URINARY COMPOSITION
AND
STONE FORMATION

By

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the requirements
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Graduate Department of Institute of Medical Science
University of Toronto

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ABSTRACT

Background: Kidney stone disease is a common and often debilitating disorder, yet its pathophysiology is poorly understood. This dissertation studies predisposition to kidney stone formation from diurnal variation in physiochemical and physiologic properties of urine and in response to increased fluid intake.

Methods: Urine volume, flow rate and constituents were measured in multiple timed specimens from healthy volunteers in a day. Further, subjects were asked to provide specimen over a period of increased fluid intake.

Results: A 24-hour specimen missed significant periods of supersaturation in individual urine samples throughout the day. Despite a significant reduction in nocturnal urine flow rate, calcium concentration as well as urine pH and divalent phosphate remained unchanged. Finally, increased water intake did not dilute urine evenly.

Conclusion: Mixing multiple urine samples obscures information about periods of increased calcium phosphate precipitation risk over 24 hours. Further, increased fluid intake does not uniformly provide risk protection.
ACKNOWLEDGMENTS & CONTRIBUTIONS

Dedicated to:

The Greatest Teacher of All

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<tr>
<td>ACE</td>
<td>Angiotensin Converting Enzyme</td>
</tr>
<tr>
<td>ADH</td>
<td>Antidiuretic Hormone (also: vasopressin)</td>
</tr>
<tr>
<td>APR</td>
<td>Activity Product Ratio</td>
</tr>
<tr>
<td>AQP</td>
<td>Aquaporin</td>
</tr>
<tr>
<td>ARA</td>
<td>Activity Product Ratio</td>
</tr>
<tr>
<td>ARB</td>
<td>Angiotensin Receptor Blocker</td>
</tr>
<tr>
<td>AVP</td>
<td>Arginine Vasopressin (also: vasopressin)</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CAHV</td>
<td>Cell Associated Herpes Virus</td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>Calcium</td>
</tr>
<tr>
<td>CaHPO4</td>
<td>Calcium Phosphate Monohydrogen</td>
</tr>
<tr>
<td>CaOx</td>
<td>Calcium Oxalate</td>
</tr>
<tr>
<td>CaOxSS</td>
<td>Calcium Oxalate Super Saturation</td>
</tr>
<tr>
<td>CaPh</td>
<td>Calcium Phosphate</td>
</tr>
<tr>
<td>CaPh SF</td>
<td>Calcium Phosphate Stone Formation</td>
</tr>
<tr>
<td>CaPhosSS</td>
<td>Calcium Phosphate Supersaturation</td>
</tr>
<tr>
<td>CaSR</td>
<td>Calcium Sensing Receptor</td>
</tr>
<tr>
<td>CNP</td>
<td>Calcifying Nanoparticles</td>
</tr>
<tr>
<td>DCT</td>
<td>Distal Convoluted Tubule</td>
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</tbody>
</table>
ECF  Extracellular Fluid
EFW  Electrolyte Free Water
ENaC  Epithelial Sodium Channel
ECaC  Epithelial Calcium Channel
ESWL  Extra-Corporal Shockwave Lithotripsy
FCV  Feline Calicivirus
FeCa  Fractional Calcium Excretion
FeNa  Fractional Sodium Excretion
FeSFV  Feline Syncytium Forming Virus
FHH  Familial Hypocalciuric Hypercalcemia
FP  Formation Production
FPR  Formation Production Ratio
FR  Fractional Reabsorption
FRCa  Fractional Reabsorption of Calcium
FRH2O  Fractional Reabsorption of water
FRNa  Fractional Reabsorption of Natrium
FRPO4  Fractional Reabsorption of Phosphate
FPR  Formation Production Ratio
FTIR  Fourier Transform Infrared Microspectroscopy
GAGs  Glycosaminoglycans
GFR  Glomerular Filtration Rate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>GI</td>
<td>Gastro Intestinal</td>
</tr>
<tr>
<td>HS</td>
<td>Hypertonic Saline</td>
</tr>
<tr>
<td>iCa</td>
<td>ionized Calcium</td>
</tr>
<tr>
<td>ICF</td>
<td>Intracellular Fluid</td>
</tr>
<tr>
<td>ICSF</td>
<td>Idiopathic Calcium Stone Former</td>
</tr>
<tr>
<td>IMCD</td>
<td>Inner Medullary Collecting Duct</td>
</tr>
<tr>
<td>IS</td>
<td>Isotonic Saline</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>K⁺</td>
<td>Potassium</td>
</tr>
<tr>
<td>Ksp</td>
<td>Solubility Product or Solubility Equilibrium Constant</td>
</tr>
<tr>
<td>Na⁺</td>
<td>Sodium</td>
</tr>
<tr>
<td>NaDC1</td>
<td>Sodium Dicarboxylate Cotransporter 1</td>
</tr>
<tr>
<td>NCC</td>
<td>Sodium Chloride Co-transporter</td>
</tr>
<tr>
<td>NHE3</td>
<td>Sodium Hydrogen Exchanger-3</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institute of Health</td>
</tr>
<tr>
<td>NKCC2</td>
<td>Sodium Potassium Chloride Co-transporter</td>
</tr>
<tr>
<td>ODS</td>
<td>Osmotic Demyelination Syndrome</td>
</tr>
<tr>
<td>OPN</td>
<td>Osteopontin</td>
</tr>
<tr>
<td>PCa</td>
<td>Plasma Calcium Concentration</td>
</tr>
<tr>
<td>PCT</td>
<td>Proximal Convoluted Tubule</td>
</tr>
<tr>
<td>pKa</td>
<td>The equilibrium constant for a chemical reaction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PKA</td>
<td>Protein Kinase A</td>
</tr>
<tr>
<td>PMCA</td>
<td>Plasma Membrane Ca(^{2+})-ATPase</td>
</tr>
<tr>
<td>PST</td>
<td>Proximal Straight Tubule</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
<tr>
<td>RAS</td>
<td>Renin Angiotensin System</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SP</td>
<td>Solubility Production</td>
</tr>
<tr>
<td>SLC26A6</td>
<td>Sulfate anion transporters involved in oxalate transporter6</td>
</tr>
<tr>
<td>SLC26A1</td>
<td>Sulfate anion transporters involved in oxalate transporter1</td>
</tr>
<tr>
<td>TAL</td>
<td>Thick Ascending Limb</td>
</tr>
<tr>
<td>TDL</td>
<td>Thick Descending Limb</td>
</tr>
<tr>
<td>TF</td>
<td>Tubule Fluid</td>
</tr>
<tr>
<td>THP</td>
<td>Tamm-Horsfall Protein (Uromodulin)</td>
</tr>
<tr>
<td>TmPi</td>
<td>Maximum Tubular Reabsorption of Phosphate</td>
</tr>
<tr>
<td>TRPV6</td>
<td>Transient Receptor Potential V6 selective for Ca(^{2+}) ions</td>
</tr>
<tr>
<td>UCreat</td>
<td>Urine Creatinine Concentration</td>
</tr>
<tr>
<td>UFR</td>
<td>Urine Flow Rate</td>
</tr>
<tr>
<td>UFCa</td>
<td>Ultra Filtrated Calcium</td>
</tr>
<tr>
<td>UK</td>
<td>Urine Potassium Concentration</td>
</tr>
<tr>
<td>UNa</td>
<td>Urine Sodium Concentration</td>
</tr>
<tr>
<td>UOsm</td>
<td>Urine Osmolality</td>
</tr>
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</table>
DEFINITIONS AND DESCRIPTIONS:

**Activity coefficient** ($g$): Ratio of the activity $a_B$ of component B of a mixture to the concentration of that component. The value of $g$ depends on the method of stating the composition. For mole fraction $x_B$, the relation is $a_B = g_B x_B$; for molarity $c_B$, it is $a_B = g_B c_B / c^\circ$, where $c^\circ$ is the standard state composition (typically chosen as 1 mol/L); for molality.

**Chelating agents**: The word chelate derives from the Greek root “chela” meaning the claw of a lobster. Chelating agents are certain organic compounds, which are capable of forming coordinate chemical bonds with metals and sequester metal ions. Chelating agents combine with metal ions and remove them from their sphere of action and holds it in solution. A chelating agent that has two coordinating atoms is called bidentate; one that has three, tridentates and so on. Organic compounds that yield sparingly soluble coordination compounds typically contain at least two functional group, each of which is capable of bonding by donation of a pair of electrons. The functional groups are located in the molecules such that a five- or six-membered ring results from the reaction. Reagents that form compounds of this are called chelating agent and their products are called Chelates.

**Chelation**: The formation or presence of bonds (or other attractive interactions) between two or more separate binding sites within the same ligand and a single central atom. A molecular entity in which there is chelation (and the corresponding chemical species) is called a 'chelate'. The terms bidentate (or didentate), tridentate, tetradentate, ... multidentate are used to indicate the number of potential binding sites of the ligand, at least two of which must be used by the ligand in forming a 'chelate'.
**Clathrates:** Clathrates are inclusion compounds which have the ability to trap many organic compounds. The organic compounds which are called guest molecules are held in a cage formed by the host molecule or by a lattice of host molecules (interpenetrating helices comprising of hydrogen-bounded). [1]

**Crystal:** A crystal is characterized by having a well-structured periodic placement of atoms. The smallest assembly of atoms that can be repeated to form the entire crystal is called a primitive cell, with a dimension of lattice constant. Crystals are solid bodies bounded by naturally formed plane faces with a regularity of external form which reflects the regular arrangement of the constituent atoms. The growth of a particular crystal depends on the chemistry of the local environment, the presence of fluids, the degree of supersaturation, and the temperature and pressure conditions. Well-formed crystals can grow. In all cases, small groups of atoms are forming and dispersing but as the physical conditions change or the chemical concentration increases these groups will grow larger and reach a critical size, at which stage a new mineral can nucleate and the atoms assume regular positions. In ionic crystals every cation is completely surrounded by anions, and vice versa. The numbers are determined by the ionic charges and the relative size of the ions; the total crystal will be electrically neutral. Slow growth with few nuclei will lead to large crystals; rapid growth with a high level of supersaturation will produce many small crystals. [3, 4]

**Epitaxy:** Epitaxy is an oriented crystal growth between two crystalline solid surfaces of different chemical composition in which the surface of one crystal offers suitable positions for deposition of a second crystal. A higher free-ion activity product will cause the solid phase (the crystals) to grow (epitaxy). [1]

**Equilibrium constant \( K \):** For a chemical reaction \( aA + bB \rightarrow cC + dD \), the equilibrium constant is defined by: [1]
Equation 1.

\[ K = \frac{a_C^c \cdot a_D^d}{a_A^a \cdot a_B^b} \]

Where \( a_i \) is the activity of component \( i \). To a certain approximation, the activities can be replaced by concentrations. The equilibrium constant is related to \(-\Delta_r G^\circ\), the standard Gibbs energy change in the reaction, by \( RT \ln K = -\Delta_r G^\circ \).

**Formation product (FP):** In the range of metastable supersaturation, if the activity products are sufficiently raised, new crystals will appear. The activity product at which new crystals form is called the formation product (FP), or the upper limit of metastability. \(^{[5,6]}\)

**Ionic Activity:** Ionic activity is a measure of the “effective concentration” of a solute in a solution. By convention, it is a dimensionless quantity. Activity depends on temperature, pressure and composition of the mixture, among other things. Activities, rather than concentrations, are needed in many chemical calculations because solutions that contain ionic solutes do not behave ideally even at very low concentrations. The activity is proportional to the concentration by a factor known as the activity coefficient \( \gamma \), and takes into account the interaction energy of ions in the solution. For a mixture of substances, the absolute activity \( l \) of substance B is defined as \( l_B = \exp(m_B/RT) \), where \( m_B \) is the chemical potential of substance B, \( R \) the gas constant, and \( T \) the thermodynamic temperature. The relative activity \( a \) is defined as \( a_B = \exp[(m_B-m_B^\circ)/RT] \), where \( m_B^\circ \) designates the chemical potential in the standard state. \(^{[1]}\)

**Ionic Strength (I):** The ionic strength of a solution is a measure of the total concentration of ions in that solution, defined by \( I = \frac{1}{2} \sum_i z_i^2 m_i \), where \( z_i \) is the charge of ionic species \( i \) and \( m_i \) is its molality. For a 1-1 electrolyte at molality \( m \), \( I = m \) \(^{[1]}\)
Kidney Stones: Kidney stones or Renal Calculi (from Latin *ren*, *renes*, "kidney" and *calculi*, "pebbles") are solid structures composed of urinary precipitates and crystals. These stones can range in size from less than a millimeters to few centimeters. [5]

Lithogen (Calculogen): Urinary constituents that can promote or undergo stone formation. [5]

Lithogenesis or Calculogenesis: promoting, or undergoing the formation of calculi. [5]

Metastable Supersaturation: If the crystals are removed from a solution at the level of the equilibrium SP and then the ion activity product is elevated, the activity product that would have caused growth of preformed crystals will not result in the appearance of a new solid phase. This solution is called metastably supersaturated. [6] The activity products of calcium salts in urine are almost constantly in the range of metastable supersaturation. [5, 6]

Nephrolithiasis: Nephrolithiasis (from Greek *nephros*, "kidney" and *λιθος* (*lithos*, "stone") refers to the condition of having kidney stones. [5]

Nucleation: Nucleation describes the process that occurs when the activity of calcium salts reaches the level at which the solid phase begins to appear. Crystals originate on a minute trace of a foreign substance acting as a nucleus. Crystals form initially in tiny regions of the parent phase and then propagate into it by accretion. [7]

pK_a: pK_a is defined in mathematical terms as -log\(_{10}\) of K_a. pK_a values are easier to remember than K_a values and pK_a values are in many cases easier to use than K_a values for fast approximations of concentrations of compounds and ions in equilibriums. In a practical sense a pK_a value refers to the pH at which you have exactly the same proportion of Neutral and Ionized species present in solution. [1]
**Precipitation:** Precipitation, in chemistry, is a process that causes dissolved substances to separate from a solution as a solid. The resulting solid, from a precipitation reaction, is referred to as a precipitate. In terms of physical processes, precipitation is the opposite of dissolution. In chemical reactions, ionic compounds that dissociate (break apart) in solution are termed ionic salts. Any resulting dissociated components (e.g., ions) that do not contribute to the formation of the precipitate are termed spectators (e.g., spectator ions). The components that react to form the precipitate are termed the precipitate’s constituent or contributing components. [4]

**Renal Colic:** Renal colic is the type of pain commonly caused by kidney stones. The pain is colicky in nature, meaning that it comes on in spasmodic waves as opposed to being a steady continuous pain. [5]

**Saturate:** Pressure has an effect only upon gases; otherwise its effect is unimportant. When a two-component system contains the maximum quantity of solute in a solvent, the system is said to be saturated. [8]

**Saturation:** Saturation is the point at which a solution of a substance can dissolve no more of that substance and additional amounts of it will appear as a precipitate. This point of maximum concentration, the saturation point, depends on the temperature of the liquid as well as the chemical nature of the substances involved. [4]

**Solubility:** Solvents have limited capacity to dissolve solutes, and that limit defines the *solubility* of the solute in the solvent (the maximum amount of solute that will dissolve in a fixed amount of solvent at a definite temperature). [8]
**Solubility Product Constant (K_{SP}):** The equilibrium constant for the dissolution of a sparsely soluble salt into its constituent ions. Solubility equilibrium is any type of chemical equilibrium relationship between solid and dissolved states of a compound at saturation. K_{SP} stands for "solubility product" or "solubility equilibrium". It is the equilibrium constant for the reaction in which a solid salt dissolves to give its constituent ions in solution. [1]

**Solution:** Solution is a chemical homogeneous mixture of two or more substances, absence of settling, and a molecular or ionic state of subdivision of the components. The dissolving medium is called the solvent, and the dissolved material is called the solute. A solution is distinct from a colloid or a suspension. In most common solutions, the solvent is a liquid, often water, and the solute may be a solid, gas, or liquid. [4, 8]

**Supersaturation:** The term supersaturation refers to a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances. A supersaturated solution is an unstable condition of a solution in which the solution contains a substance at a concentration greater than the saturation. [5, 6] An equilibrium is established between the pure solute and the dissolved solute. At the equilibrium point, the rate at which the pure solute enters the solution equals the rate at which the dissolved solute crystallizes out of the solution to return to the pure state. When a solution retains more than the equilibrium concentration of the dissolved solute (which happens under certain conditions) the solution is said to be supersaturated. [7,8]

**Undersaturated Solution:** Solutions with concentrations of salt less than the equilibrium SP are undersaturated. [5, 6]
**Unstable Solution:** Above the level of the FP, a solution is unstable, creating new crystal nuclei. [5, 6]

**Urolithiasis:** Urolithiasis refers to the condition of having calculi in the urinary tracts including kidneys, ureters, bladder and urethra. [5]
INTRODUCTION:

Kidney stone disease is common, worldwide, often debilitating disorder that has differing etiologies and pathophysiology.\[^9, 10\] Its treatment and morbidity are sources of considerable health care expenses. This condition has plagued humans for centuries, affecting populations of almost every region, culture, and race.\[^11,12\] So far, precise mechanisms for stone formation and pathophysiology of the disease have not been discovered. Furthermore, current preventative or therapeutic measures are not completely effective.\[^17\] Accordingly, kidney stone disease would be an excellent research area with great scope and opportunity.

Kidney stones are truly one of the most painful medical conditions to afflict human beings, with an approximate incidence of one in ten lifetime risk in the general population. Numerous epidemiologic studies suggest that the prevalence of stone disease over the last 35 years is increasing dramatically.\[^13, 14\] Importantly, it affects mainly productive young individuals, with a peak incidence in the third and fourth decades of life. There is a very high recurrence rate, which reduces the productivity of such individuals.\[^17\] Interestingly, the gender distribution among these patients, which originally was reported to have a ratio of three to one male to female predominance, has now been reported to be moving closer to parity.\[^15, 16, 17\]

A kidney stone begins as a tiny precipitate to form a nidus upon which the stone will subsequently grow. The etiology of the initial precipitation is heterogeneous. Detachment of the stone from the tissue will cause it to travel through the urinary tract. It is the latter event and the therapeutic modalities that cause symptoms and kidney injury. The focus of this research is the evaluation of urine composition, which is the gold standard for diagnosis of stone formation. The
study assesses the limitations of current diagnostic approach and explains ways to improve its accuracy. It attempts to provide new insights into the etiology of initial precipitate formation and new approaches to the treatment and prevention of kidney stones.\textsuperscript{[61]}

Supersaturation is the key to precipitation, crystallization and kidney stone formation. It is defined as the state of a solution that contains more than the maximum amount of solutes (in this case lithogens) that can normally be dissolved in it. Perhaps one of the reasons why research efforts to elucidate the pathophysiology of kidney stone formation have been seriously hampered is the uncertainty of the diagnostic methods for assessing supersaturation in urine composition. The problem is that the mixing of multiple urine samples over twenty-four hours obscures each individual sample’s content, which prevents the recognition of significant periods of supersaturation.\textsuperscript{[61, 68]} The thesis explores this issue.

**GENERAL STUDY OBJECTIVE:**

The overall objective of this dissertation is to study the predisposition to kidney stone formation from variation in the physicochemical and physiologic properties of urine in a 24-hour period and in response to increase fluid intake. The study will focus on calcium phosphate precipitates, which are the main precursor of calcium oxalate monohydrate nucleation and growth. Calcium oxalate stones are the most common type of kidney stones. In addition, calcium phosphate precipitates are precursor of calcium phosphate stones, which are the most common stone type with growing incidence, and they can cause renal failure by plugging Bellini’s duct.\textsuperscript{[18]}
SPECIFIC STUDY OBJECTIVES:

There are three specific study objectives:

1- **Short urine collection study**: To examine whether the individual analysis of multiple shorter urine collections will increase the ability of identifying a metabolic predisposition to calcium phosphate precipitate formation and improve risk stratification compared to the traditional 24-hour urine collection method. It is hypothesized that a 24 hour urine collection is not a good reflector of precipitate formation in each individual collection over a 24 hour period.

2- **Overnight precipitation risk study**: To assess whether the risk of calcium phosphate precipitation is increased at night, when urine flow rate is physiologically at its lowest. It is hypothesized that when urine flow rate approaches its nadir overnight, protective mechanisms come into play to prevent precipitate formation.

3- **Increased fluid intake study**: To evaluate the effect of increased oral fluid intake on urine flow rate and urinary constituents’ concentration involved in calcium phosphate precipitation. It is hypothesized that this recommendation to prevent stone recurrence results in urine flow rates that are intermittently elevated and interspersed with long periods of low flow rates.

