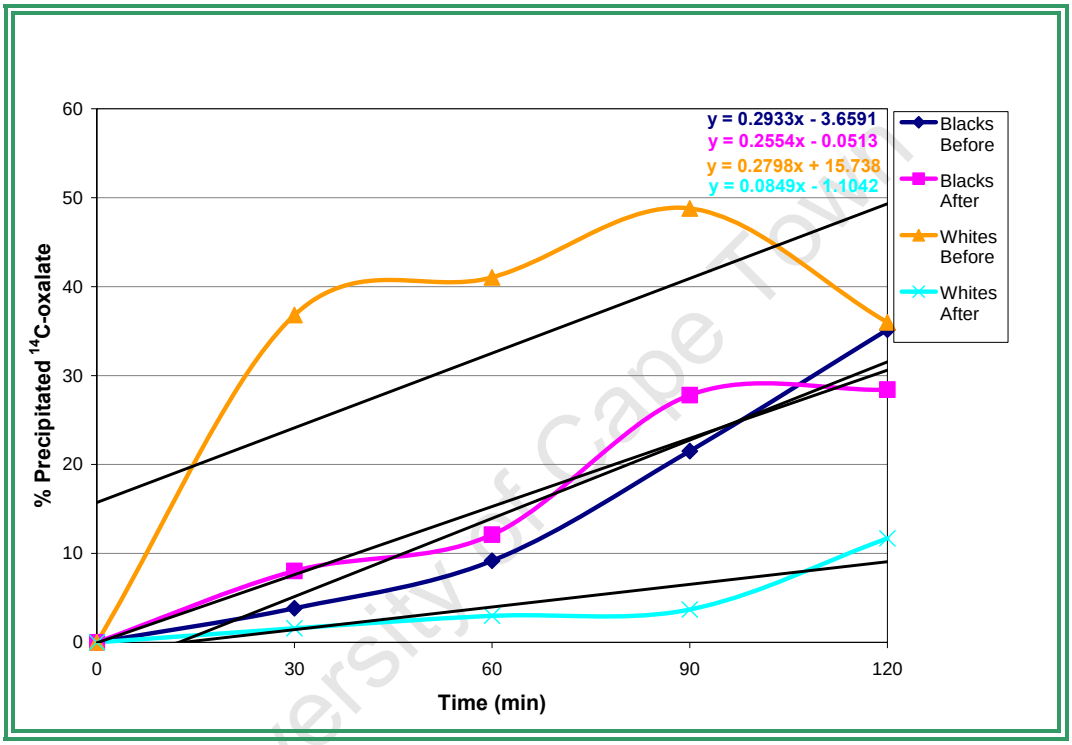


Figure 2.22: *Percentage precipitated ^{14}C-oxalate pre- and post-Xyl ingestion in urines of black and white subjects*

Crystal Aggregation

The percentages for the inhibition of aggregation (% Ia) in the urines of black and white subjects after the ingestion of Xyl relative to baseline are shown in Table 2.25. Xyl did not provoke any change in the two groups. Inter-group comparisons also revealed no changes both before and after Xyl ingestion. The corresponding plots for both groups are shown in Figure 2.23.

Table 2.25: Mean percentage inhibition of aggregation (SE) in black and white subjects before and after a Xyl load

Variables	*Blacks*		*Whites*		*Blacks vs Whites*
	% Ia (SE)	*p*	*% Ia (SE)*	*p*	*p*
Baseline	84.7 (5.52)		84.5 (5.05)		0.9907
Post Xyl	89.4 (5.08)	0.5589	72.1 (2.89)	0.7748	0.8003

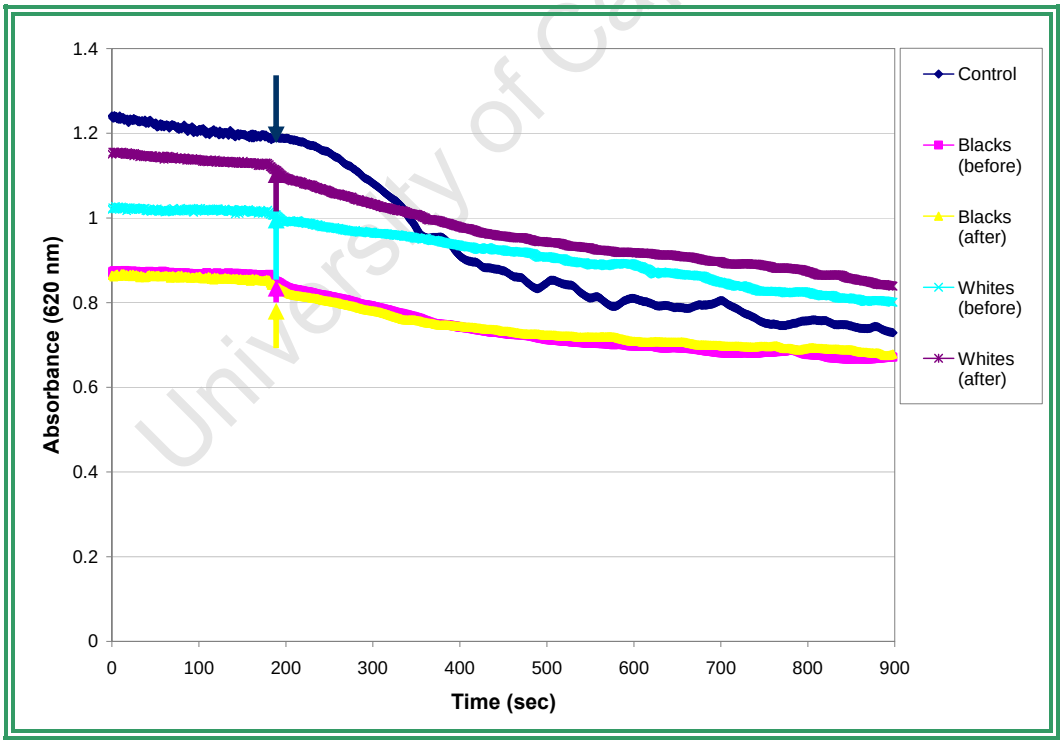


Figure 2.23: *Plot of absorbance versus time pre- and post-Xyl load in urines of black and white subjects. The time at which stirring was stopped is indicated by the arrows.*

Summary of Urine Chemistry and Crystalluria Properties After Xyl Load

	Blacks	Whites
24 h	↑ Ox, ↑ Mg	↔
Fractional (non-normalized)	↑ pH, ↑ K	↔
Fractional (normalized)	↑ Ox	↔
Particle size	↔	↓
Particle number	↓	↓
Deposition rate	↔	↓
Aggregation	↔	↔

Serum

Blood glucose results are shown in Table 2.26. As expected, Glu ingestion caused an increase in blood glucose in both groups, whereas after the intake of Sor or Xyl the blood glucose levels did not change in either of the two race groups.

Table 2.26. Mean blood glucose levels ± SE (mmol/l) at baseline and 30 min after ingestion of each sugar

Sugar	*Blacks*			*Whites*			*Black vs Whites*	
	Baseline	*30 min*	*p*	*Baseline*	*30 min*	*p*	*p Baseline*	*p 30 min*
Glu	4.67±0.48	6.02±0.83	*0.002	4.60±0.50	6.32±0.96	*0.004	1.000	0.576
Sor	4.67±0.48	4.68±0.54	0.806	4.60±0.50	4.70±0.76	0.849	0.684	0.946
Xyl	4.67±0.48	4.62±0.57	0.704	4.60±0.50	4.90±0.65	0.169	0.473	0.276

*statistically significant

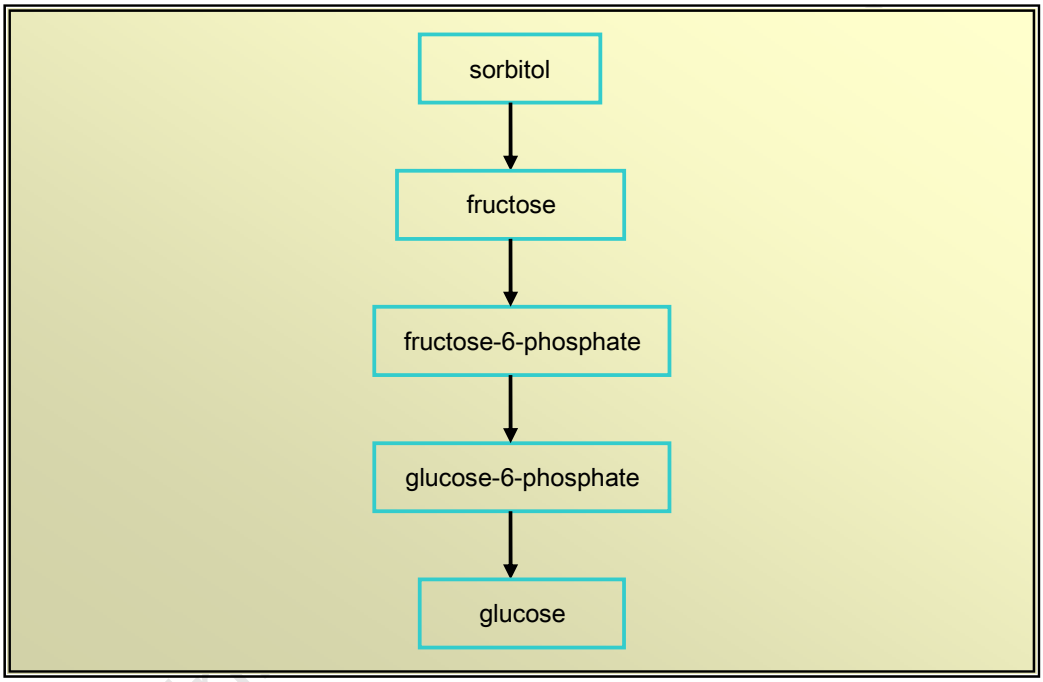
2.4 Discussion

Several intriguing observations emerged from this study which explored whether differences in renal handling of Glu and the two sugar alcohols, Sor and Xyl, exist in the two South African ethnic groups. The non-normalized excretions for the hourly fractions revealed a decrease in the urinary excretion of phosphate in blacks after the ingestion of Glu but this was not retained after 24 h. Interestingly, the normalized hourly excretions were in agreement with these findings. Moreover, Nguyen and coworkers (1993) also observed a decrease in phosphate excretion following a Glu load. The decrease in phosphaturia after Glu ingestion which was observed in black subjects in this study may be a consequence of the penetration of phosphorus into cells, which leads to a decrease in phosphoremia (Maina *et al.* 1975). This in turn reduces the renal filtered load of phosphate, thereby decreasing the urinary excretion of the phosphate (Nguyen *et al.* 1993).

In the present study changes in calciuria and oxaluria were not observed unlike the increase in both of these which has been observed in other studies (Nguyen *et al.* 1989). This can be attributed to the dose dependency effect as only 20 g of Glu was administered in this study compared to 75 g and 100 g loads in other studies. The increase in urinary citrate observed in white subjects is noteworthy. As stated in the previous Chapter, citrate is one of the most potent inhibitors of stone formation as it binds calcium ions thereby hindering CaOx and CaP precipitation. The hypercitraturic effect of Glu in this group provides explanation for the unchanged calciuria and oxaluria. With respect to the relative supersaturations, the results showed no significant changes in the RS of CaOx, brushite or uric acid within the two groups.

Similar to Glu, Sor provoked a decrease in phosphaturia in black subjects, noted in both the normalized and the non-normalized hourly excretions but not sustained after 24 h. On the other hand, their white compatriots also had reduced phosphate levels following a Sor load even though to a lesser extent, and this was observed in normalized excretions and only at 24 h in non-normalized excretions. The same proposed mechanism for the decrease in phosphaturia after Glu ingestion is thought to perhaps occur after Sor ingestion. The decrease in phosphaturia was more prominent in the black group than in the white group, suggesting that cell penetration by phosphorus which ultimately results in the reduction of urinary phosphate

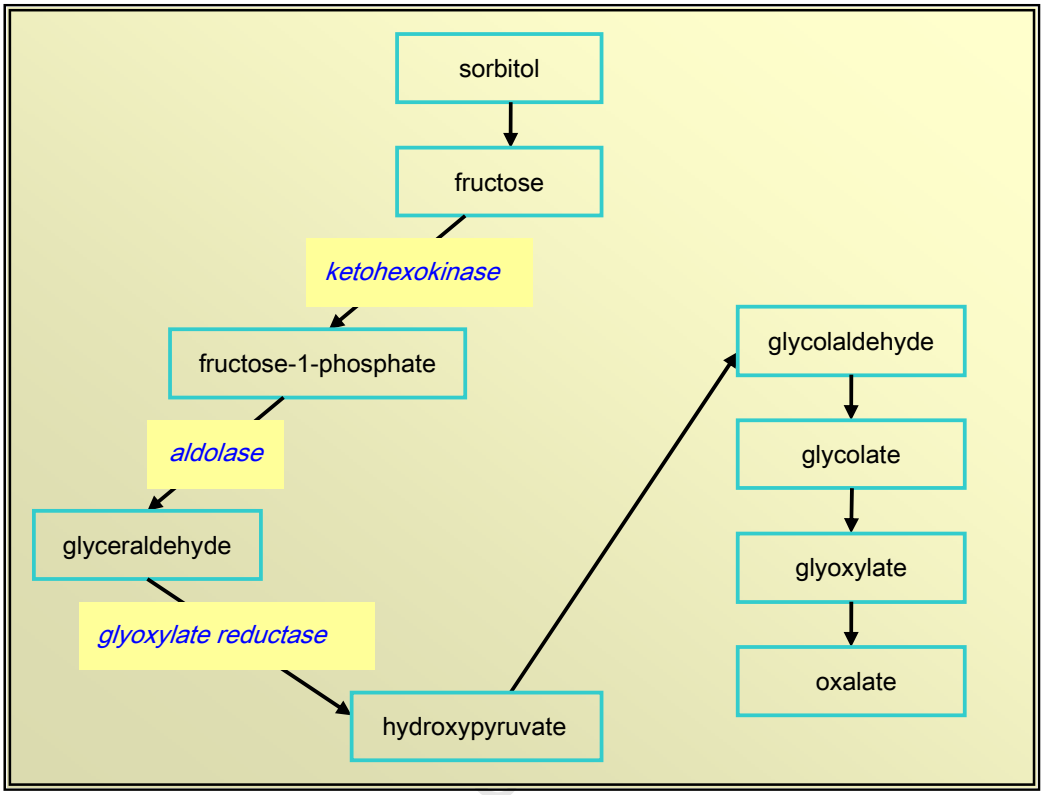
excretion occurs to a greater extent in the former. Of note is also the significant decrease in RS of brushite in black subjects which can be attributed to the decrease in phosphate excretion or to the increase in urinary pH, or both, in this group. In addition to the fall in phosphaturia, Sor overtly increased the urinary excretion of oxalate in white subjects. However, this was apparent only in normalized excretions but was not in agreement with the findings reported by Nguyen *et al.* (1993) wherein they indicated that Sor had no effect on oxalate excretion. Two possible explanations for the rise in oxaluria are firstly, the hepatic transformation of sorbitol into glucose as shown in Scheme 1.



Scheme 1: *Metabolic pathway of sorbitol leading to the production of glucose (Adcock and Gray (1957)*

It has been shown that Glu ingestion increases oxaluria at doses 20-100 g (Nguyen *et al.* 1986, Nguyen *et al.* 1989, Nguyen *et al.* 1993, Nguyen *et al.* 1998), therefore it is suggested that Sor ingestion has a similar effect due to its metabolic conversion to glucose. However, since no increase in urinary oxalate was observed after glucose ingestion in the present study, it is not likely that this mechanism can account for the rise in oxaluria following sorbitol intake. Secondly, another mechanism which can account for the rise in oxalate excretion, which has also been proposed for xylitol is

the hepatic transformation of sorbitol into glyoxylate and then into oxalate (Scheme 2).

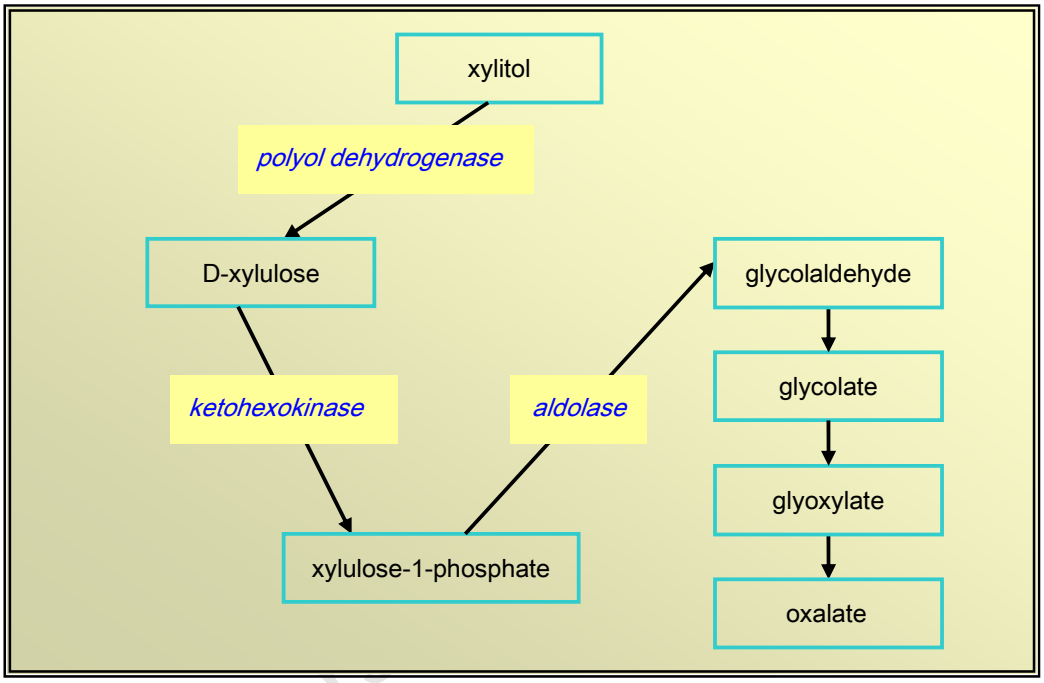


Scheme 2: *Metabolic pathway of sorbitol and enzymes involved leading to the production of oxalate (Conyers et al. 1990)*

It is therefore suggested that the increase in oxaluria in white subjects occurs via this mechanism and it seems that the baseline activity of one or more of the enzymes involved in this pathway is lower in black subjects. This provides evidence that the two groups differ in the renal handling of certain agents, implying different mechanisms as hypothesized earlier. Urinary changes in calcium excretion were apparent in the white group but only at 24 h. The decrease in excretion of this parameter was supported by the concomitant decrease in BRI in this group. Since Sor augmented the non-normalized citrate excretion in black subjects, the unchanged calcium and oxalate levels in this group can be ascribed to hypercitraturia.

Ingestion of Xyl gave rise to an increased normalized excretion of urinary oxalate in black subjects, which is in accordance with Nguyen and co-workers' results (1993).

This rise in oxaluria was also observed in non-normalized excretions at 24 h. This probably occurred via the metabolic pathway proposed by Conyers *et al.* (1990) leading to the production of oxalate, as depicted below in Scheme 3. It is believed that in the pathway, D-xylulose is converted to xylulose-1-phosphate by an enzyme known as ketohexokinases. The generated xylulose-1-phosphate is then cleaved by another enzyme called aldolase to produce glycolaldehyde, a precursor of oxalate.



Scheme 3: *Metabolic pathway of xylitol and enzymes involved leading to the production of oxalate (Conyers et al. 1990)*

The observed rise in oxaluria in black subjects but not in their white counterparts suggests that the hepatic transformation of Xyl to oxalate may be operating in the former and is hindered in the latter group. The implication of this is that one, two or all three of the enzymes in the pathway may be more active in the former group. Alternatively, the increased oxaluria in this group could have resulted from the reduction of fractional calcium reabsorption. The results showed that black subjects excreted lower urinary calcium than white subjects (normalized excretion), which could facilitate reduction of complexation with oxalate and thus increased absorption in the GI tract leading to higher urinary oxalate (Messa *et al.* 1997). The non-

normalized excretion of calcium at 24 h did not change in both groups following Xyl load, which is in accordance with the findings of a previous study (Bär 1985).
It is noted that some parameters are significantly different from the baseline within the groups after ingestion of a sugar but when intergroup comparisons are made statistical differences disappear. This is expected and not entirely surprising since such differences will depend on the extent to which each parameter changes in each group.

Crystallization experiments were performed in the reconstituted 24 h urines which were obtained pre- and post-ingestion of the three sugars. Since there were no statistically significant changes in the 24 h urinary parameters after Glu ingestion, the changes observed in the crystallization experiments must be due to subtle alterations in urine chemistry, which affect its overall crystallization capacity. It is noted that the changes in the latter were all favourable, indicating that despite earlier reports, Glu ingestion appears to have reduced the risk of stone formation. The changes in the 24 h urine parameters observed in both groups after ingestion of Sor were all such that they would have increased inhibition towards CaOx crystal formation. This is reflected in the decrease in size and volume of the crystals. The unfavourable decrease in inhibition of aggregation in black subjects again draws attention to the differences between the two groups in their respective responses to dietary challenges. The decrease in crystal volume observed in black subjects after Xyl ingestion might be attributed to the increase in the 24 h excretion of magnesium which occurred in this group. This is surprising since urinary oxalate also increased. The decrease in the deposition kinetics in whites is attributed to non-dietary subtle changes in urine chemistry.

It is rather surprising that neither of the two polyols raised the urinary calcium levels. It has been postulated that non-absorbed polyols are passed through to the lower gut where they are fermented to short-chain volatile fatty acids which give rise to acid loading in the body. This may consequently result in elevated urinary excretion of calcium (de Groot *et al.* 1995). Perhaps ingestion of larger quantities of these sugar alcohols might trigger elevated calcium excretion. Their difference in chemical structure might offer some explanation for the difference in metabolic behaviours, if any, of the two polyols.

Blood glucose levels were measured to asses the response to an oral load of Glu and the two polyols. As expected, Glu ingestion augmented blood glucose levels in the two groups, whereas Sor and Xyl did not change significantly. This is in agreement with Adcock and Gray (1957) and Nguyen *et al.* (1993). It is primarily due to the fact that unlike Glu, Sor and Xyl are more slowly and partially absorbed from the small intestine at virtually the same rate, thereby resulting in lower blood glucose and insulin response (Olefsky and Crapo 1980).

In summary, the results of the present study have demonstrated that the two ethnic groups differ in the renal handling of the carbohydrate challenges, judging by their responses to the ingestion of each of the three sugars. By consideration of established pathways, it is possible to hypothesize enhanced or diminished enzymatic activity in one (or the other) of the two groups, to account for these differences.

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Chapter Three

Investigation of the dose effect of sorbitol ingestion on the urinary risk factors of calcium oxalate kidney stone formation and the evaluation of the serum aldolase activity in black and white South African subjects

3.1 Introduction

In the study described in Chapter Two, Sor escalated the normalized urinary excretion of oxalate in the white subjects at 3 h after its ingestion. Other effects were observed in the 24 h samples from whites. In black subjects, phosphate excretion decreased and citrate excretion increased in various fractions following Sor ingestion. An obvious area for further investigation was whether dose dependency effects were operative in this mechanism. The present chapter describes a study which addresses this question.

In addition, the previous chapter also reported significant increases in oxaluria following the ingestion of Xyl in black subjects. It is now hypothesised that this elevation in oxaluria as well as that occurring in whites after Sor ingestion may be a consequence of differences in the baseline activity of one or more enzymes in the proposed metabolic polyol pathways, in the two groups. In order to lend credence to this hypothesis, a literature survey was conducted to establish which enzyme(s) might be common to both the Sor and Xyl metabolic pathways for conversion to oxalate and which might be linked to CaOx urolithiasis. Literature revealed that aldolase, which catalyses the conversion of fructose-1-phosphate to glyceraldehyde in the Sor pathway, and xylulose-1-phosphate to glycoladehyde (a precursor of oxalate) in the Xyl pathway, is indeed such an enzyme as it has been implicated in kidney stone formation (Hautman and Lutzeyer 1976, Tiktinskiĭ *et al.* 1985). As such, it was decided to investigate the respective baseline activities of this enzyme in an independent study to that mentioned above. This study constitutes the second part of the present chapter.

The main objectives of this study are:

- to investigate the potential dose dependency effects of Sor ingestion on urinary risk factors in healthy black and white male South African subjects

- to assess the physico-chemical properties of urines from the two groups before and after ingestion of two different doses of Sor

- to measure serum calcium and phosphorus with a view to comparing these with corresponding urine variables before and after the Sor loads in the two groups in an attempt to understand and elucidate the mechanistic pathways involved

- to test the hypothesis that the baseline activity of serum aldolase differs in the two groups

3.2 Subjects and Methods

Subjects

Ten black and ten white healthy male subjects (age range 18-30 years) with no history of kidney stone disease or diabetes mellitus were recruited from the student cohort of the University of Cape Town for the Sor study. This group of subjects was different from the group which was studied in Chapter Two.

For the evaluation of the aldolase activity, twenty black and twenty white healthy male subjects (age range 18-30 years) participated in the study.

The inclusion and exclusion criteria described in Chapter Two were also applied in the present studies. For the aldolase study, in particular, none of the subjects had a skeletal muscle disease, hepatitis or liver cirrhosis.

Test sugar

Sor (Sigma Aldrich, Steinheim, Germany, 97% purity).

Experimental Procedure

Sorbitol Study

The protocol which was employed in the present Sor study was identical to that described in the previous chapter. Each subject was on a self-selected diet controlled for calcium (1 g/day) and carbohydrates (300 g/day) for 7 days. On day 8, subjects consumed a strict standardized diet which was slightly modified in comparison to that shown in Chapter Two as illustrated in Table 3.1. Subjects fasted overnight over a period of 12 h. A 24 h urine sample was provided by the subjects at the end of day 8. Subjects were instructed to drink 250 ml of water (Caledon Natural Spring Water) on waking and to collect a 1 h baseline urine sample. At the end of the baseline period, a blood sample was drawn from each subject. Immediately after the blood drawing, subjects drank a solution of Sor (20 g in 250 ml water) and 30 min subsequent to that another blood sample was drawn. Three hourly urine fractions were then collected and at the end of the urine fraction collection subjects consumed a standardized diet and collected urine for the remaining 20 h of the 24 h test period. Sera and urine samples were stored at

4°C until analysis. Volumetric addition of the urine fractions was performed to reconstitute the 24 h sample. Subjects followed a wash-out period for 7 days before commencing the next cycle with 30 g of Sor in 250 ml water.

Although the 20 g dose was also administered in the previous study (Chapter Two), serum calcium and phosphorus levels were not determined. Hence, this dose was repeated in the present study.

Table 3.1: Constituents of the strict standardized diet ingested at day 8 and 9

MEAL	*DAY 8*	*DAY 9*
Breakfast: 8h00	50 g bread roll 8 g butter 25 g apricot jam 60 g cornflakes 250 ml 2% low fat milk 250 ml coffee	50 g bread roll 8 g butter 25 g apricot jam 60 g cornflakes 250 ml 2% low fat milk No coffee
Snack: 11h00	27 g muesli bar ½ cup fruit salad	27 g muesli bar ½ cup fruit salad
Lunch: 13h00	4 slices of whole wheat bread 16 g butter 28 g cheddar cheese 28 g salami pressed beef 50 g tomato	4 slices of whole wheat bread 16 g butter 28 g cheddar cheese 28 g salami pressed beef 50 g tomato
Snack: 16h00	150 g apple 175 g low fat fruit yoghurt	150 g apple 175 g low fat fruit yoghurt
Supper: 18h00	140 g beef mince* 120 g mixed vegetables 200 g pasta	140 g beef mince* 120 g mixed vegetables 200 g pasta
Snack: 20h00	1 slice of whole wheat bread 20 g peanut butter	1 slice of whole wheat bread 20 g peanut butter

* beef mince for 7 portions: 1 tbsp oil, 1 onion, 1 heaped tsp garlic, 125 g button mushrooms, 1 tin peeled tomatoes, 1 cup beef mince, salt, pepper, paprika, chilli powder

Beverages (2.25 l/day):

250 ml coffee (4 g coffee powder, 4 g creamer, 10 g sugar in 250 ml water (Day 8 only))

700 ml Caledon water until 13h00

600 ml Caledon water until 19h00

700 ml Caledon water until 22h00

Table 3.2: Nutrient content of the standardized diet

VARIABLE	*AMOUNT*
Energy (kJ)	11872
Total protein (g)	103.1
Total fat (g)	112.7
Carbohydrate (g)	301.5
Calcium (mg)	1000
Magnesium (mg)	347
Phosphorus (mg)	1554
Citric acid (mg)	509
Oxalic acid (mg)	66

Aldolase Study

Specific dietary protocols were not followed in the aldolase study since baseline values were investigated. However, subjects were instructed to abstain from the intake of dietary supplements and any medication or prescription drugs. Each subject provided a 24 h baseline urine sample and a fasting blood sample which was drawn in the morning in a gel tube and then centrifuged. Sera were stored at -70°C until analysis.

Analytical Methods

For the Sor study, urines were analyzed as previously described in Chapter Two (page 51) but chloride and sulphate were not measured. In addition, serum calcium was measured using an ion selective electrode and serum phosphorus (inorganic) using ammonium molybdate (Dryer and Routh 1963) in the blood samples.

Only oxalate and creatinine were measured in the baseline urine samples obtained in the aldolase study. Commercial kits were used to measure serum aldolase activities both colorimetrically and spectrophotometrically. The procedures were carried out according to the manuals supplied with the kits and will be described on page 112.

Sorbitol Study

Determination of Urinary Sorbitol

The concentration of Sor recovered in urine subsequent to metabolism was measured by two different techniques, that is, high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) and fluorescent assay.

HPAEC-PAD Method

I. Preparation of Samples

Urine samples (which had been kept at -80°C prior to analysis) were thawed, pooled and then diluted 50% with water. The diluted samples were filtered, firstly through 0.45 µm membranes and then C18 reverse phase solid phase extraction cartridges, to remove proteins. The following selection of internal standards: glycerol, erythritol, ribose, rhamnose and galactose were tried but all overlapped with other peaks in the chromatograms.

II. Chromatographic Procedure

Carbohydrate profiling was performed on a Dionex BioLC ion chromatograph system equipped with an AS50 autosampler (Rheodyne rotary injection valve with a 25-µL PEEK sample loop), ED50 electrochemical detector, LC30 oven and GS50 pump. The detector cell incorporated a gold working electrode and a pH (Ag/AgCl) reference electrode. Chromatographic data were recorded on a personal computer equipped with Chromeleon software.

Profiling of Sor was achieved using a CarboPac MA1 column (250 mm × 4 mm) coupled with a CarboPac MA1 guard column. Isocratic elution was performed with NaOH (250 mM for the 30 g Sor dose batch) and (470 mM for the 30 g Sor dose batch) at a flow rate of 0.4 ml/min. The concentration of Sor was determined from peak areas calculated from a Sor standard curve.

Fluorescent Assay Method

An assay method by Nakano and co-workers (2003) was applied for the determination of Sor in urine, which involved the removal of interfering ionic and non-ionic substances using an ion exchange resin column.

I. Preparation of Samples

Urine samples were thawed as previously stated and then pooled. To 2 ml of each pooled sample, distilled water (1.0 ml) was added. The mixture was then passed through an Amberlite MB-1 (Sigma-Aldrich, Germany) column. The column was washed with distilled water (20 ml) and to the eluted solution (5 ml), distilled water (1 ml), 0.475 mol NaOH (1 ml) and 0.293 mol $ZnSO_4$ (1 ml) were added to make a total volume of 8 ml. The mixture was vortexed and then centrifuged at 3000 rpm for 2 min.

II. Assay Procedure

To the supernatant (2 ml), obtained above, 0.33 M Tris buffer (0.5 ml; pH 8.6), 250 mg/dl NAD (1 ml), 0.1 M EDTA (0.1 ml) and 40 U/ml SDH (0.05 ml) were added. The mixture was incubated at 37 °C for 30 min followed by measurement of Sor using a flourimeter (Olis Inc., USA) at excitation wavelength, λ_{ex} = 366 nm and emission wavelength, λ_{em} = 452 nm.

Crystallization Experiments

The same crystallization experiments as those described in Chapter Two (page 52) were performed on the urines from the Sor study only.

Scanning Electron Microscopy

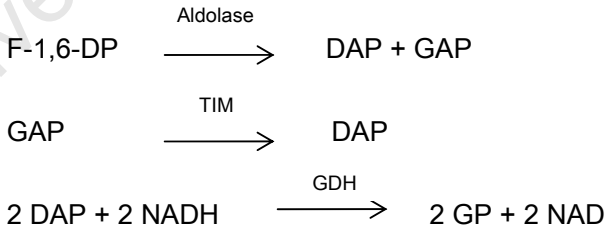
At the end of the 120 min incubation period (following induction of crystallization with Na_2Ox), 2 ml urine aliquots were filtered through a 0.22µm Millipore filter. Subsequent to drying, the filters were glued on aluminium stubs and were then splutter-coated with 3 – 5 nm of Au/Pd for 10 minutes (Bio-Rad, SEM Coating System). A scanning electron microscope (Leica S440 Scanning Electron Microscope, Leica Cambridge, England) was used to view the stubs. The microscope was operated at an accelerating voltage of 15 kV at a working distance of 10-15 mm and at a probe current of 30 pA.

Aldolase Study

It has been reported that the activity of aldolase in cleaving fructose-1-phosphate is highest in the liver and kidney (Tsunematsu and Shiraishi 1969). It is also believed that most of the serum aldolase comes from the liver, where the metabolism of the sugar alcohols (Sor and Xyl) and other sugars occurs (Tsunematsu and Shiraishi 1969, Bais *et al.* 1985). Two methods commonly used for the determination of serum aldolase activity, that is, a colorimetric method (Sibley and Lehninger 1949, Pinto *et al.* 1969a) and a spectrophotometric method (Feissli 1966), were employed in this chapter.

Colorimetric Determination of Aldolase Activity

A kit was used to measure aldolase activity by a colorimetric method (Caldon Biotech Inc., USA). The initial step is the catalysis of the cleavage of fructose-1,6-diphosphate (F-1,6-DP) to D-glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DAP). This is followed by the conversion of GAP to DAP with the addition of triosephosphate isomerase (TIM). DAP is then reduced to L-α-glycerol phosphate (GP) by α-glycerophosphate dehydrogenase and NADH is oxidised to NAD, simultaneously. These successive steps are depicted below. The absorbance is read at 340 nm and the rate of decrease is proportional to the aldolase activity.



I. Preparation of Solutions

A bottle of the reagent consisting of F-1,6-DP (0.2 mol/l), animal GDH (≥600 U/l), animal TIM (≥3000 U/l), NADH (0.32 mmol/l) and buffer; pH 7.0 was supplied with no requirement of preparation.

II. Assay Procedure

To the reagent (3.0 ml) which was pipetted into a test tube and warmed to 37°C, a serum sample (200 µl) was added and the solution was mixed and incubated at 37°C. About 2 min subsequent to addition of the sample, the initial absorbance (A_0) was measured. The mixture was left to stand at 37°C and the final absorbance (A_{10}) was measured exactly 10 min after the initial reading. The rate of the reagent blank (ΔA_b) was determined similarly using distilled water (200 µl) instead of serum and the aldolase activity (U/l) was determined by the following equation (Pinto *et al.* 1969a):

$$\frac{(\Delta A_s - \Delta A_b) \times \text{total volume} \times 1000}{6.22 \times \text{sample volume} \times 10 \times 2}$$

[2=factor to compensate for oxidation of 2 µmole of NADH]

Spectrophotometric Determination of Aldolase Activity

As with the above procedure, a commercially available kit was purchased for the determination of aldolase activity spectrophotometrically (Randox, UK). The principle of the spectrophotometric procedure is similar to that of the colorimetric procedure described above.

I. Preparation of Solutions

Three bottles of reagents were supplied with the kit. Reagent bottle 1 consisted of a buffer/substrate [collidine buffer (51 mmol/l, pH 7.4), mono-iodoacetate (0.27 mmol/l, F-1,6-DP (2.7 mmol/l)] which was reconstituted with redistilled water (20 ml). NADH (0.23 mmol/l), reagent bottle 2, was reconstituted with 1 ml of redistilled water while reagent bottle 3 containing GDH/TIM/LDH [GDH (≥326 mU/ml), TIM (≥4.35 U/ml), LDH (≥616 mU/ml), ammonium sulphate (>35%)] was ready for use. An aldolase calibrator (not provided with the kit but also purchased from Randox) was reconstituted with 1 ml of redistilled water. Lastly, 0.9% NaCl solution was prepared for the sample blank.

II. Assay Procedure

A serum sample (0.2 ml) was pipetted into a test tube followed by the successive addition of the buffer/substrate (2.50 ml), NADH (0.05 ml) and GDH/TIM/LDH (0.01 ml). The calibrator mixture was prepared in a similar manner except that the calibrator solution (0.2 ml) was used instead of a serum sample. The sample blank was prepared by adding 0.9% NaCl solution to a serum sample (0.2 ml). The three individual test tubes were each mixed followed by incubation at 37°C for 5 min. At the end of this period the initial absorbance (A_1) was read at 340 nm against the sample blank. The mixture was left to stand at 37°C for exactly 20 min after the initial reading and the final absorbance (A_2) was measured against the blank. The following equation was used to calculate the aldolase activity (Feissli *et al.* 1966):

$$\frac{(A_1 - A_2)\ \text{Sample}}{(A_1 - A_2)\ \text{Calibrator}} \times \text{Conc. of calibrator}$$

[Conc. of calibrator=16.7 U/l]

Independent Analysis of Aldolase

Both methods described above (colorimetric and spectrophotometric) were applied by the present author. In addition, all samples were sent to an external laboratory (Lancet Laboratories, Johannesburg) for independent analyses using the spectrophotometric assay.

Statistical Analysis

Data from both studies were analysed by one-way ANOVA and regarded statistically significant at $p \leq 0.05$.

3.3 Results

SORBITOL STUDY

20 g SORBITOL

24h Collections

Baseline 24 h urinary parameters are given in Table 3.3. The lower values in blacks for citrate (significant) and oxalate (approaching significance) were not observed in the previous study described in Table 2.4, Chapter Two. Conversely, several differences reported in this table were not observed in the present study.

In the 24 h samples following Sor ingestion, only one significant change occurred in the black group, namely an increase in RS of uric acid. Similarly, only the CaOx MSL significantly increased in the white group (Table 3.4). This differs markedly with the changes which occurred in the previous study (Table 2.12). Reasons for these differences will de explored in the Discussion section of this chapter.

Fractional Collections (Non-Normalized Excretion)

Prior to ingestion of Sor (corresponding to 0 h), no differences were observed in any of the urinary parameters in either group, as shown in Table 3.5. Similarly, at 1 h (Table 3.6), there were no changes within the two groups after Sor intake except for the decrease in phosphate in white subjects. The increase in urinary citrate and the decrease in urinary phosphate at 2 h (Table 3.7) were also observed in the previous study. However, the changes observed at 3 h (Table 3.8) are unique to the present study.

Table 3.3: Mean baseline 24 h urinary variables ± SE black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.53 ± 0.09	6.56 ± 0.06	0.3178
Voume (ml/24h)	1836 ± 238	2206 ± 183	0.0916
Citrate (mmol/24h)	3.21 ± 0.46	4.63 ± 0.35	*0.0041
Oxalate (mmol/24h)	0.20 ± 0.02	0.27 ± 0.02	†0.0520
Calcium (mmol/24h)	2.65 ± 0.37	3.18 ± 0.62	0.8352
Magnesium (mmol/24h)	2.33 ± 0.34	2.52 ± 0.22	0.6172
Sodium (mmol/24h)	228 ± 39.2	187 ± 30.5	0.7712
Potassium (mmol/24h)	82.8 ± 16.4	54.1 ± 7.94	0.2403
Urate (mmol/24h)	3.36 ± 0.59	3.24 ± 0.48	0.8006
Creatinine (mmol/24h)	12.7 ± 1.07	14.0 ± 1.39	0.2294
Phosphate (mmol/24h)	20.1 ± 2.05	27.2 ± 3.06	0.0726
Tiselius risk index	198 ± 24.0	265 ± 53.0	0.2506
RS Brushite	0.48 ± 0.15	0.62 ± 0.28	0.7765
RS CaOx	1.99 ± 0.54	1.74 ± 0.51	0.7914
RS Uric Acid	0.53 ± 0.16	0.33 ± 0.19	*0.0369
MSL (mol/dm³)	0.17 ±0.01	0.10 ± 0.01	*0.0066

*statistically significant; †approaching statistical significance

Table 3.4: Mean 24 h urinary variables ± SE in black and white subjects after ingestion of 20 g Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post- Sor*	*p*	*p Post- Sor*
pH	6.53±0.09	6.45±0.10	1.0000	6.56±0.06	6.70±0.06	0.4082	0.1115
Volume (ml/24h)	1836±238	2084±128	1.1177	2206±183	2175±94.9	0.9402	0.6389
Citrate (mmol/24h)	3.21±0.046	4.16±0.32	0.0740	4.63±0.35	5.03±0.48	0.9558	0.2066
Oxalate (mmol/24h)	0.20±0.02	0.28±0.02	0.1280	0.27±0.02	0.29±0.02	0.7722	0.7831
Calcium (mmol/24h)	2.65±0.37	2.82±0.37	0.7176	3.18±0.62	3.13±0.42	0.4880	0.3334
Magnesium (mmol/24h)	2.23±0.34	2.65±0.26	0.1650	2.52±0.22	2.95±0.27	0.3701	0.5355
Sodium (mmol/24h)	228±39.2	238±29.4	0.7201	187±30.5	183±15.3	0.6649	0.1743
Potassium (mmol/24h)	82.9±16.4	80.3±12.7	0.7205	54.1±7.94	53.0±11.5	0.8956	0.1890
Urate (mmol/24h)	3.36±0.59	3.51±0.42	0.2684	3.24±0.48	3.27±0.13	0.5012	0.6696
Creatinine (mmol/24h)	12.7±1.07	16.8±1.30	0.0974	14.0±1.39	16.6±0.82	0.3852	0.9090
Phosphate (mmol/24h)	20.1±2.05	26.0±2.72	0.2790	27.2±3.06	30.5±1.67	0.4242	0.2354
Tiselius risk index	198±24.0	171±20.5	0.4116	265±53.0	146±25.5	0.0867	0.4432
RS Brushite	0.48±0.15	0.41±0.16	0.7757	0.62±0.28	0.33±0.06	0.3341	0.6640
RS CaOx	1.99±0.54	1.24±0.36	0.2508	1.74±0.51	1.18±0.29	0.2536	0.8926
RS Uric Acid	0.53±0.16	1.83±1.08	*0.0351	0.28±0.19	0.31±0.05	0.2566	*0.0292
MSL (mol/dm^3)	0.17±0.01	0.14±0.01	0.2226	0.10±0.02	0.16±0.01	*0.0246	0.4224

*statistically significant

Table 3.5: Mean baseline 0 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.21 ± 0.19	6.30 ± 0.17	0.7108
Volume (ml/h)	77.0 ± 8.12	94.7 ± 21.5	0.4524
Citrate (mmol/h)	0.15 ± 0.02	0.17 ± 0.05	0.6244
Oxalate (mmol/h)	0.03 ± 0.01	0.03 ± 0.01	0.5162
Calcium (mmol/h)	0.07 ± 0.01	0.05 ± 0.02	0.2948
Magnesium (mmol/h)	0.10 ± 0.01	0.09 ± 0.02	0.9598
Sodium (mmol/h)	17.4 ± 7.07	9.73 ± 2.28	0.3142
Potassium (mmol/h)	3.92 ± 2.46	1.45 ± 1.32	0.3329
Urate (mmol/h)	0.14 ± 0.01	0.22 ± 0.04	0.1732
Creatinine (mmol/h)	0.90 ± 0.14	1.10 ± 0.22	0.4587
Phosphate (mmol/h)	1.17 ± 0.23	1.65 ± 0.44	0.3465

Table 3.6: Mean 1 h urinary variables ± SE in black and white subjects after ingestion of 20 g Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post- Sor*
pH	6.21±0.19	6.51±0.17	0.2469	6.30±0.17	6.35±0.18	0.5413	0.5334
Volume (ml/h)	77.0±8.12	173±56.0	0.1075	94.7±21.5	90.4±23.3	0.3986	0.1909
Citrate (mmol/h)	0.15±0.02	0.27±0.06	0.0788	0.17±0.05	0.21±0.06	0.5310	0.4791
Oxalate (mmol/h)	0.03±0.01	0.04±0.01	0.2507	0.03±0.01	0.03±0.01	0.2846	0.4331
Calcium (mmol/h)	0.07±0.01	0.15±0.06	0.2131	0.05±0.02	0.05±0.02	0.6098	0.1387
Magnesium (mmol/h)	0.10±0.01	0.16±0.05	0.1846	0.09±0.02	0.11±0.02	0.4721	0.3104
Sodium (mmol/h)	17.4±7.07	10.5±2.89	0.3785	9.73±2.28	5.69±1.55	0.6413	0.1586
Potassium (mmol/h)	3.92±2.46	1.84±0.52	0.4189	1.45±1.32	1.85±0.73	0.0967	0.9903
Urate (mmol/h)	0.14±0.01	0.13±0.04	0.7189	0.22±0.04	0.15±0.02	0.6506	0.8276
Creatinine (mmol/h)	0.90±0.14	1.12±0.20	0.3970	1.10±0.22	0.97±0.09	0.6218	0.5144
Phosphate (mmol/h)	1.17±0.23	1.02±0.34	0.7126	1.65±0.44	0.86±0.18	*0.0229	0.6934

*statistically significant

Table 3.7: Mean 2 h urinary variables ± SE in black and white subjects after ingestion of 20 g Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post- Sor*
pH	6.21±0.19	6.71±0.18	0.0680	6.30±0.17	6.69±0.20	0.1488	0.9299
Volume (ml/h)	77.0±8.12	241±52.7	*0.0064	94.7±21.5	173±46.3	0.1441	0.3404
Citrate (mmol/h)	0.15±0.02	0.42±0.13	*0.0480	0.17±0.05	0.41±0.14	0.1403	0.9465
Oxalate (mmol/h)	0.03±0.01	0.03±0.01	0.5759	0.03±0.01	0.04±0.01	0.5818	0.6111
Calcium (mmol/h)	0.07±0.01	0.08±0.02	0.6786	0.05±0.02	0.05±0.01	0.8265	0.2106
Magnesium (mmol/h)	0.10±0.01	0.13±0.02	0.2224	0.09±0.02	0.11±0.02	0.4466	0.6718
Sodium (mmol/h)	17.4±7.07	5.10±1.98	0.1107	9.73±2.28	5.34±1.07	0.0983	0.9179
Potassium (mmol/h)	3.92±2.46	1.27±0.59	0.3080	1.45±1.32	1.64±0.50	0.7589	0.6377
Urate (mmol/h)	0.14±0.01	0.10±0.01	0.1674	0.22±0.04	0.19±0.03	0.5112	0.1304
Creatinine (mmol/h)	0.90±0.14	0.68±0.10	0.2064	1.10±0.22	0.98±0.10	0.5498	0.0716
Phosphate (mmol/h)	1.17±0.23	0.46±0.10	*0.0111	1.65±0.44	0.46±0.11	*0.0140	0.7772

*statistically significant

Table 3.8: Mean 3 h urinary variables ± SE in black and white subjects after ingestion of 20 g Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post- Sor*
pH	6.21±0.19	6.89±0.13	*0.0085	6.30±0.17	6.74±0.14	†0.0534	0.4690
Volume (ml/h)	77.0±8.12	260±27.9	*0.0001	94.7±21.5	268±30.4	*0.0002	0.8483
Citrate (mmol/h)	0.15±0.02	0.55±0.19	†0.0516	0.17±0.05	0.45±0.07	*0.0045	0.6224
Oxalate (mmol/h)	0.03±0.01	0.03±0.01	0.7364	0.03±0.01	0.04±0.01	0.6284	0.5258
Calcium (mmol/h)	0.07±0.01	0.15±0.04	*0.0489	0.05±0.02	0.07±0.01	0.2576	†0.0512
Magnesium (mmol/h)	0.10±0.01	0.13±0.01	*0.0486	0.09±0.02	0.13±0.03	0.2381	0.8276
Sodium (mmol/h)	17.4±7.07	6.89±1.98	0.1687	9.73±2.28	5.08±1.31	0.0940	0.4555
Potassium (mmol/h)	3.92±2.46	1.78±0.44	0.4036	1.45±1.32	2.01±0.55	0.3883	0.7463
Urate (mmol/h)	0.14±0.01	0.23±0.06	0.2622	0.22±0.04	0.17±0.03	0.5048	0.5148
Creatinine (mmol/h)	0.90±0.14	0.74±0.11	0.3767	1.10±0.22	0.90±0.11	0.4239	0.3314
Phosphate (mmol/h)	1.17±0.23	0.70±0.15	0.0981	1.65±0.44	0.73±0.11	0.8854	0.8510

*statistically significant; †approaching statistical significance

Fractional Collections (Excretion normalized to Creatinine)

Figures 3.1-3.3 depict the urinary Ca, Ox and P excretion in the two groups before and after 20 g Sor intake. Similarly to Chapter Two, these are expressed as the ratio of each parameter to creatinine. No significant changes were observed in urinary calcium and oxalate excretion in either of the two groups post Sor ingestion (Figure 3.1 and 3.2). However, decreases in urinary phosphate at 1 h and 2 h in blacks (p=0.0055 and p=0.0001, respectively) at 1 h, 2 h and 3 h (p=0.0389, p=0.0016 and p=0.0291, respectively) in the white group were observed (Figure 3.3). Decreases in urinary phosphate were observed in the previous study but were more prominent in the black group.

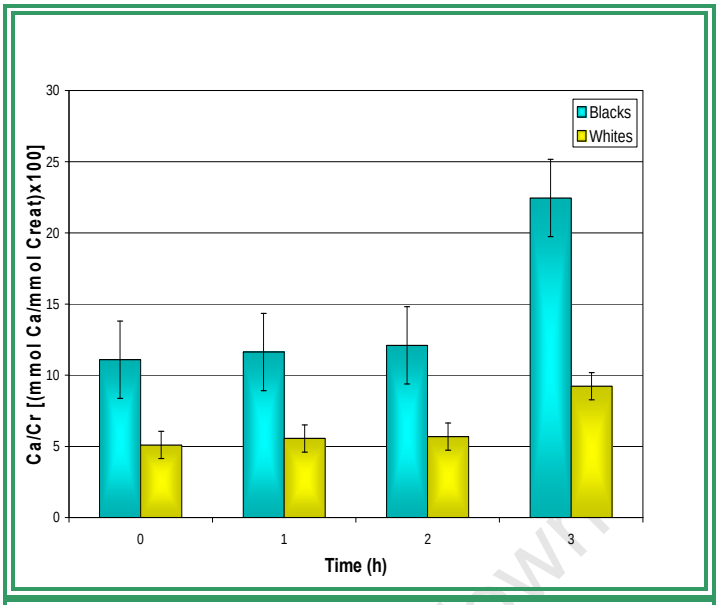


Figure 3.1: *Mean hourly values for urinary calcium excretion [(mmol Ca per mmol Creatinine) x 10^2] for black and white subjects following 20 g Sor challenge*

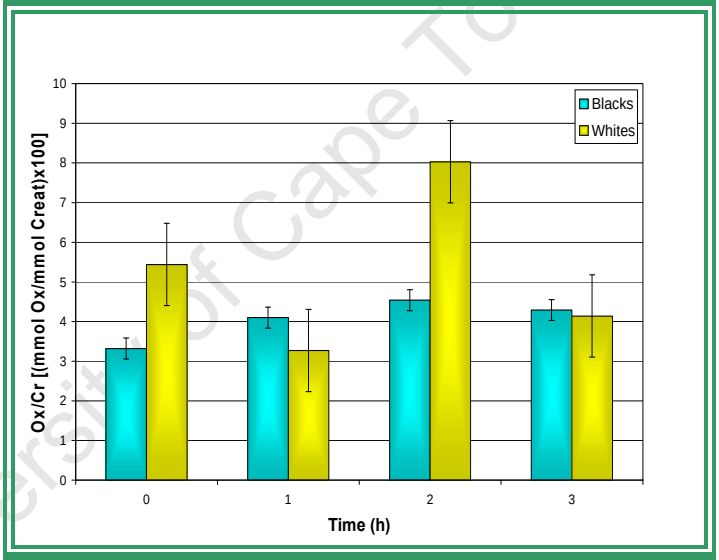


Figure 3.2: *Mean hourly values for urinary oxalate excretion [(mmol Ox per mmol Creatinine) x 10^2] for black and white subjects following 20 g Sor challenge*

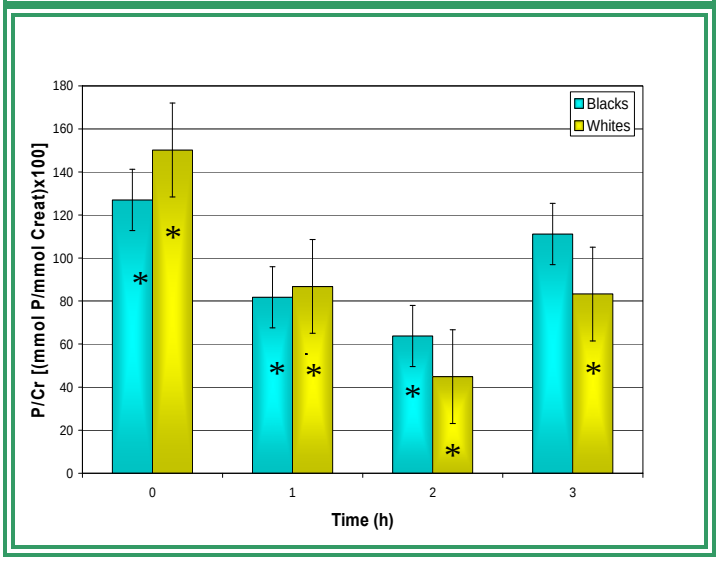


Figure 3.3: *Mean hourly values for urinary phosphate excretion [(mmol P per mmol Creatinine) x 10^2] for black and white subjects following 20 g Sor challenge*

Histograms labelled with the same symbol are significantly different ($p<0.05$)

Particle Formation Kinetics

Particle Volume-Size Distribution

The mean particle size is given in Table 3.9 before and after 20 g Sor load. Sor did not induce any change in the mean particle size in whites relative to baseline. However, in blacks, the mean particle size decreased after a Sor load. The mean particle volume-size distribution curves of urines from black and white subjects are shown in Figure 3.4.

Table 3.9: Mean particle size (µm) in urines of black and white subjects before and after ingestion of 20 g Sor

	Blacks	*Whites*
Before	4.01	3.28
After	3.28	3.28

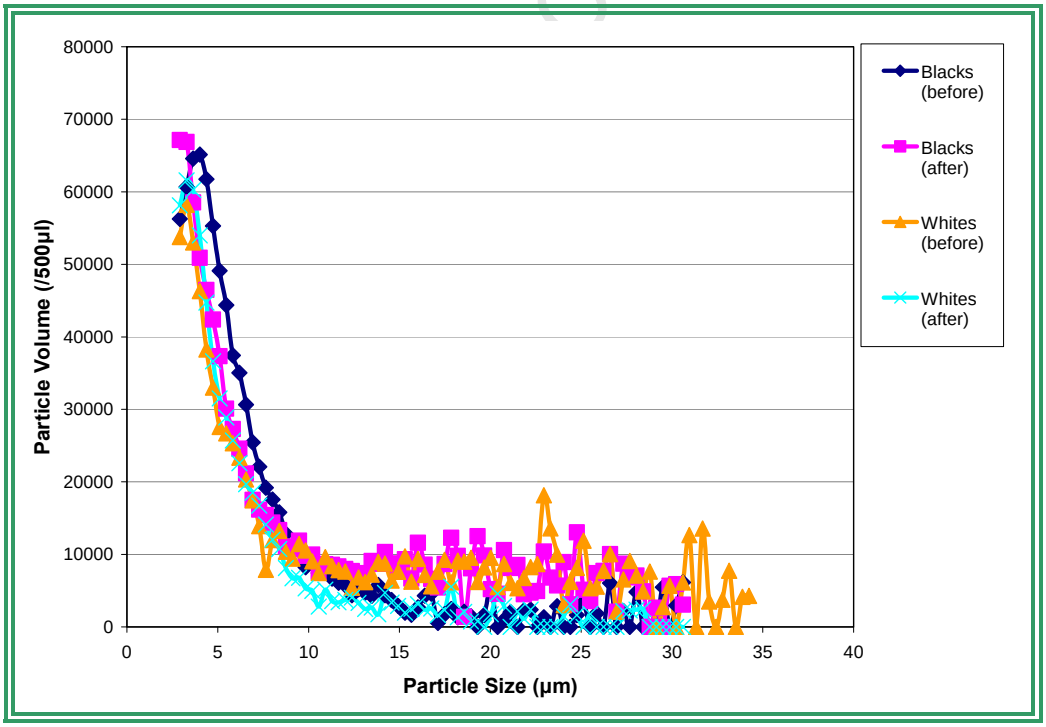


Figure 3.4: *Particle volume-size distribution pre- and post-20 g Sor ingestion in urines of black and white subjects*

Particle Number

The plots for particle number versus time are shown in Figure 3.5. At 120 min the particle number decreased in blacks post Sor load. A similar trend was noted in whites but to a much greater extent.