These research studies should improve our understanding of the physicochemical and physiological factors that may lead to supersaturation of urine, crystallization and serial stone formation.
GENERAL BACKGROUND:

PREVALENCE:

Kidney stones are very common (the commonest urologic disorder) with 7-17% prevalence in the general population. Lifetime risk of stone formation is exceeding 12% in men and 6% in women. \textsuperscript{[13, 28]} Although, the prevalence of nephrolithiasis varies by age, sex and race, the prevalence appears to have increased in the last quarter of the 20th century for men and women as well as blacks and whites in the United States \textsuperscript{[13]} (See figures 1 and 2, page 21-22). This rise in prevalence also could be due to increased detection of asymptomatic stones by increasing usage and sensitivity of imaging studies. Prevalence of stone disease has also increased in other parts of the world including Japan and Germany. \textsuperscript{[13]}
Stone disease prevalence within the United States varies by racial background. A history of stone disease is most common among older white males (approximately 10%), and lowest in younger black females (approximately 1%). The prevalence in Asians and Hispanics falls somewhere in between.\textsuperscript{[13, 28]}
INCIDENCE:

Several population-based studies have demonstrated that incidence rates, defined as the onset of an individual’s first kidney stone, vary by age, sex and race. As with prevalence, the incidence rates are highest in the white males. For men, the incidence begins to rise after the second decades of life and peaks between 40 to 60 years at approximately 3 per 1000 per year and then begins to decline \cite{13, 28}. For women, incidence rates seem to be higher in the late 20’s (2.5/1000/yr) and then decreases to 1/1000/yr by age 50. \cite{45} This rate then appears to remain relatively constant for the next several decades \cite{11, 20, 23}

CLINICAL SIGNIFICANCE:

Pain:

Without overstatement, the agonizing pain in the flank and lower abdomen from kidney stone is the most often compared with the pain of normal labour and delivery. However, kidney stone colicky pain usually appears suddenly, without any warning sign, and cripples the patient owing to intense sharp, stabbing and shooting pain. Besides, a victim of a kidney stone attack must endure pain for days to weeks. Kidney stones can also cause nausea, vomiting, fever in addition to blood in the urine, pain with urination, and probably other symptoms of infection as well. \cite{20}

Parenchymal Kidney injury:

In essence, kidney stones are not merely a mechanical disease or an extreme painful condition, but they can cause parenchymal damage and reduce kidney function by different
mechanisms including direct kidney tissue injury or indirectly by urinary obstruction, and infection. Moreover, iatrogenic renal injury by procedures needed to remove stones sometimes cause serious problem. Evidence is accumulating that nephrolithiasis is associated with decreased renal function. Two large studies reported that stone formers have slightly, but significantly, lower glomerular filtration rates and creatinine clearances than those who are not stone formers. [25, 28] Moreover, nephrolithiasis and shockwave lithotripsy may increase the risk of chronic kidney disease and hypertension. [15, 28] The relative contributions of nephrolithiasis, its treatment and its underlying predispositions to these conditions have not studied yet. [9] Fortunately, most patients with renal colic are not at imminent risk of renal failure since the stone does not completely obstruct the ureter. Furthermore, they are likely to pass the stone so that the partial obstruction is short-lived. [27]

Financial Significance:

Kidney stone disease imposes a substantial financial burden to the global health care system. [9-12] According to statistics, each year in the United States, people make almost 3 million visits to health care providers and more than half a million people go to emergency rooms for kidney stone problems. [20] Since the prevalence of nephrolithiasis is concentrated among working age adults, thus kidney stone disease places a considerable economic expenses on the community. The main burdens were related to health service costs, with personal and pharmaceutical costs representing only a small component. The economic burden of the disease on employers and their employees is considerable. The direct and indirect costs of nephrolithiasis are substantial among working-age adults. [30] The financial burden to a defined community of Christchurch, New Zealand was estimated to be $450,000 per 100,000 general population. [31]
RECURRENT RATES:

Early reports suggested that if left untreated the likelihood of forming another stone after the initial episode generally for all kind of stone has been averaged 30 to 40% at 5 years. These figures from observational studies are similar to the recurrence rates in the control arms of published randomized trials [14, 17]. Encouragingly, the treatment arms of many of the randomized trials have shown dramatic reductions in recurrence rates by 50% or more [17,26]. These reductions by medication or dietary interventions emphasize that recurrent stone disease may be preventable. [28, 45] In this regard, recurrence rates of 50% after 10 years and 75% after 20 years have been reported. [32]

GEOGRAPHIC DISTRIBUTION:

There may be a geographic predisposition to form kidney stones. For instance, regional "stone belts," have been explained in people living in the southern United States, having an increased risk of stone formation. A study of over 1 million individuals found a north-south and west-east gradient such that the highest prevalence of stone disease occurred in the southeastern United States. [13] Furthermore, it has been said that the hot climate, certain types of diet, and poor fluid intake may predispose people to form kidney stone. [28] Nonetheless, in certain areas of the world, as in the Middle East, the lifetime risk appears to be higher. [34] In other words, ten to fifteen percent of the United States population is diagnosed with urolithiasis; but in Middle East twenty to twenty five percent of population is diagnosed with urolithiasis. [34]
Individuals of Arabic, West Indian, West Asian and Latin American ancestry are more likely to have idiopathic calcium nephrolithiasis than Europeans, while the relative risk in East Asians and Africans is significantly lower. In the North America and Europe, the annual incidence is about 0.5%. In the developed world, the lifetime risk is approximately 10% to 15%, and in the Middle East, 20% to 25%. Kidney stone disease is a relatively rare condition in children from Western countries, particularly when compared with its high prevalence in adults.

RISK FACTORS:

➢ **Family history:** There are many major factors which have been linked to the development of kidney stone disease. However, some of these risk factors are modifiable but some are not modifiable (table 1, page 32). The risk of becoming a stone former is more than 2.5 times greater in individuals who have a family history of stone disease. This increased risk has been attributed both to genetic, environmental, and dietary factors.

➢ **Systemic disorders:** There are many systemic disorders, which have been linked to kidney stone formation including; primary hyperparathyroidism, renal tubular acidosis and Crohn’s disease. Interestingly, primary hyperparathyroidism may be found in 5% of stone formers. Increased body mass index (BMI > 30 kg/m²) and weight gain are now recognized risked factors for the development of kidney stones. In addition, a history of gout increases the likelihood of forming kidney stones; both uric acid and calcium oxalate (50% more likely to have a history of stones). Moreover, a history of type II diabetes mellitus increases the risk of stone formation by 30 to 50% in women but not in men.
➢ **Environmental factors:** Some environmental factors have been implicated for example: working in a hot environment, lack of access to water or lower fluid intake all have been shown to predispose to renal stone formation. [39]

➢ **Dietary Factors:** Some dietary factors have been linked to kidney stones formation. Nutrients that have been implicated include calcium, animal protein, oxalate, sodium, sucrose, magnesium, and potassium. [28]

- **Calcium:** Although high dietary calcium had been strongly suspected of raising the risk of stone disease in the past, a recent cohort study of more than 50,000 male health professionals aged 40 to 75 years, has shown that very low calcium intake can actually predispose to kidney stone formation as well. [44, 45]

- **Oxalate:** Up to one-third of patients with calcium oxalate nephrolithiasis may have increased absorption of dietary oxalate, and in some cases a deficiency of oxalate degradation by the Bacterium Oxalobacter formigenes in the gut could be the culprit. [48]

- **Other nutrients:** Several other nutrients have been reported to be implicated in the development of stone formation, including; high animal protein intake, high sodium intake, high sucrose intake, low potassium intake and low calcium intake. Furthermore, potassium supplementation decreases calcium excretion and many potassium-rich foods increase urinary citrate due to their alkali content. Recently, phytate (the principal storage form of phosphorus in many plants) was also found to reduce the
likelihood of stone formation in younger women substantially. Magnesium can make complexes with oxalate, thereby potentially reduces oxalate absorption in the gastrointestinal tract and decreases calcium oxalate supersaturation in the urine. High vitamin C intake and vitamin B6 deficiency could increase the risk of calcium oxalate stone formation as well. Vitamin B6 deficiency increases oxalate production, whereas vitamin C can be metabolized to oxalate, and both can cause hyperoxuluria.\textsuperscript{[28, 40]}

- **Fluid Intake and Beverages:** Observational studies and a randomized controlled trial in almost all types of kidney stones have demonstrated, when the urine output is less than 1 L/day, the risk of stone formation is higher than those with higher urine output. On the other hand, some beverages have been shown to increase the risk of stone formation (for instance grapefruit juice and soft drinks).\textsuperscript{[28]} Grapefruit juice intake has been associated with a 40\% higher risk of stone formation. Other studies suggested an increased risk of kidney stone formation with soda consumption.\textsuperscript{[49, 50]}

- **Urinary Factors:** Some urinary factors have been suggested as the risk factors for renal stones formation namely: hypercalciuria, hyperoxaluria and hyperuricosuria and hypocitraturia, have been associated with increased risk of stone formation.\textsuperscript{[28]} This will be discussed in detail in the urinary composition and stone formation sections.

- **Microorganisms:** Fascinatingly, a correlation between Randall’s Plaques and the presence of calcifying nanoparticles (CNP, Nanobacteria) which are similar to snowballs has been reported. These structures were discovered over a decade ago in blood and blood products. They have been detected in numerous pathological calcifications, such as in kidney stones\textsuperscript{[41,127]}, in atherosclerotic plaques, in psammoma bodies of cancer, in prostatic stones, and in
gallbladder. CNP are calcified self-propagating entities, morphologically very similar in mineral composition to spherical bodies observed in Randall’s plaques. Due to lack of their genomic evidence, CNP are controversial agents as prions. Although CNP cause specific infection, and are detected in pathological calcification, general debate over their existence continues. Methods to detect CNP include immunodetection techniques using anti-CNP mAbs, culture-techniques and electron microscopy. They precipitate apatite from media forming apatite-protein complexes on their exterior membrane. [41] Although causality has not been demonstrated, further studies should be made to explore the etiology of Randall’s Plaques formation, thus leading to a better understanding of the pathogenesis of stone formation. If supported by further work, the establishment of calcifying nanoparticles as the etiology of Randall’s Plaques will direct a fundamentally novel treatment approach for urinary stone disease. If so, inhibiting the propagation of calcifying nanoparticles would make urinary stone prevention a conceivable possibility. [41,127]

- Bacterial urea splitting including; Proteus, Pseudomonas, Klebsiella species and many other bacteria can cause urease-induced stone like Struvite and carbonate-apatite stones. [112] Even though several viruses such as: Feline Calicivirus (FCV), Feline Syncytium Forming Virus (FeSFV), and Cell Associated Herpes Virus (CAHV) has been reported as causes of urolithiasis, no research in human cases have been reported. Preliminary studies of explanted cell cultures derived from urological specimens from humans with urolithiasis suggest a need to extend these studies. The cytopathic effects observed in human explanted cell cultures paralleled those observed in the feline cell cultures infected with the herpes virus. Further, it has been documented that human populations may carry persistent infections with up to five herpesviruses. [46]
Table 1. Summarizes the most important risk factors for the development of renal stones.

<table>
<thead>
<tr>
<th>Family History and Genetics</th>
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<tbody>
<tr>
<td>Systemic Diseases</td>
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<tr>
<td>Primary Hyperparathyroidism</td>
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<td>Weight gain &amp; Obesity (Body mass index more than 30)</td>
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<tr>
<td>Diabetes Mellitus Type II</td>
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<tr>
<td>Inflammatory bowel disease, Intestinal malabsorption</td>
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<tr>
<td>Gout</td>
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<tr>
<td>Renal Tubular Acidosis</td>
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<td>Genetic monogenic diseases:</td>
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<td>Primary hyperoxaluria, cystinuria, familial hyperuricosuria,</td>
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<tr>
<td>Monogenic hypercalciuric stone forming diseases</td>
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<tr>
<td>X-linked recessive hypercalciuric diseases complex</td>
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<tr>
<td>Familial hypomagnesemia with hypercalciuria &amp; nephrocalcinosis</td>
</tr>
<tr>
<td>Bartter syndrome types III and IV</td>
</tr>
<tr>
<td>Autosomal dominant hypocalcemic hypercalciuria</td>
</tr>
<tr>
<td>Hypophosphatemia associated to hypercalciuria</td>
</tr>
<tr>
<td>Anatomic abnormalities</td>
</tr>
<tr>
<td>Medullary sponge kidney</td>
</tr>
<tr>
<td>Ureteropelvic junction stenosis</td>
</tr>
<tr>
<td>Pyelo-ureteral duplication</td>
</tr>
<tr>
<td>Polycystic renal disease</td>
</tr>
<tr>
<td>Dietary Factors</td>
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<tr>
<td>Calcium intake (very low or very high)</td>
</tr>
<tr>
<td>High oxalate intake</td>
</tr>
<tr>
<td>High animal protein</td>
</tr>
<tr>
<td>High Sodium intake</td>
</tr>
<tr>
<td>High Sucrose intake</td>
</tr>
<tr>
<td>High Vitamin C (ascorbic acid) intake</td>
</tr>
<tr>
<td>High Beverage (soft drinks) and fruit juice (grapefruit) intake</td>
</tr>
<tr>
<td>Low fluid intake</td>
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<td>------------------</td>
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<tr>
<td>Low Potassium, Magnesium, Phytate intake</td>
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<tr>
<td>Vitamin B6 deficiency</td>
</tr>
<tr>
<td>Environmental Factors</td>
</tr>
<tr>
<td>Working in a hot environment</td>
</tr>
<tr>
<td>Lack of access to water</td>
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<tr>
<td>Urinary Factors</td>
</tr>
<tr>
<td>Hypercalciuria</td>
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<tr>
<td>Hyperoxularia</td>
</tr>
<tr>
<td>Hypocitraturia</td>
</tr>
<tr>
<td>Hyperuricosuria</td>
</tr>
<tr>
<td>Urine Output &lt; 1 liter/day</td>
</tr>
<tr>
<td>Lithogenic drugs:</td>
</tr>
<tr>
<td>Triamterene, Indinavir, Sulfadiazine, Uricosuric agents</td>
</tr>
<tr>
<td>Microorganisms:</td>
</tr>
<tr>
<td>Nanobacteria, CNP, Proteus, Pseudomonas, Klebsiella, FCV, FeSFV, CAHV</td>
</tr>
</tbody>
</table>

**KIDNEY STONES MORPHOLOGY:**

There are several types of kidney stones based on the type of crystals, which they are composed of. Calcium stones are the most common type of kidney stones and constitute approximately 80-90% of all renal stones.\[^{42, 43}\] Calcium stones are generally a mixture of calcium phosphate and calcium oxalate precipitate. Depending on the main constituent (>50%) of the stone, we call them either calcium oxalate or calcium phosphate stone. In the majority of calcium kidney stones, calcium oxalate is the main constituent and calcium phosphate precipitate is present in amounts ranging from 1% to 10%. Calcium phosphate stone are less common and only about 15% of kidney stones fall in this category.\[^{10, 51, 55}\]
Figure 3. Proportion of kidney stones by types. \[51\]

Calcium phosphate crystals could be present in urinary stones as either brushite or apatite:

**Brushite:** Brushite or calcium monohydrogen phosphate with chemical formula of CaHPO$_4$·2H$_2$O. It is believed to be the precursor of apatite. \[52\]
**Brushite**

![Brushite Structure](image)

**Figure 4.** Brushite structure

**Hydroxy apatite:** \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) which is the principal constituent of bones and teeth. Up to fifty percent of bone is made up of a modified form of the inorganic mineral hydroxy apatite. Carbonated calcium-deficient hydroxy apatite is the main mineral of which dental enamel and dentin are comprised. Hydroxy apatite crystals are also found in the small soft tissue calcifications like within the pineal gland and other structures. [52]
Brushite, but not hydroxy apatite, stones are physically resistant to lithotripsy (ESWL), so repeated treatments may be needed. Brushite stones are associated with a distinctive renal disease. What drives the development of brushite versus apatite stones is not known. [28]

WHY THIS STUDY FOCUSES ON CALCIUM PHOSPHATE PRECIPITATES:

The main focus of this dissertation is to study general physicochemical and physiological changes of urinary composition that may cause calcium phosphate precipitation without any discrete metabolic or hormonal abnormality, and to provide a new insight in the understanding of factors involved in addition to sequences of events that cause supersaturation, crystallization and stone formation. The majority of calcium stones (which are the commonest type of kidney stones) are composed primarily of calcium oxalate. [53] However, calcium oxalate stone formation is linked
to increased urinary calcium and oxalate solubility products (Ksp) and their generation depends on the concentration of free calcium ions, oxalate ions and “urine flow rate” at any given moment.

On the other hand, the second most common type of kidney stones, calcium phosphate stone generation is linked to free ionic concentrations, urine flow rate and urine pH. Additionally, urine pH will have a dramatic affect on free divalent phosphate ions concentration. An analysis of urine composition focusing on calcium phosphate would be more informative because it could provide a better risk stratification, and it may perhaps grant selective preventative therapies on the basis of the following grounds:

1. Most patients with calcium phosphate kidney stones belong to a diagnostic category that has a common feature of a high urine pH rather than representing a group of specific disease entities. [10]

2. Idiopathic calcium oxalate stone formers have no evidence of renal epithelial cell injury on papillary biopsy [18], while calcium phosphate precipitates plug the inner medullary collecting ducts and ducts of Bellini with apatite crystals [54]. For this reason, formation of calcium phosphate stones may well be a clinically devastating event. Evan et al. study demonstrated that patients with calcium phosphate kidney stone develop progressive parenchymal damage and nephron loss due to plugging of the terminal collecting duct with CaHPO₄ crystals. These findings emphasize the importance of understanding the underlying pathophysiology that leads to the precipitation of these crystals so that an effective, individualized therapy could be designed. [10]
3. Unfortunately, an increase in the prevalence of calcium phosphate stones over the past two decades has been reported by different authors. Furthermore, the recurrence rate of calcium phosphate stones has been increased.\textsuperscript{[55]} \textsuperscript{[114]} In addition, this type of stone affects preferentially women; it has been found that 47% of calcium phosphate stone formers were female, compared with only 26% of calcium oxalate stone formers.\textsuperscript{[57]}

4. More importantly, interstitial calcium phosphate precipitates (hydroxy apatite crystals) are the main precursors of both types of calcium stones\textsuperscript{[52]} and promote both calcium phosphate and calcium oxalate stones formation on the surfaces of the renal papillae over collections of interstitial sub-urothelial calcium phosphate particles\textsuperscript{[18]} named Randall’s plaque. The number of calcium oxalate stones generation, adjusted for the duration of stone formation, varies directly with the CaHPO$_4$ plaque surface coverage\textsuperscript{[56]}, as it is anticipated if plaques act like a surface that promotes calcium oxalate overgrowth.\textsuperscript{[52]}

Other types of kidney stones including; uric acid, struvite, and cystine stones, which are much less common causes of kidney stones (See also page 107, literature review).
SHORT URINE COLLECTION STUDY

Objective:

To examine whether the individual analysis of multiple shorter urine collections throughout the day will increase the ability of identifying a metabolic predisposition to calcium phosphate precipitate formation and improve risk stratification compared to the traditional 24-hour urine collection method.