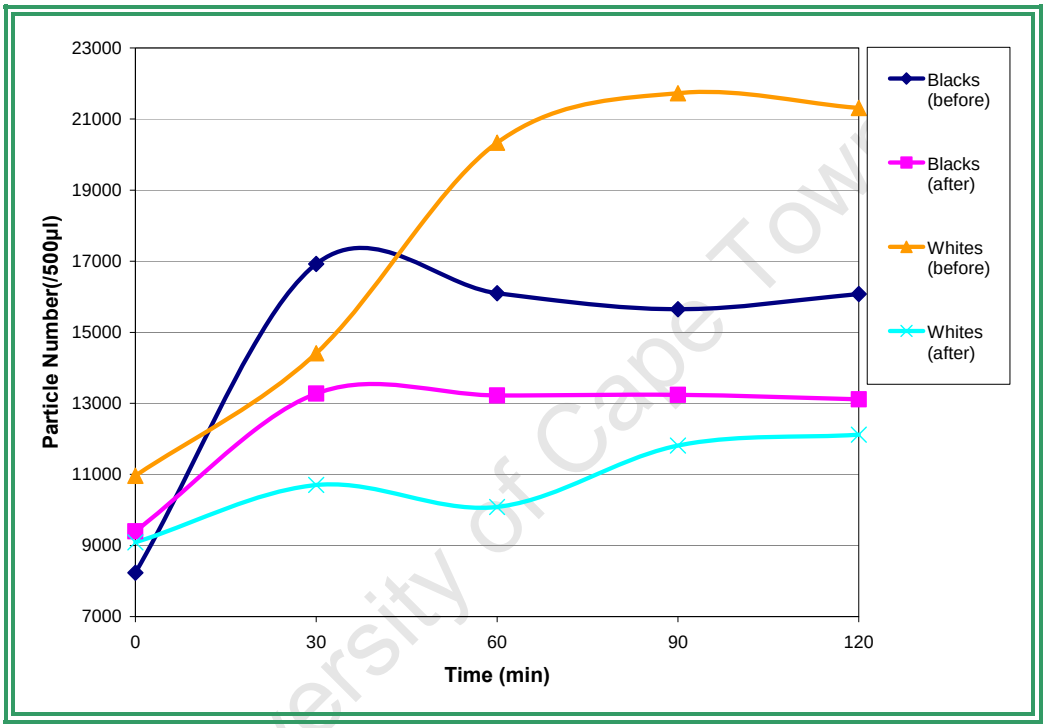


Figure 3.5: *Particle number in urines of blacks and whites before and after ingestion of 20 g Sor*

Scanning Electron Microscopy

The scanning electron micrographs obtained before and after a 20 g Sor load are shown in Figure 3.6 for black (a and b) and white (c and d) subjects. In all micrographs, the dominating crystals are the coffin-shaped calcium oxalate monohydrate crystals (COM). No changes in the number of crystals were observed in black subjects. However, in white subjects the number of crystals after the intake of Sor was lesser.

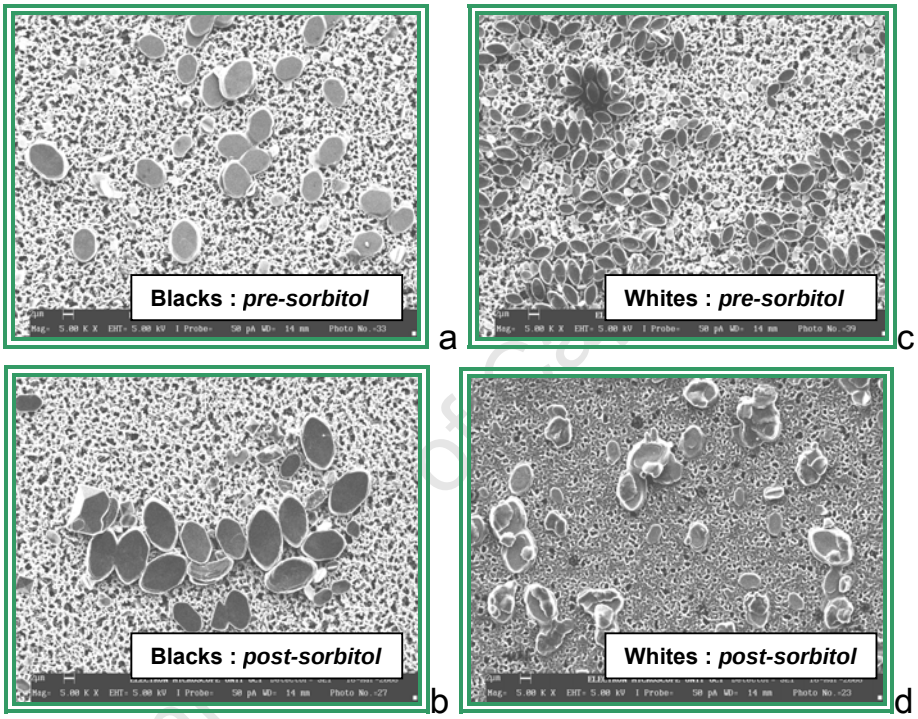


Figure 3.6: *Scanning electron micrographs of CaOx crystals pre- and post-ingestion of 20 g Sor in blacks (a: pre; b: post) and whites (c: pre; d: post) at 5 K magnification*

Crystallization Experiments

^{14}C-Oxalate Crystal Deposition

The rate of CaOx deposition is indicated by the gradient of each curve (top right-hand corner) for black and white subjects pre- and post-intake of 20 g Sor (Figure 3.7). There was no change in CaOx deposition rate in both groups after Sor load.

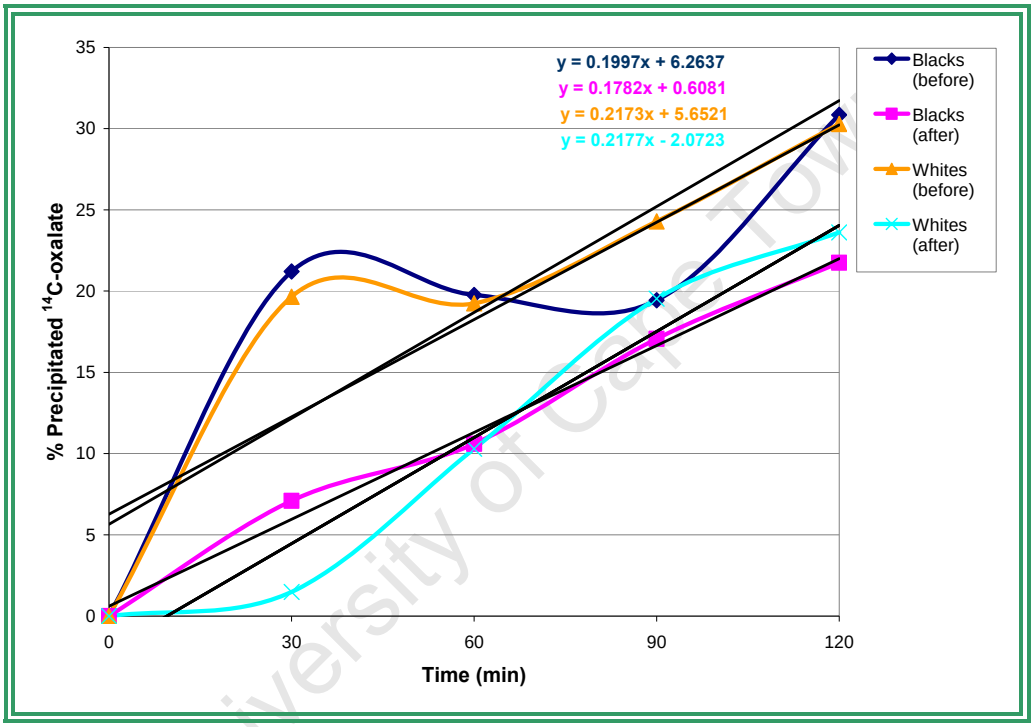


Figure 3.7: *Percentage precipitated ^{14}C-oxalate pre- and post-20 g Sor ingestion in urines of black and white subjects*

Crystal Aggregation

The mean percentages for the inhibition of CaOx crystal aggregation in black and white subjects are given in Table 3.10, corresponding to the plots shown in Figure 3.8. Within each group no significant changes were noted after 20 g Sor load. Inter-group comparisons also revealed no changes both before and after Sor ingestion.

Table 3.10: Mean percentage inhibition of aggregation in black and white subjects before and after a 20 g Sor load

Variables	*Blacks*		*Whites*		*Blacks vs Whites*
	% Ia (SE)	*p*	*% Ia (SE)*	*p*	*p*
Before	54.1 (4.25)		67.4 (6.75)		0.1021
After	63.8 (2.29)	0.0618	66.5 (5.41)	0.9134	0.6255

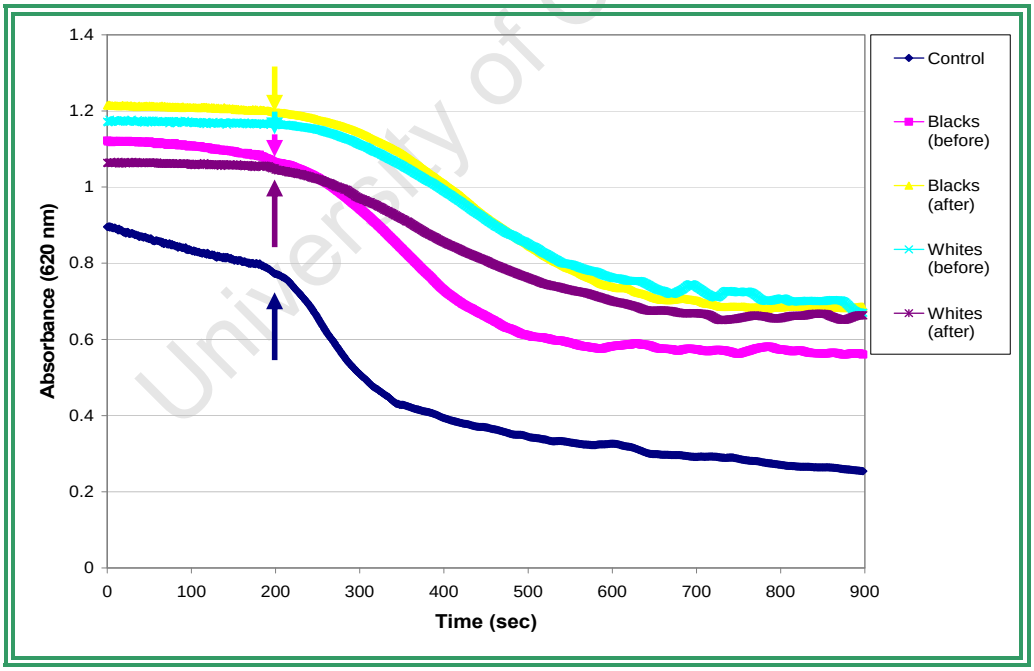


Figure 3.8: *Plot of absorbance versus time pre- and post-20 g Sor load in urines of black and white subjects. The time at which stirring was stopped is indicated by the arrows.*

Summary of Urine Chemistry and Crystalluria Properties After 20 g Dose

	Blacks	Whites
24 h: RS uric acid	↑	↔
Fractional (non-normalized)	↑ pH, ↑Cit, ↑Ca, ↑ Mg, ↓P	↑ Cit, ↓P
Fractional (normalized)	↓ P	↓P
Particle size	↓	↔
Particle number	↓	↓
SEM	↔	↓ Particle number
Deposition rate	↔	↔
Aggregation	↔	↔

30 g SORBITOL

24h Collections

Table 3.11 shows the excretions of 24 h urinary variables before a 30 g Sor load in the two race groups. The urinary citrate was significantly different between the two groups, with blacks a having lower mean excretion than whites. In addition, the former group had a higher CaOx MSL compared to the latter group. It is also noteworthy that urinary phosphate was approaching a significant difference, with the excretion lower in the black group. Sor did not change any of the 24 h parameters after its ingestion in blacks but it increased the CaOx MSL in whites (Table 3.12).

Fractional Collections (Non-normalized Excretion)

At 0 h before a 30 g Sor load the urinary pH was approaching significance, with whites having a higher mean, as shown in Table 3.13. Sor provoked a decrease in the excretion of urinary phosphate in both groups at 1 h (Table 3.14). In blacks, at 2 h (Table 3.15) the urinary potassium and phosphate levels decreased and the latter was also noted in the white group. A number of statistically significant changes were observed at 3 h (Table 3.16), in the black group the urinary pH, urine volume and urinary citrate increased, while urinary sodium and potassium decreased. On the other hand, in the white group the urine volume also increased but urinary sodium and creatinine decreased. No additional changes were observed within the two groups.

Table 3.11: Mean baseline 24 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.64 ± 0.15	6.66 ± 0.08	0.8161
Volume (ml/24h)	1859 ± 226	2179 ± 148	0.2793
Citrate (mmol/24h)	3.04 ± 0.36	4.57 ± 0.55	*0.1023
Oxalate (mmol/24h)	0.23 ± 0.03	0.26 ± 0.02	0.6795
Calcium (mmol/24h)	2.58 ± 0.61	2.59 ± 0.58	0.8761
Magnesium (mmol/24h)	1.60 ± 0.22	2.35 ± 0.28	0.1090
Sodium (mmol/24h)	287 ± 53.9	217 ± 42.6	0.1266
Potassium (mmol/24h)	88.9 ± 16.7	57.2 ± 12.8	0.0642
Urate (mmol/24h)	2.72 ± 0.23	3.28 ± 0.29	0.1500
Creatinine (mmol/24h)	13.6 ± 1.29	15.6 ± 1.37	0.4247
Phosphate (mmol/24h)	17.8 ± 1.73	28.8 ± 2.96	†0.0513
Tiselius risk index	194 ± 33.0	168 ±34.4	0.4016
RS Brushite	0.87 ± 0.08	0.66 ± 0.10	0.9226
RS CaOx	2.19 ± 1.20	1.41 ± 0.90	0.5313
RS Uric Acid	0.39 ±0.05	0.32 ± 0.07	0.8792
MSL (mol/dm³)	0.16 ± 0.01	0.11 ± 0.01	*0.0363

*statistically significant; †approaching statistical significance

Table 3.12: Mean 24 h urinary variables ± SE in black and white subjects after ingestion of 30 g Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post- Sor*
pH	6.64±0.15	6.60±0.15	0.6609	6.66±0.08	6.68±0.07	0.9709	0.6884
Volume (ml/24h)	1859±226	1977±133	0.8829	2179±1.48	1979±151	0.2299	0.9929
Citrate (mmol/24h)	3.04±0.36	4.84±0.70	0.1150	4.57±0.55	3.53±0.55	0.2059	0.2337
Oxalate (mmol/24h)	0.23±0.03	0.27±0.02	0.3872	0.26±0.02	0.23±0.02	0.4361	0.1846
Calcium (mmol/24h)	2.58±0.61	2.35±0.41	0.8817	2.59±0.58	1.74±0.25	0.1642	0.3075
Magnesium (mmol/24h)	1.60±0.22	1.73±0.29	0.8083	2.35±0.28	2.38±0.26	0.7809	0.1524
Sodium (mmol/24h)	287±53.9	224±28.1	0.3282	217±42.6	164±19.8	0.6457	0.1614
Potassium (mmol/24h)	88.9±16.7	74.4±12.1	0.4998	57.2±12.8	50.1±10.3	0.8354	0.2315
Urate (mmol/24h)	2.72±0.23	3.10±0.20	0.3868	3.28±0.29	3.68±0.41	0.6420	0.4329
Creatinine (mmol/24h)	13.6±1.29	16.1±1.03	0.3066	15.6±1.37	16.0±1.40	0.8543	0.9538
Phosphate (mmol/24h)	17.8±1.73	24.6±2.62	0.1538	28.8±2.96	30.6±2.70	0.7412	0.2072
Tiselius risk index	194±33.0	157±13.8	0.2777	168±34.4	99.9±12.4	0.1248	*0.0090
RS Brushite	0.87±0.08	0.34±0.08	0.4902	0.66±0.10	0.57±0.25	0.6036	0.5148
RS CaOx	2.19±1.20	1.46±0.33	0.6428	1.41±0.90	1.15±0.21	0.4529	0.4217
RS Uric Acid	0.39±0.05	0.65±0.15	0.5006	0.32±0.07	0.38±0.12	0.8528	0.3081
MSL (mol/dm^3)	0.16±0.01	0.15±0.01	0.6411	0.11±0.02	0.17±0.01	*0.0335	0.3405

*statistically significant

Table 3.13: Mean baseline 0 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.26 ± 0.13	6.69 ± 0.16	†0.0533
Volume (ml/h)	123 ± 28.6	115 ± 31.2	0.8449
Citrate (mmol/h)	0.17 ± 0.06	0.18 ± 0.04	0.9488
Oxalate (mmol/h)	0.03 ± 0.01	0.02 ± 0.01	0.4778
Calcium (mmol/h)	0.11 ± 0.04	0.08 ± 0.03	0.5505
Magnesium (mmol/h)	0.07 ± 0.02	0.15 ± 0.03	0.0617
Sodium (mmol/h)	12.5 ± 3.56	12.1 ± 4.11	0.9355
Potassium (mmol/h)	2.97 ± 0.74	3.18 ± 1.70	0.9141
Urate (mmol/h)	0.17 ± 0.04	0.16 ± 0.03	0.9310
Creatinine (mmol/h)	1.08 ± 0.25	1.02 ± 0.14	0.8498
Phosphate (mmol/h)	1.45 ± 0.37	1.31 ± 0.21	0.7396

†approaching statistical significance

Table 3.14: Mean 1 h urinary variables ± SE in black and white subjects after ingestion of 30 g Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post- Sor*
pH	6.26±0.13	6.39±0.18	0.5413	6.69±0.16	6.65±0.18	0.8793	0.3129
Volume (ml/h)	123±28.6	94.1±17.5	0.3986	115±31.2	90.4±27.2	0.5640	0.9101
Citrate (mmol/h)	0.17±0.06	0.13±0.03	0.5310	0.18±0.04	0.12±0.03	0.2829	0.7793
Oxalate (mmol/h)	0.03±0.01	0.02±0.00	0.2846	0.02±0.01	0.03±0.00	0.5069	0.3306
Calcium (mmol/h)	0.11±0.04	0.08±0.04	0.6098	0.08±0.03	0.06±0.01	0.3931	0.5105
Magnesium (mmol/h)	0.07±0.02	0.06±0.01	0.4721	0.15±0.03	0.08±0.01	0.0800	0.2357
Sodium (mmol/h)	12.5±3.56	10.4±2.81	0.6413	12.1±4.11	4.77±1.37	0.1099	0.0910
Potassium (mmol/h)	2.97±0.74	1.45±0.44	0.0967	3.18±1.70	0.71±0.15	0.1658	0.1301
Urate (mmol/h)	0.17±0.04	0.13±0.02	0.6506	0.16±0.03	0.14±0.03	0.6792	0.8908
Creatinine (mmol/h)	1.08±0.25	0.95±0.05	0.6218	1.02±0.14	0.86±0.10	0.3821	0.4739
Phosphate (mmol/h)	1.45±0.37	0.51±0.07	*0.0229	1.31±0.21	0.53±0.10	*0.0033	0.8719

*statistically significant

Table 3.15: Mean 2 h urinary variables ± SE in black and white subjects after ingestion of 30 g Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post- Sor*
pH	6.26±0.13	6.57±0.33	0.3708	6.69±0.16	6.62±0.11	0.7203	0.8876
Volume (ml/h)	123±28.6	91.9±11.6	0.3460	115±31.2	140±21.1	0.5065	0.0684
Citrate (mmol/h)	0.17±0.06	0.18±0.02	0.8961	0.18±0.04	0.19±0.04	0.8708	0.9106
Oxalate (mmol/h)	0.03±0.01	0.04±0.01	0.0658	0.02±0.01	0.03±0.00	0.0710	0.3833
Calcium (mmol/h)	0.11±0.04	0.07±0.01	0.3323	0.08±0.03	0.08±0.01	0.9189	0.5124
Magnesium (mmol/h)	0.07±0.02	0.06±0.01	0.6261	0.15±0.03	0.12±0.01	0.5042	*0.0002
Sodium (mmol/h)	12.5±3.56	7.65±2.51	0.2910	12.1±4.11	3.75±1.20	0.0682	0.1662
Potassium (mmol/h)	2.97±0.74	1.00±0.27	*0.0289	3.18±1.70	0.98±0.22	0.2169	0.9605
Urate (mmol/h)	0.17±0.04	0.10±0.00	0.3596	0.16±0.03	0.35±0.16	0.3700	0.4193
Creatinine (mmol/h)	1.08±0.25	0.78±0.03	0.2816	1.02±0.14	0.89±0.08	0.4560	0.2266
Phosphate (mmol/h)	1.45±0.37	0.40±0.03	*0.0231	1.31±0.21	0.75±0.16	*0.0487	0.0676

*statistically significant

Table 3.16: Mean 3 h urinary variables ± SE in black and white subjects after ingestion of 30 g Sor

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Sor*	*p*	*Baseline*	*Post-Sor*	*p*	*p Post- Sor*
pH	6.26±0.13	6.88±0.12	*0.0037	6.69±0.16	7.06±0.12	0.0875	0.3401
Volume (ml/h)	123±28.6	314±32.3	*0.0005	115±31.2	254±37.7	*0.0109	0.2567
Citrate (mmol/h)	0.17±0.06	0.52±0.12	*0.0251	0.18±0.04	0.39±0.11	0.0998	0.4554
Oxalate (mmol/h)	0.03±0.01	0.04±0.01	0.1585	0.02±0.01	0.04±0.01	0.1138	0.8137
Calcium (mmol/h)	0.11±0.04	0.11±0.03	0.9810	0.08±0.03	0.08±0.02	0.9248	0.3354
Magnesium (mmol/h)	0.07±0.02	0.07±0.01	0.9956	0.15±0.03	0.10±0.02	0.1972	0.2889
Sodium (mmol/h)	12.5±3.56	3.90±0.58	*0.0373	12.1±4.11	3.16±0.75	*0.0472	0.4631
Potassium (mmol/h)	2.97±0.74	1.01±0.12	*0.0248	3.18±1.70	0.79±0.19	0.1805	0.3575
Urate (mmol/h)	0.17±0.04	0.10±0.00	0.3385	0.16±0.03	0.13±0.02	0.4919	0.4224
Creatinine (mmol/h)	1.08±0.25	0.73±0.07	0.2239	1.02±0.14	0.62±0.08	*0.0260	0.3273
Phosphate (mmol/h)	1.45±0.37	1.51±0.42	0.9207	1.31±0.21	0.88±0.12	0.0902	0.1677

*statistically significant

Fractional Collections (Excretion normalized to Creatinine)

Figures 3.9-3.11 show the urinary Ca, Ox and P excretion as creatinine ratios before and after 30 g Sor ingestion. No statistically significant changes were observed in calcium excretion within the black group, whereas in the white group it increased significantly at 3 h relative to baseline (Figure 3.9). With regard to oxalate excretion, there were no changes in blacks following a Sor load but in whites it increased at 2 h and 3 h as shown in Figure 3.10. Similarly, phosphate excretion did not change in blacks but it decreased significantly in whites at 1 h and 2 h (Figure 3.11).

Figure 3.9: *Mean hourly values for urinary calcium excretion [(mmol Ca per mmol Creatinine) x 10^2] for black and white subjects following 30 g Sor challenge*

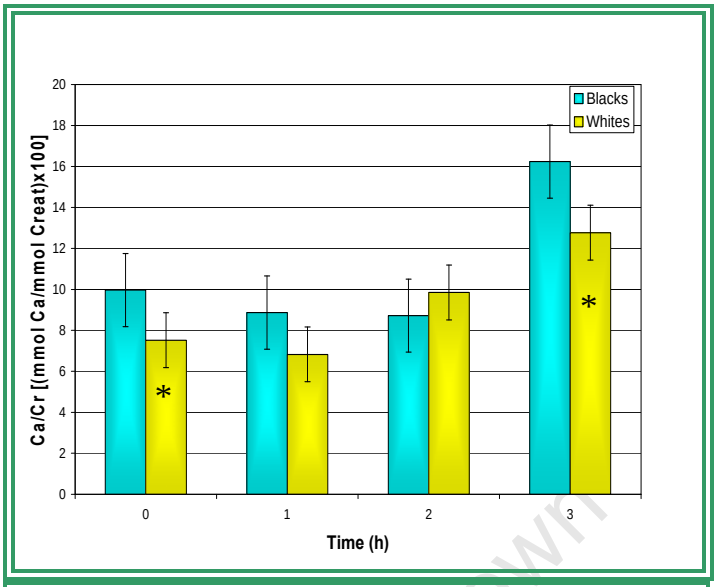


Figure 3.10: *Mean hourly values for urinary oxalate excretion [(mmol Ox per mmol Creatinine) x 10^2] for black and white subjects following 30 g Sor challenge*

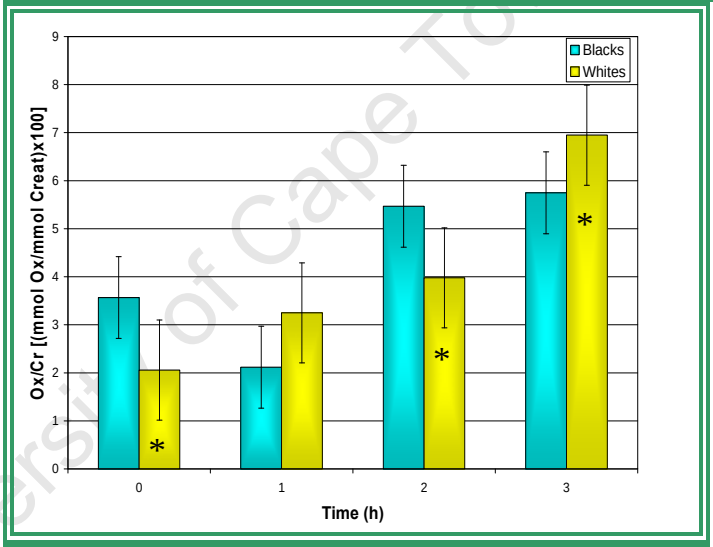
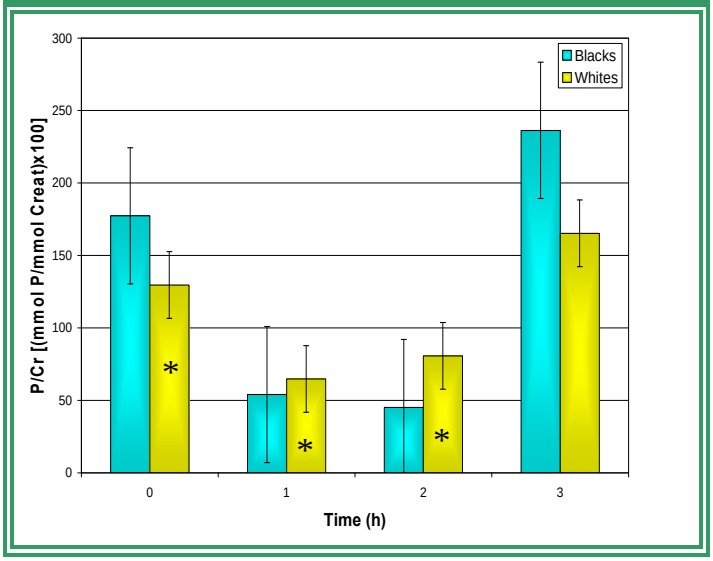


Figure 3.11: *Mean hourly values for urinary phosphate excretion [(mmol P per mmol Creatinine) x 10^2] for black and white subjects following 30 g Sor challenge*



Histograms labelled with the same symbol are significantly different ($p<0.05$)

Particle Formation Kinetics

Particle Volume-Size Distribution

No significant changes were observed in the mean particle size after the ingestion of 30 g of Sor in blacks (Table 3.17). In whites, the mean particle size increased. The pattern of the curves in Figure 3.12 is of note. The curves for white subjects are higher than the curves for black subjects, indicating a larger mean particle volume in the urine from the former group.

Table 3.17: Mean particle size (µm) in urines of black and white subjects before and after ingestion of 30 g Sor

	Blacks	*Whites*
Before	4.01	3.64
After	4.01	4.01

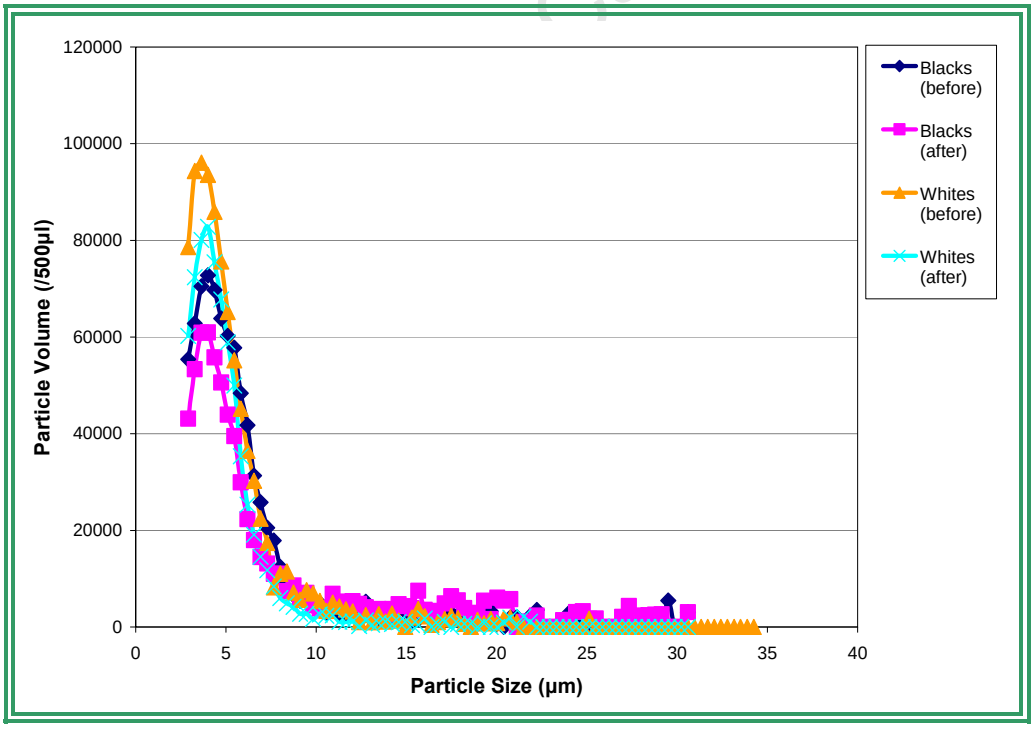


Figure 3.12: *Particle volume-size distribution pre- and post-30 g Sor ingestion in urines of black and white subjects*

Particle Number

Figure 3.13 shows the plots for particle number versus time before and after ingestion of 30 g Sor. At 120 min the particle number decreased in blacks but remained the same in whites following the Sor load.