Study specific background:

Metabolic work up of urine composition for stone formation in a 24-hour collection originally simulated from measurements of creatinine and protein in a 24-hour urine collection. Over a century ago, 24-hour collections of urine were introduced to estimate the GFR. Subsequently, 24-hour urine collections were used to standardize the amount of urinary protein, as well as urinary hormones, physiologic compounds and ions or pharmacologic substances. Much later, the collection of urine over 24 hours replicated as an important part of the investigation of kidney stone patients and metabolic stone risk analysis.\[106]\n
At the present time analysis of calcium, magnesium, phosphate, oxalate, uric acid, citrate and creatinine in 24-hour urine collections is considered to be essential for the selection of the most appropriate intervention to prevent kidney stone recurrence.\[37, 107]\ Thus, 24-hour urine collection has become a central aspect of metabolic work up of stone former patients and considered to be a gold standard approach for patients with kidney stones to discover if lithogens exceed K_{SP}.\[107, 108]\
A single 24 hour urine sample is not sufficient to evaluate patients before metabolic treatment for stone prevention, because misdiagnosis is common, leading to inappropriate treatment. Even with considerable attention to improve the methods of collection and analysis of 24 hour urine samples, it is still not capable of identifying the metabolic abnormality that can explain stone formation in a significant proportion of kidney stone patients. [107]

Therefore, some investigators recommend collection of two to four 24-hour urine samples, to increase the diagnostic value of the test. Nonetheless, this protocol would be inconvenient to the patient and has a higher potential for improper collections and erroneous values may be obtained. [107]

**Rationale:**

Since exceeding the $K_{SP}$ of free ionic products of calcium and divalent phosphate is considered to be essential for precipitation, experimental study of supersaturation of free ionic products of calcium and divalent phosphate required to illustrate how the composition of the urine must change to increase the risk of precipitation. Randall’s plaques, calcium phosphate deposits found in almost 100% of idiopathic Calcium stone formers, arise in unique anatomical regions of the thin-limb basement membranes of ascending limb of Henles’ loop of the kidney. [90] These plaques anchored to renal tissue in the renal papillae are an ideal site on which overgrowths of Calcium oxalate or calcium phosphate could grow into stones. [18] In fact, this region is not bathed in a retained 24-hour urine, but rather it is exposed to continuous tubular flow. Therefore, any period that ionic product activity exceeds formation product and it is long enough to be transmitted to the interstitium and basement membrane of TAL Henles’ tiny precipitate of calcium and phosphate can form. Upon precipitation and crystal formation, the formed crystal would not easily dissolve even if urinary concentrations of lithogens decrease below the $K_{SP}$. [10]
Nevertheless, principle risk factors for supersaturation and stone formation may vary and take place over a period shorter than 24 hours. In essence, physiologic mechanisms of excretion of lithogens and renal handling of stone formation inhibitor factors are regulated by many different physiologic and hormonal factors and their diurnal excretion may vary significantly causing significant fluctuation of their concentration. As a result, the average concentration in a 24 hour urine collection may fail to show peaks and troughs of concentrations. This finding holds true for supersaturation even more than individual factors because supersaturation depends on many urine ligands and their interactions. [109]

As a result, it is quite possible that free ionic activity of calcium and divalent phosphate exceeds formation product over shorter periods of time and causes precipitation and crystal formation, but may not be long enough to be detected in a 24 hour collection. This new precipitate hardly ever redissolves even when the free ionic activity product is below K_{SP}. Furthermore, it can not be excreted if it is anchored but it can function as a nidus for further precipitation and eventually it can grow to become a stone. In summary, it seems that a 24 hour collection can underscore the complexity and changes of urinary composition, and can miss the danger zones, therefore it is not an ideal predictor of the stone formation risks. [10]

Hypothesis:

It is hypothesized that there may be times during the day that urine composition favours precipitation; while it is not revealed by a 24 hour urine collection. Furthermore it is to identify any time of the day that free ionic calcium and divalent phosphate product exceed their K_{SP}. This study will determine how common and how long the duration of urine collections that have a composition with supersaturation of the sparingly water soluble substances in healthy subjects are by having volunteers void every 2-3 hours while awake plus an overnight sample in a 24-hour
period. If this does occur shorter collections of urine would be superior to discover occult risk periods where calcium phosphate could precipitate.

**Material and methods:**

**Subjects:** The Research Ethics Board of St. Michael’s Hospital approved the protocols described herein. Twenty six healthy subjects (16 males and 10 females) provided their urine samples every 2-3 hours while awake. To allow restful sleep, only one nocturnal urine sample was collected. Volunteers were in good health and did not take any medication in the week prior to study. Each subject recorded the time and volume of each voiding, and a small sample was kept under refrigeration for analysis.

**Procedures:** Urine was obtained by voluntary voiding; the time and volume of each sample was recorded. The rate of excretion of creatinine was used to assess completeness of collection. To ensure consistency, each subject performed 2 or more 24 hour urine collections 2 to 7 days apart with unrestricted oral intake and activity. The subjects’ urine flow rate was calculated in each collection by dividing each sample volume in milliliters over the time in minutes.

**Measurements:**

Urinary constituents for calcium phosphate precipitation including sodium potassium, chloride, calcium, phosphate, citrate, magnesium, urea, creatinine and pH were measured. A simulated 24-hour collection was recalculated by the totaling of all shorter urine collections volume and urinary constituents excretions throughout the day.
Analytic techniques:

Urine sodium and potassium were measured by flame photometry method (Radiometer, FLM-3; London, ON, Canada). Urine chloride was measured by electromimetic titration (Chloride meter, CMT 10; London Scientific Ltd., London, Ontario). The concentrations of calcium, phosphorus, magnesium, and creatinine in urine were measured by standard laboratory methods using a Synchron CX5CE analyser (Beckman Instruments, Brea, CA, U.S.A.). Urine citrate was measured enzymatically with citrate lyase. Urine pH was measured using a glass electrode pH meter (Corning 178 blood pH analyzer; Corning Glass Works, Corning, NY, USA). Urine osmolality was measured by freezing point depression (Advanced Instruments, Inc., Needham Heights, MA, USA). Urea and creatinine in urine were measured by using a Kodak Ektachem analyser. All the measurements were done at Dr. Halperin’s lab at St. Michael’s Hospital, Toronto, Ontario, Canada.

Statistical analysis:

Results are reported as the mean ± SEM (standard error of the mean). Comparisons among short collections and 24 hour collections were done by using McNemar's test, a non-parametric method on nominal data. It was applied to $2 \times 2$ contingency tables of matched pairs of standard 24h collections and multiple shorter collections, to determine whether the row and column marginal frequencies are equal ("marginal homogeneity"). Calcium concentration 2 mmol more than citrate concentration was used as significant high free calcium (mainly to count for Mg which has higher avidity for HPO4 (For more detail see page 140-149). A paired T test was used for comparison of matched groups of data. For either test a P value that was < 0.05 was considered to be statistically significant.
RESULTS:

Studies were performed in 10 female and 16 male volunteers aged between 21 and 54 years (mean ± SEM, 31 ± 1.5). Number of collections per day was 8 ± 1. Table 2 indicates the subjects’ characteristics and number of diurnal collections, in addition to any significant free ionic calcium concentration as well as any significant free ionic calcium and divalent phosphate products, which were not evident in 24 hour collections when they were present in shorter collections. To analyze the statistical significance a concentration difference of more than 2mmol/l was chosen arbitrarily, for calcium and citrate and the ionic product of free calcium times divalent phosphate.

Table 2: Diurnal subjects Characteristics:

<table>
<thead>
<tr>
<th>ID</th>
<th>Age (years)</th>
<th># of Collections per day</th>
<th># of Ca&gt;Cit by 2mmol in short but not in 24 collections</th>
<th># of iCa*PO₄²⁻ by 2 in short not 24 collections</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>33</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F2</td>
<td>35</td>
<td>7</td>
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<td>1</td>
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<td>F3</td>
<td>54</td>
<td>9</td>
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<td>F4</td>
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<td>M1</td>
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<tr>
<td>M2</td>
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<td>8</td>
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**Diurnal creatinine excretion to confirm collections accuracy:**

To confirm the collection precision of each sample in subjects, creatinine concentration of each sample was measured and creatinine excretion was calculated. The rate of excretion of creatinine in each subject confirmed the completeness of collections [118].
Figure 6. Creatinine excretion of male and female throughout 24 hours (mean ± SEM)

Examples of calcium minus citrate difference in short collections along with 24 hour collection:

The following are individual examples of significant calcium citrate differences representing possible risk zones that were missed on a 24-hour urine collection.
Figure 7. An individual example of a 24-hour urine collection for calcium and citrate concentrations.

In this graph, a 24-hour urine collection for calcium and citrate concentrations from a male subject is illustrated. As can be seen the citrate concentration (green bar graph) is well above the calcium concentration (red bar graph), suggesting there would not be any free calcium left in the urine and therefore the risk of precipitation would be almost zero.
Figure 8. The same individual shorter urine collections for calcium and citrate concentrations.

This graph shows the same subject’s shorter urine collections for calcium and citrate concentrations. The time of the day is on the horizontal axis and the concentrations of calcium and citrates are on the vertical axis. As can be seen, there is a period that calcium concentration, is well above citrate concentration indicating potential risk of precipitation. This risk of precipitation (red oval) was not apparent in a 24 hour urine collection analysis shown on the previous page.
Figure 9. An individual example of 24 hour urine collection for calcium and citrate concentrations.

In this graph, a 24-hour urine collection for calcium and citrate concentrations from another male subject is illustrated. As can be seen again the citrate concentration (green bar graph) is above the calcium concentration (red bar graph), suggesting free calcium concentration of almost zero and a negligible risk of precipitation.
Figure 10. The same individual shorter urine collections for calcium and citrate concentrations.

This graph compares a male’s subject shorter urine collections for calcium and citrate concentrations. The time of the day is on the horizontal axis and the concentrations of calcium and citrates are on the vertical axis. The second vertical axis depicts urine flow rate (blue line). As can be seen, there are periods where calcium concentration is well above citrate concentration. The risk of precipitation (red ovals) is increased in these periods, which were not evident in the 24-hour collection. Urine flow rate did not change significantly (blue linear graph).
Figure 11. An individual example of the comparison of a 24 hour urine collection with shorter urine collections for calcium and citrate concentrations.

This graph compares a 24-hour urine collection for calcium and citrate concentrations with shorter collections of another male subject. Again different times of the day are on the horizontal axis and concentrations of calcium and citrates are on the vertical axis. The second vertical axis shows urine flow rate (blue line). In the 24-hour collection calcium is minimally higher than citrate. Therefore, one might think that this subject would not be at risk of precipitation; however, as it can be seen from shorter collections, there are significant periods when calcium concentration is much higher than citrate concentration. These periods of increased precipitation risk (red ovals) were not evident in a 24-hour collection. Urine flow rate did not change significantly (blue linear graph).
Figure 12. An individual example of the comparison of a 24 hour urine collection with shorter urine collections for calcium and citrate concentrations.

This graph compares a 24-hour urine collection for calcium and citrate concentrations with shorter collections of another male subject. Again different times of the day are on the horizontal axis and concentrations of calcium and citrates are on the vertical axis. The second vertical axis shows the urine flow rate (blue line). In the 24-hour collection calcium is clearly higher than citrate. However, in shorter collections, there are significant periods when those calcium concentrations are greatly higher than citrate concentrations. These risks of precipitation (red ovals) were not as evident in a 24 hour collection. As it can be seen, the urine flow rate does not change significantly (blue linear graph).
Figure 13. An individual example of the comparison of a 24 hour urine collection with shorter urine collections for calcium and citrate concentrations.

This graph compares a 24-hour urine collection for calcium and citrate concentrations with shorter collections of another male subject. Once more, the time of day is on the horizontal axis and the concentrations of calcium and citrates are on the vertical axis. The second vertical axis shows the urine flow rate (blue line). In the 24 hour collection calcium is slightly higher than citrate, hence, one might think that the risk of precipitation would be negligible. However, as it can be seen from shorter collections, there are significant periods where calcium concentration is much higher than citrate concentration. These risks of precipitation (red ovals) were not visible in the 24 hour collection. Furthermore, urine flow rate does not change significantly (blue linear graph).
Figure 14. A female individual example of the comparison of a 24 hour urine collection with shorter urine collections for calcium and citrate concentrations.

This graph compares a 24-hour urine collection for calcium and citrate concentrations with shorter collections in a female subject. Time of the day is on the horizontal axis and concentrations of calcium and citrates are on the vertical axis. Although in the 24-hour collection calcium concentration is slightly higher than citrate concentration in shorter collections, there are significant periods where calcium concentration is significantly higher than citrate concentration. These risks of precipitation (red ovals) were not evident in a 24-hour collection.
Statistical analysis:

After analysis of 26 standard 24-hour urine collections, only high free calcium concentration ([Ca]-[Cit]>2mmol/l, as discussed in page 43) was found in 2 subjects. When this was tested using short collections in a day, 11 additional subjects had high free ionized calcium and the other 13 subjects did not have high free calcium. A contingency table made of high and low calcium minus citrate more than 2 mmol/l of standard 24-hour collections with multiple shorter collections (Table 3). Multiple short urine collections were superior in identifying periods of high free Ca$^{2+}$ (P<0.001, using McNemar's test).

<table>
<thead>
<tr>
<th>Multiple Short duration Urine Collections</th>
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<tr>
<td>Standard 24h</td>
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Table 3. McNemar’s test for paired proportion of high [Ca]-[Cit] (P < 0.001)

After analysis of 26 standard 24-hour urine collections, only free calcium concentration times divalent phosphate products ([Ca]-[Cit]*[HPO$_4^{2-}$]) exceeded KSP in 5 subjects, and the other 21 urine collections [Ca]-[Cit]*[HPO$_4^{2-}$] products did not exceed KSP. When this was tested using short collections in a day, 12 additional subjects [Ca]-[Cit]*[HPO$_4^{2-}$] products exceeded KSP. A contingency tables made of high and low (Ca-Cit*HPO$_4^{2-}$) of matched pairs of standard 24h
collections with multiple shorter collections (Table 4). Multiple short urine collections were superior in identifying periods of high Ca-Cit*HPO_{4}^{2-} product exceeding KSP (P<0.0005, using McNemar's test).

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<th>Multiple Short duration Urine Collections</th>
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<td>Urine Collections</td>
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Table 4. McNemar’s test for paired proportion of high [Ca]-[Cit]*[HPO_{4}^{2-}] product (P < 0.0005)

As it can be seen in table 3 (page 55), there are eleven subjects who would have a “change in management” if you acted on the multiple short duration results and two whose management would be the same no matter which results you used. This represents 42% of patients (95% CI: 36-81%). This confidence interval is precise enough that fewer than 36% of patients would have their management changed.
SECONDARY RESULTS:

Our secondary results illustrate some typical diurnal changes of different lithogens, stone formation inhibitors concentration and their effects on supersaturation. The renal handling plus diurnal variation in excretion of the most important urinary constituents are discussed.

Figure 15. Urine flow rate in our subjects throughout 24 hours.
Figure 16 compares the diurnal variation of urine flow rate and urine osmolarity. As can be seen, the nadir in urine flow rate was in the overnight collection period in healthy subjects. Furthermore, urine osmolality remains high throughout the day and over night.

**Figure 16.** Diurnal pattern for the urine flow rate and osmolality, representing means ± SEM. Urine flow rate is shown by the blue line and urine osmolarity (Uosm) is shown by the brown line. The nadir in the urine flow rate is in the overnight period, but no appreciable diurnal variation in the Uosm can be seen.
Figure 17. Diurnal pattern for the excretion of electrolytes and urea, representing means ± SEM. The rate of excretion of sodium is shown by the green line and the rate of excretion of urea by the orange line. The nadir in the sodium excretion rate is in the overnight period, but no appreciable diurnal variation in the rate of excretion of urea can be seen.

Figure 17 compares the diurnal excretion of sodium and urea. As we can see the sodium excretion is higher during day and lowest in the overnight period. Nevertheless, the urea excretion does not change significantly throughout the day.
Figure 18. Diurnal pattern of urine calcium versus citrate in female subjects. Data represent means ± SEM for the urine citrate and calcium. The red line depicts urine calcium values in female subjects and the green line depicts the urine citrate in female subjects.

Figure 18 compares urine concentrations of calcium and citrate. Citrate concentration is generally more than calcium concentration.
Figure 19. Diurnal pattern of urine calcium versus citrate in male subjects. Data represent means ± SEM for the urine citrate and calcium. The red line depicts urine calcium values in female subjects and the green line depicts the urine citrate in the female subjects.

As can be seen in Figure 17 (page 59) sodium excretion is lower overnight and highest around noon or in the evening. This is also true for calcium excretion, which we and others have previously shown. \cite{10,42,88,91}

Urinary citrate excretion does not depend on citrate intake or plasma citrate concentration. Instead, the final urinary excretion of citrate is determined by proximal tubule cell pH. Acid loading increases citrate absorption by four different mechanisms (Figure 50) see more detail in literature review (page 141). \cite{68}
Even though there were no specific and unique patterns in our subjects’ excretion pattern for phosphate, in most collections phosphate excretion was lower in the morning and higher in the evening and overnight (Figure 20).

**Figure 20.** Diurnal pattern of urine phosphate concentration in female and male subjects. Data represent means ± SEM for the urine phosphate. The red line depicts urine phosphate values in female subjects and the blue line depicts the urine phosphate in male subjects.
Figure 21. Diurnal pattern for the urine pH. Data represent means ± SEM for the urine pH. The red line depicts urine pH values in female subjects and the blue line depicts the urine pH in male subjects.

In our analysis of the urine pH of 26 healthy subjects, the urine pH is typically around 6 but can vary from 5 to 7 throughout the day. The lowest urine pH is usually overnight and the highest around noon or in the evening time, which is usually called alkaline tide (Figure 21). [10, 113] Alkaline tide refers to a condition, normally encountered around a mealtime. In a 24-hour period urine collections pH is usually around 6.0 and the variations in a 24-hour period are obscured.
General discussion:

The purpose of this study was to examine the usefulness of a 24 hour urine collection to identify metabolic derangements that would predispose to calcium phosphate precipitation. The principal results of the study revealed that while it is currently the widely used test in stone former, 24 hour urine collection can miss significant periods of supersaturation that happen throughout the day. This perhaps can partly explain why still in a significant proportion of kidney stone patients where a 24 hour collection was performed, no justification as a basis of precipitate formation can be identified. [107]

Furthermore, the results of the study overemphasize the diurnal complexity of urinary composition. As far as can be determined there are no previous studies specifically examining the impact of collection periods (24 hours versus shorter periods) on illuminating urine metabolic derangements. However, one can speculate that these observations may account, at least partially, for the lower rate of incident metabolic abnormality in stone formers, which have been previously reported. [107]

In essence, calcium phosphate precipitates in peritubular interstitium (actually thin basement membrane of loop of Henles) are precursors of almost 100% of calcium stone formation; hence, this region of plaque precipitation is not immersed in a 24-hour urine. Rather, it is exposed to continuous tubular fluctuation in lithogen and stone forming inhibitor concentrations. Therefore, multiple shorter period collections throughout the day is a logical physiologic approach to identify periods of risk.
Furthermore, multiple 24-hour urine collections which are suggested by some authors \cite{107}, seem to dismay clinicians since they are too hard to collect, cumbersome for practices and expensive. Consequently one uses aliquots or tries to determine the minimum possible to increase patient compliance and maintain costs in line. Hence, this research study provided a reasonable set of data to justify using multiple shorter collections instead of a single 24-hour urine sample.

Randall’s plaques, interstitial papillary calcium phosphate precipitates, are the main precursors of both calcium oxalate and calcium phosphate stones. Randall’s plaque coverage fraction as it has been measured correlates strongly with urine measurements made at an entirely different point in time, thus confirming that the urinary milieu and interstitial environment around the loops of Henle have important pathophysiologic significance in the case of calcium nephrolithiasis.\cite{94}

**Secondary Discussion:**

**Diurnal variations in the urine flow rate and osmolarity:**

When we compare the diurnal variation of urine flow rate and urine osmolarity, it can be seen that the nadir in urine flow rate was in the overnight collection period in healthy subjects (Figure 12, page 52). As we can see urine osmolality remains high throughout the day and over night. Therefore, the main reason for the low urine flow rate overnight is low osmoles excretion rate. The peak urine flow rate was usually around noon to evening.\cite{113}
**Diurnal variations in urinary excretion of electrolytes and urea:**

Our central blood volume is likely to be highest in the overnight period, because this follows the meal with our largest intake of sodium chloride, and also because we are no longer in an upright posture. Thus it is reasonable to suggest that the rate of excretion of sodium should be highest in the overnight period. Nevertheless, the signal to excrete sodium is related more directly to pressure than to central blood volume (Figure 16, page 58). Therefore, it appears that the signal for the renal excretion of sodium is not simply due to an increased central blood volume; perhaps what is sensed is not a rise in volume, but a rise in central venous pressure. A possible explanation for the decrease in ‘pressure’ is that adrenergic stimulation, which increases venous tone, is lower during sleep. This lower excretion of sodium in the overnight period will permit undisturbed sleep, because it will slow the filling of the urinary bladder. [69,113]

Guyton et al have described the close link that exists between blood pressure and the renal handling of fluid and electrolytes. [116] The pressure natriuresis relationship tends to prevent any sustained increase in arterial blood pressure since a rise in pressure immediately enhances the renal output of sodium and thereby reduces extracellular volume and cardiac output. [115,116]

**Diurnal variations in urinary excretion of calcium:**

The renal handling of calcium has been discussed in detail in the previous chapter, but in summary kidneys are responsible for the excretion of the absorbed dietary calcium (4-8 mmol/d) to maintain the body in balance. [68, 91] The fractional excretion of calcium into the final urine is only about 1% to 2%. [68]
Upon volume expansion with normal saline, the fractional excretion of calcium increases in parallel with that of sodium. This is not surprising because calcium reabsorption is dependent on sodium reabsorption in the proximal convoluted tubule and the thick ascending limb of Henle. Furthermore, it seems that the increased calcium excretion could be actually due to decreased reabsorption in a segment downstream of the distal convoluted tubule, presumably either the connecting tubule or cortical collecting tubule. [68]

Building on well accepted concepts, the majority of authors believe calcium absorption in nephron is mainly coupled and correspondent to sodium absorption for the most part (table 9, page 127). Calcium excretion after meals usually increases significantly. [68, 91] Moreover, effective circulatory volume, blood pressure, and rennin-angiotensin-aldosterone system are the main determining factors for sodium excretion and thereby calcium excretion. As we can see from figure 12 (page 52) sodium excretion is lower overnight and highest around noon or in the evening. This perhaps can be true for calcium but needs to be tested. This has been shown in our previous study and others as well. [68, 91]

**Diurnal variations in urinary excretion of phosphate:**

The relationship between plasma phosphate and phosphate excretion has been shown. When the plasma phosphate exceeds a certain level, the renal threshold phosphate begins to appear in the urine, increasing in proportion to the filtered load. This indicates that tubular reabsorption of phosphate is saturable. In humans, with a glomerular filtration rate (GFR) above 40 ml/min, the maximum tubular reabsorption rate of phosphate (TmP) varies proportionately with GFR. Thus,
TmP/GFR, which is the theoretical renal threshold, is kept constant and is a reliable index of tubule reabsorptive capacity.\textsuperscript{[68]}

Clearance studies have demonstrated that phosphate excretion is remarkably responsive to antecedent phosphate intake. Fractional excretion of phosphate increases with a high phosphate diet and decreases with a low phosphate diet, independent of any effect on plasma phosphate, calcium, or PTH concentration. By micropuncture, the major site of adaptation is the proximal tubule, though the distal tubule also shows upregulation of phosphate reabsorption during phosphate deprivation.\textsuperscript{[68]}

Effective circulatory volume expansion increases and volume contraction decreases, phosphate excretion by several mechanisms. First, volume expansion increases GFR and the filtered load of phosphate. Second, volume expansion inhibits proximal tubule sodium and water reabsorption, diluting the concentration of luminal phosphate available for reabsorption. Third, volume expansion decreases plasma calcium and increases PTH, which inhibits proximal tubule phosphate reabsorption (see later). Finally, there is probably a direct effect of volume expansion to inhibit tubule phosphate reabsorption, which is independent of filtered load, plasma calcium, or PTH.\textsuperscript{[68]}

Parathyroid hormone is the major hormonal regulator of renal phosphate handling. It inhibits tubule phosphate reabsorption, primarily in the proximal convoluted tubule. PTH also inhibits phosphate transport in the proximal straight tubule (PST), distal collecting tubule, and the cortical collecting duct.\textsuperscript{[68]} Even though there is no specific excretion pattern for phosphate, but usually phosphate excretion is lower in the morning and higher in evening and overnight.\textsuperscript{[10]}
**Diurnal variations in urinary excretion of citrate:**

As it has been discussed in detail in the literature review, urinary citrate excretion does not depend on citrate intake or plasma citrate concentration. Instead, the final urinary excretion of citrate is determined by proximal tubule cell pH. Acid loading increases citrate absorption by four mechanisms: (Figure 50, page 141) [68] In other words, intracellular acidosis increases expression of the NaDC1 transporter and insertion of NaDC1 into the apical membrane. Furthermore, NaDC1 is also gated by pH such that low pH acutely stimulates its activity. In addition, low luminal pH titrates citrate$^{3-}$ to citrate$^{2-}$ which is the preferred transported species. Finally, intracellular acidosis stimulates enzymes that metabolize citrate in the cytoplasm and mitochondria. This is a well concerted response and an appropriate response of the proximal tubule to cellular acidification is hypocitraturia. Although perfectly adaptive from an acid-base point of view, this response is detrimental to prevention of calcium chelation. [68]

Therefore, all conditions that lead to proximal tubular cellular acidification (e.g., distal renal tubular acidosis, high-protein diet, potassium deficiency) are clinical risk factors for calcareous nephrolithiasis. Hypocitraturia can cause kidney stones by itself or by acting with other risk factors such as hypercalciuria, and therapy with potassium citrate has been shown to reverse the biochemical defect and reduce stone recurrence. [68]

**Diurnal variations in urinary excretion of magnesium:**

Although several hormones have nonspecific effects on magnesium homeostasis, and no single hormone appears to regulate magnesium excretion merely. The active metabolites of vitamin D have a slight, stimulatory effect on intestinal magnesium absorption. PTH induces a
modest decrease in urinary magnesium excretion. Magnesium is also an agonist at the calcium sensing receptor (CaSR), but its affinity is relatively weak. \[68\]

Therefore, there is no definite excretion pattern for magnesium, but it has an appreciable diurnal variation, with a minimum excretion rate during the morning hours and higher excretion in evening and overnight. \[120\]

**Diurnal variations of urine pH:**

The ideal urine pH to prevent calcium phosphate stones or uric acid is 6.0. From our previous study the typical urine pH in the healthy subjects was close to 6.0, but it may vary from 5 to 7 throughout the day. The lowest urine pH is usually overnight and the highest around noon or in the evening time (alkaline tide). \[10, 113\] Alkaline tide refers to a condition, normally encountered around a mealtime, when stomach acid is released into the stomach causing a temporary increase in blood bicarbonate and a minimal increase in blood pH. During hydrochloric acid excretion in the stomach, the blood bicarbonate will increase. This increase in bicarbonate is to maintain the plasma's electrical balance, as the chloride anions have been extracted. Even normal physiology response is to maintain effective circulatory volume, and the kidneys would not excrete bicarbonate in this condition, however, the urine pH trends upward probably due to intracellular pH and the reduction of ammoniogenesis. A more pronounced alkaline tide results from vomiting. \[113\]

In the analysis of the urine pH of normal subjects, there could be large and reproducible variations. The lowest urine pH is usually found overnight, while higher values were observed close to noon. \[113\] Hence mixing urine samples with a low pH and others with a high pH could
mask times in the 24-hour period when the urine pH is high enough to change the proportion of \( \text{HPO}_4^{2-} \) to \( \text{H}_2\text{PO}_4^- \) causing calcium phosphate precipitation. \[^{10}\]

In summary, while excretion of creatinine is relatively constant over a 24-hour period, there is a diurnal variation in the excretion of water and electrolytes. A larger amount of the calcium excreted daily occurs close to noon while the rate of excretion of phosphate is different. Ideally, this diurnal variation should be taken into account when interpreting urine tests, but in practice this is rarely done. There are no studies, to our knowledge, addressing the possible shortcomings of not taking into account the diurnal variation in the interpretation of urine composition metabolic disorder causing precipitate and possible stone formation. \[^{97}\]

Due to this uncertainty, it is advisable to check shorter urine collections for the sake of uniformity and as a way to circumvent this problem. For practical purposes, however, a spot urine sample for electrolytes can be ordered any time of the day. It is advisable to order sodium, potassium, and chloride together.

Urine flow rate, calcium, phosphate, oxalate, citrate, magnesium excretion as well as urine pH are regulated by many different hormonal and physicochemical mechanisms. Therefore, their urinary concentrations throughout the day are not only constant, but also might fluctuate significantly. Furthermore, these fluctuations in concentrations might cause drastic changes in their solubility product and exceed \( K_{sp} \). In addition changes in pH will have exponential effects on concentration of divalent phosphate (Table 7). This can cause a period of supersaturation of tubular fluid in loop of Henle above the formation product, which may be long enough to be transmitted to the interstitium and promote calcium phosphate precipitations as Randall’s plaques in papillary interstitium. Nevertheless, a 24 hour urine sample which is actually a mixture of many smaller voided urine may not be able to discover these periods of supersaturation.
OVERNIGHT PRECIPITATION RISK STUDY

Objective:

To assess whether the risk of calcium phosphate precipitation is increased at night when urine flow rate is physiologically at its lowest.

Study Specific Background:

Restful sleep is essential for the normal functioning of our body, and it should not be interrupted by an urge to void. Therefore, urine produced overnight should not exceed the bladder capacity. In order to achieve this goal, the urine flow rate (UFR) should be low in this period. Nevertheless, this urine ideally must contain all the waste products, excess fluid and minerals to maintain body in balance. Conceivably, a lower urine volume may lead to supersaturation of lithogen in the renal collecting system. If ionic product of constituents of calcium stones exceeds their KSP by any appreciable amounts, precipitation may form. Some author suppose that the overnight period is one of the maximal water conservation period and it is a time of maximal urinary precipitation risk and perhaps a target for specific clinical intervention. [81]

From part one of our study (objective 1) and prior studies, in the healthy subjects, the nadir in urine flow rate was in the overnight collection period (Figure 15, page 57). The average urine flow rate, sodium excretion, osmole excretion, and calcium excretion were lower overnight and higher during the day peaking around noon to afternoon (Figure 16-19, pages 58-61).
Hence urine osmolarity equals number of excreted osmoles over urine volume in unit of time (urine flow rate), UFR can be calculated by dividing number of excreted osmoles over time (mOsm/min) by urine osmolarity (mOsm/l). Furthermore, urine flow rate can be calculated by dividing number of excreted effective osmoles over time (mOsm/min) divided by urine effective osmolarity (mOsm/l) (equation 2). Since the osmolarity in 24 hours does not change significantly (Figure 26), urine flow rate depends on the number of effective osmoles excreted over a given period of time. Because effective osmole excretion is at its lowest overnight (Figure 17), urine flow rate would be at its nadir.

\[
\text{Urine Flow Rate} = \frac{\text{Number of excreted effective osmoles over time (mOsm/min)}}{\text{Urine effective Osmolarity (mOsm/l)}}
\]

Guyton et al have described the close link that exists between blood pressure and the renal handling of fluid and electrolytes. The pressure natriuresis relationship tends to prevent any sustained increase in arterial blood pressure since a rise in pressure immediately enhances the renal output of sodium and thereby reduces extracellular volume and cardiac output.

At a superficial level, if urine flow rate were the only variable, the effect of volume on supersaturation is amplified by the fact that it would raise both ionized calcium and divalent phosphate concentrations. This amplification has been illustrated in table 5 (page 109). Ionic product of calcium phosphate rise 4-fold when the urine flow rate halves to 0.6 ml/min if the excretion rates for these ions remains constant.

The core research question was: "Is there a danger of supersaturation and thereby urinary precipitate formation during oliguria in healthy subjects?" The aim of this part of the study is to define
the urinary composition and excretion rate of calcium, phosphate, pH, and recognize the likelihood of supersaturation, when the urine flow rate is at nadir overnight.

**Rationale:** Low urine flow rate is necessary to have a restful sleep; however, urinary constituents in healthy subjects, independent of diet and fluid intake, must always be kept soluble to avoid excessive rise in supersaturation and crystal formation of lithogen substances.

**Hypothesis:** It is hypothesized that healthy subjects will not have excessive rise in the ionic product of constituents of calcium precipitate, when urine flow rate approaches its nadir overnight, and therefore the risk of precipitation would not increase.

**Material and methods:**

Twenty six healthy subjects (16 males and 10 females) provided their urine samples during daylight (07:00-23:00), every 2-3 hours as they were able to do so. To allow restful sleep, only one nocturnal urine sample was collected. The subject recorded the time and volume of each specimen, and a small sample was kept under refrigeration for analysis at a later time.

**Subjects:** The protocol described herein was approved as explained in objective 1. Volunteers were in good health and did not take any medication in the week prior to the study.

**Procedures:** Urine was obtained by voluntary voiding; the time and volume of each sample were recorded. The rate of excretion of creatinine is used to assess completeness of collection.\[118\] To ensure consistency, each subject performed 2 or more 24 hour urine collections 2 to 7 days apart with unrestricted oral intake and activity. Subjects’ urine flow rate was calculated in each collection by dividing each sample volume in milliliters over the time in minutes.
Measurements:

Then the important urinary constituents for calcium phosphate precipitation including electrolytes, calcium, phosphate, citrate, and pH were measured as explained in objective 1.

Analytic techniques:

Urine sodium and potassium were measured by flame photometry (Radiometer, FLM-3; London, ON, Canada); chloride was measured by electromimetic titration (Chloride meter, CMT 10; London Scientific Ltd., London, Ontario); osmolality was measured by freezing point depression (Advanced Instruments, Inc., Needham Heights, MA, USA); urea and creatinine in plasma and urine \[^{110}\] and vasopressin in plasma \[^{111}\] were measured as previously described.

Statistical analysis:

Results of daytime versus overnight urinary concentrations as well as excretion rate of sodium, calcium, citrate, total and divalent phosphate in addition to urine flow rate, urine creatinine excretion rate, and urine pH are reported as the mean ± SEM. Comparisons within individuals were done by paired t test. Again a P value that was < 0.05 was considered to be statistically significant.
RESULTS:

Daytime and overnight creatinine excretion rates in umol/min as well as daytime and overnight urine flow rates in ml/min are illustrated in Figure 22. As can be seen excretion of creatinine was relatively constant over daytime and nocturnal periods (no statistical difference). On the other hand, overnight urine flow rate dropped significantly and reached to its trough average 0.6 ± 0.10 ml/min, in comparison to daytime urine flow rate average of 1.00 ± 0.04 ml/min (P < 0.05).

Figure 22. Daytime and overnight creatinine excretion rate (umol/min) vs. daytime and overnight urine flow rate (ml/min).
However, the urine sodium excretion rate significantly plunged to $54 \pm 16$ umol/min from its daytime average of $127 \pm 12$ umol/min ($P$ value $<0.05$). Even overnight urine sodium concentrations reduced somewhat to $122 \pm 16$ mmol/l from diurnal of $160 \pm 12$ mmol/l, but this reduction was not statistically significant (Figure 23).

Corresponding to the significant plunge urine sodium excretion rate overnight, urine calcium excretion dropped significantly from $2.4 \pm 0.2$ umol/min to its overnight average of $1.5 \pm 0.3$ umol/min ($P <0.05$) (Figure 23).

![Figure 23. Daytime and overnight urine sodium excretion rate (umol/min) in comparison to daytime and overnight urine calcium excretion (umol/min).]
Nevertheless, both overnight urine sodium and calcium concentrations reduced slightly, which were not significant statistically. Urine calcium concentration reduced somewhat from $3.0 \pm 0.3$ mmol/l daytime to $2.5 \pm 0.5$ mmol/l overnight (Figure 24).

Figure 24. Daytime and overnight urine sodium concentration (mmol/l) in comparison to daytime and overnight urine calcium concentration (mmol/l).
Corresponding to the overnight urine sodium excretion drop, the urine citrate excretion rate also significantly reduced to $2.4 \pm 0.2$ umol/min from a daytime average of $1.6 \pm 0.3$ umol/min ($P < 0.05$). However, citrate concentration remained almost the same (Figure 25).

Figure 25. Daytime and overnight citrate excretion rate (umol/min) vs. daytime and overnight urine citrate excretion concentration (mmol/l).
A comparison of corresponding daytime or overnight calcium and citrate concentration showed that the average daytime urine calcium concentration (3.0 ± 0.3 mmol/l) was not higher than citrate concentration (3.1 ± 0.5 mmol/l), meaning that the urine free ionized calcium was almost zero. This parity of concentrations of citrate (3.0 ± 0.4 mmol/l) and calcium (2.5 ± 0.5 mmol/l) were even more apparent overnight, indicating the estimated free calcium of zero (Figure 26).

Figure 26. Daytime and overnight comparison of urine calcium (Ca) and citrate (Cit) concentrations (mmol/l).
Surprisingly, following a significant reduction of urine flow rate overnight, urine phosphate excretion rate did not reduce significantly; therefore, overnight total urine phosphate concentration rose significantly (daytime average of 18.7 ± 1.4 umol/min versus nocturnal average of 20.9 ± 1.7 umol/min) (Figure 27).

Figure 27. Daytime and overnight urine phosphate excretion (umol/min) vs. daytime and overnight urine total phosphate concentration (mmol/l).
Notwithstanding, overnight urine pH was slightly lower (statistically non-significant) at an average of 5.9 ± 0.1 versus daytime average of 6.1 ± 0.1 (Figure 28).

Utilizing urine pH in each collection, divalent phosphate (HPO$_4^-$) was calculated in each subject. The average concentration of nocturnal divalent phosphate was compared to the daytime divalent phosphate, which was very similar (4.9 ± 0.9 versus the daytime average of 4.9 ± 1.0) (Figure 28).

Figure 28. Daytime and overnight urine pH vs. daytime and overnight urine divalent phosphate (mmol/l).

General Discussion:
This study clearly showed that in healthy subjects, when urine flow rate approaches its nadir overnight, ionic product of calcium and divalent phosphate would not increase to cause supersaturation. Nocturnal urine flow rate is significantly lower mainly because of lower sodium excretion. This can allow a restful sleep without any urge to wake up for urination. Furthermore, calcium excretion rate falls significantly overnight, in parallel with sodium excretion, preventing nocturnal calcium concentration from increasing, despite a lower urine flow rate. Even though citrate excretion is lower overnight, citrate concentration remains above calcium concentration, therefore, free ionic calcium would be negligible. Interestingly, total phosphate excretion does not fall overnight and therefore, total phosphate concentration increases significantly. Nevertheless, since urine pH drops overnight (by change in proportion of monovalent to divalent phosphate) the concentration of divalent phosphate does not rise appreciably overnight.

Furthermore, the analysis of the pattern of excretions of the individual urine osmoles overnight shows that approximately half of osmoles would be urea and the other half would be electrolytes. As shown in Figure 16 (page 58), there is little variation in the excretion of urea throughout the 24-hour cycle, but there is a lower electrolyte excretion rate overnight. The important issue that needs to be addressed is; why is overnight salt excretion rate so low, if most of our salt intake usually occurs in the evening?\[113\]

Our central blood volume is likely to be highest in the overnight period, because this follows the meal with our largest intake of sodium chloride, and also because we are no longer in an upright position. Based on this fact alone, the rate of excretion of sodium should be highest in the overnight period. Nevertheless, the signal to excrete sodium is related more directly to pressure than to central
blood volume (Figure 15). Therefore, it appears that the signal for the renal excretion of sodium is not simply due to an increased central blood volume; perhaps what is sensed is not a rise in volume, but a rise in central venous pressure. A possible explanation for the decrease in ‘pressure’ is that adrenergic stimulation, which increases venous tone, is lower during sleep. This lower excretion of sodium in the overnight period will permit undisturbed sleep, because it will slow the filling of the urinary bladder. [113,122]

In essence, lower free ionized calcium and divalent phosphate is a consequence of the following main mechanisms:

1. The fall in central pressure overnight, which lowers the sodium excretion rate and therefore calcium excretion rate
2. The low urinary pH overnight, which changes the proportion of divalent to monovalent phosphate significantly

These two issues will be discussed further.

Physiologic circadian variation of blood pressure:

Blood pressure varies over a 24 hour period with a peak during the day and nadir during the night. [125] The decline in BP during sleep requires major physiological adjustments. Intuitively, assuming the supine posture increases preload which would logically increase cardiac output and BP. However, BP falls during sleep due to a coordinated and active switching off of autonomic activity in part evoked by baroreflex adjustments. [124,125]

Lower urine pH overnight:
The pH of a solution is dependent on two factors, the rate of addition of free H\(^+\) and the availability of acceptors (A) that can bind H\(^+\) at the pH of that solution (proper pKa). Applying this principle, one can deduce that there are two groups of causes for a low urine pH. First, there may be a higher rate of H\(^+\) secretion in the distal nephron. Second, there may be diminished availability of acceptors for H\(^+\) in the lumen of the collecting duct. The common acceptors of H\(^+\) in tubular fluid are NH\(_3\) and HCO\(_3^-\). Since at typical urine pH of 6, virtually there is no HCO\(_3^-\), the main urinary acceptor is NH\(_3\). \(^{[113]}\)

Measuring the rate of the acceptor (usually NH\(_4^+\)) excretion would separate these two possible etiologies. Acceptor excretion should be high if distal H\(^+\) secretion is elevated, but low if acceptor availability is low. Therefore, to have a lower overnight urine pH the excretion of acceptor is expected to be relatively low and or the H\(^+\) secretion in the distal nephron should relatively be high. The other possible cause of the lower urine pH overnight is usually because of the higher excretion of sulfate overnight. \(^{[113, 114]}\)

Figure 29. Causes for a low urine pH. A low urine pH could be due to high activity of H\(^+\) secretion in the distal nephron; or a low entry of H\(^+\) acceptor.
Conclusion:

Decrease in urine flow rate overnight is essential to have a restful sleep. This is mainly due to a decrease in osmole excretion rate (mainly a decrease in sodium excretion rate). Since, both urine flow rate and calcium excretion reduce; therefore, there is no significant change in calcium concentration overnight. Furthermore, in healthy subjects, citrate concentration remains higher than calcium mutually overnight and during the day. Even though total phosphate concentration rises significantly, because of the fall in urine pH overnight, there is no significant change in divalent phosphate concentration. In conclusion, even though urine flow rate is lower overnight to allow restful sleep, there is no evidence that risk of calcium phosphate precipitate formation will increase.
INCREASED FLUID INTAKE STUDY

Objective:

To determine the effect of increased oral fluid intake on urine flow rate and urinary constituents’ concentration involved in calcium phosphate precipitation.