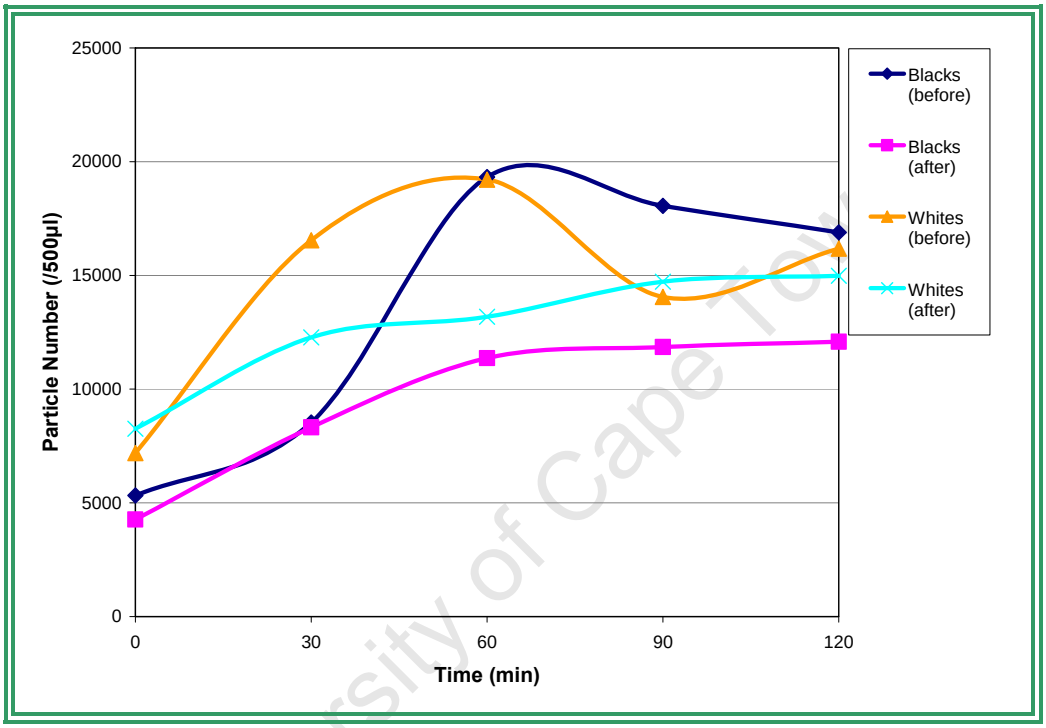


Figure 3.13: *Particle number in urines of blacks and whites before and after ingestion of 30 g Sor*

Scanning Electron Microscopy

The scanning electron micrographs obtained before and after a 30 g Sor load are shown in Figure 3.14 for black (a and b) and white (c and d) subjects. In all micrographs, the dominating crystals are the coffin-shaped COM crystals. Fewer crystals were observed after ingestion of Sor in the urines from black subjects and the opposite was noted in the urines from white subjects. In both cases the size of the crystals was unchanged.

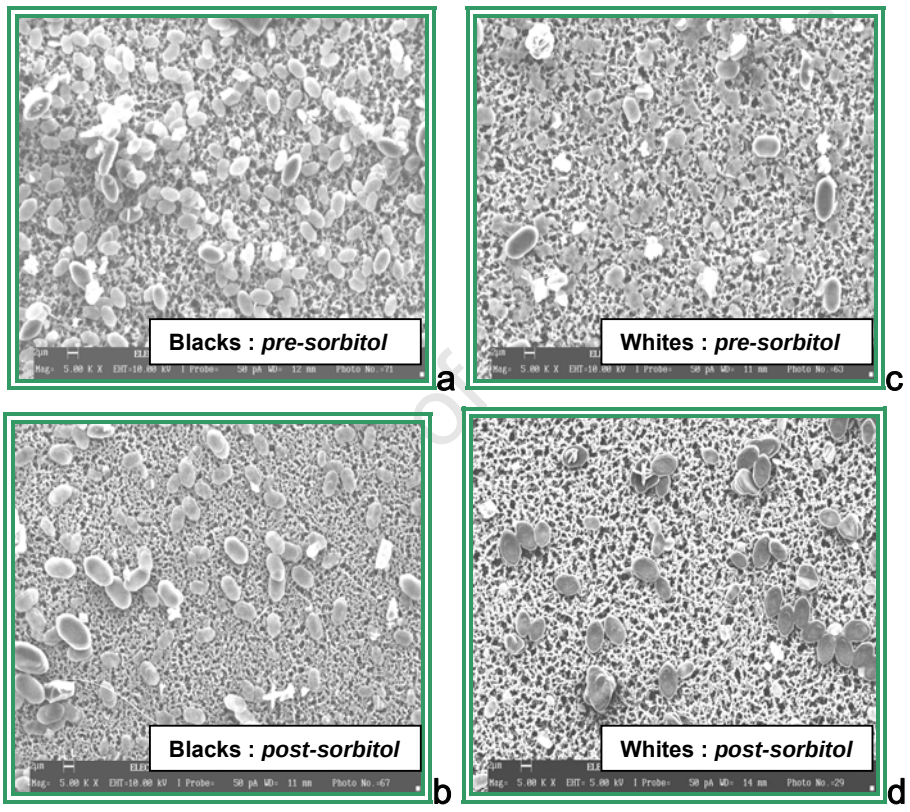


Figure 3.14: *Scanning electron micrographs of CaOx crystals pre- and post-ingestion of 30 g of Sor in blacks (a: pre; b: post) and whites (c: pre; d: post) at 5 K magnification*

Crystallization Experiments

^{14}C-Oxalate Deposition

The rate of CaOx deposition is indicated by the gradient of each curve (top-right hand corner) for black and white subjects pre- and post 30 g Sor ingestion (Figure 3.15). A reduction in CaOx deposition rate was observed in blacks after the Sor load and conversely, the crystal deposition increased in whites. The percentage of precipitated ^{14}C-oxalate was higher in whites compared to blacks both before and after ingestion of Sor.

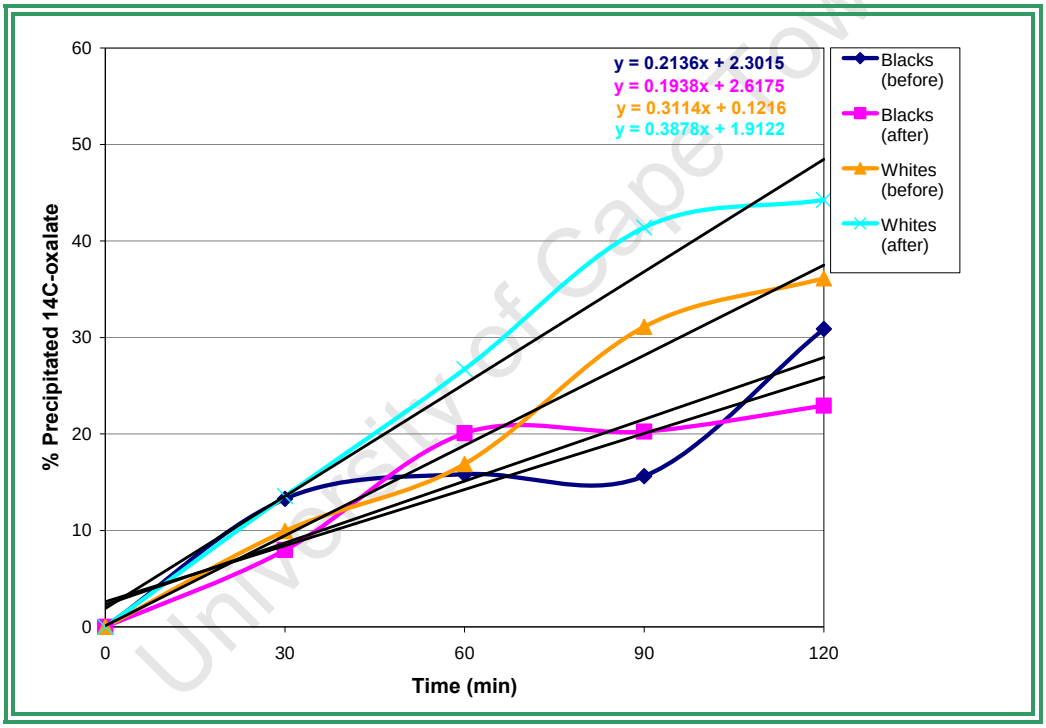


Figure 3.15: *Percentage precipitated ^{14}C-oxalate pre- and post-30 g Sor ingestion in urines of black and white subjects*

Crystal Aggregation

The mean percentages for the inhibition of CaOx crystal aggregation in black and white subjects are given in Table 3.18, corresponding to the plots shown in Figure 3.16. Within each group no significant changes were noted after 30 g Sor load. Inter-group comparisons also revealed no changes both before and after Sor ingestion.

Table 3.18: Mean percentage inhibition of aggregation in black and white subjects before and after a 30 g Sor load

Variables	*Blacks*		*Whites*		*Blacks vs Whites*
	% Ia (SE)	*p*	*% Ia (SE)*	*p*	*p*
Before	55.9 (4.09)		57.7 (9.94)		0.8581
After	69.1 (6.27)	0.0943	58.7 (8.38)	0.9395	0.3246

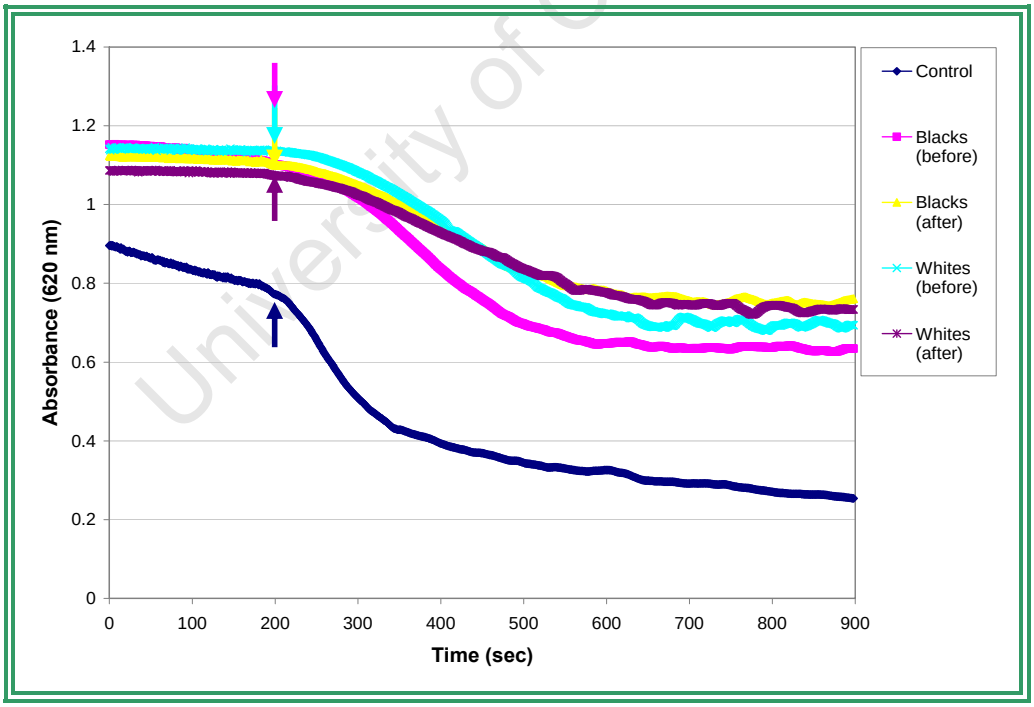


Figure 3.16: *Plot of absorbance versus time pre- and post-30 g Sor load in urines of black and white subjects. The time at which stirring was stopped is indicated by the arrows.*

Summary of Urine Chemistry and Crystalluria Properties After 30 g Dose

	Blacks	Whites
Fractional (non-normalized)	↑ pH, Cit, ↓ Na, ↓ K, ↓ P	Na, ↓ Creat, ↓ P
Fractional (normalized)	↔	↑ Ca, ↑ Ox, ↓ P
Particle size	↔	↑
Particle number	↓	↔
SEM	↓ Particle number	↑ Particle number
Deposition rate	↓	↑
Aggregation	↔	↔

Recovery of Urinary Sorbitol

HPAEC-PAD Method

The typical chromatograms obtained from the HPAEC-PAD method for one of the standard solutions of Sor and for one of the urine samples are depicted in Figures 3.17 and 3.18, respectively.

The actual concentrations of Sor after the two doses in the urines of black and white subjects are given in Table 3.19. In blacks, the levels of Sor peaked at 2 h subsequent to the 20 g dose, whereas in whites they peaked at 1 h, with the concentrations in the latter group being higher than in their black compatriots from 0 to 24 h.

After the 30 g dose, a similar effect was observed, with Sor concentrations again peaking at 2 h in black subjects and at 1 h in white subjects.

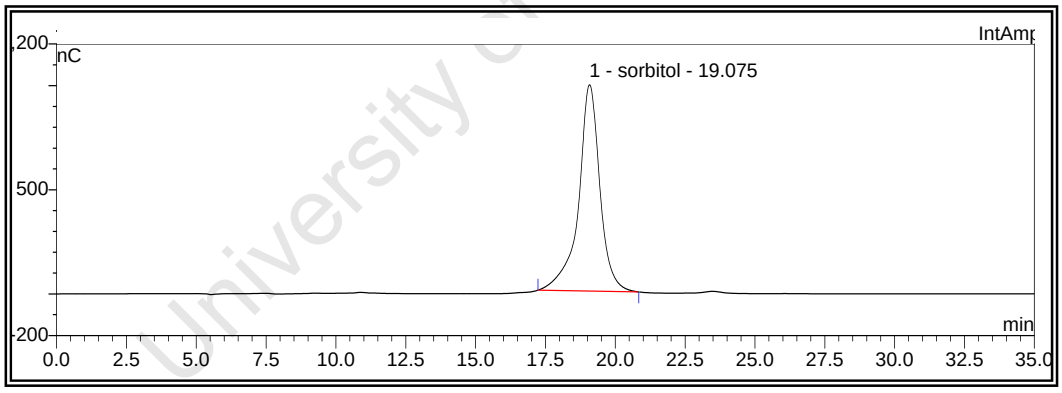


Figure 3.17: *Typical chromatogram for a Sor standard solution*

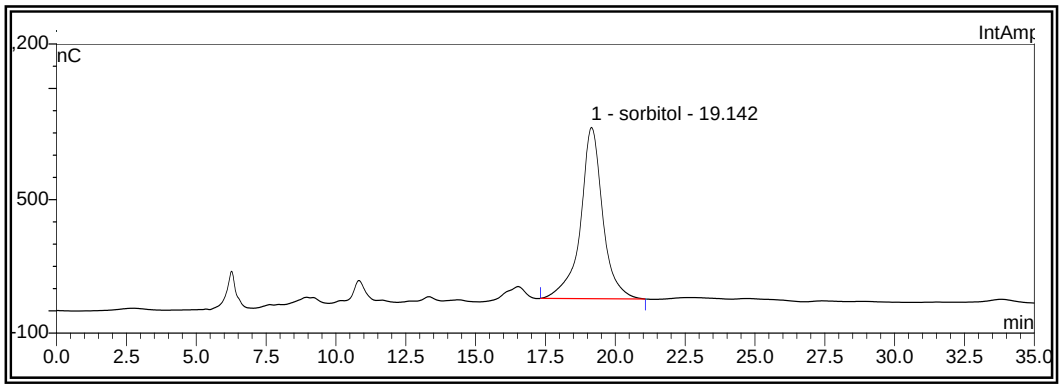


Figure 3.18: *Typical chromatogram for a urine sample*

Table 3.19: Concentration of Sor (µg/ml) in the urines of black and white subjects after the two doses using the HPAEC-PAD method

	Blacks		*Whites*	
Time (h)	*20 g dose*	*30 g dose*	*20 g dose*	*30 g dose*
0	*10.0*	*4.600*	*6.60*	*4.40*
1	*45.6*	*118.4*	*61.4*	*326*
2	*46.0*	*131.0*	*21.4*	*64.0*
3	*2.00*	*11.40*	*6.80*	*9.40*
24	*1.60*	*10.20*	*6.80*	*3.20*

Fluorescent Assay Method

Table 3.20 shows the urinary concentrations of Sor measured fluorimetrically in the urines of the two groups. The levels of Sor peaked at 2 h in both groups following a 20 g dose. After the 30 g dose blacks had the highest concentration of urinary Sor at 3 h, whereas in whites it was at 1 h and 24 h. The concentrations did not differ after the two doses in both groups, contrary to the observed differences in Table 3.19 when using the HPAEC-PAD method. In addition, it is noted that the levels of Sor for each dose obtained from the two methods are not similar. These results are surprising and will be dealt with in depth in the discussion section of this chapter.

Table 3.20: Concentration of Sor (µg/ml) in the urines of black and white subjects after the two doses using the Fluorescent Assay method

	Blacks		*Whites*	
Time (h)	*20 g dose*	*30 g dose*	*20 g dose*	*30 g dose*
0	*21.7*	*12.0*	*18.0*	*11.0*

1	*55.0*	*75.0*	*95.6*	*93.6*
2	*93.0*	*87.3*	*105.7*	*85.7*
3	*87.3*	*92.3*	*103.3*	*82.6*
24	*77.0*	*92.0*	*93.3*	*100*

Serum

The mean serum Ca and P values before and after ingestion of 20 g and 30 g Sor are given in Table 3.19. Both Ca and P did not change significantly within the black group after 20 g or 30 g of Sor ingestion. However, in whites Ca was approaching a significant increase after the 30 g load but no changes were observed in P. Inter-group comparisons showed lower Ca levels in this group prior to 30 g Sor ingestion.

Table 3.19: Mean ± SE serum calcium and phosphate excretion (mmol/l) at before and after intake of 20 g and 30 g of Sor in blacks and whites

Variable	*Blacks*			*Whites*			*Black vs Whites*	
	Before	*After*	*p*	*Before*	*After*	*p*	*p Before*	*p After*
Ca (20 g)	2.21±0.02	2.23±0.02	0.446	2.21±0.01	2.22±0.02	0.651	0.903	0.816
P (20 g)	1.25±0.03	1.20±0.04	0.258	1.24±0.05	1.17±0.04	0.238	0.807	0.606
Ca (30 g)	2.25±0.02	2.24±0.02	0.680	2.19±0.01	2.23±0.01	†0.057	*0.004	0.477
P (30 g)	1.23±0.03	1.23±0.03	0.173	1.18±0.07	1.17±0.04	0.928	0.486	0.960

* statistically significant; † approaching statistical significance

ALDOLASE STUDY

The relevant 24 h urinary variables for black and white subjects are shown in Table 3.20. Urinary oxalate and creatinine were similar in the two groups. These results, most importantly with regard to urinary oxalate, are in agreement with those obtained in the previous study (Chapter Two) as well as in the present Sor study.

Table 3.20: Mean urinary variables ± SE in black and white subjects

Variables	*Blacks (n=20)*	*Whites (n=20)*	*Blacks vs Whites p*
Volume (ml)	1436 ± 132	1471 ± 164	0.8689
Oxalate (mmol/24h)	0.24 ± 0.01	0.23 ± 0.02	0.8361
Creatinine (mmol/24h)	13.9 ± 0.82	14.1 ± 1.29	0.8501

The mean aldolase activities are shown in Figure 3.19. It is noted that the difference in the activity between the black group and the white group was approaching statistical significance when determined by the spectrophotometric method carried out by the author at the Kidney Stone Research Laboratory (blacks: 4.39 ± 0.53, whites: 3.25 ± 0.24; p=0.0553), with white subjects having a lower activity than black subjects. However, neither the results from Lancet Laboratories (blacks: 6.26 ± 0.62, whites: 5.79 ± 0.45; p=0.5536) nor those obtained using the colorimetric method by the author (blacks: 5.34 ± 0.58, whites: 4.80 ± 0.52; p=0.4962) demonstrated any statistically significant differences.

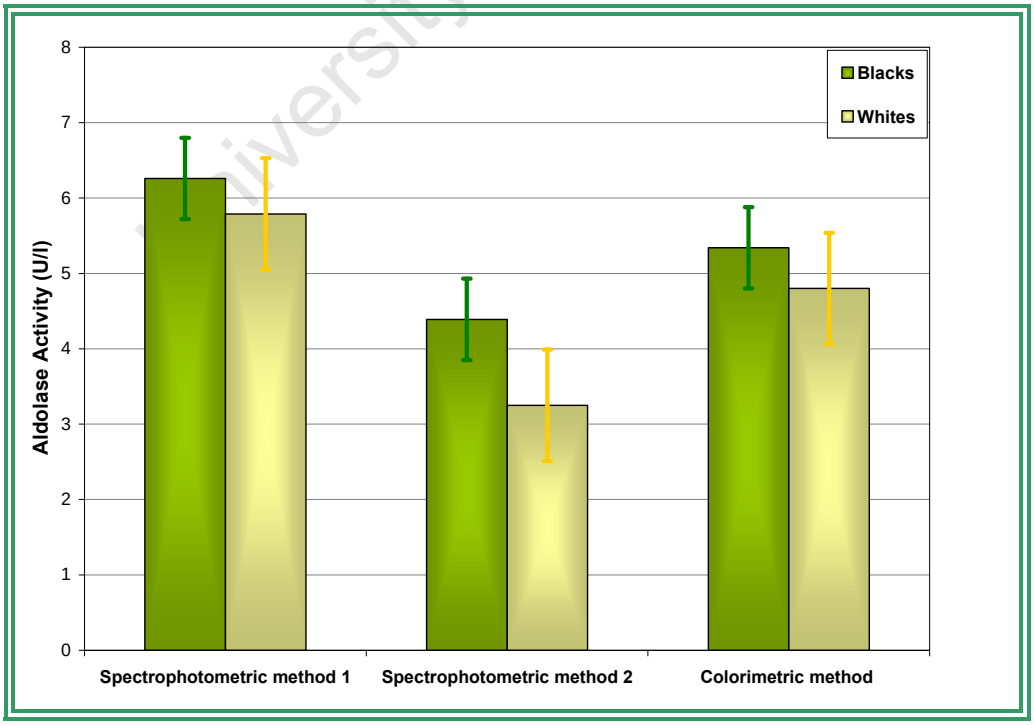


Figure 3.19: *Mean aldolase activities in blacks and whites using Spectrophotometric and Colorimetric methods. Spectrophotometric method 1: Lancet Laboratories, Spectrophotometric method 2: Kidney Stone Research Laboratory.*

In order to test the correlation between the methods, scatter plots were created and a bivariate correlation test was performed as depicted in Figures 3.20 – 3.22. The spectrophotometric methods undertaken at the two laboratories correlated positively. Similarly, a positive correlation was noted between the colorimetric method and the spectrophotometric method (from each laboratory).

Figure 3.20: *Evaluation of the correlation between the data obtained using the spectrophotometric method from two laboratories.*

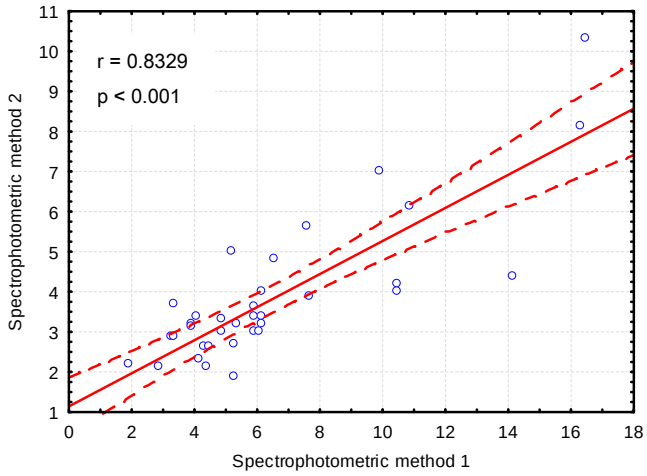


Figure 3.21: *Evaluation of the correlation between the data obtained using the colorimetric method and the spectrophotometric method (1) from Lancet Laboratories.*

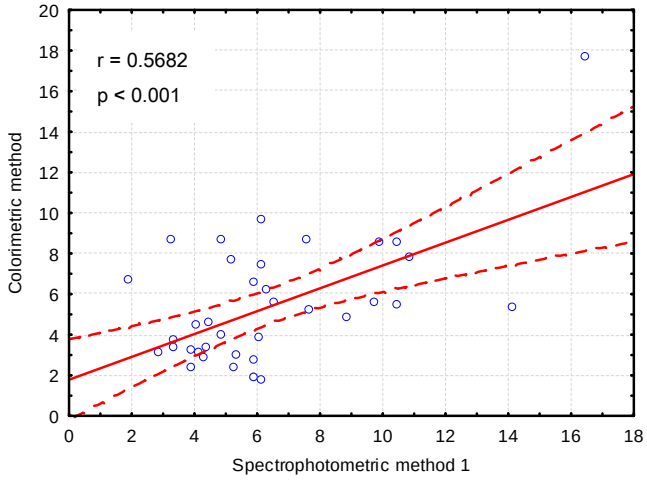
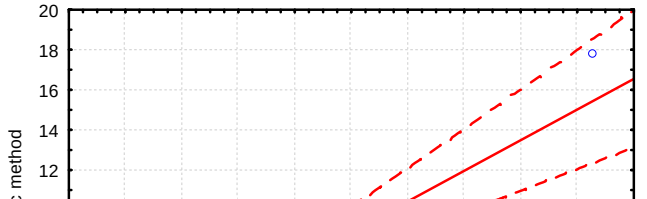


Figure 3.22: *Evaluation of the correlation between the data obtained using the colorimetric method and the spectrophotometric method*



r = 0.7695
p < 0.001

3.4 Discussion

Subsequent to the ingestion of 20 g of sorbitol, the urinary excretion of calcium in the non-normalized fractions increased concomitantly with magnesium and citrate in black subjects but was, however, not maintained up to 24 h. The role of magnesium and citrate as inhibitors of stone formation has been well established. The former forms a soluble complex with oxalate, resulting in decreased urinary excretion of this parameter. The unchanged levels of oxalate in blacks could have been influenced by elevation of magnesium levels. It is surprising, on the other hand, that the increase in calciuria was manifested in this group, in spite of the increased citrate excretion. An opposite effect would have been anticipated, as citrate binds with calcium, leading to a lesser excretion of latter in the urine. However, citrate influenced the increased urinary pH, thereby hindering the supersaturation of uric acid. This effect did not continue to 24 h, on the contrary, as a rise in RS of uric acid was observed during this period. The rise in RS of uric acid is also surprising because it normally occurs at pH below 5.5 (Coe *et al.* 1980), as stated in Chapter One, and this was not the case in the present study. Increasing the dose to 30 g resulted in the rise in urinary pH, also accompanied by an increase in citrate in the black group and similarly this was not sustained in the 24 h fraction. In addition, the excretion of urinary phosphate and the proposed mechanism for the reduction in urinary phosphate levels was explained in Chapter Two. The decreased urinary phosphate was observed in the previous study (Chapter Two) after the 20 g load but this was not the case in the present study.

The non-normalized excretions in white subjects revealed a decrease in urinary phosphate after the administration of the 20 g dose. This is in accordance with the results in Chapter Two except that the effect in that study was delayed until 24 h,

whereas it occurred earlier in the present study. In contrast, the decreased RS of brushite which accompanied the reduced phosphate levels in the previous study was not observed in this study in this group. Additionally, decreased urinary calcium levels were noted previously in the white group at 24 h, presently, this effect was not reflected. These inconsistencies may be ascribed to slight modifications in the standardized diets provided in the two studies and also the individual variation in the metabolism of Sor. However, apart from the common decrease in urinary phosphate, the non-normalized excretion of oxalate did not change in the two studies in the white group as well as in the black group. The 30 g dose induced a decrease in urinary phosphate in white subjects, analogous to that observed after the 20 g dose. Serum phosphate levels, however, did not change in both groups but Nguyen ad co-workers (1993) noted similar findings in their study with the 20 g dose.

The normalized fractional excretions revealed that, post 20 g load, the oxalate excretion was not changed in either group but was significantly increased in the white group in the previous study (Chapter Two). The discrepancy in the results of the two studies is surprising and one possible explanation for this inconsistency is probably the difference in the metabolism of sorbitol in the subjects recruited in the two studies, as the two groups were not the same. Nevertheless, the current finding is in agreement with the results of Nguyen *et al.* (1993), as they also did not observe any effect after 20 g administration of sorbitol on the urinary excretion of oxalate. However, contrary to these observations, the 30 g load in the present study increased the excretion of this parameter in the white group. In the black group, both doses of sorbitol did not induce any significant changes in oxalate excretion. This is consistent with the previous study (Chapter Two) in which no increase in oxalate excretion occurred in black subjects after the 20 g dose.