Study Specific Background:

The standard recommendation (suggested by many stone prevention clinics) for recurrent stone formers to prevent recurrence of kidney stones is consumption of generous amounts of fluid approximately 2.5 to 3 liters of water per day. The main idea is to dilute sparingly soluble urinary constituents and thereby prevent their supersaturation and their potential precipitate formation.\textsuperscript{[21,130]}

Nevertheless, despite increased oral fluid intake, significant proportion of the recurrent stone former patients continue to present with kidney stones, indicating the inconsistency of this paradigm.\textsuperscript{[22]} To scrutinize this paradigm, the key question that needs to be answered is whether drinking 2.5 to 3 liters of water, whether gulped (swallowed quickly) intermittently or sipped continuously, could maintain a constant and even high urine flow rate and dilute the urinary lithogenic substances, or if it only provides intermittent transient periods of high urine flow rates, followed by long periods of low flow rates. Our previous study has shown that gulping (swallowing quickly) large amounts of water 10ml/kg would provide a sudden and transient rise in the urine flow rate.\textsuperscript{[126]} Obviously, to be able to evaluate this paradigm, short collections of urine is needed. Furthermore, to understand the paradigm, it is essential to apply our prior study finding that the main signal leading to a water diuresis is a large fall in the arterial plasma sodium.\textsuperscript{[19, 24]} Nonetheless, the other important question that needs to be answered is whether this signal (large fall in the arterial plasma sodium) can be kept activated constantly.
Rationale:

Gulping significant amounts of water can cause sudden brisk water diuresis, followed by sudden drop in the urine flow rate. Although, by sipping, water will initially be retained until it suppresses the release of Anti-diuretic hormone (ADH). When this happens, there will be a period of brisk water diuresis. Following by brisk water diuresis urine flow rate will fall. In this regard, if the urine sample is collected over a 24-hour period, it would contain a mixture of concentrated and diluted urine and it would not reflect the risk of precipitate formation very well.

Hypothesis:

Sipping 2ml of water per minute can induce water retention initially but if water consumption continues, periods of brisk water diuresis will follow. Although the average UFR would be 2 ml/min, this does not reflect UFR at any individual time. In order to demonstrate this fact we need many shorter collections.

Material and methods:

Seventeen healthy subjects, 11 male and 6 female initially provided two diurnal collections. On the day of the sipping experiment, each subject started with an early morning void followed by drinking 2ml/kg water. Then they sipped 2 ml of water per minutes (120 ml per hour) over the next five hours. Urine samples were collected as frequently as subjects were able to provide them. During the experiment food was withheld to prevent the effect of food and its solutes on the ADH release and therefore on the urine flow rate. The subject recorded the time and volume of each specimen, and a
small sample was kept under refrigeration for analysis at a later time. Urine measurements were compared with each subjects’ baselines diurnal urine collection.

**Subjects:**

The Research Ethics Board of St. Michael’s Hospital approved the protocols described herein. Volunteers were in good health and did not take any medication in the week prior to study.

**Procedures:**

Urine was obtained by voluntary voiding; the time and volume of each sample were recorded. The rate of excretion of creatinine was used to assess completeness of collection. The subjects’ urine flow rate was calculated in each collection by dividing each sample volume in milliliters over the time in minutes.

**Measurements:**

The important urinary constituents for calcium phosphate precipitation including electrolytes, calcium, phosphate, citrate, and pH were measured.

**Analytic techniques:**

Urine sodium and potassium were measured by flame photometry (Radiometer, FLM-3; London, ON, Canada). Urine chloride was measured by electromimetic titration (Chloride meter, CMT 10; London Scientific Ltd., London, Ontario). Urine osmolality was measured by freezing point depression.
(Advanced Instruments, Inc., Needham Heights, MA, USA, and urea and creatinine in urine were measured as previously described. [110]

**Statistical analysis:**

Results of baseline versus sipping period urinary concentrations as well as excretion rate of calcium, Citrate, total and divalent phosphate in addition to urine flow rate, urine creatinine excretion rate, and urine pH are reported as the mean ± SEM. Comparisons within individuals were done by paired t test. Again a P value that was < 0.05 was considered to be statistically significant.
RESULTS:

Studies were performed in 6 female and 11 male volunteers. Figure 30 illustrates the subjects’ creatinine excretion to confirm accuracy of collections. Horizontal axis represents time in hour and vertical axis represents creatinine excretion in umol/min. Blue diamonds are mean ± SEM of different voids during sipping period. The yellow rectangles are indicators of average length of each urination. As can be seen, there was no significant change in creatinine excretion throughout the sipping period.

Figure 30. The urine creatinine excretion rate (umol/min) during sipping period. Different time of the urination is on the horizontal axis and the creatinine excretion on the vertical axis. The yellow rectangles are indicators of average length of each urination.
Figure 31 illustrates the subjects’ urine flow rate. Horizontal axis represents time in hour and vertical line is representing urine flow rate in ml/min. Blue diamonds are mean ± SEM of different UFR in each void during sipping period. The yellow rectangles are indicators of average length of each urination (Figure 31). Urine flow rate increased significantly from 0.8 ± 0.07 ml/min to an average of 2.88 ± 0.44 ml/min over a period lasting 45 minutes and then it fell in the next 105 minutes to an average of 0.93±0.25 ml/min (very close to baseline UFR) (P < 0.05).

Figure 31. The urine flow rate (ml/min) during sipping period. Different time of the urination is on the horizontal axis and the urine flow rate is on the vertical axis. The yellow rectangles are indicators of average length of each urination. Three paired T tests between brief period of high urine flow rate (# sign) and each period of low urine flow rate (* sign) were significant (P < 0.05).
Figure 32 illustrates changes in calcium concentration during sipping period. Horizontal axis represents time in hour and vertical axis represents calcium concentration in mmol/l. Orange diamonds are mean ± SEM of different concentration in each void during sipping period. Again the yellow rectangles are indicators of average length of each urination (Figure 32). Urine calcium concentration dropped notably from 2.50 ± 0.40 mmol/l over a period lasting 45 minutes and then it rose in the next 105 minutes to an average of 3.00 ± 1.00 ml/min (P < 0.05).

Figure 32. The urine calcium concentration (mmol/l) during sipping period. Different time of the urination is on the horizontal axis and the calcium concentration is on the vertical axis. The yellow rectangles are indicators of average length of each urination. Three paired T tests between brief period of low calcium concentration (# sign) and each period of high calcium concentration (* sign) were significant (P < 0.05).
Figure 33 illustrates changes in urine citrate concentration during sipping period. Horizontal axis represents time in hour and vertical axis represents citrate concentration in mmol/l. Green diamonds are mean ± SEM of different concentration in each void during sipping period. Again the yellow rectangles are indicators of average length of each urination (Figure 33). Urine citrate concentration changed appreciably from 2.70 ± 0.50 mmol/l to an average of 1.2 ± 0.20 mmol/l over a period lasting 45 minutes and then it rose in the next 105 minutes to an average of 3.20 ± 0.80 ml/min (P < 0.05).

Figure 33. The urine citrate concentration (mmol/l) during sipping period. Different time of the urination is on the horizontal axis and the citrate concentration is on the vertical axis. The yellow rectangles are indicators of average length of each urination. Three paired T tests between brief period of low citrate concentration (# sign) and each period of high citrate concentration (* sign) were significant (P < 0.05).
Figure 34 illustrates changes in the difference between calcium and citrate during sipping period, which roughly can indicate free calcium concentration. Horizontal axis represents time in hour and vertical axis represents calcium minus citrate concentration in mmol/l. Brown diamonds are mean ± SEM of different concentration in each void during sipping period. Again the yellow rectangles are indicators of average length of each urination (Figure 34). As can be seen urine calcium minus citrate concentration did not changed significantly throughout this period.

Figure 34. The urine calcium minus citrate concentration (mmol/l) during sipping period. Different time of the urination is on the horizontal axis and the urine calcium minus citrate concentration is on the vertical axis. The yellow rectangles are indicators of average length of each urination. Three paired T tests between baseline calcium minus citrate concentration and other periods of calcium minus citrate concentration were not significant (P > 0.05).
Figure 35 illustrates changes in urine total phosphate concentration during sipping period. Horizontal axis represents time in hour and vertical axis represents total phosphate concentration in mmol/l. Brown diamonds are mean ± SEM of different concentration in each void during sipping period. Again the yellow rectangles are indicators of average length of each urination (Figure 35). Urine total phosphate concentration changed considerably from 17.40 ± 2.40 mmol/l to an average of 7.2 ± 1.10 mmol/l over a period lasting 45 minutes and then it fell in the next 105 minutes to an average of 14.84 ± 4.80 ml/min (P < 0.05).

Figure 35. The urine total phosphate concentration (mmol/l) during sipping period. Again different time of the urination is on the horizontal axis and the phosphate concentration is on the vertical axis. The yellow rectangles are indicators of average length of each urination. Three paired T tests between brief period of low total phosphate concentration (# sign) and each period of high total phosphate concentration (* sign) were significant (P < 0.05).
Figure 36 illustrates changes in the urine pH during sipping period. Horizontal axis represents time in hour and vertical axis represents urine pH. Blue diamonds are mean ± SEM of different urine pH in each void during sipping period. Again the yellow rectangles are indicators of average length of each urination (Figure 36). As can be seen, the urine pH progressively increased but the changes were not statistically significant from $5.9 \pm 0.20$ to $6.50 \pm 0.40$ throughout this period.

Figure 36. Urine pH during sipping period. The yellow rectangles are indicators of average length of each urination. Three paired T tests between baseline urine pH and other period’s urine pH were not significant ($P > 0.05$).
Figure 37 illustrates changes in the urine divalent phosphate concentration during sipping period, which roughly can indicate free calcium concentration. Horizontal axis represents time in hour and vertical axis represents calcium minus citrate concentration in mmol/l. Brown diamonds are mean ± SEM of different concentration in each void during sipping period. Again the yellow rectangles are indicators of average length of each urination (Figure 34). As can be seen, the urine divalent phosphate concentration did not change appreciably throughout this period.

Figure 37. Urine divalent phosphate concentration (mmol/l) during sipping period. Different time of the urination is on the horizontal axis and divalent phosphate concentration is on the vertical axis. The yellow rectangles are indicators of average length of each urination. Three paired T tests between baseline divalent phosphate concentration and other periods of divalent phosphate concentration were not significant (P > 0.05).
Figure 38 illustrates changes in the free calcium concentration (difference between calcium and citrate) * divalent phosphate product during sipping period. Horizontal axis represents time in hour and vertical axis represents calcium minus citrate concentration in mmol/l * divalent phosphate product in mmol/l. Brown diamonds are mean ± SEM of different concentration in each void during sipping period. Again the yellow rectangles are indicators of average length of each urination (Figure 38). As can be seen urine calcium minus citrate concentration * divalent phosphate product did not changed significantly throughout this period.

Figure 38. Urine free calcium and divalent phosphate product (mmol²/l² during sipping period. Different time of the urination is on the horizontal axis and the free calcium (calcium minus citrate) times divalent phosphate concentration is on the vertical axis. The yellow rectangles are indicators of average length of each urination. Three paired T tests between baseline free calcium * divalent phosphate concentration and other periods of free calcium * divalent phosphate concentration were not significant (P > 0.05).
General discussion:

Our study confirms that after sipping water, urine flow does not remain constantly high; rather it increases and causes brisk water diuresis after 2-3 hours, followed by a sudden appreciable drop in urine flow rate. Corresponding statistically significant changes were observed in the urine concentration of calcium, citrate, and total phosphate in addition to non-significant changes of urine pH and urine divalent phosphate. In this study only the changes in urine flow rate were significant and the rest of the changes were not statistically significant, nevertheless it confirms periodic and transient changes in concentrations of lithogenic substances and inhibiting factors like citrate. Diurnal variations of excretion of lithogenic substances and inhibiting factors could add to the physicochemical complexity of precipitation risk. Furthermore, as it has been discussed before, changes of urine pH can cause dramatic change in proportion of monovalent to divalent phosphate (Table 10, page 137).

Our previous study [126] revealed that the signal perceived by the hypothalamic water control system was a significant fall in the arterial plasma sodium. Furthermore, we clearly showed that gulping significant amounts of water around 5-10ml per kilogram always cause a brisk water diuresis followed by significant drop in urine flow rate. Nevertheless, when the same amount of water load was consumed slowly, initially it can be retained for future sweat, however, eventually can cause a water diuresis.

In fact, four factors can influence the ability of an oral water load to lower circulating vasopressin levels sufficiently to induce a water diuresis. First, there are events that occur before water enters the systemic circulation. Second, enough water must enter cells of the central osmostat to suppress the release of vasopressin. Third, nonosmotic stimuli for the release of vasopressin and V2 receptor up-regulation must be taken into account. Fourth, an increased ability to shift water rapidly into
muscle cells can decrease the degree of fall in the arterial plasma sodium and thereby the ability of a given water load to induce a water diuresis.\textsuperscript{126}

1. Events acting prior to the absorption of water

Oropharyngeal factors may cause a rapid small suppression of the release of vasopressin independent of arterial plasma sodium or effective osmolarity. This fall mainly is influenced by the temperature of the ingested fluids.\textsuperscript{129} In addition, the rate of water absorption from the gastrointestinal tract depends on the rates of water ingestion, stomach emptying, and factors such as poorly absorbed solutes in this fluid, which could influence the rate of absorption of water in the small intestine. In our previous study, the ingestion of sodium chloride and fructose, but not glucose, delayed the water diuresis.\textsuperscript{126}

2. Degree of cell swelling in the central osmostat

Again our previous study has confirmed that a large water diuresis was consistently seen following the ingestion of 20mL of water per kg in <15 minutes presumably because this caused a sufficient degree drop in arterial plasma sodium. There are a number of factors that can influence the arterial plasma sodium after water is ingested. For example, the content of electrolytes in the lumen of the stomach or small intestine prior to the ingestion of water can influence the amount of osmole-free water.

Another factor that may prevent constant urine flow rate is a strong aversion to drink after the ingestion of significant water load developed \textsuperscript{130}. Secondly, after a big water load, 30 minutes after water intake had stopped, almost 50% of subjects were complaining of thirst despite the retention of approximately 50% of the positive water balance. The possible mechanism for thirst has been explained
as a rise in the renal venous plasma sodium and thereby in arterial blood due to an ongoing water diuresis when water absorption from the intestinal tract had declined. [126]

3. Nonosmotic stimuli for the release of vasopressin and V₂ receptor levels

With a large non-osmotic stimulus, such as pain and anxiety for the release of vasopressin [97], a water diuresis might not occur despite achieving a low arterial plasma sodium. Furthermore, subjects with a higher intake of sodium might require a larger positive water balance to suppress the release of vasopressin sufficiently to cause a water diuresis. [126]

4. Ability to shift water rapidly into muscle cells

The skeletal muscle mass of a 70 kg adult is approximately 24 kg and its relative blood supply at rest is approximately 1 L/min, therefore the capacity to draw the extra water from the arterial plasma into muscle cells will not be saturated at early times. More ingested water could be ‘stored’ quickly in muscle cells, with a smaller early fall in the arterial plasma sodium for a given positive water balance. This water becomes ‘occult’ to the central osmostat, and it would be less able to induce a water diuresis. [126,136]

Explanation of the sequence of the events after water load:

Following water ingestion in the first phase, water will be absorbed from the gastrointestinal tract and it can be retained as long as it would not cause a significant fall in arterial plasma sodium.
However, if one continues to ingest water, in the second phase, eventually it can cause a significant fall in arterial plasma sodium. This will cause ADH suppression and brisk water diuresis.

Figure 39. First phase in sipping water

Figure 40. Second phase of sipping water
Further explanation of in a different illustration, in the initial phase, water will be absorbed from the gastrointestinal tract; yet, it can be retained as long as it would not cause a significant fall in arterial plasma sodium.

Figure 41. First phase of sipping water (ADH On), the amount of water added by gastrointestinal tract is not adequate to fall arterial plasma sodium.
Figure 42. Second phase of sipping water (where ADH is turned off), the amount of water added by the gastrointestinal tract is larger than the amount of salt added by the kidney to the venous pool.
Figure 43. Third phase of sipping water, where ADH is turned back on, the amount of water added by the gastrointestinal tract is smaller than the amount of salt added by the kidney to the venous pool.

CONCLUSION

The signal perceived to initiate a water diuresis is closely related to an initial fall in the arterial plasma sodium. Hence, almost all collected urine samples’ osmolarity were above plasma osmolarity. The normal water control system is poised for water retention, and rarely for water excretion. [126] Ingested water could be initially retained if it is consumed slowly but eventually will cause brisk water diuresis as it suppresses arterial plasma sodium. Following suppressing ADH and water diuresis, the kidney will excrete free water and add sodium to the renal vein. In summary, it seems that increased water intake might not be successful in diluting urine evenly and preventing precipitate formation. This needs to be evaluated further in kidney stone former patients.
ADDITIONAL LITRATURE REVIEW

URINARY COMPOSITION:

Background:

Human body cellular metabolisms generate numerous waste compounds, many of them rich in nitrogen that require elimination from the bloodstream and are not volatile as to be exhaled through the lungs. Additionally, the surplus of ingested fluid, electrolytes, and minerals must be excreted to maintain body equilibrium. Urine is composed of all of the body’s daily waste products excreted by the kidneys. In essence, from the body perspective urine should contain all daily waste products to maintain balance, though; all these materials should be excreted safely, and kept water-soluble from the urine perspective to prevent any precipitation, crystallization or possible kidney stone formation.

Despite detailed metabolic evaluation, the detection of individuals with an enhanced risk of precipitate is often challenging. Although many patients with calcium urolithiasis excrete more lithogenic which they are sparingly water soluble such as calcium, oxalate, and phosphate … and less stone-inhibiting factors like citrate, and magnesium… in urine than the healthy individuals. However, there is no single urinary parameter able to discriminate lithogenic and stone inhibiting factors sufficiently, partially because stone formers constitute an extremely heterogeneous group with respect to urolithiasis etiology. In terms of metabolic factors, typical lithogenic abnormalities, such as hypercalciuria, hyperoxaluria, or hypocitraturia, occur in different proportions and only in
a portion of all patients as measured by 24 hour urine collections. Therefore, some recurrent stone-formers have no obvious predisposing factors that can be detected by the standard metabolic evaluation techniques. In contrast, many hypercalciuric individuals, never develop kidney stones. These inconsistencies indicate the complexity of physicochemical and biochemical processes as well as the inadequacy of our metabolic assessment of lithogenesis. A variety of risk formulas have been proposed to estimate the metabolic derangement in urine composition causing stone formation. The aim of this research study is to determine the basic metabolic disorder causing calcium phosphate precipitation, by assessing precipitation risk at any given time in different parts of urinary tubules or interstitium.

Kidney stones form as a consequence of tubular or interstitial supersaturation. Supersaturation occurs as a result of elevated concentrations of urinary solutes. Many different processes alter urine flow rate or solute excretion rate and thereby urinary solute concentrations at any given time. In this chapter a review of urine flow rates and factors involved in the activities of solutes causing supersaturation will be discussed.