The mechanisms by which sorbitol is converted to oxalate in the liver are described in Chapter Two. Briefly, the first mechanism involves the dehydrogenation of sorbitol to fructose, which is in turn transformed to glucose (Adcock and Gray 1957). Alternatively, sorbitol undergoes a pathway whereby it is transformed to glyoxylate resulting in the production of oxalate (Conyers *et al.* 1990). The increased oxalate excretion in the white group and not in the black group suggests inactivity of one or more of the three enzymes involved in this pathway, that is, ketohexokinase, aldolase or glyoxylate reductase in the latter group as seen also in

the previous chapter (Chapter Two). Since the increase in oxaluria in white subjects was observed only after the bigger dose, it can be suggested that the effect is dose dependent. However, further studies involving a wider range of dose levels are required before a definite conclusion can be drawn.

Following a 20 g sorbitol challenge, the urinary excretion of calcium did not change in both studies in the white group. The results from the two studies are thus consistent and are in accordance with observations by Nguyen and co-workers (1993). Increasing the dose to 30 g resulted in an increase in the excretion of this parameter in this group. Seemingly, the 20 g load was not enough to provoke an increase in urinary calcium excretion, as it escalated following a 30 g load. In black subjects, the excretion of calcium did not change in both studies after the administration of 20 g of sorbitol nor did it increase after the 30 g load in the present study. It is apparent that increasing the dose from 20 g to 30 g affected the white subjects but not the black subjects. A possible explanation for the increased calcium levels in the former group following the 30 g sorbitol load is that, non-absorbed polyols are usually passed through to the lower gut where they are fermented to short-chain volatile fatty acids. These fatty acids then give rise to acid loading in the body and may consequently trigger the elevation of urinary calcium excretion (de Groot *et al.* 1995). Serum calcium levels after the 30 g load also increased with increase in urinary excretions. Generally, about 50 % of serum calcium is available for filtration in the kidney and excess calcium is absorbed and excreted in the urine (Sayer *et al.* 2004). In principle, high concentrations of serum calcium contribute to elevated urinary levels, hence observed effect of the 30 g dose. The concomitant increase in urinary calcium and oxalate in this group will dramatically increase the risk of CaOx crystallization in the fractional collections. The effect disappears in the 24 h collection, as can be seen in Table 3.12, in which no change in the RS values for CaOx are reflected. This result suggests the findings of other studies which have shown that during a 24 h cycle, peak periods of crystalluria (and hence stone risk) occur. Hence, ingestion of a 30 g dose of sorbitol is a risk factor for CaOx stone formation in white subjects but not in black subjects.

The urinary phosphate excretion decreased in the white group after a 20 g load in both the present and the previous study. Similarly, ingestion of 30 g induced a decrease in this parameter in this group. In the present study, the overall decrease

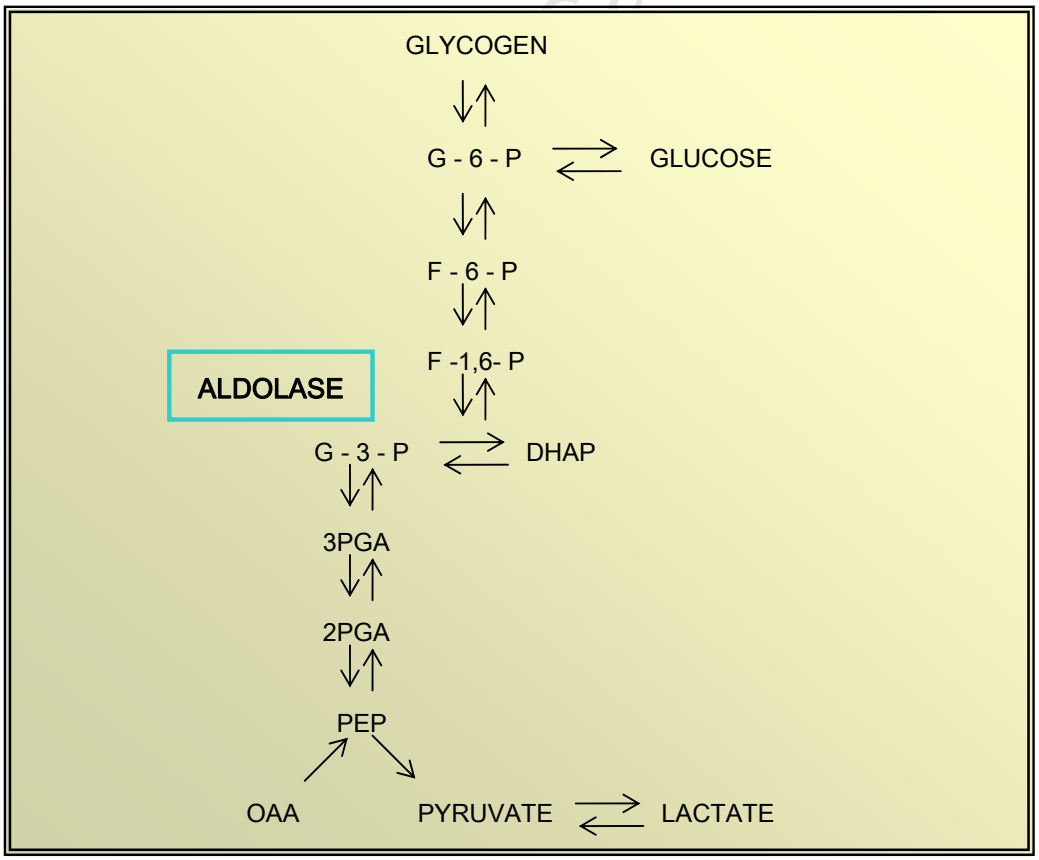
was 156 % after the 20 g dose while after the 30 g dose it was 88 %, which implies that it is dose independent. In black subjects, urinary phosphate decreased in both studies after the 20 g dose. However, after the intake of 30 g, no statistically significant changes in phosphaturia were observed. The explanation for the failure of the results for the 30 g dose to be statistically significant is the wide spread of the values which led to high standard errors as a consequence of the variation and thus lowered the precision. The anomaly in these results suggests either an inconclusive effect of the 30 g load or dose independency in this group.

The recovery of sorbitol in the urine after the ingestion of the two doses was measured. Polyols are partially absorbed from the small intestine and it is believed about 80 % of sorbitol can be absorbed (Livesey 1992, Langkilde *et al.* 1994). The unabsorbed portion undergoes bacterial fermentation in the colon to produce short-chain fatty acids such as acetic and butyric acids as well hydrogen and methane gases. A small amount (10 to 20 %) of the absorbed portion is excreted in the urine (Livesey 1992). The results from the two methods used for the determination of the concentration of the excreted urinary sorbitol were not consistent. In the chromatographic method, after the 20 g dose, the white group had higher levels compared to the black group, implying a higher absorption in the former group. A similar effect was observed after the 30 g dose. Of important note is also the earlier transit time in white subjects compared to black subjects. The fluorescent assay method, on the other hand, showed poor reproducibility and widely varied results. It is possible that the resin column (with no indicator) that was used in this study failed to eliminate interference by the ionic and non-ionic factors. The stability of the enzyme, SDH, is also of consideration. It is apparent that the results obtained from the chromatographic method are more reliable as there were no interfering limitations.

In the studies on the crystalluria properties, the results were generally varied and inconsistent. However, particle numbers decreased consistently in black subjects in response to both doses (20g: 18 %, 30 g: 28 %). Moreover, the observation after the 30 g dose was confirmed semi-quantitatively by SEM measurement.

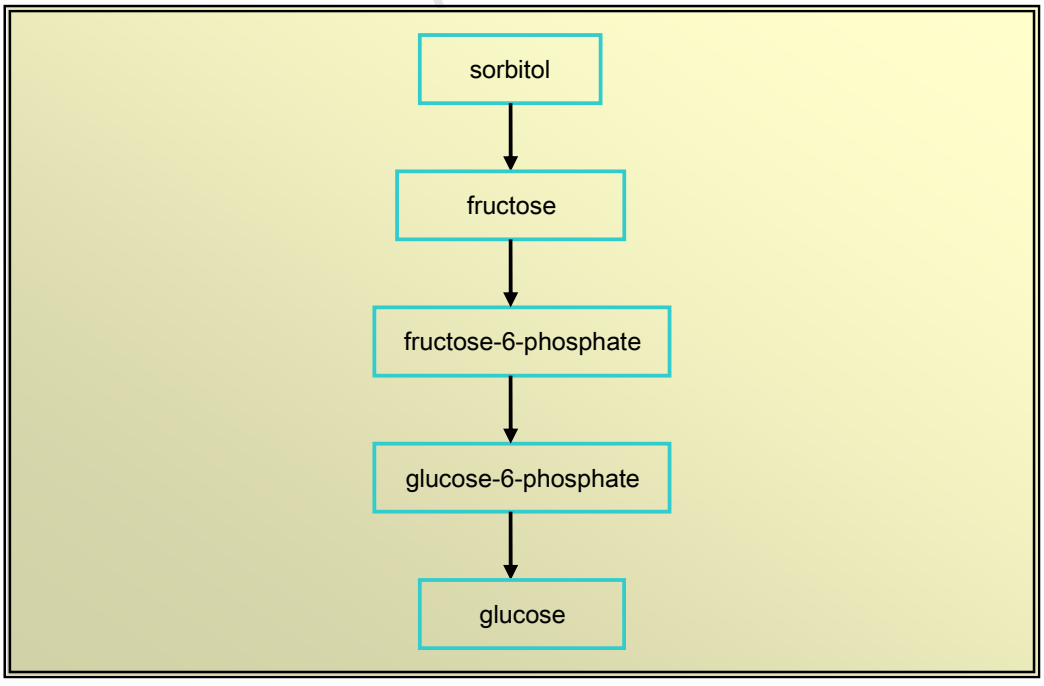
As hypothesised in this discussion and in the previous chapter, the increase in oxalate production following a sorbitol load in white subjects and not in their black compatriots might be indicative of the difference in baseline activity of one or more

enzymes that are involved in the metabolic pathway of sorbitol (and xylitol) in one group and not the other. It was therefore deemed necessary to test this hypothesis by determining the serum activity of aldolase, one of the enzymes common to both pathways and also associated with CaOx kidney stone disease. As shown in Scheme 1 below, aldolase catalyses the equilibrium conversion of fructose-1,6-phosphate (F-1,6-P) to glyceraldehyde-3-phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP). Hautmann and Lutzeyer (1976) discovered that, when compared to healthy kidneys, those from oxalate stone formers had a significantly lower aldolase activity. The authors attributed this deficiency to a decrease in glyconeogenesis because in the kidney, unlike in other organs, glucose synthesis is favoured thereby resulting in oxaloacetate accumulation (OAA) which may lead to oxalate synthesis. However, as mentioned in the introduction of this chapter, it has been suggested that most of the serum aldolase comes from the liver, where the activity of this enzyme is greatest. The sorbitol and xylitol pathways proposed in Chapter Two for the production of oxalate possibly occur in the liver as well as in the kidney.



Scheme 1: *Metabolic pathway for glucose in the cytoplasm of the kidney (Hautmann and Lutzeyer 1976)*

In the present study, the serum aldolase activity in white subjects was generally lower when compared to that of black subjects and was approaching statistical significance when measured spectrophotometrically by the present author at the Kidney Stone Research Laboratory. In light of the lower activity of aldolase observed in the former group, the increased urinary excretion of oxalate following sorbitol ingestion can be explained in terms of the above metabolic pathway for glucose (Scheme 1). Hautmann and Lutzeyer (1976) demonstrated that in the kidney the metabolism of glucose favours the direction of glucose synthesis and a reduced activity of aldolase results in the ultimate production of oxalate. The metabolic conversion of sorbitol into glucose via the following pathway (Scheme 2) was proposed in Chapter Two. It is therefore suggested that in the white group, due to the low baseline activity of aldolase, the metabolism of sorbitol results in glucose synthesis thereby increasing urinary oxalate excretion and is likely to occur in the kidneys. In other words, this implies that the aldolase activity is inversely proportional to oxalate excretion.



Scheme 2: *Metabolic pathway of sorbitol leading to the production of glucose (Adcock and Gray 1957)*

The increase in oxalate excretion in black subjects after xylitol ingestion (Chapter Two) and the observation of a relatively lower baseline activity of aldolase (present chapter) suggests that in this group the liver is the main site for the metabolism of xylitol and that aldolase activity is directly proportional to urinary oxalate excretion.

The inconsistency in the values obtained for the aldolase activity using the spectrophotometric method from the two laboratories can be attributed to the wider spread in the individual results noted in the data provided by Lancet Laboratories. It was therefore necessary for outliers to be eliminated. Pinto *et al.* (1969b), when comparing serum aldolase activities obtained by a spectrophotometric method from two laboratories, noted differences in the results even though they also observed a positive correlation, as is the case in the present study. The colorimetric correlated positively as well with the spectrophotometric method performed in both laboratories.

Thus, summarizing the two studies described in this chapter, it is clear that different renal handling mechanisms of sorbitol are present in the two ethnic groups and indeed dose dependency effects are operative. It has also been established that the baseline activity of aldolase differs in the two groups.

Finally, it is apparent that further research is required to explore the effects of sorbitol and xylitol in CaOx urolithiasis. For now, caution in the consumption of these polyols is recommended.

3.5 References

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Chapter Four

Investigation of the effect of dietary oxalate challenges on renal injury and oxidative stress in black and white South African subjects

4.1 Introduction

As stated in the General Introduction, an increased urinary excretion of oxalate has been identified as one of the major risk factors for CaOx stone formation (Finlayson 1974, Robertson *et al.* 1978, Ito *et al.* 1992). Oxalate can be produced mainly endogenously, through sources like ascorbic acid, protein and precursors such as glycolate or to a lesser extent exogenously, through dietary sources. It is believed that oxalate that is produced endogenously and that which is absorbed from dietary sources are excreted largely in the urine (Holmes *et al.* 2005). The role of dietary oxalate in CaOx stone formation has been reviewed quite extensively by various researchers (Finch *et al.* 1981, Massey *et al.* 1993, Holmes 2001). It has been known for some time that some foods are particularly high in oxalate content and these include rhubarb, spinach, beetroot, parsley, soya products, wheat bran, nuts, peanut butter, chocolate, tea, etc. Of particular interest is the high ratio of oxalate to calcium possessed by spinach and rhubarb which contributes to the elevated urinary excretion of oxalate as a result of the diminished availability of calcium to bind oxalate (Noonan and Savage 1999). Brogren and Savage (2003) examined the changes in urinary oxalate after a spinach load under a controlled diet. Their results showed an increase in the urinary excretion of oxalate after the ingestion of spinach.

The absorption of oxalate is thought to occur along the intestinal tract but this process is not well understood. There are few factors which have been implicated and are believed to influence oxalate absorption, namely, oxalate-degrading bacteria, bile acid malabsorption and co-ingestion of calcium and magnesium (Holmes and Assimos 2004). The percentage of oxalate absorbed may vary widely depending also on the intake of oxalate and the composition of the remainder of the diet (Jaeger and Robertson 2004). An important consideration is the bioavailability of oxalate in foods for intestinal absorption. Brinkley *et al.* (1981) tested the oxalate bioavailability in normal subjects of 7 different oxalate-rich foods including spinach, instant tea, vegetable cocktail juice, cranberry juice, orange juice, pecans and chocolate, and in addition, three standard solutions of Na_2Ox. Out of the 7 food items, spinach had the highest oxalate content; however, the most concentrated Na_2Ox standard solution (20 mmol) had even higher oxalate content than spinach. With regard to oxalate bioavailability, spinach had a lower percentage compared to the standard oxalate solutions. In comparison to other food items, the bioavailability of oxalate in spinach (higher oxalate content) was within the same range as chocolate and canned

vegetable cocktail juice (moderate oxalate content). However, pecans and orange juice (lower oxalate content) had a much higher bioavailability with respect to oxalate compared to spinach. In other studies, the bioavailability of oxalate in rhubarb was lower compared to that of the sodium oxalate standard solutions reported in the aforementioned study by Brinkley (Prenen *et al.* 1984, Honow and Hesse 2002).

There has been remarkable evidence which suggests that tubular injury prevails in idiopathic CaOx stone formers (Baggio *et al.* 1983). It is posited that hyperoxaluria in stone-forming patients may lead to calcium oxalate crystallization in the kidney and ultimately tubular injury, which arises from crystal-cell interactions (Hackett *et al.* 1990, Scheid *et al.* 1995, Lieske *et al.* 1992, Sarica *et al.* 2001). Subsequent to hyperoxaluria, renal tubular damage can be manifested by an increase in the urinary excretion of marker enzymes such as N-acetyl-β-glucosaminidase (NAG) (Baggio *et al.* 1983, Jaeger *et al.* 1986, Khan *et al.* 1989, Scheid *et al.* 1996, Tungsanga *et al.* 2005) and β-galactosidase (GAL) (Huang *et al.* 2003). NAG is found mainly in the proximal tubular cells of the kidney and due to its sensitivity in detecting nephrotoxicity and stability in a urinary environment, it is considered as one of most important markers of tubular damage (Sikora *et al.* 2003).

In a previous study, a positive correlation between NAG and oxalate excretion was observed in children with urolithiasis (Balla *et al.* 1998). Similarly, a study conducted in CaOx stone patients and healthy control subjects showed an elevated NAG excretion in patients with increased oxalate, among other urinary parameters (Winter *et al.* 1996). Cell injury occurs as a result of binding of oxalate or CaOx crystals and their entry into the proximal tubular cells thereby provoking inflammation and consequently cell damage (Khan and Thamilselvan 2000). Subsequently, NAG is excreted in the urine (Balla *et al.* 1998). Evidence from animal (Baggio *et al.* 1983, Khan *et al.* 1979, Selvam *et al.* 1992) and cell culture (Hackett *et al.* 1994, Scheid *et al.* 1995) studies suggests that oxygen free radical generation as a result of high oxalate concentrations causes damage to the renal tubular cells. It is also suggested that lipid peroxidation, a process whereby free radicals are generated via the degradation of polyunsaturated fatty acids plays a role in cell injury (Tungsanga *et al.* 2005).

There have been contradicting observations in other studies with regard to the association of NAG and urinary oxalate excretion in both healthy subjects and stone formers (Sikora *et al.* 2003, Knight *et al.* 2007). Holmes and co-workers (2005)

observed changes in urinary and plasma oxalate after administering different increasing doses of sodium oxalate (0-8 mmol) to healthy subjects. However, no changes were observed in NAG levels.

An association of renal stone disease with renal tubular damage and oxidative stress has been also been reviewed. Hyperoxaluria-induced oxidative stress has been shown in various studies (Khan 1991, Selvam 2002). Oxidative damage of DNA has been reported to be triggered by reactive oxygen species. A biomarker of oxidative DNA damage known as 8-hydroxydeoxyguanosine (8-OHdG), which is excreted in the urine upon DNA repair, has been used as an important tool to assess the level of cellular oxidative stress (Chiou *et al.* 2003, Shimoi *et al.* 2002). Boonla *et al.* (2007) undertook a study on healthy controls and nephrolithiasis patients in which they measured the urinary NAG activity along with urinary 8-OHdG. They found a higher excretion of both markers in patients than in controls. Moreover, they observed a positive correlation between urinary NAG activity and urinary 8-OHdG.

Since the aforementioned studies on the association between urinary NAG and urinary oxalate are inconclusive, further investigation of the effects of high oxalate dietary challenges is warranted. In addition, the different renal handling responses which have been reported in previous studies for South African black and white subjects provides a further motivation for undertaking a study of this nature.

The main objectives of this study are:

- to investigate the effect of oral oxalate loading in the form of rhubarb, spinach and Na_2Ox on the renal handling of oxalate in healthy black and white male South African subjects

- to examine whether oxalate-induced renal injury occurs in the two ethnic groups by measuring the activity of urinary NAG, the marker enzyme for tubular injury and to assess the physiological mechanisms involved, after ingestion of rhubarb, spinach and Na_2Ox challenges

- to examine whether any evidence of cellular oxidative stress is apparent following oxalate loading in the two groups by measuring the levels of the

urinary oxidative DNA biomarker, 8-OHdG, after ingestion of the Na_2Ox challenge

- to examine whether a correlation exists between urinary NAG and urinary 8-OHdG in the two groups after ingestion of the Na_2Ox challenge

- to investigate the consequences of the rhubarb challenge on the CaOx crystallization properties in the urine of the two groups

- to use the results of the above investigations to test the hypothesis that the renal handling of oxalate is less injurious in black subjects than in whites

4.2 Subjects and Methods

Subjects

Three groups comprising ten black and ten white healthy males aged between 18 and 30 years were recruited from the student cohort of the University of Cape Town. None of the subjects had any history of kidney stones or any metabolic disorder which could influence the absorption or excretion of oxalate. Moreover, supplements or medication and tobacco products were forbidden.

Experimental Procedure

The three groups of subjects were studied at different periods of time for three different protocols comprising rhubarb, spinach and sodium oxalate, respectively. The Ethics Committee of the University of Cape Town granted approval for the protocols. Three days prior to the commencement of each protocol, subjects were instructed to avoid calcium- and oxalate-rich foods (Appendix CD: Chapter 2).

Protocol 1: Rhurbarb juice

On day 4, that is, three days after the subjects had been on the aforementioned calcium and oxalate restricted diets, subjects consumed a strict standardized diet (Table 4.1) controlled in content for calories, fat, protein, carbohydrate, calcium, magnesium, sodium, phosphorus and oxalate. The amounts of the nutrients are given in Table 4.2. Urine fractions were collected during the first 6 h period and during the following 18 h period and were pooled to reconstitute the full 24 h collection. During day 5, subjects continued with the standardized diet but coffee was replaced with 130 ml of rhubarb juice at breakfast. Urine fractions were again collected over the 6 h period and a further 18 h period. These were then pooled.

Protocol 2: Spinach

In order to stabilize the baseline urinary oxalate levels, a strict standardized diet, also controlled in content for calories, was consumed by the subjects for two days (day 4 and day 5) prior to ingestion of spinach (day 6). This is different to protocol 1 in which the standardized diet was consumed one day before (day 4) the intake of rhubarb juice (day 5). The composition of the standardized meals is shown in Table 4.1 and the nutrient content is shown in Table 4.2. Urine samples were collected on

day 5 during the first 6 h period and during the following 18 h period. On day 6, subjects continued consumption of the standardized diet but were given 160 g of spinach at breakfast and they collected urine fractions (6 h and 18 h).

Protocol 3: Sodium Oxalate

In week 1, a strict standardized diet controlled in content of calories, was provided to the subjects for daily consumption for a period of three days (days 4 to 6) (Table 4.1). The nutrient content of the standardized diet is shown in Table 4.2. Analogous to protocol 2, the extension of the days for the consumption of the standardized diet was done in order to stabilize the baseline urinary oxalate levels. On day 6, baseline urine fractions were collected from each subject over a period of 1 h (discarded), 5 h and a further period of 18 h. During days 7 to 10, subjects consumed calcium and oxalate restricted diets as they did initially three days prior to the study.

On days 11, 12 and 13, the standardized diet was continued but in addition the subjects each ingested a solution of Na_2Ox (10 mmol) (Sigma-Aldrich, Germany, 99% purity) (Holmes *et al.* 2005), in 200 ml water during these days at breakfast. Urine fractions were collected over 1 h (discarded), 5 h and 18 h on day 13.

Table 4.1: Constituents of the strict standardized diet for the three protocols

MEAL	*DAY 4 and/or DAY 5, 6*	*DAY 5 or 6 or 11-13*
Breakfast: 8h30	100 g bread rolls 10 g margarine 25 g strawberry jam 30 g salami pressed beef 130 ml coffee	100 g bread rolls 10 g margarine 25 g strawberry jam 30 g salami pressed beef 130 ml rhubarb juice/ 160 g spinach/ 10 mmol sodium oxalate
Snack: 11h00	27 g muesli bar 150 g banana	27 g muesli bar 150 g banana
Lunch: 13h00	60 g turkey ragout 140 g creamy sauce 120 g mixed vegetables 120 g pasta	60 g turkey ragout 140 g creamy sauce 120 g mixed vegetables 120 g pasta
Snack: 17h00	150 g apple 175 g low fat fruit yoghurt	150 g apple 175 g low fat fruit yoghurt
Supper: 19h00	3 slices of whole wheat bread 10 g margarine 30 g cheddar cheese 17 g cream cheese 50 g tomato 150 g apple	3 slices of whole wheat bread 10 g margarine 30 g cheddar cheese 17 g cream cheese 50 g tomato 150 g apple
Snack: 22h00	1 slice of whole wheat bread 5 g margarine 17 g cream cheese 50 g tomato	1 slice of whole wheat bread 5 g margarine 17 g cream cheese 50 g tomato

Beverages: (2 l/day):

700 ml caledon water until 13h00

600 ml caledon water until 19h00

700 ml caledon water until 22h00

Table 4.2: Nutrient content of the standardized diet for the three protocols

VARIABLE	*AMOUNT*
Energy (kJ)	7700
Total protein (g)	60.4
Total fat (g)	56.8
Carbohydrate (g)	193
Calcium (mg)	827
Magnesium (mg)	432
Phosphorus (mg)	1320
Citric acid (mg)	876
Oxalic acid (mg)	74

Analytical Methods

Fractional urine samples were analyzed as previously described in Chapter Two (page 51). However, in protocol 1, the reconstituted 24 h samples were also analyzed.

Urinary NAG and 8-OHdG

In all three protocols, the NAG activity was measured immediately in the baseline and urine fractions. Urinary 8-OHdG was measured only in the urine samples (5 h fraction) from protocol 3. Samples were stored at -80°C before analysis. Both markers (NAG and 8-OHdG) were measured only in those samples where a significant increase in urinary oxalate excretion was observed.

Determination of Urinary NAG

A commercially available NAG test kit was used to determine the urinary NAG excretion (Roche, Germany). The method involved hydrolysis of a substrate, 3-cresolsulfonphthaleinyl-N-acetyl-β-D-glucosaminide by NAG, with the release of 3-cresolsulfonphthalein, which was measured photometrically at 580 nm. Three bottles were provided with the kit. Bottle 1 was a buffer consisting of citric acid and potassium citrate, bottle 2 contained a lyophilized substrate consisting of sodium 3-cresolsulfonphthaleinyl--N-acetyl-β-D-glucosaminide and borax and bottle 3 consisted of a stop reagent, sodium carbonate. The first step of the assay required preparation of the solutions as described below.

I. Preparation of Solutions

The contents of bottle 1 were dissolved in 55 ml of redistilled water to make up a buffer solution. The substrate solution was prepared by dissolving the contents of bottle 2 in 55 ml of the buffer solution. The contents of bottle 3 were dissolved in 110 ml of redistilled water to make up a stop reagent solution.

II. Assay Procedure

The substrate solution (1 ml) was pipetted into test tubes and incubated at 37°C for 5 minutes. Urine samples (0.05 ml) were then added and the mixture was vortexed and incubated for 1 h at 37°C, followed by the addition of a stop reagent (2 ml). The mixture was vortexed and then allowed to stand for 10 minutes. The reagent blank was prepared in the same way with the elimination of a urine sample. The absorbance of the each sample solution was measured at 580 nm against the blank and the NAG activity (U/l) was calculated using the following equation (Yakata *et al.* 1983):

$$\frac{1000 \times 3.05}{40.67 \times 1 \times 0.05 \times 60} \quad X \quad A_{sample}$$

[A_{sample} = absorbance of the sample]

Determination of Urinary 8-OHdG

Urinary 8-OHdG was determined by an enzyme-linked immunosorbent assay (ELISA) kit (New 8-OHdG Check, JaICA, Japan). The kit consisted of a microtiter plate with wells which were precoated with 8-OHdG, a primary antibody (anti 8-OHdG, a primary antibody solution (phosphate buffered saline), a secondary antibody (HRP-conjugated anti mouse antibody) and a secondary antibody solution (phosphate buffered saline). In addition, the kit included a chromatic solution (3,3',5,5'-tetramethylbenzidine, a diluting solution (hydrogen peroxide/citrate-phosphate buffered saline), a washing solution (5x concentrated phosphate buffered saline), a reaction terminating solution (1M phosphoric acid) and standard 8-OHdG solutions (purified 8-OHdG (0.5-200 ng/ml)).

I. Principles of the Procedure

Subsequent to addition of the primary antibody into the microtiter plate, the antibody reacts competitively with the 8-OHdG bound on the plate and the 8-OHdG in urine sample. The bound antibodies in 8-OHdG in the sample are washed away from the antibodies that have bound to the 8-OHdG coated on the plate. Addition of the secondary antibody to the plate leads to the binding of the antibody to the monoclonal antibody which is bound to the 8-OHdG coated on the plate. Washing of the plate removes the unbound HRP-conjugated secondary antibody. The substrate solution is then added which results in colour development in proportion to the amount of the bound anti 8-OHdG antibody in the plate. Finally, phosphoric acid is added to terminate the reaction followed by measurement of the absorbance at 450 nm.