**URINE FLOW RATE:**

To prevent supersaturation urine volume must be large enough and excessive oliguria must be avoided to decrease the risk of precipitation of poorly soluble constituents in the urine. Nevertheless, preserving effective circulatory volume is essential; therefore, control of the rate of water excretion is important for survival. When water must be conserved, the antidiuretic hormone (ADH) increases the permeability for water in the distal part of the kidney, the late distal convoluted tubule, the cortical collecting duct, and throughout the medullary collecting duct.
The effect of volume on supersaturation is amplified by the fact that it would affect both free calcium and free divalent phosphate concentrations. To illustrate the importance of urine volume, the concentrations of Ca\(^{2+}\) and phosphate are assigned values of X mmol/l and Y mmol/l, respectively, at a urine flow rate of 1.2 ml/min. Their ion products rise 4-fold and 16-fold when the urine flow rate halves to 0.6 ml/min and halves again to 0.3 ml/min despite the fact that the excretion rates for these ions are constant.\textsuperscript{[22]}

<table>
<thead>
<tr>
<th>Flow rate (ml/min)</th>
<th>Ca(^{2+}) (mmol/L)</th>
<th>HPO(_4^{2-}) (mmol/L)</th>
<th>(\text{Ca}^{2+} \times \text{HPO}_4^{2-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>X</td>
<td>Y</td>
<td>XY</td>
</tr>
<tr>
<td>0.6</td>
<td>2-X</td>
<td>2-Y</td>
<td>4-XY</td>
</tr>
<tr>
<td>0.3</td>
<td>4-X</td>
<td>4-Y</td>
<td>16-XY</td>
</tr>
</tbody>
</table>

To maintain the minimum urine flow rate, when ADH is acting the number of ‘effective’ osmoles must not be too low in the urine. This is not an issue while consuming a sufficient amount of NaCl. On the other hand, oliguria may occur if the urine contains few electrolytes. Urea is the major urine osmole, and under normal circumstances, when ADH acts it continues to be very permeable in the inner medulla, it remains as an ‘ineffective’ osmole. Notwithstanding, it has been found that the inner medullary collecting duct is not sufficiently permeable to urea when
vasopressin acts if few electrolytes are excreted as there is a large concentration difference for urea (higher in the lumen than in the renal papilla) as demonstrated in the rat on a low salt diet \[88\]. Hence urea becomes an ‘effective’ osmole in the urine in this setting. This strategy depends on having a rate of excretion of an extra 300 ‘effective’ mOsmoles per day (0.3 liter * 1000 mOsm/L), a value similar to the usual rate of excretion of urea. The mechanism could be due to internalization of urea transporters, an effect that is mediated by Protein Kinase A (PKA). \[22\]

Equation 2. Urine Flow Rate = \frac{\text{Number of excreted effective osmoles over time (mOsm/min)}}{\text{Urine effective Osmolarity (mOsm/l)}}

SOLUTES INFLUENCING URINARY SUPERSATURATION:

The multiple factors influencing urinary supersaturation in a clinical setting are shown in Table 3. The renal excretion of calcium salts that precipitate and take part in stone formation in addition to urine flow rate are primary determinants of urinary supersaturation in calcium containing stones. Thus, urinary volume, calcium, oxalate, and phosphate ions all participate in the risk of calcium precipitation. In addition, urine pH significantly changes the ratio of mono to divalent phosphate (CaHPO\(_4^2\)-) and has great importance in regulating saturation (See table 7). This is one of the reasons why hypercalciuria, oxaluria, unduly alkaline urine, and low urinary volumes are not sufficient to explain urinary precipitation and stone formation at all times. Binding of the components of calcium salts also complicates the measurement of urine saturation, and simple concentration measurements give only small clues to actual free ion activity products. \[61\]
Alternative divalent cations to calcium may be considered as inhibitors of urinary supersaturation. These contribute to the ability of urine to hold salts in a solution to a much greater extent than does a simple aqueous solution. The known inhibitors of urinary saturation include the divalent cation magnesium, which forms oxalate, and phosphate salts, which are more soluble compared to those of calcium. In addition, citrate and sulfate are anions that calcium forms soluble complexes as alternatives to phosphate or oxalate. Urine also contains substances to which calcium binds, thereby reducing the free ion activity. Pyrophosphate, nephrocalcin, and osteopontin are other inorganic and organic crystal inhibitory calcium-binding sites, and are discussed in greater detail later in the stone formation inhibitors and chelating agents sections. In addition, certain substances to which calcium salts may bind to actually promote precipitation like uric acid and sodium urate, which I will not address in this dissertation.
Measurements of urinary supersaturation

Since simple concentration measurements do not provide adequate information regarding the activity of specific ions in urine, several strategies have been designed to estimate urinary supersaturation. These approaches are computer-based calculations of urinary free ion activity for calcium, oxalate, and phosphate derived from their concentrations and their known tendencies to form soluble complexes with each other and with other ligands such as citrate and sulfate.\[61]\n
The effect of ionic strength on ionic interaction:

The ionic strength of a solution is a fraction of the concentration of ions in that solution. In other words, ionic compounds, when dissolved in water, dissociate into ions and they would have a certain ionic strength depending on the concentration and valence of ions. Accordingly, the ionic strength (I), of this solution would be a function of the molar concentration of all ions present in a solution.\[63, 64]\n
**Equation 3.**

\[
I_c = \frac{1}{2} \sum_{B=1}^{n} c_B z_B^2
\]

Where \( c_B \) is the molar concentration of ion B (mol/l), \( z_B \) is the charge number of that ion, and the sum is taken over all ions in the solution. For a 1:1 electrolyte such as sodium chloride, the ionic strength is equal to the concentration, but for MgSO\(_4\) the ionic strength is four times higher. Generally multivalent ions contribute strongly to ionic strength. This equation demonstrates that ionic strength is calculated as the squared sum of all ions in the solution. The concentration metric used is usually the molar concentration (mol/l), but actually the most accurate is the molal concentration (mol/kg). In most cases it is easier to use molarities and usually the difference is insignificant. For example the ionic strength of a solution of 5.0 mmol/l Na\(_2\)SO\(_4\) and 2.0 mmol/l NaCl is.\[64]\n
Equation 4. \[ I_c = \frac{1}{2}(2 \times (+1)^2 \times 0.0050) + (+1)^2 \times 0.0020 + (-2)^2 \times 0.0050 + (-1)^2 \times 0.0020 = 0.017 \text{ M} \]

Table 7. Typical Urinary Composition and Calculated Ionic Strength

<table>
<thead>
<tr>
<th>Typical Urinary Composition</th>
<th>Concentration mmol/l</th>
<th>Valence</th>
<th>Concentration *(Valence)^2/1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>300</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Na</td>
<td>150</td>
<td>+1</td>
<td>150</td>
</tr>
<tr>
<td>Cl</td>
<td>150</td>
<td>-1</td>
<td>150</td>
</tr>
<tr>
<td>K</td>
<td>60</td>
<td>+1</td>
<td>60</td>
</tr>
<tr>
<td>NH₄</td>
<td>40</td>
<td>+1</td>
<td>40</td>
</tr>
<tr>
<td>HPO₄</td>
<td>3</td>
<td>-2</td>
<td>12</td>
</tr>
<tr>
<td>H₂PO₄</td>
<td>27</td>
<td>-1</td>
<td>27</td>
</tr>
<tr>
<td>SO₄</td>
<td>20</td>
<td>-2</td>
<td>80</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.4</td>
<td>-2</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca</td>
<td>4</td>
<td>+2</td>
<td>16</td>
</tr>
<tr>
<td>Citrate</td>
<td>4</td>
<td>-3</td>
<td>27</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2</td>
<td>+2</td>
<td>8</td>
</tr>
<tr>
<td>Ionic strength</td>
<td></td>
<td></td>
<td>= 0.572</td>
</tr>
</tbody>
</table>

Table 7 demonstrates an example of typical urine constituents and calculated ionic strength. Ionic strength plays a central role in ionic activities. As it has been explained before, ionic activities, rather than simple concentrations, are needed in many chemical calculations because solutions that contain ionic solutes do not behave ideally even at very low concentrations. The activity is proportional to the concentration by a factor known as the activity coefficient \( \gamma \), and takes into account the interaction energy of ions in the solution. The ionic activity coefficient depends on the ionic strength of the solution (Debye–Hückel theory, which describes the strong deviations from ideality typically encountered in ionic solutions). The Debye-Hückel limiting law enables one to determine the activity coefficient of an ion in a dilute solution of known ionic
strength. It is important to note that because the ions in the solution act together, the activity coefficient obtained from this equation is actually a mean activity coefficient. \([63, 64]\)

Ionic strength is also important for the theory of double layer and related electrokinetic and electroacoustic phenomena in colloids and other heterogeneous systems. That is, the Debye length, which is the inverse of the Debye parameter (\(\kappa\)), is inversely proportional to the square root of the ionic strength. Debye length is characteristic of the double layer thickness. Increasing the concentration or valence of the counterions compresses the double layer and increases the electrical potential gradient. \([64]\)

Urine milieus with high ionic strength are used in stability constant determination in order to minimize changes, during a titration, in the activity quotient of solutes at lower concentrations. Natural waters such as seawater have a non-zero ionic strength due to the presence of dissolved salts which significantly affects their properties.

**Ion-activity of Ca\(^{2+}\) and HPO\(_4^{2-}\) product:** The ion-activity product of CaP was estimated by means of the AP(CaP)-index \([65]\):

\[
2.7 \times 10^{-3} \times \text{Ca}^{1-07} \times \text{PO}_4^{0.70} \times (\text{pH} - 4.5)^{6.8} = \frac{\text{CaP}_{\text{ia}}}{\text{Citr}^{0.20} \times \text{V}^{1.31}}
\]

**Equation 5.** \(\text{CaP}_{\text{ia}}\)
**Ksp of Hydroxyapatite and Fluorapatite:**

Reported values for the solubility products of these salts scatter over quite large ranges. The reasons for this are complex and not fully understood. There is, however, general agreement that the value for fluorapatite is lower than that of hydroxyapatite. The solubility product (Ksp) is the ionic product when the system is in equilibrium. The values cited below are those of McDowell et al [66] and Moreno et al [67].

**Table 8. Reported solubility product for different urinary precipitates**

<table>
<thead>
<tr>
<th>Salt</th>
<th>Ionic Composition</th>
<th>Solubility Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brushite</td>
<td>Ca(HPO$_4$)$_2$.2H$_2$O</td>
<td>2.32*10$^{-7}$</td>
</tr>
<tr>
<td>Tricalcium diphosphate</td>
<td>Ca$_3$(PO$_4$)$_2$</td>
<td>2.83*10$^{-30}$</td>
</tr>
<tr>
<td>Octacalcium phosphate</td>
<td>Ca$_8$(HPO$_4$)$_2$(PO$_4$)$_4$.5$H_2$O</td>
<td>2*10$^{-49}$</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>Ca$_3$(PO$_4$)$_3$.OH</td>
<td>2.34*10$^{-59}$</td>
</tr>
<tr>
<td>Fluorapatite</td>
<td>Ca$_5$(PO$_4$)$_3$.F</td>
<td>3.16*10$^{-60}$</td>
</tr>
<tr>
<td>Calcium Oxalate hydrate</td>
<td>CaC$_2$O$_4$.H$_2$O</td>
<td>1.96*10$^{-8}$</td>
</tr>
<tr>
<td>Calcium hydrogen phosphate</td>
<td>Ca$_4$HPO$_4$</td>
<td>1*10$^{-7}$</td>
</tr>
</tbody>
</table>
Supersaturation of solutes:

Consider calcium phosphate crystals exposed to a solution with ample amount of calcium and phosphate dissolved in water, which is well mixed and at a stable temperature. The crystals have been bathed in the solution for a long time and neither grow nor shrink. When the system reaches equilibrium, the calcium and phosphate concentrations in the solution would not change, because the crystals are of a stable mass. The product of the free ionized calcium and phosphate concentrations in such a solution is called the equilibrium solubility product. (Figure 44) [68]
Now remove the crystals from the equilibrium system and raise the ionic activity product by adding calcium, phosphate, or both. In the absence of a solid phase, initially nothing appears to happen: the solution remains clear, free of crystals. A solution that will cause growth of preformed crystals, but not the appearance of new solid phase is called metastable. (Figure 45) [68]

Figure 45. Removing crystals and adding calcium, and phosphate. Without having crystals no precipitation would occur and the solution is supersaturated metastable.
By increasing the activity product sufficiently, the new crystals will appear. This point is often called the formation product, or the upper limit of metastability. Above the formation product, a solution is unstable, prone to creating new crystal nuclei. Urine may be undersaturated, metastable, or unstable with respect to calcium oxalate or the stone-forming calcium phosphate crystals. (Figure 46) \[68\]

\[
\begin{align*}
\text{H}_2\text{PO}_4^- & \quad \text{Ca}^{2+} & \quad \text{HPO}_4^{2-} & \quad \text{Ca}^{2+} \\
\text{HPO}_4^{2-} & \quad \text{Ca}^{2+} & \quad \text{H}_2\text{PO}_4^- & \quad \text{HPO}_4^{2-} \\
\text{H}_2\text{PO}_4^- & \quad \text{Ca}^{2+} & \quad \text{HPO}_4^{2-} & \quad \text{Ca}^{2+} \\
\text{Ca}^{2+}\text{HPO}_4^{2-} & \quad \text{Ca}^{2+}\text{HPO}_4^{2-} & \quad \text{Ca}^{2+}\text{HPO}_4^{2-} \\
\end{align*}
\]

**Figure 46.** By increasing the activity product sufficiently, the new crystals will appear eventually. This solution called the formation product, or the upper limit of metastability.
Activity Product Ratio and Formation Product:

In comparison of urine to any other aqueous solution, clearly urine is able to hold much higher levels of calcium salt in solution than water. If one considers an aqueous solution containing crystals of a calcium salt when the crystal neither grows nor shrinks, the solution is in equilibrium. The product of the free ion concentrations (activity product) at this equilibrium determines the equilibrium solubility product of the salt. Solutions with concentrations of salt less than the equilibrium solubility product are undersaturated. A higher free-ion activity product will cause the solid phase (the crystals) to grow (epitaxy). The activity products of calcium salts in urine are almost constantly in the range of metastable supersaturation. In the range of metastable supersaturation, if the activity products are sufficiently raised, new crystals will appear. The activity product at which new crystals form is called the formation product, or the upper limit of metastability. Above the level of the FP, a solution is unstable, creating new crystal nuclei. Urine may be undersaturated, metastably supersaturated, or unstable with respect to calcium oxalate or the stone-forming calcium phosphate crystals (brushite, octacalcium phosphate, hydroxyapatite, and apatite). However, most of the time, it is metastably supersaturated and, particularly for brushite, close to the FP. [61]

A calculated free ion activity product such as the calcium oxalate ion product, when divided by the corresponding equilibrium solubility product (SP), yields an activity product ratio (APR), which estimates the degree of saturation. [61]

**Equation 6.** Activity product ratio (APR) = \[ \frac{\text{Calculated Free Ia product (iCa}^{2+}\text{*Ox)}}{\text{Ca}^{2+}\text{*Ox Solubility Product}} \]
A ratio above 1 indicates urinary supersaturation. Ratios below 1 represent undersaturation. The upper limit of metastable supersaturation can be determined by raising the APRs to the point at which precipitation or solid phase formation begins to appear. The APR at this point is called the formation product ratio (FPR). [61]

**Observations of Urinary Supersaturation**

Numerous investigators with different approaches have accumulated evidences indicating that urine from stone formers is more supersaturated than normal. [61] As a result, stone formers, whether hypercalciuric or normocalciuric, had higher average values of urine saturation than did those who did not form stones. This held whether saturation was measured with respect to calcium oxalate, brushite, octacalcium phosphate, or hydroxyapatite. An important observation common to approaches both with and without the use of seed crystals is that activity products of normal urine, on average, are above the equilibrium SP (Figure 45) or oversaturated, except with respect to brushite. The urine measurements performed in 24-hour collections to assess supersaturation may be insufficient to reveal the full precipitation potential that exists in the renal tubule at any given moment. [61]

Detailed studies of urine activity product ratio (APR) at the limit of metastability, the (FPR) both for calcium phosphate and calcium oxalate showed urine is abnormally supersaturated in stone formers. The values of APRs lie close enough to the FPR, at least for calcium oxalate, so that new crystal formation would be expected. Most urine, even from those without stone formations, is metastably supersaturated with respect to calcium oxalate, so that growth of crystal nuclei into a significant mass is predictable. [61, 70]
Urine Supersaturation Profile:

Urine supersaturation profile is a calculation to estimate the biochemical risk for the formation of calcium oxalate, calcium phosphate (brushite), and uric acid calculi. This test predicts the formation of calcium oxalate, calcium phosphate, and uric acid calculi using concentrations of analytes measured in a 24-hour urine specimen. Measured individual components and calculated supersaturation of stone-forming complexes are displayed graphically to depict relative risk for calculi formation. Test results are used in the evaluation and management of renal stone disease. Analyte concentrations as a function of urolithiasis risk are plotted on a chart. Numbers at the far left and far right on each line of the chart provide a scale. Normal reference values fall in the middle of this scale. The values determined for this sample are placed on the scale to indicate the approximate risk associated with the particular concentrations. Increased risk is to the right of center, decreased risk to the left. Relative supersaturation calculated for calcium oxalate, calcium hydrogen phosphate, and uric acid calculi is displayed in a second section of the chart. Relative risk increases from the middle to the right side of this chart.\[^{[71]}\] The test flaw is that supersaturation profile was calculated in a mixture of voids in one 24 hour urine collection, which may not represent the supersaturation profile at the interstitium of the TAL of Henle in any short period of time to predict precipitate formation.

Role of urine electrolytes:

Numerous authors believe that increased sodium intake will promote nephrolithiasis by hypercalciuric effects of high sodium intake. However, equivocal data exist on whether increased urine sodium actually increases the nephrolithiasis risk. The relationship between urine sodium and urine risk factors for stone formation appears to be more complicated and requires further review.\[^{[72]}\]
The first scheme, limiting dietary intake of sodium to decrease hypercalciuria and prevent recurrent urine stones, has long been a standard recommendation of primary care physicians, nephrologists and urologists. This is based on physiological evidence that sodium and calcium transport are coupled in the renal proximal tubule. In other words, following sodium reabsorption in the proximal tubule, water will be absorbed and cause concentration gradient for calcium, causing calcium absorption. Also, the landmark prospective, a randomized study by Borghi et al of patients with idiopathic hypercalciuria showed a significantly decreased incidence of recurrent nephrolithiasis in patients on a low sodium, low animal protein diet vs. a normal sodium, low calcium diet. These data questioned the case for sodium restriction in patients with nephrolithiasis.

The remaining literature continues to be equivocal on the potential deleterious effects of sodium on urine stone risk. While in some studies increasing urine sodium excretion is associated with nephrolithiasis, others do not show a relationship between these two urine parameters. Several studies revealed an increase in urine calcium with increased salt intake in patients with and without a nephrolithiasis history. On the other hand, epidemiological studies in large cohorts of men and women did not show an increased risk of nephrolithiasis with increasing dietary sodium. Finally, although Borghi et al noted decreased stone recurrence in patients on a low sodium intake, and a low animal protein diet, the change in urine calcium excretion from baseline to year 5 was not different between the groups despite significant differences in dietary and urine sodium.

A study by Eisner et al showed that significant relationship between urine calcium and volume increased with increasing urine sodium (each p <0.01) but urine calcium oxalate supersaturation decreased with increasing urine sodium (p <0.01). Multivariate linear regression
was adjusted for age, sex, body mass index and urine constituents. Urine sodium excretion was positively associated with urine calcium excretion but negatively associated with urine calcium oxalate supersaturation. There was a trend toward a positive association of urine sodium and volume.\textsuperscript{[72]}

In summary, increasing urine sodium excretion does not appear to increase the risk of calcium nephrolithiasis. Global sodium restriction may not necessarily alter the risk of stone formation, for instance because of changes in calcium oxalate urine supersaturation, in patients with a history of nephrolithiasis.\textsuperscript{[72]}

Various studies have suggested that potassium depletion leads to intracellular acidosis and hypocitraturia. In Northeastern Thailand, for example, mild hypokalemia and mild hyperoxaluria are observed in most stone formers. However, there are limited reports about the direct link between different potassium salts depletion and the formation of urinary calcium stones. Moreover, the accompanied anion depleted with potassium could have a more important role than potassium per se. Normally potassium from fruits is usually in the form of potassium citrate and the source of potassium phosphate is meat. Yachantha et al\textsuperscript{[73]} in a study of seventy two rats found that potassium depletion caused a rapid decrease in the urinary concentrations of potassium, citrate, magnesium, and phosphorus (in rats citrate is the main anion ingested with potassium). There was no detectable renal damage, renal calcium deposition, and no significant increase of urinary oxalate or calcium. However, the urinary supersaturation index of calcium oxalate increased significantly in rats with potassium depletion. These findings indicate that potassium deficiency may increase the risk of stone formation through enhanced supersaturation.\textsuperscript{[73]} However, this study did not address the significance of accompanied depleted anion with potassium.
Role of urine urea:

Urea or carbamide is an organic compound with the chemical formula \((\text{NH}_2\text{)}_2\text{CO}\). The molecule has two amine (-\text{NH}_2) residues joined by a carbonyl (-\text{CO}-) functional group. Even though urea is one of the major ingredients of urine and contributes significantly to the osmolarity of the urine, it has received little attention in nephrolithiasis literature. Notwithstanding, urea's high aqueous solubility reflects its ability to engage in extensive hydrogen bonding with water. It is neither acidic nor alkaline. Walker & Hambly (1895) showed that the isomeric transformation of ammonium cyanate is formed spontaneously from urea in an aqueous solution into urea (Wohler, 1828) and was spontaneously reversible in aqueous solutions.\(^{[119]}\)

Equation 7: \((\text{NH}_2\text{)}_2\text{CO} \Leftrightarrow \text{NH}_4\text{CNO}\)^{[119]}

Hence urea minimally dissociates and ionized, theoretically it will have a negligible effect on the ionic strength of calcium or divalent phosphate. However, by virtue of its tendency to form porous frameworks, urea has the ability to trap many organic compounds and functions as a clathrate. The organic "guest" molecules are held in channels formed by interpenetrating helices comprising of hydrogen-bonded urea molecules. This behavior can be used to separate mixtures, e.g. in the production of aviation fuel and lubricating oils, and in the separation of paraffins.\(^{[1]}\)

Furthermore, urea has an important role in the countercurrent exchange system of the nephrons that allows for reabsorption of water and electrolytes from the excreted urine. Urea is reabsorbed in the inner medullary collecting ducts of the nephrons, thus raising the osmolarity in the medullary interstitium surrounding the thin ascending limb of the loop of Henle. By action of
the urea transporter 2, some of this reabsorbed urea will eventually flow back into the thin ascending limb of the tubule, through the collecting ducts, and into the excreted urine. This mechanism, which is controlled by the antidiuretic hormone, allows the body to excrete urine in with high osmolality. This mechanism is very important to preserve water and maintain effective circulatory volume. On the other hand, it is well known that urine can have a high osmolality and very few electrolytes when the effective circulatory volume is contracted. In this setting, urea is virtually the sole urinary solute. Moreover, when sodium and chloride are being conserved maximally in a water-deprived subject, there is a trade-off, that is, a little larger deficit of water, but a lesser likelihood of near-anuria and thereby renal stone formation because urea becomes an effective urinary osmole.

Soroka et al. showed that during water deprivation in normal subjects, the ingestion of urea caused a twofold rise in urine flow rate, a fall in the non-urea osmolality, and a rise in the rate of excretion of non-urea osmoles. The non-urea osmolality of the urine, and presumably the medullary interstitial fluid as well, was inversely related to the urea excretion rate. In chronic fasting, the nature, but not the quantity, of non-urea osmoles changed. The similar minimum urine volume was predictable from an analysis based on non-urea osmole considerations.

**ROLE OF URINE CALCIUM IN SUPERSATURATION:**

In order to prevent calcium supersaturation and precipitation in the urinary tubules or interstitium, calcium absorption should match water absorption in each area of the nephron (see figure 47). Hence, there is unmatched calcium to water absorption in the thick ascending limb of Henle’s (TAL), Calcium sensing receptor (CaSR) modulation of calcium absorption in tAL seems to be an appealing mechanism of prevention of a rise in interstitial calcium concentration and
protection against supersaturation. \cite{81} This will be discussed further after a brief overview of renal calcium handling and factors involved in supersaturation.

The average daily dietary intake of calcium, of which the majority is obtained from dairy products, is 15-20 mmol for adults. Approximately 20% to 25% of dietary calcium intake (4-8 mmol/d) is absorbed by the intestine. Ultimately, the kidneys are responsible for the excretion of this amount per day to maintain the body in balance. More importantly, regulation of renal calcium excretion is one of the principal ways in which the body regulates extracellular calcium balance. \cite{68, 90}

The ionized calcium (iCa) concentration in plasma is meticulously maintained within a very narrow range, therefore, in a healthy individual calcium never supersaturates or precipitates in blood. \cite{68} Calcium in plasma occurs in three forms: approximately 46% of total calcium is bound to protein, 6% is complexed with various anions, and 48% is in the form of free calcium ions. \cite{91} Only the ionized and complexed forms of plasma calcium are ultrafiltered at the glomerulus; generally 60% to 70% of the total plasma calcium concentration. Renal clearance studies have demonstrated that 98% to 99% of the filtered load of calcium is reabsorbed by the renal tubules, so that only about 5 mmol/d is ultimately excreted. The contribution of individual nephron segments in the reabsorption of calcium in comparison to sodium are summarized in Table 9. \cite{68}
<table>
<thead>
<tr>
<th>Nephron Segment</th>
<th>FRNa</th>
<th>FRH₂O</th>
<th>FRCa</th>
<th>FRPO₄</th>
<th>PO₄ Transport Mechanism</th>
<th>Ca Transport Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal tubule</td>
<td>65%</td>
<td>65%</td>
<td>60-70%</td>
<td>80%</td>
<td>Active transcellular transport</td>
<td>Passive follow Na absorption, Angiotensin II</td>
</tr>
<tr>
<td>Thin descending &amp; ascending limbs</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thick ascending limb of Henle</td>
<td>30%</td>
<td>0</td>
<td>20%</td>
<td>0</td>
<td></td>
<td>Positive lumen following K re-entry, stimulated by PTH</td>
</tr>
<tr>
<td>Distal convoluted tubule/Connecting tubule</td>
<td>10%</td>
<td>10-20%</td>
<td>10-15%</td>
<td>5%</td>
<td>Active transcellular transport</td>
<td>Active, transcellular</td>
</tr>
<tr>
<td>Collecting duct</td>
<td>&lt;5%</td>
<td>10%</td>
<td>&lt;5%</td>
<td>5%</td>
<td>Active transcellular transport</td>
<td>ECaC Aldosterone?</td>
</tr>
</tbody>
</table>

Table 9. Segmental Handling of Sodium, Calcium, Phosphate, and water along the Renal Tubule [68, 91] Fractional Reabsorption (FR) of Sodium (FRNa), Calcium (FRCa), phosphate (FRPO₄) and Water (FRH₂O).

Most of the filtered load of calcium (65%) is reabsorbed in the proximal tubule largely by the paracellular route, driven by the lumen positive potential difference in the S3 (pars recta) segment of the proximal tubule. [92] Tubular and interstitial calcium concentrations are sensed along the length of the nephron by CaSR (calcium-sensing receptor). In the rat proximal tubule, CaSR expression is localized to apical membrane, where its activation by luminal calcium may limit PTH stimulated 1,25-dihydroxyvitamin D3 production and phosphate excretion, and regulate proximal tubule volume reabsorption.[92]

The ratio of the concentration of calcium in tubule fluid to its concentration in the glomerular ultrafiltrate (TF/UFCa), as determined by micropuncture, is 1.0 in the early
convolutions of the superficial PCT, rising to 1.1 to 1.2 in the later convolutions. The finding that the proximal tubule fluid calcium concentration is close to UFCa suggests that calcium is absorbed in parallel with sodium and water. The modest increase in the later convolutions could be attributed either to a lag in the reabsorption of calcium relative to water, creating a favorable concentration gradient for diffusive reabsorption downstream, or a rising concentration of non-absorbable complexed calcium. [68]

Beyond the proximal tubule, there is no evidence for calcium transport (even of limited passive permeability) in the thin descending limb or the thin ascending limb of Henle, whereas the TAL (thick ascending limb) of Henle accounts for 20–25% of the filtered load of calcium. [92] In this segment, active reabsorption of sodium chloride and luminal membrane recycling of potassium generate a lumen-positive potential difference. Magnesium transport in the thick ascending limb of Henle is also similar to calcium primarily mediated by passive paracellular diffusion. [68]
Figure 47. Concept of safe Calcium absorption: Calcium, Phosphate, Sodium, and water absorption in different area of nephron. Calcium absorption needs to be coupled with water absorption to prevent supersaturation in tubules or interstitium.

Figure 47 demonstrates the concept of safe calcium absorption. In other words in order to prevent calcium supersaturation in tubular fluid or interstitium, calcium absorption must be coupled with water absorption in each area. In the proximal tubule and cortical convoluted tubule calcium absorption almost matches water absorption. Calcium sensing receptor (CaSR) modulation of calcium absorption in TAL of Henle appears to be an important mechanism of protection against stone formation, since there is no significant water absorption in thin descending or ascending limb of Henle. [22] CaSR expression on the basolateral (blood) side of TAL allows regulation of calcium absorption. Stimulation of the basolateral CaSR in the medullary TAL serves
to impair NaCl reabsorption and less lumen positive charge, which cause lower calcium absorption and reduces the risk of interstitial calcium precipitation. Stimulation of the cortical TAL CaSR, where most of the TAL calcium reabsorption takes place, serves to limit potassium recycling via PLA2 (phospholipase A2)-mediated arachidonic acid production and its metabolism to 20-HETE (20-hydroxyeicosatetraenoic acid), thus decreasing NaCl uptake and subsequently divalent cation reabsorption.⁹²

Terranegra et al have reported that the genotypes of the 5’UTR tract of the CaSR gene (a region that regulates CaSR gene transcription) and the haplotype of the first block of CaSR gene are associated with idiopathic calcium nephrolithiasis and increase the risk of stones by as much as 3.36 times.⁹³ One can hypothesize that an alteration of CaSR expression could favour the formation of calcium precipitation within kidney papilla through an influence on calcium absorption uncoupled with water. Further exploration of the alteration of CaSR expression might provide additional information regarding calcium precipitation in papillary interstitium.⁹³

Finally, regulated reabsorption of 8–10% of filtered calcium occurs in the distal tubule, which includes the distal convoluted tubule, connecting tubule, and collecting tubule. Here, calcium is reabsorbed actively by transcellular processes against a transtubular electrochemical gradient and may be regulated independently of sodium reabsorption. Calcium enters the cell via apical TRPV5 (also known as ECaC1) entry channels, a member of the transient receptor potential channel superfamily V. In human kidneys, TRPV6 (also known as CAT1 and ECaC2) may also participate as an apical entry pathway, but this is controversial. There is evidence of co-expression of TRPV5 (ECaC1) with the sodium influx channel ENaC in the mouse late distal tubule. The collecting duct likely plays a minor role in renal calcium reabsorption. Here, a high-sodium load leading to high sodium influx would depolarize the apical membrane and lead to urinary calcium
wasting. Having entered into the cell, calcium is moved across the cell by calcium-binding proteins, such as calbindin, and exits the cell basolaterally, via NCX (Na+/Ca2+ exchanger) and PMCA (plasma membrane Ca2+-ATPase). Excessive influx above the efflux capacity of the combined NCX and PMCA pathways would not only increase transtubular transport, but also result in calcium overload and intracellular calcium precipitates. In the Inner medullary collecting duct, CaSR expression is apical and serves to regulate calcium and water reabsorption. Although of minor quantitative significance to the absorption of the filtered tubular load of calcium, transport of calcium at the IMCD is of special significance for delivery of calcium to the papillary interstitium. At last, the fractional excretion of calcium into the final urine is only about 1% to 2%. [68, 92]

High calcium flow through tubules, as occurs in hypercalciuria, if coupled with increased water extraction, could increase calcium concentrations along the nephron, and create conditions favorable to calcium precipitation in the interstitial spaces that would, in turn, lead to calcium solid phase deposits. [94] Urinary calcium excretion rates among idiopathic calcium phosphate stone formations cluster at the high end of this distribution. Lowering urinary calcium levels prevents supersaturation and stone recurrence. [52]

Hypercalciuria is one of the main risk factors for idiopathic calcium stone formation. [95] Other investigators also showed that higher plaque severity appeared related to hypercalciuria. These studies highlight a possible association between calcium nephrolithiasis, especially in the setting of hypercalciuria, and Randall’s plaque. [94] Urinary calcium excretion as measured on random diets varies in humans, and excretion rates among idiopathic Calcium Phosphate Stone Formations cluster at the high end of this distribution. Lowering urinary calcium levels prevents stone recurrence. Data linking calcium excretion to stone risk are supportive of the idea that it is a
graded risk factor. [52] Many findings support the idea that urine volume and calcium are the main correlates of plaque coverage. [94]

Kuo et al [94] clearly showed that Randall’s plaque coverage fraction strongly correlates with urine measurements made at an entirely different point in time, thus confirming that the urinary milieu has important pathophysiologic significance in the case of calcium nephrolithiasis. The present results lend support to the idea that interstitial plaque deposits arise from driving forces that are reflected in urine calcium excretion and urine volume. Plaque coverage, as expressed as a percentage of total papillary area, varies inversely with urine volume, and directly with urine calcium concentration, and the two variables have independent contributions to the regression as analyzed using standard general linear modeling. [94]

**ROLE OF URINE PHOSPHATE IN SUPERSATURATION:**

Phosphate at urine and blood pH exists as monovalent (HPO\(_4^{2-}\)) and divalent phosphate (HPO\(_4^{3-}\)), but HPO\(_4^{2-}\) is the only form of phosphate that can bind with calcium and precipitate. Considering phosphate dissociation pK\(_a\), at pH of 6.8 half of the amount of phosphate would be monovalent and the other half would be divalent phosphate. (See table 10). Therefore the concentration of tubular fluid HPO\(_4^{2-}\) depends on total excreted phosphate concentration and pH which indicate the proportion of HPO\(_4^{2-}\) to H\(_2\)PO\(_4^{-}\). [10]

Plasma phosphate exists in ionized, complexed and protein-bound forms. Measured ultrafilterable phosphate to plasma phosphate ratios have been found to range from 89 to 96%. Only the ionized and complexed forms of plasma phosphate are ultrafiltered at the glomerulus, so that the phosphate concentration of fluid in Bowman space is approximately 90% of the total
plasma phosphate concentration. Of ultrafilterable phosphate, approximately 60% is ionized and 40% is complexed to the major plasma cations, chiefly calcium, magnesium, and sodium.\textsuperscript{[68]}

The daily dietary intake of phosphate is 30-50 mmol. Approximately 65% of ingested phosphate is absorbed. Dietary polyvalent cations such as calcium, magnesium, and aluminum bind to intestinal luminal phosphate and decrease its absorption. Ultimately, the kidneys are responsible for the excretion of a substantial excess of phosphate, about 20-30 mmol per day. Thus, renal phosphate excretion is the principal mechanism by which the body regulates extracellular phosphate balance.\textsuperscript{[68]}

Renal clearance studies have demonstrated that 80% to 90% of the filtered load of phosphate is reabsorbed by the renal tubules. The contribution of individual nephron segments to fractional reabsorption of phosphate includes 80% by proximal tubule through active transcellular transport, in addition to 5% by distal collecting tubule and connecting tubule through active transcellular transport. There is likely no reabsorption of phosphate after the proximal straight tubules in the loop of Henle. The collecting duct accounts for very little, if any, phosphate reabsorption.\textsuperscript{[68]}

Hyperphosphaturia in patients with urolithiasis has been reported by several authors. A decrease of the renal phosphate threshold normalized for the glomerular filtration rate (TmPi) is more frequently observed in stone formers without hyperparathyroidism than in control subjects. Hyperphosphaturia, by increasing urinary calcium excretion and urinary saturation, may predispose the patient to calcium stone formation.\textsuperscript{[95]}
The product of free ionized calcium and free divalent phosphate will determine the precipitation at any given time. For example it has been reported that in hypercalciuric rats, a low-phosphate diet decreased phosphaturia, preventing stone formation, although hypercalciuria was augmented further. [112] Regardless of the mechanism involved, the increase of phosphate excretion may augment urine saturation, and hence promote calcium stone formation. Conversely, decreasing phosphaturia may contribute to the prevention of calcium renal stone recurrences. [95]

Calcium Phosphate can precipitate either as brushite CaHPO$_4$·$2$H$_2$O (calcium monohydrogen phosphate simply HPO$_4^{2-}$) or hydroxy apatite Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ (the principal constituent of bones and teeth simply PO$_4^{3-}$). Many studies indicate initial precipitation of calcium phosphate as Brushite crystals, then conversion to appetite crystals. [28] This will be discussed further in the stone formation part.

**Urine pH:**

The main role of pH in calcium phosphate precipitation is its effect on the proportion of Divalent/Monovalent phosphate. At blood pH around 7.4, approximately 80% of total phosphate exist as HPO$_4^{2-}$. Considering the amount of phosphate excretion (20-30 mmol/d), the excretion of urine with this pH could imply a significant risk of precipitation. Nonetheless, the typical urine pH in healthy individuals is around 6.0. [10] At urine pH of 6.0 only 10% of total phosphate exist as HPO$_4^{2-}$ (Table 10). Even though the measurement of tubular fluid pH in vivo at all different parts of nephron is very tricky and not reliable, usually tubular fluid pH would reduce from 7.4 at Bowman’s capsule to 6.0 at the distal collecting duct as tubular fluid moves distally (Figure 48). This transition of tubular fluid pH from 7.4 to average urine pH of 6.0, alters the proportion of
HPO$_4^{2-}$ to H$_2$PO$_4^{-}$ [10] which can be a protective mechanism as tubular fluid calcium and phosphate concentration rises, by reducing the proportion of HPO$_4^{2-}$ significantly. [96]

Figure 48. Changing in tubular pH from Bowman capsule 7.4 to typical urine pH of 6.0.

The proximal convoluted tubule secretes H$^+$ ions that acidify the luminal fluid leading to the reabsorption of approximately 75% of filtered bicarbonate and decreasing luminal bicarbonate concentration to 5 to 10 mEq/l and luminal pH from 7.4 to 6.8. However, the loop of Henle reabsorbs 10% to 20% of filtered HCO$_3^-$ via reabsorption of HCO$_3^-$ by the thick ascending limb (TAL) of Henle. [97]
Micropuncture studies demonstrated that approximately 50 to 70% of the HCO3 remaining at the end of the accessible superficial proximal tubule is reabsorbed prior to the superficial distal tubule. The apical H extrusion process is predominantly sodium-dependent and amiloride-sensitive (i.e., Na-H exchange). The predominant apical membrane Na-H exchanger in the TAL is the NHE3 isoform. However, NHE2 is also present on the apical membrane of the TAL. A luminal vacuolar type of H-ATPase is also present but its role in transepithelial HCO3 transport in the TAL is not established. Similarly, K-ATPase activity and a K+-dependent HCO3 transport pathway (possibly a K+-HCO3 cotransporter) have been found in TAL but their roles are not clear either. The basolateral extrusion of HCO3 probably occurs via a Na/HCO3 cotransporter as in the proximal tubule. In addition, basolateral Cl/HCO3 exchange (AE2, anion exchanger-2) and K-HCO3 transport are present and may be involved in transepithelial HCO3 transport. AE2 may mediate some of the adaptive changes that occur in acid-base transport in the TAL. [97]

Acute and chronic metabolic acidosis stimulates, and chronic chloride depletion metabolic alkalosis inhibits, HCO3⁻ reabsorption in the TAL. Acidosis increases NHE-3 and basolateral Na-HCO3 cotransport. NHE-3 decreases with metabolic alkalosis. However the effects of metabolic alkalosis on HCO3⁻ reabsorption in vivo appear to vary with some experimental models perhaps due to opposing influences of the acid-base status and sodium and HCO3⁻ delivery. HCO3⁻ reabsorption increases as luminal HCO3⁻ concentration increases. Sodium chloride or sodium bicarbonate loading in vivo increase HCO3⁻ reabsorption in the TAL; and sodium depletion reduces HCO3⁻ reabsorption. These effects of sodium balance have been thought to result from changes in sodium delivery rather than from changes in mineralocorticoids. However, aldosterone has been shown to inhibit TAL HCO3⁻ reabsorption by a nongenomic mechanism inhibition of NHE3. Glucocorticoids and aldosterone at high doses have been reported to stimulate HCO3⁻
reabsorption in vivo. Ang II inhibits TAL HCO$_3^-$ reabsorption via a P-450–dependent mechanism. Increase in osmolality, signaling via tyrosine kinase pathways, decreases apical Na/H exchange and HCO$_3^-$ reabsorption; in vivo, this may be an additive factor with ADH during states of antidiuresis. Hypoosmolality stimulates HCO$_3^-$ reabsorption via a PI3 kinase mechanism.\textsuperscript{[97]}

Luminal fluid delivered to the distal nephron is normally low in HCO$_3^-$ and pH, usually 5 to 7 mEq/l and 6.5 to 6.7, respectively. The distal tubule reabsorbs this remaining HCO$_3^-$ via H secretion, as in the proximal tubule. In contrast to the proximal tubule, however, net H secretion continues after reabsorption of virtually all of luminal HCO3, lowering luminal and urine pH to less than 5.5 under appropriate conditions.\textsuperscript{[97]}

On the other hand, patients with calcium phosphate kidney stones belong to a diagnostic category that has a common feature of high urine pH.\textsuperscript{[96]} Urine pH rises progressively with increasing calcium phosphate percentage in stones. Blood bicarbonate levels are normal, and net acid excretion is not low. High ammonia production and excretion could raise urinary pH but was not obvious in the study patients.\textsuperscript{[52]} Table 10 illustrates the effect of raising the urine pH on the HPO$_4^{2-}$ concentration.\textsuperscript{[133]}