II. Assay Procedure

To each 50 µl of urine sample per well, 50 µl of a reconstituted primary antibody was added and incubated at 37°C for 1 h. Subsequent to discarding the mixture, the wells were washed with 250 µl of the washing solution. 100 µl of the secondary antibody was then added to the wells and incubated at 37°C for another hour. Wells were washed again as previously described at the end of the incubation period. After washing, 100 µl of the substrate solution was added to each well and incubated in the dark at room temperature for 15 minutes and then finally 100 µl of the reaction terminating solution was added and the absorbance was measured at 450 nm using an ELx 800 microtiter plate reader (BioTek, USA).

Crystallization Experiments

Crystallization experiments were performed in urines obtained from protocol 1 only as described in Chapter Two (page 52).

Statistical Analysis

All data were analysed by one-way ANOVA at statistical significance of $p \leq 0.05$.

4.3 Results

PROTOCOL 1

The results for the baseline 24 h urinary excretion of various parameters for protocol 1 before the ingestion of rhubarb juice are presented in Table 4.3. Urinary citrate excretion was significantly higher in black subjects compared to white subjects while, on the other hand, higher urinary calcium, creatinine and phosphate levels were observed in the latter group relative to the former. Furthermore, white subjects had higher RS of both brushite and CaOx and lower MSL. These baseline results are consistent with those obtained in the other studies described in the previous chapters, with the exception of creatinine which was not previously significantly different in the two groups. No additional significantly different urinary variables were observed between the two race groups.

After the ingestion of rhubarb juice, no statistically significant changes were observed in black subjects (Table 4.4). However, in white subjects rhubarb juice provoked an increase in urinary pH, a decrease in RS of uric acid and an increase in MSL.

Table 4.3: Mean baseline 24 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.11 ± 0.13	6.06 ± 0.10	0.7650
Volume (ml/24h)	2018 ± 135	1861 ± 211	0.5408
Citrate (mmol/24h)	3.28 ± 0.23	1.84 ± 0.26	*0.0007
Oxalate (mmol/24h)	0.48 ± 0.03	0.45 ± 0.02	0.5521
Calcium (mmol/24h)	1.86 ± 0.55	4.11 ± 0.49	*0.0069
Magnesium (mmol/24h)	2.23 ± 0.37	2.96 ± 0.29	0.1415
Sodium (mmol/24h)	172 ± 51.1	132 ± 37.8	0.5427
Potassium (mmol/24h)	42.5 ± 9.10	53.4 ± 8.43	0.4159
Urate (mmol/24h)	2.87 ± 0.36	3.63 ± 0.27	0.1115
Creatinine (mmol/24h)	12.8 ± 1.09	16.4 ± 0.81	*0.0167
Phosphate (mmol/24h)	18.2 ± 1.98	27.8 ± 1.97	*0.0029
Chloride (mmol/24h)	189 ± 27.6	185 ± 18.6	0.9058
Tiselius risk index	327 ± 53.8	418 ± 57.6	0.2632
RS Brushite	0.23 ± 0.10	0.88 ± 0.14	*0.0011
RS CaOx	2.30 ± 0.47	8.60 ± 1.35	*0.0003
RS Uric Acid	1.04 ± 0.24	1.59 ± 0.32	0.1848
MSL	0.11 ± 0.02	0.06 ± 0.01	*0.0138

*statistically significant

Table 4.4: Mean 24 h urine variables ± SE in black and white subjects after ingestion of rhubarb juice

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Ox*	*p*	*Baseline*	*Post-Ox*	*p*	*p Post-Ox*
pH	6.11±0.13	6.29±0.10	0.2943	6.06±0.10	6.62±0.11	*0.0016	*0.0440
Volume (ml/24h)	2018±135	2167±154	0.3047	1862±211	2285±196	0.1585	0.6421
Citrate (mmol/24h)	3.28±0.23	2.83±0.42	0.3188	1.84±0.26	2.00±0.34	0.7163	0.1401
Oxalate (mmol/24h)	0.48±0.03	0.50±0.06	0.7303	0.45±0.02	0.51±0.05	0.3414	0.8997
Calcium (mmol/24h)	1.86±0.55	1.26±0.24	0.3292	4.11±0.49	3.71±0.58	0.5995	*0.0011
Magnesium (mmol/24h)	2.23±0.37	2.30±0.26	0.9965	2.96±0.29	3.11±0.44	0.7666	0.1242
Sodium (mmol/24h)	172±51.1	116±20.7	0.2525	132±37.8	164±35.4	0.5517	0.2569
Potassium (mmol/24h)	42.5±9.10	35.3±7.66	0.6720	53.4±8.43	59.5±9.02	0.6290	†0.0564
Urate (mmol/24h)	2.87±0.36	3.39±0.23	0.2829	3.63±0.27	4.35±0.31	0.0983	*0.0221
Creatinine (mmol/24h)	12.8±1.09	13.4±0.90	0.6451	16.4±0.81	20.7±3.93	0.3005	0.0855
Phosphate (mmol/24h)	18.2±1.98	17.0±2.02	0.8222	27.8±1.97	28.9±3.32	0.7655	*0.0066
Chloride (mmol/24h)	189±27.6	173±14.1	0.5710	185±18.6	226±27.7	0.2417	0.1056
Tiselius risk index	327±53.8	261±36.1	0.3231	418±57.6	304±47.0	0.1475	0.4783
RS Brushite	0.23±0.10	0.17±0.07	0.6097	0.88±0.14	1.24±0.24	0.2124	*0.0005
RS CaOx	2.30±0.47	1.91±0.46	0.5575	8.60±1.35	5.50±1.50	0.1411	*0.0346
RS Uric Acid	1.04±0.24	0.86±0.28	0.6136	1.59±0.32	0.49±0.10	*0.0043	0.2328
MSL	0.11±0.02	0.13±0.01	0.1512	0.06±0.01	0.09±0.02	*0.0132	*0.0287

*statistically significant; †approaching statistical significance

Urinary NAG

The values for the mean urinary NAG activity ± SE for the two groups are shown in Table 4.5. It is noted that, in general, values in white subjects were greater, albeit that these were not statistically significant. The NAG activity did not change within each group.

Table 4.5: Mean urinary NAG activity (SE) [U/l] in black and white subjects before and after ingestion of rhubarb juice

	Blacks (n=10)		*Whites (n=10)*		*Blacks vs Whites*
	NAG (SE)	*p*	*NAG (SE)*	*p*	*p*
Before	0.25 (0.04)		0.53 (0.18)		0.1433
After (6 h)	0.25 (0.05)	1.0000	0.58 (0.15)	0.9138	†0.0548
After (18 h)	0.25 (0.07)	1.0000	0.33 (0.07)	0.2868	0.4478

†approaching statistical significance

Crystallization Experiments

^{14}C-Oxalate Crystal Deposition

Figure 4.1 shows the rate of CaOx deposition obtained from the urines of black and white subjects before and after the ingestion of rhubarb juice. The gradients for each curve (top right-hand corner) reveal an increase in CaOx deposition in both groups, with whites having a higher percentage of precipitated ^{14}C-oxalate compared to blacks following a rhubarb juice load.

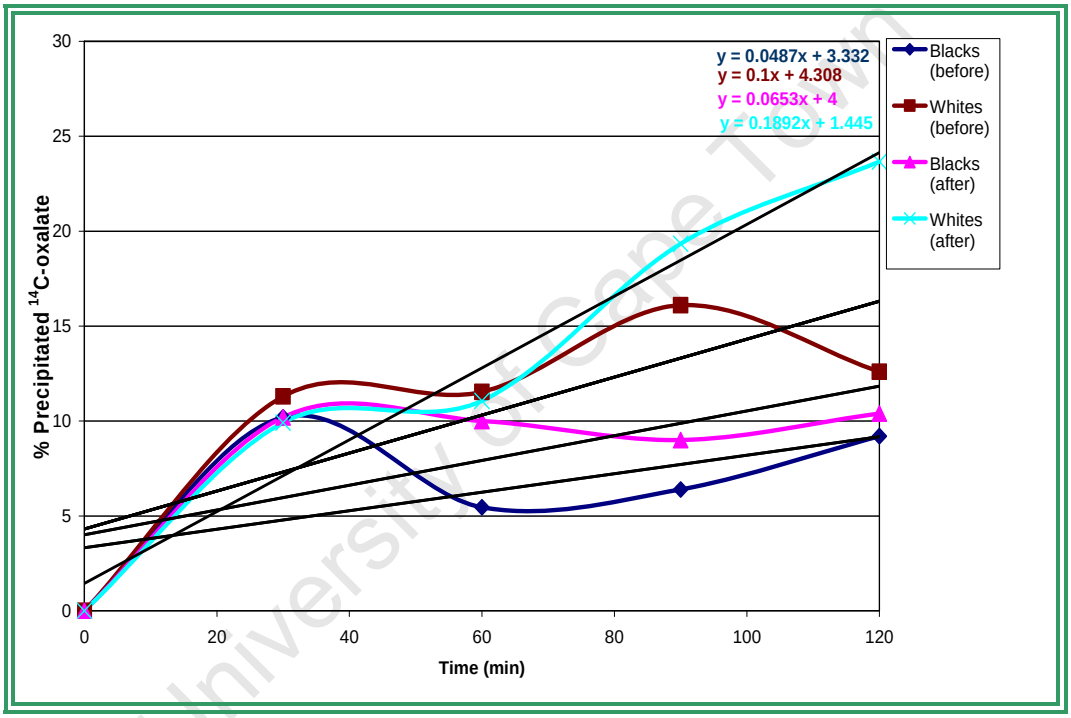


Figure 4.1: *Percentage precipitated ^{14}C-oxalate pre- and post-rhubarb juice ingestion in urines of black and white subjects*

Crystal Aggregation

Table 4.6 shows the mean percentages for the inhibition of crystal aggregation in the two groups and the corresponding plot of absorbance versus time before and after the ingestion of rhubarb juice (Figure 4.2). No statistically significant changes were observed in either group.

Table 4.6: Mean percentage inhibition of aggregation (%Ia) in black and white subjects before and after a rhubarb juice load

Variables	*Blacks*		*Whites*		*Blacks vs Whites*
	% Ia (SE)	*p*	*% Ia (SE)*	*p*	*p*
Before	81.3 (6.32)		74.5 (6.94)		0.8002
After	78.4 (4.62)	0.8879	76.3 (6.46)	0.9548	0.9193

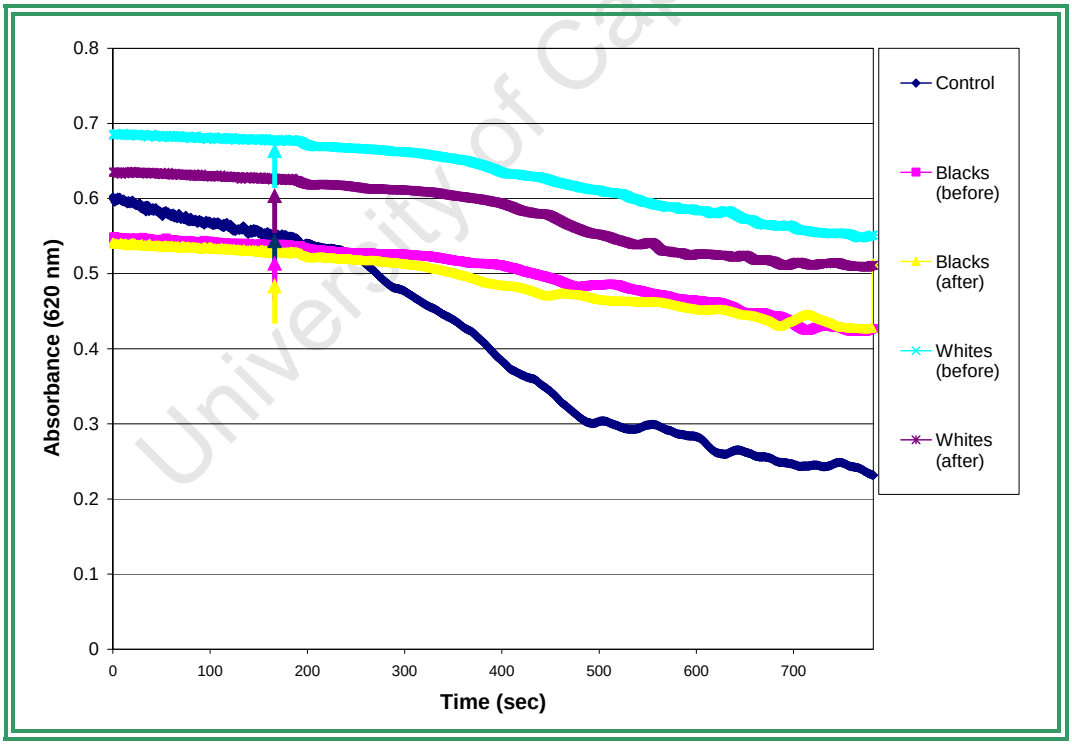


Figure 4.2: *Plot of absorbance versus time pre- and post-rhubarb juice load in urines of black and white subjects. The time at which stirring was stopped is indicated by the arrows.*

Summary of Urine Chemistry and Crystalluria Properties After Rhubarb Juice Load

	Blacks	Whites
RS uric acid / MSL	↔	↓
Non-Fractional	↔	↑ *pH*
Deposition rate	↑	↑
Aggregation	↔	↔
NAG	↔	↔

PROTOCOL 2

6 h Fraction

Table 4.7 shows the 6 h urinary excretion of various variables for protocol 2 in black and white subjects before the ingestion of spinach. The urinary oxalate and magnesium excretion were higher in black subjects than in white subjects. None of the other variables were significantly different.

Spinach provoked an increase in urinary pH in blacks 6 h after ingestion as shown in Table 4.8. In addition, citrate excretion was decreased in this group. In whites, spinach augmented the excretion of urinary urate only.

Table 4.7: Mean baseline 6 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.30 ± 0.12	6.22 ± 0.13	0.6873
Volume (ml/6h)	802 ± 76.9	848 ± 55.6	0.7008
Citrate (mmol/6h)	1.45 ± 0.16	0.97 ± 0.20	0.0924
Oxalate (mmol/6h)	0.13 ± 0.01	0.08 ± 0.01	*0.0226
Calcium (mmol/6h)	0.78 ± 0.12	0.94 ± 0.35	0.6007
Magnesium (mmol/6h)	0.69 ± 0.12	0.24 ± 0.06	*0.0263
Sodium (mmol/6h)	25.1 ± 12.4	19.9 ± 6.95	0.7836
Potassium (mmol/6h)	5.61 ± 2.17	4.38 ± 1.53	0.7152
Urate (mmol/6h)	0.97 ± 0.07	0.82 ± 0.07	0.2207
Creatinine (mmol/6h)	4.56 ± 0.32	4.36 ± 0.25	0.6865
Phosphate (mmol/6h)	4.38 ± 0.52	2.88 ± 0.36	0.0802
Chloride (mmol/6h)	62.5 ± 7.51	51.0 ± 7.83	0.3577

*statistically significant

Table 4.8: Mean 6 h urinary variables ± SE in black and white subjects after ingestion of spinach

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Ox*	*p*	*Baseline*	*Post-Ox*	*p*	*p Post-Ox*
pH	6.30±0.12	6.84±0.18	*0.0237	6.22±0.13	6.75±0.22	0.0727	0.7676
Volume (ml/6h)	802±76.9	705±101	0.4538	848±55.6	856±22.7	0.8974	0.3195
Citrate (mmol/6h)	1.45±0.16	0.72±0.18	*0.0077	0.97±0.20	1.08±0.25	0.7250	0.2677
Oxalate (mmol/6h)	0.13±0.01	0.10±0.01	0.0875	0.08±0.01	0.14±0.04	0.2411	0.3042
Calcium (mmol/6h)	0.78±0.12	0.58±0.10	0.2310	0.94±0.35	0.67±0.22	0.5395	0.6725
Magnesium (mmol/6h)	0.69±0.12	0.58±0.12	0.5366	0.24±0.06	0.20±0.03	0.6056	†0.0552
Sodium (mmol/6h)	25.1±12.4	32.9±6.78	0.5884	19.9±6.95	60.7±20.9	0.1014	0.1314
Potassium (mmol/6h)	5.61±2.17	6.98±2.54	0.6875	4.38±1.53	10.1±4.12	0.2284	0.5080
Urate (mmol/6h)	0.97±0.07	1.13±0.11	0.2373	0.82±0.07	1.66±0.29	*0.0243	†0.0571
Creatinine (mmol/6h)	4.56±0.32	4.73±0.31	0.7043	4.36±0.25	4.38±0.60	0.9761	0.5707
Phosphate (mmol/6h)	4.38±0.52	4.51±0.52	0.8624	2.88±0.36	4.84±0.99	0.1007	0.7496
Chloride (mmol/6h)	62.5±7.51	75.0±4.51	0.1708	51.0±7.83	78.9±8.82	0.3043	0.6697

*statistically significant; †approaching statistical significance

18 h Fraction

There was a significant difference in the mean 18 h baseline urine volume between the two groups, with blacks having lower levels compared to whites (Table 4.9). No other differences were observed between the two groups.

After the ingestion of spinach, there was a rise in citrate and oxalate excretion in black subjects (Table 4.10). However, no significant changes were noted in the excretion of any of the urinary parameters in white subjects.

Table 4.9: Mean baseline 18 h urinary variables ± SE in black and white subjects after ingestion of spinach

Variables	*Blacks*	*Whites*	*p*
pH	6.06 ± 0.10	6.11 ± 0.10	0.8071
Volume (ml/18h)	990 ± 125	1710 ± 253	*0.0069
Citrate (mmol/18h)	1.23 ± 0.30	1.99 ± 0.36	0.5056
Oxalate (mmol/18h)	0.13 ± 0.02	0.19 ± 0.05	0.2342
Calcium (mmol/18h)	1.30 ± 0.35	1.87 ± 0.51	0.3438
Magnesium (mmol/18h)	1.18 ± 0.29	0.81 ± 0.42	0.4421
Sodium (mmol/18h)	63.7 ± 17.0	32.9 ± 7.26	0.3827
Potassium (mmol/18h)	16.1 ± 4.81	11.3 ± 2.82	0.4908
Urate (mmol/18h)	1.62 ± 0.22	1.53 ± 0.52	0.9298
Creatinine (mmol/18h)	8.98 ± 0.97	11.8 ± 2.96	0.2285
Phosphate (mmol/18h)	17.3 ± 2.46	21.0 ± 3.15	0.3059
Chloride (mmol/18h)	101 ± 16.7	92.3 ± 16.4	0.9496

*statistically significant

Table 4.10: Mean 18 h urinary variables ± SE in black and white subjects after ingestion of spinach

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Ox*	*p*	*Baseline*	*Post-Ox*	*p*	*p Post-Ox*
pH	6.06±0.10	6.16±0.11	0.5371	6.11±0.10	6.30±0.11	0.2102	0.4690
Volume (ml/18h)	990±125	1165±138	0.3584	1710±253	1444±267	0.4177	0.3180
Citrate (mmol/18h)	1.23±0.30	2.06±0.21	*0.0358	1.99±0.36	2.21±0.57	0.4422	0.7595
Oxalate (mmol/18h)	0.13±0.02	0.24±0.02	*0.0005	0.19±0.05	0.27±0.04	0.1043	0.4264
Calcium (mmol/18h)	1.30±0.35	1.43±0.31	0.7761	1.87±0.51	1.78±0.32	0.8777	0.4888
Magnesium (mmol/18h)	1.18±0.29	0.97±0.20	0.5645	0.81±0.42	0.46±0.10	0.4172	0.1070
Sodium (mmol/18h)	63.7±17.0	30.0±6.23	0.0792	32.9±7.26	71.0±27.5	0.3314	0.0695
Potassium (mmol/18h)	16.1±4.81	12.0±2.93	0.4745	11.3±2.82	18.0±5.35	0.2733	0.3025
Urate (mmol/18h)	1.62±0.22	1.37±0.12	0.3575	1.53±0.52	1.58±0.23	1.0000	0.3983
Creatinine (mmol/18h)	8.98±0.97	8.51±0.96	0.7418	11.8±2.96	9.02±1.59	0.3837	0.7814
Phosphate (mmol/18h)	17.3±2.46	14.4±1.53	0.3472	21.0±3.15	16.1±2.63	0.1919	0.5621
Chloride (mmol/18h)	101±16.7	94.9±10.4	0.7654	92.3±16.4	114±14.2	0.5240	0.3232

*statistically significant

Urinary NAG

At 6 h, following spinach load, the urinary excretion of oxalate did not change in both groups. NAG was therefore not measured on those samples as shown in Table 4.11. However, at 18 h oxalate excretion increased in the majority of black subjects and in few white subjects but NAG did not change significantly in either group. Unlike in the rhubarb study, the mean NAG levels in white subjects were lower.

Table 4.11: Mean urinary NAG activity (SE) [U/l] in black and white subjects before and after ingestion of spinach

	Blacks (n=9)		*Whites (n=3)*		*Blacks vs Whites*
	NAG (SE)	*p*	*NAG (SE)*	*p*	*p*
Before	1.40 (0.46)		0.61 (0.28)		0.3445
After (6 h)	-	-	-	-	-
After (18 h)	0.71 (0.16)	0.1604	0.33 (0.15)	0.4261	0.2295

Summary of Urine Chemistry After Spinach Load

	Blacks	Whites
Fractional (6 h)	↑ pH, ↓ Cit	↑ Ur
Fractional (18 h)	↑ Cit, ↑ Ox	↔
NAG (18 h)	↔	↔

PROTOCOL 3

5 h Fraction

The mean baseline excretions of the various urinary variables at 5 h before the administration of Na_2Ox are presented in Table 4.12. Urinary pH and citrate were higher in blacks as well as the urine volume.

In Table 4.13, urinary concentrations measured at baseline and 5 h after administration of Na_2Ox are compared. It is seen that urinary pH, citrate and potassium decreased significantly in blacks. Of note is the increase in oxalate excretion in this group which approached statistical significance. No significant changes were observed in whites.

Table 4.12: Mean baseline 5 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	7.06 ± 0.13	6.68 ± 0.11	*0.0400
Volume (ml/5h)	840 ± 68.3	616 ± 68.0	*0.0319
Citrate (mmol/5h)	1.80 ± 0.12	0.94 ± 0.15	*0.0004
Oxalate (mmol/5h)	0.08 ± 0.01	0.06 ± 0.01	0.0954
Calcium (mmol/5h)	0.73 ± 0.12	0.84 ± 0.09	0.4478
Magnesium (mmol/5h)	0.57 ± 0.07	0.60 ± 0.05	0.6707
Sodium (mmol/5h)	9.43 ± 0.81	13.4 ± 3.85	0.3238
Potassium (mmol/5h)	4.54 ± 0.48	5.55 ± 1.81	0.5948
Urate (mmol/5h)	0.84 ± 0.08	0.96 ± 0.11	0.5403
Creatinine (mmol/5h)	4.54 ± 0.62	4.40 ± 0.69	0.8818
Phosphate (mmol/5h)	3.54 ± 0.42	3.64 ± 0.37	0.9023

*statistically significant

Table 4.13: Mean 5 h urinary variables ± SE in black and white subjects after ingestion of sodium oxalate

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Ox*	*p*	*Baseline*	*Post-Ox*	*p*	*p Post-Ox*
pH	7.06±0.13	6.41±0.13	*0.0024	6.68±0.11	6.50±0.44	0.6916	0.6585
Volume (ml/5h)	840±68.3	645±85.7	0.0913	616±68.0	742±95.1	0.2952	0.8655
Citrate (mmol/5h)	1.80±0.12	1.07±0.21	*0.0088	0.94±0.15	1.39±0.26	0.1596	0.8065
Oxalate (mmol/5h)	0.08±0.01	0.17±0.05	†0.0528	0.06±0.01	0.08±0.01	0.1039	0.1475
Calcium (mmol/5h)	0.73±0.12	0.63±0.09	0.5180	0.84±0.09	0.68±0.08	0.2082	0.4560
Magnesium (mmol/5h)	0.57±0.07	0.45±0.07	0.2597	0.60±0.05	0.50±0.05	0.1755	0.2333
Sodium (mmol/5h)	9.43±0.81	7.63±0.84	0.1430	13.4±3.85	22.5±6.87	0.2641	0.5985
Potassium (mmol/5h)	4.54±0.48	2.35±0.41	*0.0027	5.55±1.81	6.08±1.75	0.8371	0.9467
Urate (mmol/5h)	0.84±0.08	1.27±0.29	0.3367	0.96±0.11	0.77±0.07	0.2775	0.0832
Creatinine (mmol/5h)	4.54±0.62	4.62±0.91	0.9425	4.40±0.69	2.79±0.33	0.0605	*0.0100
Phosphate (mmol/5h)	3.54±0.42	3.25±0.20	0.3905	3.64±0.37	2.57±0.34	0.1304	†0.0516

*statistically significant; †approaching statistical significance

18 h Fraction

Table 4.14 shows the urinary excretions of the parameters 18 h before the ingestion of Na_2Ox. The white subjects had a significantly higher urinary excretion of sodium compared to their black counterparts. No further observations were made.

In Table 4.15, urinary concentrations measured 18 h after administration of Na_2Ox are compared with the control values. Urinary oxalate increased significantly in blacks while no significant changes were noted in any of the parameters in whites.

Table 4.14: Mean baseline 18 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.21 ± 0.08	6.29 ± 0.14	0.8418
Volume (ml/18h)	1576± 214	1628 ± 214	0.4565
Citrate (mmol/18h)	2.93 ± 0.49	2.78 ± 0.35	0.3660
Oxalate (mmol/18h)	0.14 ± 0.01	0.20 ± 0.04	0.0667
Calcium (mmol/18h)	1.79 ± 0.29	2.70 ± 0.30	0.6770
Magnesium (mmol/18h)	1.82 ± 0.32	2.34 ± 0.27	0.6066
Sodium (mmol/18h)	39.1 ± 12.9	48.9 ± 12.2	*0.0456
Potassium (mmol/18h)	17.0 ± 5.92	17.5 ± 3.68	0.9522
Urate (mmol/18h)	1.38 ± 0.24	2.40 ± 0.24	0.1852
Creatinine (mmol/18h)	7.12 ± 0.96	12.1 ± 1.36	0.0926
Phosphate (mmol/18h)	15.7 ± 2.22	29.0 ± 2.98	0.1261

*statistically significant

Table 4.15: Mean 18 h urinary variables in black and white subjects after ingestion of sodium oxalate

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-Ox*	*p*	*Baseline*	*Post-Ox*	*p*	*p Post-Ox*
pH	6.21±0.08	5.90±0.15	0.0826	6.29±0.14	6.18±0.28	0.7438	0.3720
Volume (ml/18h)	1576±214	1267±198	0.3033	1628±214	1676±242	0.8833	0.2065
Citrate (mmol/18h)	2.93±0.49	3.45±0.50	0.4638	2.78±0.35	2.67±0.44	0.8531	0.2603
Oxalate (mmol/18h)	0.14±0.01	0.35±0.04	*0.0001	0.20±0.04	0.24±0.03	0.3533	†0.0565
Calcium (mmol/18h)	1.79±0.29	1.45±0.30	0.4322	2.70±0.30	2.85±0.43	0.7791	*0.0156
Magnesium (mmol/18h)	1.82±0.32	1.40±0.14	0.2444	2.34±0.27	2.31±0.40	0.9606	*0.0446
Sodium (mmol/18h)	39.1±12.9	28.8±7.28	0.4958	48.9±12.2	95.8±27.3	0.1539	*0.0295
Potassium (mmol/18h)	17.0±5.92	14.1±4.71	0.7067	17.5±3.68	36.6±12.1	0.1492	0.1014
Urate (mmol/18h)	1.33±0.24	1.15±0.11	0.1716	2.40±0.24	2.55±0.26	0.7844	0.0913
Creatinine (mmol/18h)	7.12±0.96	8.45±1.22	0.4197	12.1±1.36	14.9±0.97	0.1305	*0.0009
Phosphate (mmol/18h)	15.7±2.22	11.9±2.88	0.5596	29.0±2.98	30.5±3.04	0.7920	*0.0218

*statistically significant; †approaching statistical significance

Urinary NAG

The mean urinary NAG levels before and after (5 h and 18 h) the ingestion of Na_2Ox are given in Table 4.16. The NAG activity did not change within each group at 5 h or 18 h relative to baseline. However, whites had a significantly higher activity than blacks when making an inter-group comparison.

Table 4.16: Mean urinary NAG activity (SE) [U/l] in black and white subjects before and after ingestion of sodium oxalate

	Blacks (n=5)		*Whites (n=4)*		*Blacks vs Whites*
	NAG (SE)	*p*	*NAG (SE)*	*p*	*p*
Before	0.42 (0.19)		0.61 (0.10)		0.0987
After (5 h)	0.31 (0.18)	0.8003	0.78 (0.22)	0.6508	*0.0403
After (18 h)	0.32 (0.16)	0.7669	0.57 (0.18)	0.8855	0.1004

*statistically significant

Urinary 8-OHdG

Table 4.17 shows of the mean urinary 8-OHdG levels before and 5 h after the ingestion of Na_2Ox. No statistical differences were observed in the black group or in the white group. However, it is noted that the value after 5 h in the black group is greater than that in the white group and that the difference is approaching statistical significance.

Table 4.17: Mean urinary 8-OHdG levels (SE) [ng/ml] in black and white subjects before and after ingestion of sodium oxalate

	Blacks (n=5)		*Whites (n=4)*		*Blacks vs Whites*
	8-OHdG (SE)	*p*	*8-OHdG (SE)*	*p*	*p*
Before	7.42 (2.11)		6.98 (2.33)		0.9122
After (5 h)	9.18 (3.03)	0.7434	5.60 (1.09)	0.8989	0.0605

Correlation between NAG and 8-OHdG

A bivariate correlation test was performed as shown in Figure 4.3 to test whether there is a correlation between urinary NAG and urinary 8-OHdG levels after the ingestion of Na_2Ox. This test was performed using the urinary data obtained from both groups. There was no correlation between the two markers as indicated by the r and p values.

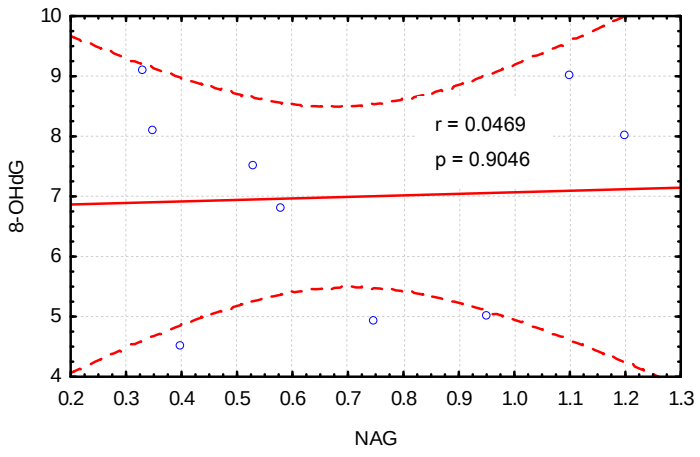


Figure 4.3: *Correlation analysis of NAG and 8-OHdG*

Summary of Urine Chemistry After Sodium Oxalate Load

	Blacks	Whites
Fractional (5 h)	↓ pH, ↓ Cit, ↑ Ox, ↓ K	↔
Fractional (18 h)	↑ Ox	↔
NAG (5 h/18 h)	↔	↔
8OHdG (5 h)	↔	↔

4.4 Discussion

As stated in the introduction, the main objective for the three studies presented in this chapter was to measure the response to dietary oxalate challenges in black and white South African subjects and to assess the association, if any, of the biomarkers for renal tubular damage (NAG) and cellular oxidative stress (8-OHdG) with urinary oxalate. Since previous studies have identified a link between increased urinary oxalate and NAG excretions after an oral oxalate load and others did not, the present study was imperative, especially in the context of the perceived differences in the renal handling of oxalate in South Africa's black and white population groups.

In protocol 1, rhubarb juice did not have any significant effect on oxalate excretion within either group. However, urinary pH increased and RS of uric acid decreased in white subjects. The rise in urinary pH is consistent with the observed decrease in RS of uric acid as a low pH poses a risk of uric acid supersaturation. Of interest is the higher urinary excretion of calcium in the white group compared to the black group following ingestion of rhubarb juice. The unchanged levels of urinary oxalate observed in the former group can be ascribed to the complexation of oxalate with calcium thereby limiting the oxalate available for absorption in the intestine (Sutton *et al.* 1994, Messa *et al.* 1997, Seiner *et al.* 2003). However, neither urinary oxalate nor calcium excretion changed in black subjects was observed following a rhubarb juice load, this result is inconsistent with Lewandowski et al.'s findings (2001) whereby they noted an increase in oxalate excretion after an oxalate load this group. The oxalate content of rhubarb juice used in the present study was therefore later analysed routinely by a high-performance liquid chromatography (HPLC) method with a view to elucidate the unchanged urinary oxalate levels. The results indicated that the rhubarb juice contained a total of 374.4 mg of oxalate /100 ml of rhubarb juice, of which 150.4 mg/100ml is soluble, which was indeed not sufficient to cause any significant increase in the urinary oxalate excretion.

Rhubarb juice did not change the urinary NAG excretion in either group. This can be attributed to the unchanged urinary oxalate concentrations in both groups. However, the higher baseline levels in whites, which were approaching statistical significance is noteworthy, especially since this observation was consistently observed in all three protocols.

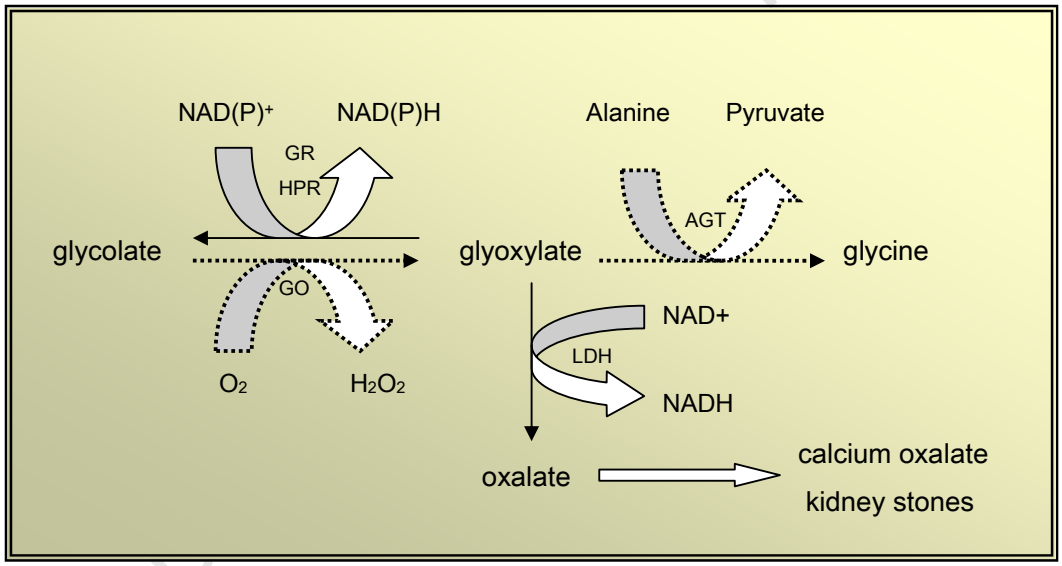
The crystalluria properties of the urines from the two groups revealed lower levels of CaOx deposition in black subjects in conjunction with lower urinary NAG activities as well as the RS of CaOx. The white subjects, on the other hand, had a higher deposition rate which was supported by the higher urinary NAG levels in this group. However, the increased MSL in the white group subsequent to rhubarb juice load implies increased hindrance of CaOx crystallization, which is inconsistent with the higher RS of CaOx and its deposition observed in this group. Regardless of this discrepancy, it seems plausible to conclude that the oxalate load had an adverse effect in white subjects, with regard to all the aforementioned risk factors except for the MSL.

Subsequent to the ingestion of spinach in protocol 2, the urinary citrate excretion in black subjects decreased at 6 h but this did not influence any rise in urinary oxalate excretion. This finding is not in agreement with the reported results of oxalate excretion peaking between 4-6 hours after ingestion of an oxalate-rich meal (Tiselius and Almgard 1977). One possible explanation for this anomaly is the presence of calcium in the standardized meals which the subjects consumed, even though it was present in negligible amount. Moreover, the calcium in spinach could not have had an effect due to its lack of bioavailability (Brinkley *et al.* 1981). Similar to rhubarb juice in protocol 1, the oxalate content was measured routinely at a later stage using a HPLC method. The results revealed that the spinach used in the present study contained a total of 818.4 mg of oxalate/100 g of spinach of which 175.3 mg /100g is soluble. It is therefore surprising that the urinary oxalate did not increase in either group as the oxalate content in spinach was sufficient to trigger a rise.

At 18 h, the urinary oxalate excretion was augmented in the black group. The delayed response in the excretion of urinary oxalate is interesting and it could mean that oxalate absorption occurred in the large intestine rather than in the small intestine in this group. Lewandowski *et al.* (2001) observed higher urinary oxalate levels in black South African subjects compared to their white compatriots at 24 h after an oxalate-rich snack which included spinach. In the present study, spinach did not have any significant effect on the urinary oxalate excretion in white subjects. The metabolic pathway for oxalate production in the liver leading to CaOx stone formation and the enzymes involved is depicted in Scheme 1. The conversion of glycolate to glyoxylate is catalyzed by glycolate oxidase (GO), also known as glycolic acid oxidase (GAO) and the oxidation of the latter to oxalate is catalyzed by lactate

dehydrogenase (LDH). It seems therefore logical to suggest that, apart from the increased absorption of oxalate obtained from spinach, which resulted in the elevated excretion in the urine in black subjects and not in white subjects, there is also a possibility of a higher activity of the aforementioned enzymes in the former group which could have promoted the endogenous synthesis of oxalate.

The concomitant increase in citrate levels with oxalate subsequent to ingestion of spinach in black subjects is surprising. Since high levels of citrate normally affect oxalate excretion by reducing its absorption and thus its excretion, the contradictory effect observed in the black group is not understood. The NAG activity was not significantly different in both groups, in spite of the increased oxalate concentrations in the black group induced by spinach suggesting absence of renal tubular injury.



Scheme 1: *The metabolic pathway of glycolate and enzymes involved leading to oxalate in the human liver (Behnam et al. 2006)*

Sodium oxalate, administered in protocol 3 provoked an increase in urinary oxalate in black subjects. This finding indicates a higher risk of CaOx crystallization in this group. The white subjects did not respond to the sodium oxalate load as opposed to what was expected, which is also contrary to what was hypothesized earlier. In fact, at 18 h, lower oxalate levels were observed in this group compared to their black counterparts. On the other hand, statistically significant changes in urinary NAG within each group were not apparent subsequent to sodium oxalate ingestion.

However, the white group had a higher mean activity in comparison with the black group, which is contradictory to the lower oxalate levels noted in the former group. The failure of sodium oxalate to raise NAG excretion, though, is consistent with the observations from other studies, where no differences were found in NAG levels after sodium oxalate intake (Holmes *et al.* 2005, Knight *et al.* 2007). In addition, sodium oxalate had no significant effect on the urinary excretion of 8-OHdG in the two groups. Moreover, there was no correlation between these two markers, contrary to the observations by Boonla *et al.* (2007). This can be attributed to the sample size in which the correlation test was performed. A larger size could provide a conclusive estimation.

In retrospect, the two oxalate-rich foods, rhubarb juice and spinach did not induce any changes to the urinary NAG excretion within the black and the white groups. Similarly, sodium oxalate triggered no response in both groups. It was hypothesized earlier that exposure to high concentrations of oxalate would induce cell injury in white subjects and this would trigger the release of NAG. The reason for this expectation was that, aggravated dietary oxalate levels would provoke an increase in the urinary excretion oxalate since, as mentioned before, studies have demonstrated that this group is more prone to CaOx kidney stone formation than the black group. It is intriguing that in all three protocols the white subjects did not respond as expected to the oxalate challenges, as no statistically significant changes were observed in the urinary excretion of oxalate, either. Khan and Thamilselvan (2000) posited that the mechanism by which NAG is excreted is that, cell injury, as a result of binding of oxalate and/or calcium oxalate crystals into the renal tubular cells and their entry thereof, induces the excretion of NAG in the urine via the tubular lumen, which manifests damage to the cells. The failure for the two groups to present elevated NAG levels implies that cell injury did not occur.

Oxidative damage to DNA due to attack by reactive oxygen species has been implicated in high urinary levels of 8-OHdG observed in stone formers (Boonla *et al.* 2007). Hyperoxaluria may induce oxidative stress and elevate 8-OHdG. In the present study neither group showed a correlation between 8-OHdG and urinary oxalate after an acute sodium oxalate load, possibly implying absence of oxidative stress.

In view of the results presented in this chapter, it can be concluded that even though the two South African race groups were subjected to high oxalate loads and elicited different handling responses, the hypothesis put forward initially that the white subjects will adversely demonstrate cell injury and oxidative stress remains unproven.

4.5 References

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Chapter Five

Investigation of the effect of the ingestion of taurine on the excretion of urinary glycolate in black and white South African subjects

5.1 Introduction

Oxalate can be synthesized in animal models from endogenous metabolism of various precursors including glycolate, glyoxylate and hydroxypyruvate (Ogawa *et al.* 2000a). In plants, glycolate is generated from phosphoglycolate. The diffusion of glycolate across the chloroplast envelope as it moves into the peroxisomes, facilitates its metabolism by glycolate oxidase (GO) to glyoxylate (Ogawa *et al.* 2000b). The metabolic origin of glycolate in the body is not clearly understood but it is believed that a larger portion of it is endogeneously synthesized. It is suggested that the metabolism of glyoxylate from glycolate to urinary oxalate occurs in the hepatocytes in the presence of enzymes (Ogawa 2000b). The ingested or synthesized glycolate is either metabolized to glyoxylate by GO in the liver or excreted in the urine (Nakai and Ichiyama 2000).

Taurine, a β-amino acid is one of the most abundant amino acids present in tissues. It is synthesized from dietary cysteine and methionine and is present in certain foodstuffs such as dairy, meat, eggs and poultry (Kerai *et al.* 2001). Red Bull, a well known energy drink is also a rich source of taurine (Kim 2003). It has been shown that taurine has a beneficial effect as an antioxidant in biological systems due to its ability to scavenge reactive oxygen species, and to reduce the peroxidation of unsaturated membrane lipids (Nandhini *et al.* 2005). The kidney is one of the most important organs involved in taurine regulation and there is considerable evidence to demonstrate that the kidney regulates the whole body homeostasis of taurine (Rozen *et al.* 1979). In the kidney, there is a high affinity, low capacity, Na^+-dependent, β-amino acid specific transport system, which is responsible for the re-absorption of taurine across the renal tubular brush border membrane (Rozen *et al.* 1979). The effect of taurine on urinary oxalate excretion after intravenous loading with glycolic acid in rats has been investigated (Ogawa *et al.* 1999). The results from this study showed a significant decrease of oxalate synthesis from glycolate after loading with glycolate to taurine-treated rats. Other studies have also shown that an oral administration of taurine reduces urinary excretion of glycolate (Talwar *et al.* 1985). Bile acids which are not absorbed in the body may react with intestinal calcium which may lead to the formation of soaps. These limit oxalate binding with free calcium and the consequence of this is the absorption of oxalate in the intestine and its increased urinary excretion (Earnest *et al.* 1974). In a study of hyperoxaluric patients with intestinal bypass, taurine reduced glycine, which is available for bile salts, thereby

decreasing the production of glyoxylate and oxalate (Dowling *et al.* 1971, Admirand *et al.* 1971). However, this therapeutic effect of taurine was not confirmed in a later study (Smith *et al.* 1972a). Since then, no other human studies have been undertaken, particularly with a view to investigate the role of taurine in stone inhibition similar to that demonstrated in animal studies.

Having noted in the previous chapters the different renal handling of various dietary agents in the South African black and white subjects, the present study was undertaken to investigate whether the two groups will have a different response to an oral taurine load.

The main objectives of this study are:

- to determine the urinary concentrations of glycolate in healthy black and white male South African subjects after a taurine load and to explore any evidence of its inhibitory effect on the excretion of glycolate and oxalate.

- to determine the crystallization properties in the urine of the two ethnic groups following the ingestion of taurine.

5.2 Subjects and Methods

Subjects

Ten black and ten white healthy South African males (aged 18-30) recruited from the student cohort of the University of Cape Town participated in the study. Approval for carrying out the study was granted by the Ethics Committee of the University.

Experimental Procedure

All subjects avoided calcium- and oxalate-rich foods for 3 days prior to the commencement of the study, as instructed. On day 4, subjects consumed a strict standardized diet. The actual composition of the standardized meals and the nutrient content are shown in Table 5.1 and Table 5.2, respectively. A 24 h urine sample was collected on day 5 while subjects continued with the standardized diet. On day 6, 250 ml Red Bull (Red Bull GmbH, Austria) containing carbonated water, sucrose, glucose, sodium citrate, taurine (1000 mg), glucuronolactone (600 mg), caffeine (80 mg), inositol, niacin, D-pantothenol, vitamin B12, artificial flavours and colours was ingested with the snack at 11h00. During the following 24 h, a urine sample was collected while consuming the standardized diet.

Table 5.1: Constituents of the strict standardized diet

MEAL	*DAY 4 AND 5*	*DAY 6*
Breakfast: 8h30	100 g bread rolls 10 g margarine 30 g salami pressed beef 50 g tomato 250 ml coffee	100 g bread rolls 10 g margarine 30 g salami pressed beef 50 g tomato 250 ml coffee
Snack: 11h00	27 g muesli bar	27 g muesli bar 250 ml Red Bull
Lunch: 13h00	90 g steamed chicken breast 100 g tomato 20 g lettuce 50 g hamburger roll 20 g sauce 30 g lightly salted potato crisps 150 g banana	90 g steamed chicken breast 100 g tomato 20 g lettuce 50 g hamburger roll 20 g sauce 30 g lightly salted potato crisps 150 g banana
Snack: 16h00	1 slice of whole wheat bread 20 g cream cheese 50 g tomato	1 slice of whole wheat bread 20 g cream cheese 50 g tomato
Supper: 20h00	120 g mince 120 g carrots 100 g tomato 50 g green pepper 240 g pasta 30 g cheese	120 g mince 120 g carrots 100 g tomato 50 g green pepper 240 g pasta 30 g cheese
Snack: 22h00 pm	175 g low fat strawberry yoghurt 150 g apple	175 g low fat strawberry yoghurt 150 g apple

Beverages: (2.25 l/day):

250 ml coffee (4 g coffee powder, 4 g creamer, 10 g sugar in 250 ml water)

700 ml caledon water until 13h00

600 ml caledon water until 19h00

700 ml caledon water until 22h00

Table 5.2: Nutrient content of the standardized diet

VARIABLE	*AMOUNT*
Energy (kJ)	12063
Total protein (g)	110.3
Total fat (g)	85.0
Carbohydrate (g)	383.5
Calcium (mg)	785
Magnesium (mg)	364
Phosphorus (mg)	1385
Citric acid (mg)	1385
Oxalic acid (mg)	258

Analytical Methods

Urine parameters were routinely measured in each of the 24 h urine samples obtained on day 5 and day 6 during the protocol as described in Chapter Two (page 51). Urine aliquots from each subject were kept at -80°C for the determination of urinary glycolate until analysis.

Determination of Urinary Glycolate

The method described by Van hee *et al.* (2004) was followed. Urine samples were prepared prior to analysis (described below) and the concentration of glycolate in each sample was then measured using gas chromatography tandem mass spectrometry (GC/MS). The pre-treatment of the samples involved a derivatization step. The objective for this step was to chemically modify the active hydroxyl sites in the glycolate molecule in urine samples by adding an excess of the derivatizing agent bis-N,O-trimethylsilyl trifluoroacetamide (BSTFA) in order to enhance chromatographic detectability of glycolate. Derivatization is crucial as it aids in increasing not only the detectability of compounds but also their volatility and stability. Moreover, differences in the sample compounds are accentuated through this procedure to facilitate chromatographic separation.

I. Preparation of Standards

A 100 mg/l glycolic acid (Sigma-Aldrich, Germany) stock solution prepared in redistilled water was used to make up six dilute standard solutions of varying concentrations (0.5, 10, 15, 30, 40 and 50 mg/l). The internal standard was prepared by adding 1,3-propylene (7 mg) (Aldrich, Bornem, Belgium) to acetonitrile (10 ml) (Merck, Darmstad, Germany) and the solution was stored at 4°C until use.

II. Derivatization Assay

20 µl of the urine sample was transferred by a micropipette into a screw-cap vial and to it 20 µl of the internal standard solution 1,3-propylene glycol in acetonitrile (7mg/10 ml), 20 µl of dimethylformamide (DMF) (Merck, Darmstad, Germany) and 750 µl of BSTFA (Sigma-Aldrich, Germany) were added. The standard solutions (20 µl) were pre-treated in the same manner. The vial was vortexed immediately followed by incubation at 70°C for 15 min and then cooled.

III. Chromatographic Procedure

1 µl of the solution was injected into the GC system (Agilent Technologies, USA), at a set temperature of 280°C, on an HP-5MS (Agilent part no: 19091S-433) fused silica capillary column (30 m x 0.25 mm I.D, 0.25 µm film thickness) with helium as carrier gas at linear velocity of 36 cm/min, with a 1/60 split ratio. The samples were analysed for 10.50 min.

Crystallization Experiments

Crystallization experiments were performed as described in Chapter Two (page 52) and all data were statistically analyzed by one-way ANOVA at statistical significance of $p \leq 0.05$.

Scanning Electron Microscopy

Urine samples were prepared, filtered and mounted on stubs and the stubs were viewed under a scanning electron microscope as described in Chapter Three (page 111).

5.3 Results

Prior to the administration of taurine, there were no statistically significant differences in the urinary excretion of any of the urinary variables in either the black or the white group as shown in Table 5.3. These results are not in agreement with the baseline differences observed in the earlier studies in the previous chapters between the two groups. This anomaly will be discussed in Chapter Six.

The intake of a taurine load did not have a significant effect within either of the two race groups except for the increase in BRI in whites after a taurine load (Table 5.4). However, inter-group comparisons demonstrated that whites had higher urinary oxalate and creatinine levels as well as the RS of brushite compared to their black compatriots after taurine ingestion.

Table 5.3: Mean baseline 24 h urinary variables ± SE in black and white subjects

Variables	*Blacks*	*Whites*	*p*
pH	6.61 ± 0.11	6.47 ± 0.07	0.3999
Volume (ml/24h)	1980 ± 197	2204 ± 169	0.3209
Citrate (mmol/24h)	3.96 ± 0.67	4.75 ± 0.39	0.3847
Oxalate (mmol/24h)	0.28 ± 0.03	0.32 ± 0.03	0.5286
Calcium (mmol/24h)	2.60 ± 0.38	3.75 ± 0.77	0.2723
Magnesium (mmol/24h)	2.63 ± 0.39	2.83 ± 0.26	0.8897
Sodium (mmol/24h)	185 ± 32.0	132 ± 25.3	0.3503
Potassium (mmol/24h)	51.0 ± 10.2	64.8 ± 13.4	0.3029
Urate (mmol/24h)	3.33 ± 0.25	3.68 ± 0.29	0.9759
Creatinine (mmol/24h)	12.9 ± 1.72	16.6 ± 0.99	0.1418
Phosphate (mmol/24h)	20.0 ± 1.15	25.6 ± 2.05	0.2720
Glycolate (mg/l)	12.0 ± 2.25	12.6 ± 6.11	0.8472
Tiselius risk index	319 ± 58.9	247 ± 32.5	0.3001
RS Brushite	0.14 ± 0.08	0.43 ± 0.11	0.0808
RS CaOx	3.43 ± 0.56	2.36 ± 0.30	0.1725
RS Uric Acid	0.30 ± 0.23	0.39 ± 0.12	0.7804
BRI (/l)	1.16 ± 0.88	0.49 ± 0.33	0.4969
MSL (mol/dm³)	0.11 ± 0.02	0.08 ± 0.02	0.3116

Table 5.4: Mean 24 h urinary variables ± SE in black and white subjects after ingestion of taurine

	Blacks			*Whites*			*Blacks vs Whites*
Variables	*Baseline*	*Post-taurine*	*p*	*Baseline*	*Post-taurine*	*p*	*p Post-taurine*
pH	6.61±0.11	6.27±0.21	0.2346	6.47±0.07	6.35±0.19	0.5507	0.7934
Volume (ml/24h)	1980±197	2094±204	0.6669	2204±169	2393±108	0.3606	0.2034
Citrate (mmol/24h)	3.96±0.67	4.41±0.70	0.8095	4.75±0.39	4.09±0.72	0.4284	0.8710
Oxalate (mmol/24h)	0.28 ±0.03	0.26±0.02	0.1908	0.32±0.03	0.31±0.02	0.6904	*0.0194
Calcium (mmol/24h)	2.60±0.38	2.44±0.27	0.5832	3.75±0.77	3.97±0.62	0.8287	†0.0520
Magnesium (mmol/24h)	2.63 ±0.39	2.29±0.41	0.3843	2.83±0.26	2.86±0.40	0.9446	0.2943
Sodium (mmol/24h)	185 ±32.0	146±30.8	0.1878	132±25.3	142±38.6	0.2312	0.6139
Potassium (mmol/24h)	51.0±10.2	41.8±4.65	0.5263	64.8±13.4	41.8±13.2	0.2366	0.8746
Urate (mmol/24h)	3.33±0.25	3.25±0.33	0.4462	3.68±0.29	3.89±0.34	0.7070	0.2550
Creatinine (mmol/24h)	12.9±1.72	11.2±1.78	0.3322	16.6±0.99	17.4±1.42	0.6512	*0.0124
Phosphate (mmol/24h)	20.0± 1.15	19.5±2.84	0.6988	25.6±2.05	27.2±1.94	0.6868	0.0923
Glycolate (mg/l)	12.0±2.25	11.9±2.02	0.5023	12.6±6.11	12.3±1.90	0.8355	0.8283
Tiselius risk index	319±58.9	410±92.2	0.4162	247±32.5	253±38.6	0.9046	0.1355
RS Brushite	0.14±0.08	0.17±0.08	0.7990	0.43±0.11	0.58±0.17	0.5248	*0.0163
RS CaOx	3.43±0.56	2.37±0.34	0.1452	2.36±0.30	3.04±0.48	0.3285	0.3094
RS Uric Acid	0.30±0.23	0.57±0.34	0.5369	0.39±0.12	0.50±0.15	0.6050	0.8720
BRI (/l)	1.16±0.88	3.21±0.92	0.1458	0.49±0.33	2.97±0.78	*0.0194	0.8495
MSL (mol/dm³)	0.11±0.02	0.09±0.04	0.4484	0.08±0.02	0.09±0.03	0.6606	0.9030

*statistically significant; †approaching statistical significance

Particle Formation Kinetics

Particle Volume-Size Distribution

Taurine did not induce any change in the mean particle size in blacks relative to baseline (Table 5.5). However, in whites, the mean particle size increased after a taurine load. The mean particle volume-size distributions in urines from black and white subjects before and after taurine loading are shown in Figure 5.1.

Table 5.5: Mean particle size (µm) in urines of black and white subjects before and after ingestion of taurine

	Blacks	*Whites*
Before	4.01	3.64
After	4.01	6.92

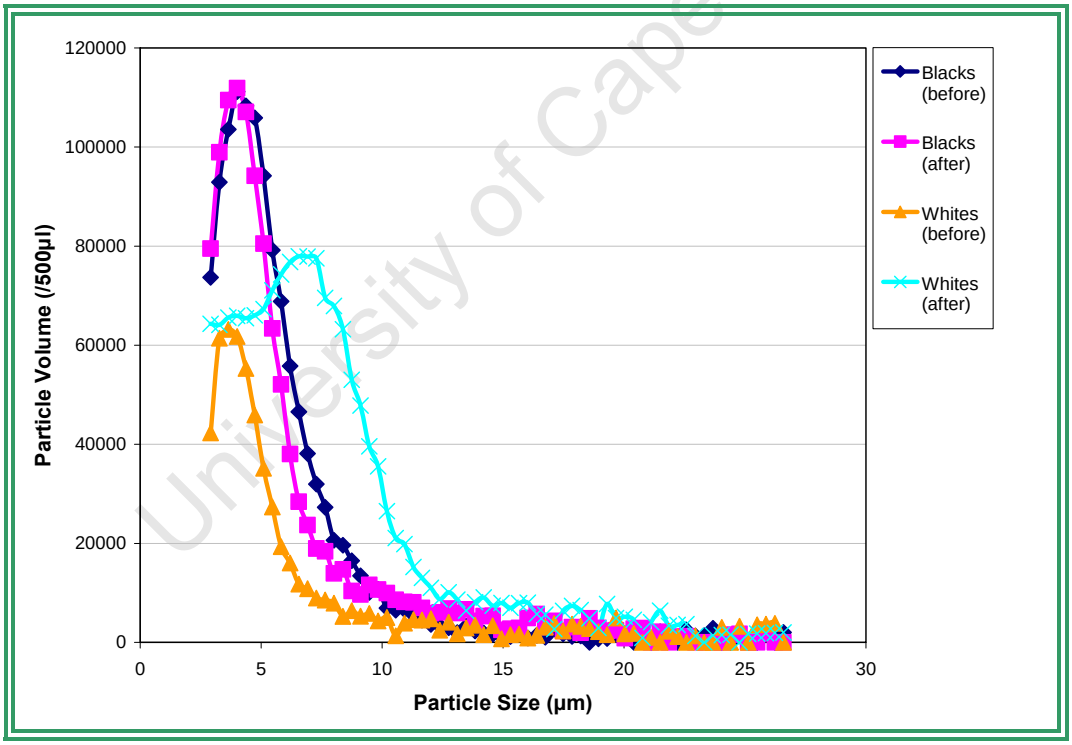


Figure 5.1: *Particle volume-size distribution after a taurine load in urines of black and white subjects*

Particle Number

The curves for particle number versus time are shown in Figure 5.2. At 120 min the particle number increased in blacks post a taurine load. In whites taurine did not provoke any changes in particle number.

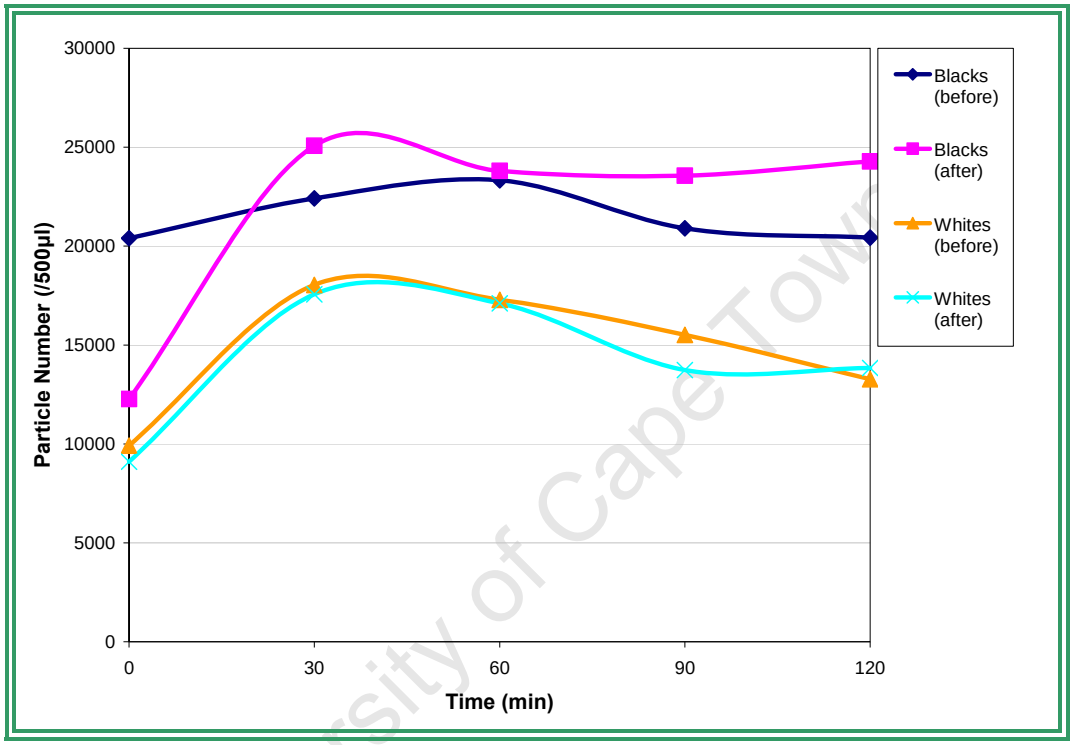


Figure 5.2: *Particle number in urines of blacks and whites before and after ingestion of taurine*

Scanning Electron Microscopy

The scanning electron micrographs obtained before and after a taurine load are shown in Figure 5.3 for urines from black and white subjects. In all micrographs, the dominating crystals are the coffin-shaped COM crystals. The number of crystals increased in the black group but remained unchanged in the white group. An increase in particle size was noted in the white group.

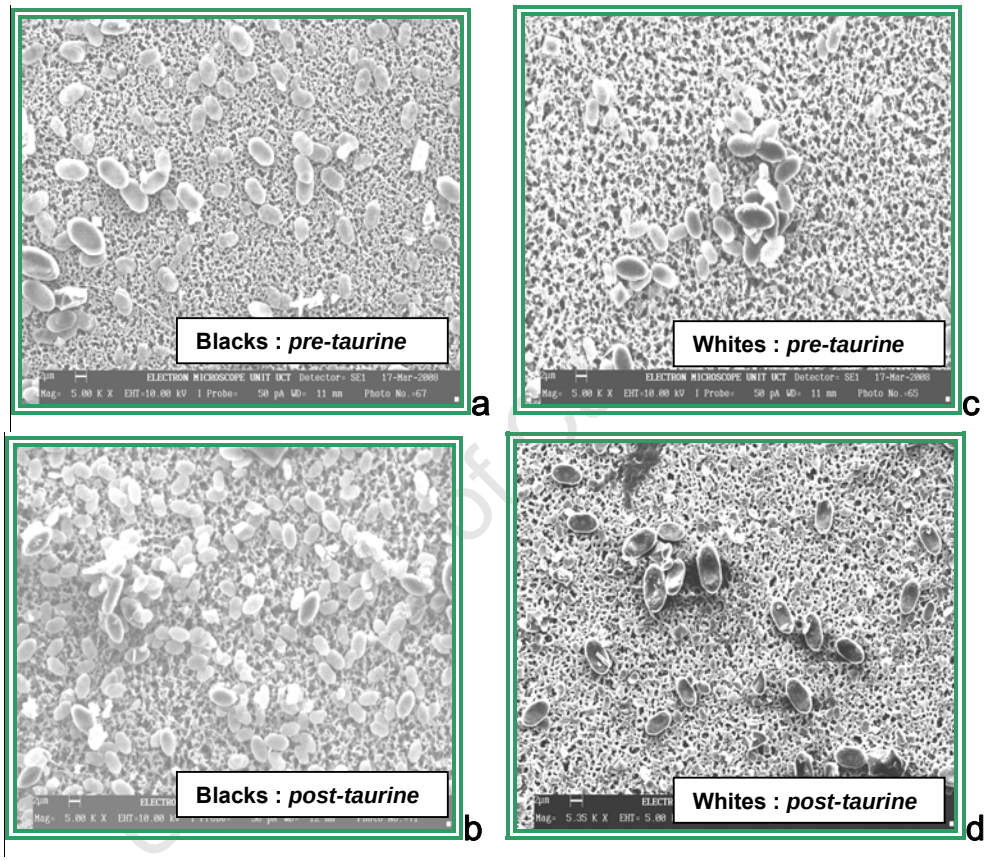


Figure 5.3: *Scanning electron micrographs of CaOx crystals pre and post ingestion of taurine in blacks (a: pre; b: post) and whites (c: pre; d: post) at 5 K magnification*

Crystallization Experiments

^{14}C-Oxalate Crystal Deposition

The rate of CaOx deposition is indicated by the gradient of each curve (top right-hand corner) for black and white subjects pre- and post-intake of taurine (Figure 5.4). A reduction in CaOx deposition rate was observed in both groups after a taurine load.

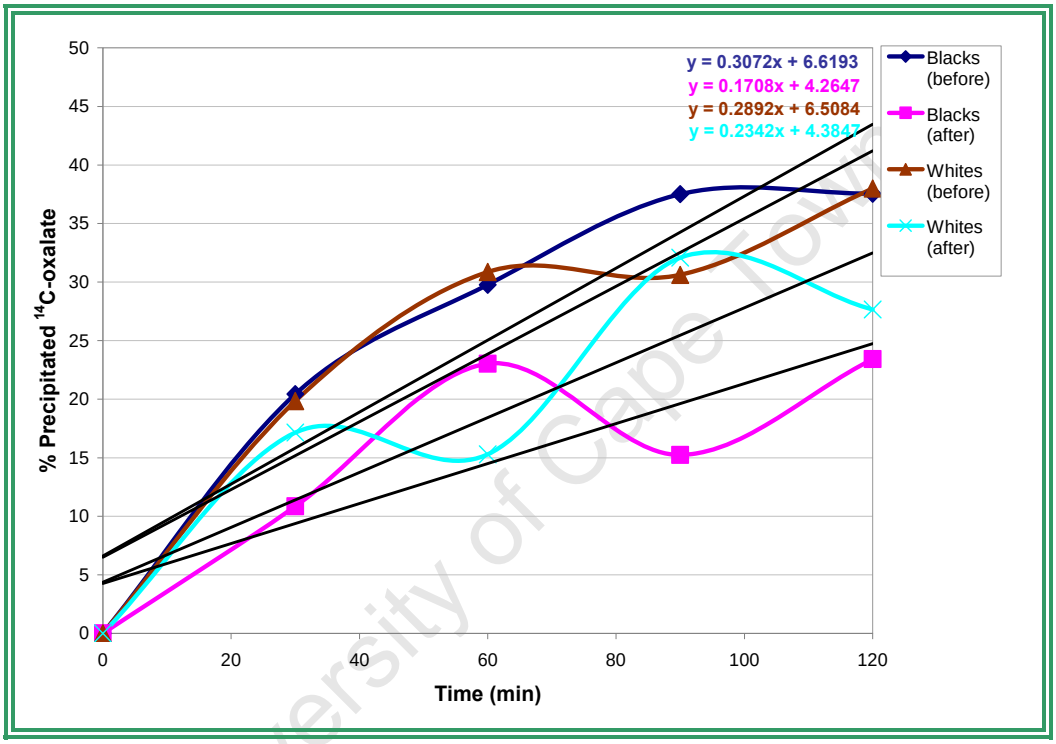


Figure 5.4: *Percentage precipitated ^{14}C-oxalate pre- and post-taurine ingestion in urines of black and white subjects*

Crystal Aggregation

The mean percentages for the inhibition of CaOx crystal aggregation in black and white subjects are given in Table 5.6, corresponding to the plots shown in Figure 5.4. Within each group no significant changes were noted after a taurine load in either of the two groups. Inter-group comparisons also revealed no changes both before and after taurine ingestion.

Table 5.6: Mean percentage inhibition of aggregation in black and white subjects before and after a taurine load

	Blacks		*Whites*		*Blacks vs Whites*
	% Ia (SE)	*p*	*% Ia (SE)*	*p*	*p*
Before	62.0 (8.92)		58.4 (7.01)		0.7502
After	56.5 (10.4)	0.6972	57.3 (5.85)	0.9113	0.9450

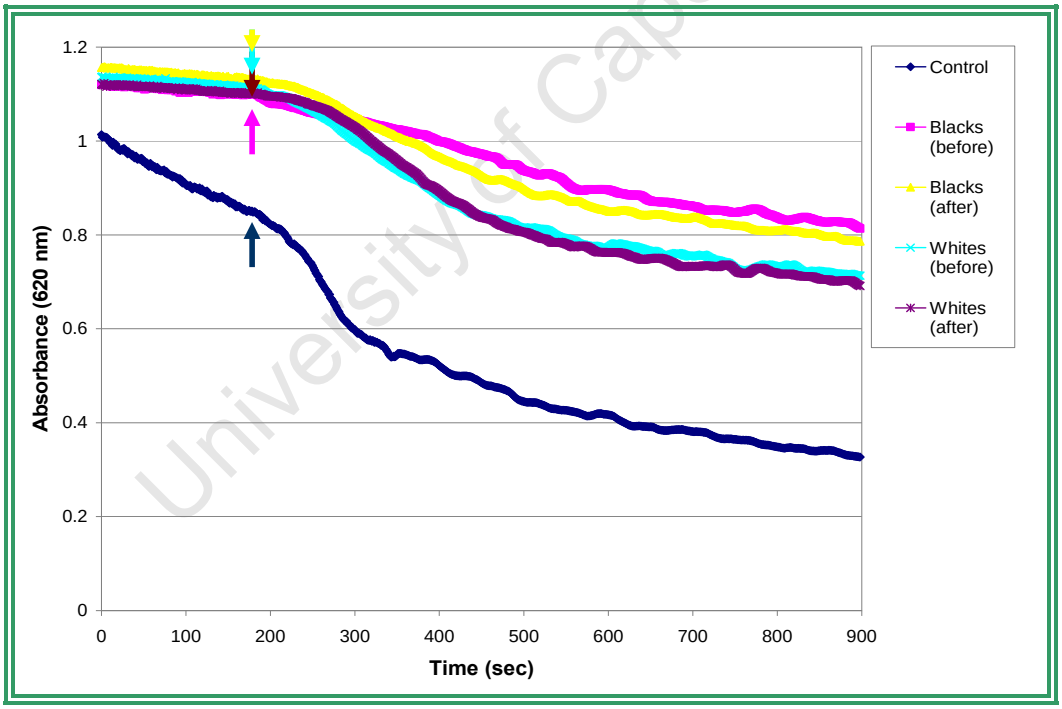


Figure 5.4: *Plot of absorbance versus time pre- and post-taurine load in urines of black and white subjects. The time at which stirring was stopped is indicated by the arrows.*

Summary of Urine Chemistry and Crystalluria Properties After Taurine Load

	Blacks	Whites
24 h: BRI	↔	↑
Oxalate / Glycolate	↔	↔
Particle size	↔	↑
Particle number	↑	↔
SEM	↑ Particle number	↔ Particle number
Deposition rate	↓	↓
Aggregation	↔	↔

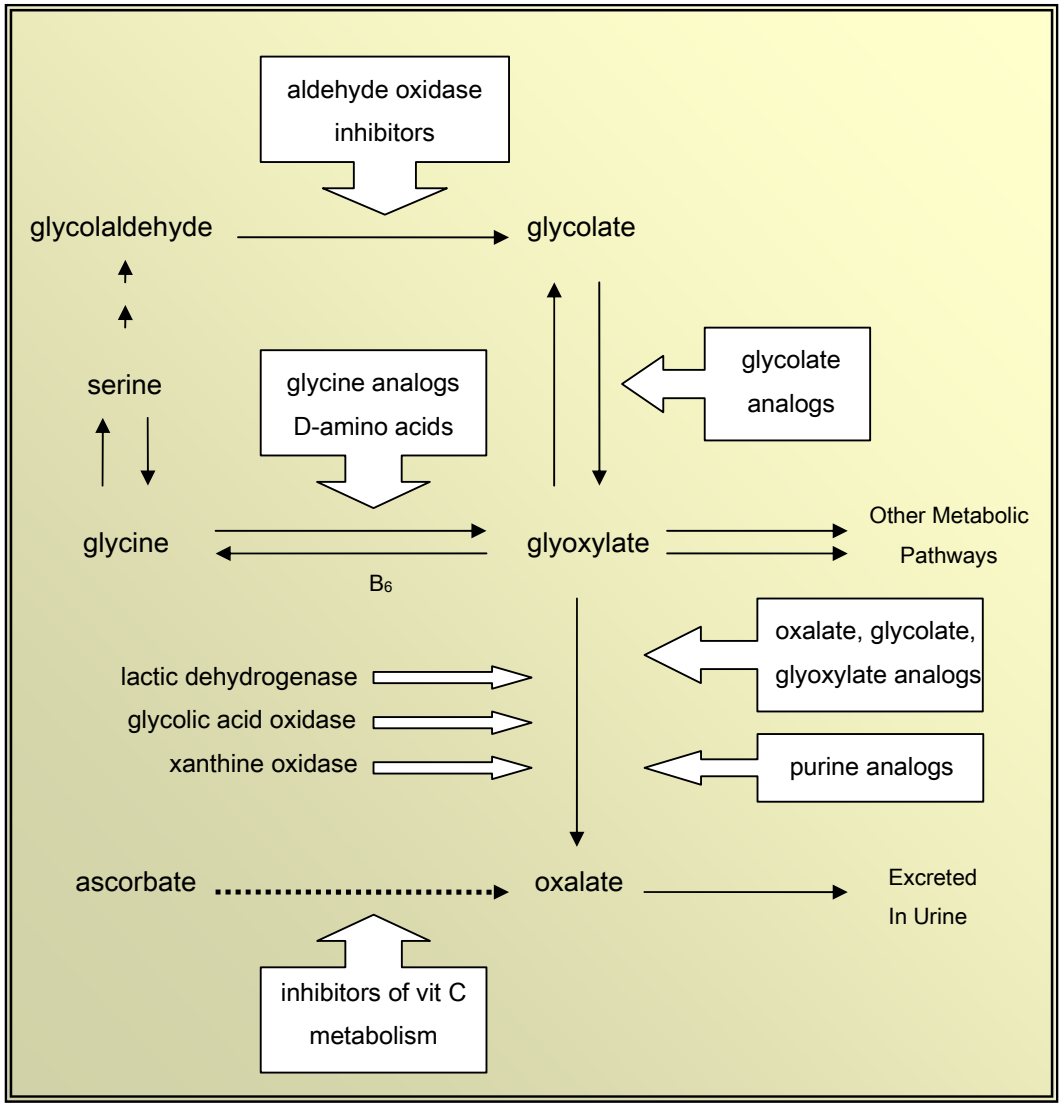
5.4 Discussion

The inhibitory effect of taurine on urinary glycolate and thus oxalate excretion was investigated in this chapter. The two groups did not show any statistically significant changes in the two urinary parameters following a taurine load. However, inter-group comparisons showed significantly higher levels of oxalate in white subjects. As stated earlier, glycolate is a well-known precursor of oxalate which is matabolized mainly in the liver to produce glyoxylate and is in turn converted to oxalate. Even though some of the glycolate in the body is derived from the diet, a large portion is synthesized endogenously (Harris and Richardson 1980). Studies on rats have reported a high excretion of oxalate in the urine after the administration of glycolate and other precursors of oxalate such as, glyoxylate, hydroxypyruvate and hydroxyproline (Ogawa *et al.* 1999, Ogawa *et al.* 2000). The ingestion of the latter has been shown both in animal (Bushinsky *et al.* 2002, Takayama *et al.* 2003, Bushinsky *et al.* 2005, Ogawa *et al.* 2007) and human (Knight *et al.* 2006) studies to induce an increase in urinary glycolate and oxalate.

Several potential inhibitors of oxalate synthesis have been studied both in vitro and in vivo (Smith *et al.* 1972b, O'Keeffe *et al.* 1973). The NAD-dependent oxidation of glyoxylate to oxalate which is catalyzed by lactate dehydrogenase (LDH) (Scheme 1, Chapter Four) has been identified as the most important pathway for the synthesis of oxalate (Smith *et al.* 1972b). In the in vitro study undertaken by Smith and co-workers (1972b) in search of finding the most potent inhibitor(s) of oxalate synthesis, fourteen oxalate or glycolate analogues were examined and hydroxymethanesulfonate (sulfonic acid analogue of glycolate) was the most effective inhibitor of the two enzymes, glycolic acid oxidase (GAO) and LDH in the erythrocytes of rats. Earlier human studies have also supported this finding (Gibbs and Watts 1967). In addition, oxalatehydrazide (oxalate analogue) was demonstrated to be also the most potent inhibitor of LDH (Smith *et al.* 1972b). Scheme 1, below, is a depiction of the possible sites of oxalate synthesis inhibition.

Elevated excretion of urinary oxalate and its precursors has been reported in vitamin B_6 deficiency due to taurine depletion (Gershoff 1964, Swan *et al.* 1964, Miller *et al.* 1978, Nishijima *et al.* 2003). The potency of pyridoxine in reducing the synthesis of oxalate has been reported (Gibbs and Watts 1970). A study by Talwar *et al.* (1985)

demonstrated that administration of taurine together with glycolate in rats lowered oxalate excretion compared to rats treated with glycolate alone.



Scheme 1: *Possible sites of oxalate synthesis inhibition (broken arrow from ascorbate to oxalate indicates pathway not defined and may go via glyoxylate)* *(Smith et al. 1972b)*

In spite of the findings described above, taurine displayed no effect towards the aforementioned urinary risk factors in both groups. Since taurine neither promoted nor inhibited the excretion of oxalate or glycolate significantly in the two groups, it appears that it did not alter the activity of the enzymes glycolate oxidase (GO) and LDH in Scheme 1, Chapter Four. It is possible that the administered dose was not

sufficient to induce significant changes within the two groups. However, inter-group comparisons revealed that a subtle change may indeed have occurred. This would need further investigation.

Talwar and co-workers (1985) performed an in vitro experiment with 0.5 mmol/l of taurine and they observed no effect of this substance on COM crystal growth. However, the data was not published. The increased number of precipitated particles in the black group (substantiated by Coulter Counter and SEM studies), indicates that taurine failed to inhibit crystal growth. On the other hand, taurine induced a decrease in the rate of crystal deposition in both groups. This implies a protective role exhibited by taurine. However, this is inconsistent with the urine chemistry results and its relevance is not clear.

In summary, the role of taurine in reducing urinary glycolate and oxalate, which has been reported in other human and animal studies, was not demonstrated in the present study. Furthermore, the two South African population groups did not differ with respect to other urinary parameters in the handling of the taurine challenge. There is one possible explanation for the apparent inconsistency with the other studies. The source of taurine which was used in the present study, Red Bull Energy Drink, contains many other ingredients, any one of which may have induced confounding effects. Future studies involving humans are indeed warranted, but taurine in a simpler matrix than Red Bull may have to be used.

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Chapter Six

Discussion and Conclusion

6.1 General Discussion

The studies described in this thesis addressed the effects of various lithogenic and antilithogenic dietary supplements on the urinary risk factors for calcium oxalate kidney stone formation and whether these effects were different in subjects from South Africa's black and white population groups. It is appropriate now to interrogate the results which have been obtained in these studies and to assess the effects of each of the different supplements.

Refined Carbohydrate: Significance in Urolithiasis Risk Factors

The impact of refined carbohydrates on the key risk factors for kidney stone formation has been established and firmly understood. Consumption of refined carbohydrates has risen over the years with the evolution of affluent lifestyles, so has the incidence of urolithiasis. Low digestible carbohydrates such as sorbitol and xylitol which belong to the class of polyols/sugar alcohols have also been studied and their influence on the urinary constituents is intriguing. The summarized effects of ingestion of some sugars on urinary calcium, oxalate and phosphate excretion which have been investigated by various researchers are given in Table 6.1.

Table 6.1: Effects of ingestion of certain sugars on urinary Ca, Ox and P excretion

Sugar	*Ca*	*Ox*	*P*	*References*
Glucose	↑	↑	↓	*Gluszek 1988, Nguyen et al. 1989*
Sucrose	↑	↔	↓	*Lemann et al. 1969, Nguyen et al. 1994*
Fructose	↑	↑	↔	*Nguyen et al. 1995*
Sorbitol	↔	↔	↔	*Nguyen et al. 1993*
Xylitol	↑	↑	↑	*Bar 1985, Nguyen et al. 1993*

In Chapter Two of this thesis, the effects of oral glucose, sorbitol and xylitol loads on the excretion of the aforementioned urinary parameters were examined in black and white South African male subjects. The highlights which emerged from the study were, firstly, the reduction of phosphate in the black group after glucose intake.

Additionally, ingestion of 75 or 100 g of glucose has been shown in previous studies to increase the urinary excretion of oxalate, whereas no significant change has been observed after a 20 g load. Similar findings were observed in the present study after the latter load in both race groups. Secondly, an analogous decrease in phosphate subsequent to the ingestion of sorbitol occurred in each group, with an additional increase in oxalate in the white group. Thirdly, xylitol overtly increased oxalate in the black group and even though the white group responded in a similar manner, the increase was not statistically significant. Plausible proposed mechanisms for the occurrence of these influences in one group and not the other or in a greater or lesser extent were provided. An apparent conflict arose when attempting to correlate the results from the series of crystallization experiments to the urine chemistry results, but it was recognized that subtle changes in the latter can influence the crystallization capacity of the urine.

The second aforesaid highlight stipulated that sorbitol caused significant changes in urinary phosphate (both groups) and oxalate (white group) and this was intriguing since a previous study reported no changes in the two parameters including calcium. It was therefore critical to explore this further by increasing the dose with a view to establishing whether the excretion of oxalate, in particular, would be aggravated in the white group (Chapter Three). The 30 g sorbitol load did indeed intensify the urinary excretion of oxalate in the white group and it also induced a remarkable increase in urinary calcium which correlated with the almost significant increase in serum concentrations. The SEM photographs confirmed the increase in the size and number of deposited CaOx crystals, implying enhanced CaOx crystallization in this group. Yet again no changes were observed in the black group in either urinary parameter. On the other hand, urinary phosphate did not change in this group following a 30 g load but a similar decrease previously observed after the 20 g load was sustained in the white group despite the unchanged serum levels in either group. Interestingly, Nguyen co-workers (1993) did not note any changes as well in serum phosphate levels. Nevertheless, the results of the present study have demonstrated that sorbitol does affect the urinary phosphate excretion.

Thus, the significance of sorbitol influencing the risk factors of urolithiasis has been demonstrated by the two studies for the first time. The unfavourable effect of this sugar in the stone-prone white group but not in the black group suggests a superior renal handling of this nutrient by the latter group.

In addition, the assessment of the baseline activity of serum aldolase, an enzyme implicated in the metabolic conversion of sorbitol and xylitol to oxalate, revealed a difference in the two groups. The implication of this is that the renal handling of the two polyols is possibly controlled by this enzyme. The higher activity demonstrated by black subjects relative to their white compatriots supports the hypothesis that the baseline activity of aldolase is different in the two ethnic groups.

Oxalate: Significance in Cellular and Oxidative Injury

Elevated urinary levels of markers of tubular damage such as NAG, GAL, gamma-glutamyl transpeptidase (GGT) and angiotensin I converting enzyme have been noted in stone-forming patients compared to healthy individuals (Baggio *et al.* 1983). However, in some loading studies, oral administration of acute loads of oxalate had no adverse effects on cellular injury, as indicated by the unchanged excretion of NAG, despite increased urinary oxalate concentrations (Holmes *et al.* 2005, Knight *et al.* 2007). In both studies, the administered loads of oxalate ranged from 2-8 mmole. It is therefore possible that extreme concentrations of oxalate are required to trigger the desired response.

In Chapter Four, three different oxalate challenges in the form of rhubarb, spinach and sodium oxalate were investigated in order to examine whether they would have an effect on the urinary excretion of oxalate and NAG in the two South African race groups. Significant increases in oxalate excretion were observed in the black group after ingestion of spinach and sodium oxalate whereas rhubarb did not affect the excretion of this parameter in any of the groups. It is rather surprising that increases in urinary oxalate were observed in the black group but not in the white group, since it was indicated in Chapter One that Lewandowski and co-workers (2005) reported lower levels in this group despite excessive consumption of dietary oxalate. The concentrations, however, were not high enough to cause an increase in the relative supersaturation of CaOx. With respect to urinary NAG excretion, all three challenges did not have a significant impact on this variable even though the white group demonstrated higher levels after rhubarb and sodium oxalate ingestion compared to their black compatriots. The latter did not provoke any changes in the biomarker for oxidative stress, 8-OHdG.

It can be speculated that the dietary oxalate loads administered in these studies were possibly not sufficient to trigger either cellular injury or oxidative stress in the two groups.

Taurine: Significance in Stone Inhibition

Animal studies have repeatedly demonstrated that taurine plays a significant role in the reduction of urinary oxalate and its precursors and thus inhibits CaOx crystallization as discussed in the previous chapter. The present study was the first to administer taurine and to examine the urine chemistry in the two South Africa's race groups in response to this challenge. The results showed that taurine did not provoke any changes in urinary oxalate or its precursor, glycolate in either group. Thus, it can be concluded that in the metabolic pathway for the production of oxalate from glycolate, taurine neither suppressed nor activated the hepatic enzymes LDH and GAO, thereby culminating in unaltered urinary composition. The ability of taurine to retard the CaOx deposition in both groups was of note as it demonstrated a possible inhibitory role even though this was not reflected by the urine chemistry. Evidently, further investigations are warranted to clarify the potency of taurine in inhibition of urolithiasis in humans.

Baseline 24 h Urinary Variable Comparisons

The urinary excretion of calcium and phosphate were commonly lower in black subjects compared to white subjects prior to the ingestion of the dietary challenges (Chapters Two to Four). The lower calcium excretion in the former is in accordance with previous studies which have reported similar observations, as stated in Chapter One. In addition, the metastable limit was higher in black subjects (Chapters Two to Four) and the relative supersaturation of CaOx was lower (Chapter Four) in this group than in white subjects. The higher metastable limit observed in the former group signifies that black subjects' urine can withstand elevated concentrations of supersaturation exclusive of CaOx crystallization. These observations support the lower incidence of kidney stone formation in this group than in their white compatriots. In Chapter Five none of the urinary parameters were significantly different. This and other inconsistencies can be ascribed to the fact that the controlled standardized diets administered in all the studies were different and were designed for the specific protocols in each study. The 24 h baseline urinary data in each study were not consolidated into a single table, as each study was considered

independently, due to the small numbers of subjects who participated in each of them.

6.2 Conclusions

The pathogenesis of urolithiasis is governed by a wide spectrum of factors which are crucial in the understanding of this condition. As stated earlier, diet plays a fundamental role in the excretion of the key stone risk factors. This thesis was conducted with the aim of gaining more insight to the renal response to several lithogenic and antilithogenic dietary agents in South Africa's white "stone-prone" and black "stone-resistant" population groups. A significant attempt was also made to clarify some contradictions in research literature.

The results from the different studies in this thesis have not only demonstrated different renal handling mechanisms of the dietary challenges in the two groups but they have also provided some insight into the complexity associated with the rarity of stone formation in the black group. This was highlighted, for instance, by the increased oxaluria in this group subsequent to the administration of the spinach and sodium oxalate protocols, which did not cause cell injury, as seen by the unaltered excretion of the markers for cellular and oxidative damage. Thus, the conceptualized idea in the research literature that even a slight elevation of urinary oxalate excretion poses a risk of injury to renal epithelial cells thereby facilitating stone formation was invalidated.

Lastly, the dual role demonstrated by sorbitol in increasing urinary oxalate excretion and concomitantly decreasing phosphate excretion in white subjects is intriguing as the former promotes calcium oxalate kidney stone formation, while the latter inhibits calcium phosphate kidney stone formation. Previous studies have reported that sorbitol has no effect on the excretion of these urinary parameters. However, the present study has for the first time revealed that it does indeed have an effect.

It is recognised that in the studies a small number of subjects participated, hence these studies are limited in their statistical power. Nevertheless, differences which have been identified are worthy of noting. Thus the studies described in this thesis have made original and meaningful contributions towards the understanding of

kidney stone disease in general, and have provided insights into unravelling the anomalies of the rarity of this disease in the South African black population.

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