<table>
<thead>
<tr>
<th>pH</th>
<th>HPO$_4^{2-}$</th>
<th>H$_2$PO$_4^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.9</td>
<td>10%</td>
<td>90%</td>
</tr>
<tr>
<td>6.2</td>
<td>20%</td>
<td>80%</td>
</tr>
<tr>
<td>6.5</td>
<td>33%</td>
<td>67%</td>
</tr>
<tr>
<td>6.8</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>7.1</td>
<td>67%</td>
<td>33%</td>
</tr>
<tr>
<td>7.4</td>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
<td>7.7</td>
<td>89%</td>
<td>11%</td>
</tr>
</tbody>
</table>
Furthermore, urinary ionic strength will influence of on phosphate pKa₂ and the determination of titratable acid with the following equations.

$$\text{Equation 8. Phosphate } \text{pK}_2: \quad \text{pK}_2 = \frac{1.57 \sqrt{\mu}}{1 + 1.49 \sqrt{\mu}} - \log \frac{[\text{H}_2\text{PO}_4^-]}{[\text{HPO}_4^{2-}]} = \text{pK}_2'$$

The ionic strength of the solution, $\mu$, is calculated by multiplying the concentration of each ion, $C_i$, by the square of its valence $Z_i^2$ adding all such products, and dividing by 2. $^{[84]}$

$$\text{Equation 9. Ionic strength of the solution } \quad \mu = \frac{\sum C_i Z_i^2}{2}$$

$$I = \frac{1}{2} \sum C_i Z_i^2$$

$$\text{Equation 11. Activity coefficients } \quad \log f_z = \frac{-0.51 z^2 \sqrt{I}}{1 + \sqrt{I}}$$
Activity of any ion (with charge $z$) \[ a_{ion} = f_z C_{ion} \]

\[ 2.7 \times 10^{-3} \times Ca^{1.07} \times PO_4^{0.70} \times (pH - 4.5)^{6.8} \]

Equation 12. Ionic Strength = 
\[ \frac{Cit^{0.20} \times V^{1.31}}{} \]

The other important finding is that activity of $Ca^{2+}$ in the urine of healthy adults was found to be quite variable throughout the day.
STONE FORMATION INHIBITORS AND CHELATING AGENTS:

CITRATE:

Citrate is the most important calcium chelator. It binds calcium and inhibits nucleation and growth of calcium crystals. It chelates calcium at 1:1 stoichiometry and when citrate concentration is more than calcium concentration virtually free Ca$^{2+}$ activity is nil. Hypocitraturia is a significant risk factor for developing calcium stones. By citrate excretion, urinary bases can be excreted without raising urine pH; thus it maintains divalent phosphate concentration low and prevents calcium phosphate precipitation. \[^{[43]}\]

Citrate has multiple functions in mammalian urine and the two most important ones are as a chelator for urinary calcium, and as a physiologic urinary base. It is a tricarboxylic acid cycle intermediate and the majority of citrate reabsorbed by the proximal tubule is oxidized to electroneutral end products so H$^+$ is consumed in the process rendering citrate a major urinary base (Figure 49). Calcium associates in a one-to-one stoichiometry. The highest affinity and solubility is a monovalent anionic (Ca$^{2+}$Citrate$^3-$) complex. \[^{[68]}\]

Figure 49. Citrate molecule.
Citrate exists mostly as a tricarboxylate at plasma pH, but in the proximal tubule lumen, because of apical H\(^+\) transport, citrate\(^3-\) is titrated (citrate\(^3-\)/citrate\(^2-\) pK 5.7–6.0) and is taken up in protonated form as citrate\(^2-\). The Km for dicarboxylates ranges between 0.3 \(\mu\)M and 1 \(\mu\)M. Transport of one divalent anion substrate is coupled to three Na\(^+\) ions. (Figure 50)\(^{[68]}\)

**Equation 13:** \[ \text{Citrate}^{3-} + H^+ \rightarrow \text{Citrate}^{2-} \quad \text{pK 5.7–6.0} \]

![Diagram](image)

**Figure 50.** Proximal tubule citrate absorption and metabolism. The Na\(^+\)K\(^+\)ATPase generates the low cell [Na\(^+\)]. As a secondary active transporter NaDC1 uses the electrochemical gradient to pick up filtered citrate, which metabolized in the cytoplasm or the mitochondria. Ambient and cytoplasmic pH increase citrate uptake and metabolism. (1) Acidification of urinary lumen titrates citrate to the divalent transported species; (2) NaDC1 is directly activated by pH and chronic low pH increases expression of NaDC1 (circled arrow); (3) Intracellular acidification increases the expression of ATP citrate lyase and aconitase (circled arrows).\(^{[68]}\)
Sodium Dicarboxylate Cotransporter 1 (NaDC1) is found on apical membranes of the renal proximal tubule cells where it mediates absorption of tricarboxylic acid cycle intermediates from the glomerular filtrate or the intestinal lumen. The preferred substrates of NaDC1 are 4-carbon dicarboxylates such as succinate, fumarate, and α-ketoglutarate.

Sodium Dicarboxylate Cotransporter 3 (NaDC3) has a wider tissue distribution and much broader substrate specificity than NaDC1. NaDC3 is expressed on basolateral membranes in renal proximal tubule cells, as well as the liver, brain, and placenta. The basolateral location of NaDC3 was mapped to a motif in its amino-terminal cytoplasmic domain. Like NaDC1, NaDC3 is sodium-coupled and electrogenic so it is very unlikely that NaDC3 will mediate citrate efflux from the proximal tubule into the peritubular space. [68]

Acidic proximal cells favor tubular reabsorption of citrate and thus hypocitraturia, while alkalosis reduces tubular reabsorption and increases urinary citrate excretion. Potassium-magnesium citrate has been used as prophylaxis against recurrent calcium oxalate nephrolithiasis and it has been recommended as a treatment for nephrocalcinosis to increase urinary pH and citrate concentration, but citrate after absorption will be metabolized to bicarbonate by the liver. [65] Hence plasma ionized calcium around 1-1.5 mmol/l is needed for many physiologic reactions, plasma concentration of citrate is very low around 0.16 mmole/l. Therefore, the final urinary excretion of citrate is determined by reabsorption in the proximal tubule and the most important regulator of citrate reabsorption is proximal tubule cell pH. Acid loading increases citrate absorption by four mechanisms: (Figure 50)
1. Low luminal pH titrates citrate\(^3^-\) to citrate\(^2^-\) (pK of 5.7-6.0) which is the preferred transported species;

2. NaDC1 is also gated by pH such that low pH acutely stimulates its activity;

3. Intracellular acidosis increases expression of the NaDC1 transporter and insertion of NaDC1 into the apical membrane;

4. Intracellular acidosis stimulates enzymes that metabolize citrate in the cytoplasm and mitochondria. This is a well concerted response and an appropriate response of the proximal tubule to cellular acidification is hypocitraturia. Although perfectly adaptive from an acid-base point of view, this response is the detrimental to the prevention of calcium chelation.

All conditions that lead to proximal tubular cellular acidification (e.g., distal renal tubular acidosis, high-protein diet, potassium deficiency) are clinical risk factors for calcareous nephrolithiasis. Hypocitraturia can cause kidney stones by itself or by acting with other risk factors such as hypercalciuria. Therapy with potassium citrate has been shown to reverse the biochemical defect and reduce stone recurrence.\(^{[68,134]}\)

Citrate also increases the activity of some macromolecules in the urine like Tamm-Horsfall protein that inhibit calcium oxalate aggregation further. Citrate seems able to increase the expression of urinary osteopontin as well.\(^{[43]}\)
TAMM-HORSFALL PROTEIN:

Tamm-Horsfall protein, also known as uromodulin, is expressed by the thick ascending limb of Henle’s epithelial cells. Tamm-Horsfall protein is the most abundant protein found in urine. Humans produce up to 100 mg of Tamm-Horsfall protein daily in urine (1.5umol/d). There is a significant correlation between the concentration of Tamm-Horsfall protein and citrate in stone former patients. Tamm-Horsfall protein plays dual roles in the formation of Calcium oxalate stones. The inhibitory effect of Tamm-Horsfall protein on crystal aggregation and growth has been described both for calcium oxalate and hydroxyapatite stones. In a rat model of nephrolithiasis, Tamm-Horsfall protein was specifically associated with reduced renal crystal deposits. Approximately, 16% of mice deficient for Tamm-Horsfall protein spontaneously developed calcium crystals in the kidneys. Calcium overload in these mice resulted in an aggravation of calcium crystal formation (76% of the Tamm-Horsfall protein mice), whereas wild-type littermates were still without calcium crystals. Interestingly, Osteopontin expression (see below) is induced in Tamm-Horsfall protein exposed to calcium overload, suggesting a synergistic action of both these proteins. In some humans with calcium oxalate nephrolithiasis, a molecular abnormality of THP could be detected. Other studies showed decreased urinary levels of Tamm-Horsfall protein in patients with nephrolithiasis. In a recent analysis, it could be shown that urinary macromolecular inhibition of crystal adhesion to renal epithelial cells was impaired in male stone formers and related to a relative Tamm-Horsfall protein deficiency. [43]
Osteopontin:

Osteopontin, also known as uropontin or nephropontin, is a major component of renal stones. An average of 4 mg of Osteopontin is secreted into urine per day. Osteopontin can inhibit nucleation, growth, and aggregation of calcium oxalate crystals in vitro. Interestingly, Osteopontin can increase the adhesion force between a carboxylate tip and a specific crystal surface. Using immunogold labeling, Osteopontin was shown to be localized mainly on the surfaces of the apatite crystal phase at the junction of crystal organic layers. In vitro experiments revealed that Osteopontin concentrations ranging from 16 to 28 nM are sufficient for a 50% reduction in crystal growth rate and aggregation of calcium oxalate monocrystals, respectively. Mean urine Osteopontin concentrations of 131 nM therefore indicate that Osteopontin may also act in vivo. Mice deficient for Osteopontin develop renal calcium oxalate stones when exposed to high levels of oxalate, but not under normal conditions. In that study, hyperoxaluria in wild-type littermates resulted in an up-regulation of renal Osteopontin expression.\[43\]

To what extent Osteopontin is involved in human nephrolithiasis is less clear, as there are reports of reduced Osteopontin concentration in stone formers, while other researchers could not detect a difference in urinary Osteopontin levels between stone formers and healthy individuals.\[43\]
Glycosaminoglycans:

Chondroitin sulfate, heparan sulfate, and hyaluronic acid are the best studied glycosaminoglycans with respect to nephrolithiasis. Chondroitin sulfate delays nucleation, while dermatan sulfate inhibits nucleation. Hyaluronic acid, which is found on pericellular matrices, is thought to be the key binding substance for crystals at the surfaces of renal tubular cells. In primary cultures of human tubular cells, intact distal tubular epithelium could not bind crystals, while crystal retention by damaged distal tubular epithelium depended on the expression of hyaluronic acid-, CD44-, and osteopontin-rich cell coats. A rat study showed that during the process of nephrolithiasis, there was an increased expression of heparan sulfate in both distal and proximal tubules. In a canine tubular cells study it was found that synthesis of glycosaminoglycans may increase protection from toxic insults of calcium oxalate crystals and oxalate ions. Some studies in humans show that decreased urinary glycosaminoglycan levels are more common in patients with stone formation. However, other studies could not demonstrate a major relationship between urinary glycosaminoglycan excretion and calcium stone formation. [43]

Renal Handling and Diurnal excretion Variation of magnesium:

The average daily dietary intake of magnesium in adults is 12 mmol, with the minimum daily dietary intake of 0.5 to 4 mmol. Of the dietary magnesium intake, 30% to 50% is normally absorbed, but this can increase to 75% on a low magnesium diet, and decrease to 24% on a high magnesium diet. Urinary excretion normally accounts for about 4 mmol of magnesium output per day. [68]
Seventy percent to 80% of serum magnesium is freely filtered at the glomerulus, of which most is reabsorbed along the length of the nephron. Only 5-15% of filtered magnesium will be absorbed in the proximal tubule. However, 60-70% of filtered magnesium will be absorbed in the thick ascending limb of Henle and 5-10% of it will be absorbed in the distal collecting tubule and connecting tubule. Only about 3% of filtered magnesium normally appears in the final urine. During severe dietary magnesium deprivation, the kidney avidly retains magnesium. Under these circumstances, urinary magnesium excretion may be reduced to less than 1 mmol/day (and often less than 0.5 mmol/day), and the fractional excretion of filtered magnesium (FeMg) to less than 1%. Magnesium reabsorption is not, in fact, saturable with respect to luminal magnesium concentration in any segment of the nephron, but is inhibited in a concentration-dependent manner by increasing peritubular magnesium levels in the thick ascending limb of Henle, giving rise to an apparent Tm effect in studies of whole kidney clearance.\[^{68}\]

**Other Factors:**

Nephrocalcin belongs to the osteocalcin family, which constitutes up to 1–2% of total bone protein. Nephrocalcin binds strongly to apatite and calcium. It is expressed in the kidney and depends on vitamin K availability for \(\gamma\)-carboxylation and thus activation. Nephrocalcin inhibits nucleation of calcium oxalate monohydrate crystals in vitro. Some patients with renal stones produce an abnormal nephrocalcin lacking the \(\gamma\)-carboxyglutamic acid and thus failing to inhibit crystallization functionally. Calgranulin, also known as calprotectin, is a member of the calcium binding S100 family. Calgranulin is a potent inhibitor of calcium oxalate crystal growth and aggregation and can be detected within urinary calcium stones. Urinary prothrombin fragment 1 is present in calcium stones as well and is an inhibitor of calcium oxalate crystallization in urine in vitro. During blood coagulation, prothrombin is ultimately degraded to three fragments: thrombin, fragment 1, and fragment 2, respectively. In patients
with calcium oxalate calculi, the e-carboxyglutamic acid composition of urinary prothrombin fragment 1 and its ability to inhibit calcium oxalate crystal growth was described to be significantly decreased. Bikunin, the light chain of inter-a-inhibitor, prevents the adhesion of calcium oxalate crystal to renal tubular cells in human urine. Normally, bikunin expression is mostly limited to the proximal tubules; however, hyperoxaluria in rats resulted in an increased expression of bikunin. Urinary bikunin levels were approximately 50% lower in patients who form calcium oxalate stones compared with normal volunteers. Phytate, the principal storage molecule of phosphorous in many plants, can inhibit calcium oxalate crystal formation in vitro. In the Nurses Health Study II, women in the highest quintile of phytate intake had a reduced relative risk to develop kidney stones, 37% compared with those in the highest quintile. Magnesium can inhibit calcium oxalate and calcium phosphate crystal growth and aggregation in vitro. The inhibitory activity of magnesium is positively related to urinary pH. However, magnesium oxide therapy could not show any therapeutic benefit in recurrent calcium stone formers. Pyrophosphates are found in hydroxyapatite and oxalate calculi in the urine as well. As pointed out above, pyrophosphates are natural inhibitors that block hydroxyapatite precipitation in vitro. In clinical studies, calcium stone formers had reduced urinary pyrophosphate concentrations. Urinary trefoil factor 1 is predominantly found in the stomach overlying the gastrointestinal mucosa. Recently, it could also be identified as a novel calcium oxalate crystal growth inhibitor in human urine by mass spectrometry, with nearly the same inhibitory potential as nephrocalcin.  

Recently, the so-called crystal adhesion inhibitor, which is constitutively secreted by renal cells, could be identified. This 39 kDa protein blocked the adhesion of calcium oxalate crystals to the cell surface of epithelial cells. A summary of actions of the described urinary calcium-inhibitory factors is given in Table 11.
Table 11. Calcification inhibitors in urine [43]

<table>
<thead>
<tr>
<th><strong>Citrate</strong></th>
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<tbody>
<tr>
<td>Most abundant organic anion in the urine</td>
<td></td>
</tr>
<tr>
<td>Hypocitraturia: risk factor for calcium stone development</td>
<td></td>
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<tr>
<td>Alkali citrate significantly reduces stone formation</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Tamm-Horsfall protein</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Most abundant protein in urine</td>
<td></td>
</tr>
<tr>
<td>Tamm-Horsfall Protein mice on calcium overload develop renal calcium crystals</td>
<td></td>
</tr>
<tr>
<td>Decreased urinary levels in patients with nephrolithiasis</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Osteopontin</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Major component of renal stones</td>
<td></td>
</tr>
<tr>
<td>Experimental hyperoxaluria resulted in OPN upregulation</td>
<td></td>
</tr>
<tr>
<td>Osteopontin mice with hyperoxaluria develop calcium oxalate stones</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Glycosaminoglycans</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondroitin sulfate, heparan sulfate, hyaluronic acid display different degrees of inhibitory effects</td>
<td></td>
</tr>
<tr>
<td>Increased expression in animal models of nephrolithiasis</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Other factors</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium, pyrophosphates, phytate, nephrocalcin, calgranulin, prothrombin fragment 1, bikunin, trefoil factor 1, crystal adhesion inhibitor</td>
<td></td>
</tr>
</tbody>
</table>

**Table 11. Adopted with permission from Schlieper et al; Inhibitors of calcification in blood and urine.** [43]

**Summary:** Even numerous stone formation inhibitors have been explained, nevertheless from many functional and clinical studies, it can be concluded that citrate is the most important calcium chelator and main determinant of calcium precipitate. Citrate is needed in urine at equimolar concentrations to calcium in order to prevent the formation of large crystal aggregates.
Calcium Phosphate Precipitation:

The lower the pH, the higher the proportion of monovalent phosphate (H$_2$PO$_4^-$), the risk of crystallization would be lower. Conversely, the higher the pH, the higher the proportion of divalent phosphate (HPO$_4^{2-}$), the precipitation risk will be higher. Studies have evaluated the effect of pH on calcium and phosphate limit concentrations in a typical parental nutrition regimen. Figure 46 depicts the borderline between solubility and crystallization.\textsuperscript{[10]}
STONE FORMATION:

The mechanism of stone formation is a complex physicochemical process occurring as a result of an imbalance between promoting and inhibiting factors of crystallization and aggregation in urine that remains poorly understood despite considerable efforts over many centuries. According to the generally accepted theory, the process is a cascade of events beginning with calcium salt precipitating from a solution (nucleation) forming a crystal in supersaturated urine (crystallization), followed by crystal growth and aggregation leading to a stone nidus. When the aggregate adheres to the tubulopelvic uroepithelium, continued epitaxial growth of the crystal aggregate eventually leads to a detectable size, making it a renal stone.

As a result, stones may occur when the urine is supersaturated leading to early microcrystallizations, which subsequently aggregate into overt stones. Supersaturation of urine is the key point in forming urinary precipitate, crystallization and likely kidney stone formation. Therefore, assessment of urinary composition in addition to stone analysis has been an essential part in understanding the cause of kidney stone formation. Urinary stones are crystal aggregates embedded in a small amount of glycoprotein matrix.

As it has been discussed in the urine composition chapter in detail, urine concentration of lithogens lie above their solubility point (supersaturation) in most urine samples from even normal humans and lie even higher than normal in the urine of nephrolithiasis patients. This was further demonstrated in a study by Pak et al showing the visible fact that added crystals will grow in urine from most of normal subjects. Calcium phosphate supersaturations may occur in the thin segment, and they can produce a calcium phosphate solid phase in the form of “apatite-like” materials. Asplin et al study supports the notion that the loop of Henle may produce fluid
supersaturated with respect to calcium-phosphate phases and produce a solid-phase material. Since apatite can nucleate calcium oxalate monohydrate, the loop nuclei could potentially promote calcium oxalate monohydrate crystallizations in the collecting ducts. [101] (This will be discussed further in this chapter)

**Nucleation:**

Nucleation describes the process that occurs when the activity of calcium salts reaches the level at which the solid phase begins to appear. As we discussed earlier, a solution in equilibrium is a solution where the existing crystals neither grow nor shrink. The activity product of the ions at this equilibrium determines the equilibrium solubility product of the salt (Figure 44). Solutions with concentrations of salt less than the equilibrium SP are undersaturated. A higher free-ion activity product will cause the solid phase (the crystals) to grow (epitaxy). However, if the crystals are removed from a solution at the level of the equilibrium SP and then the ion activity product is elevated, the activity product that would have caused growth of preformed crystals will not result in the appearance of a new solid phase (metastably supersaturated) (Figure 45). The activity products of calcium salts in urine are almost constantly in the range of metastable supersaturation. In the range of metastable supersaturation, if the activity products are sufficiently raised (called formation product), new crystals will appear (Figure 46). Above the level of the formation product, a solution is unstable, creating new crystal nuclei. Urine may be undersaturated, metastably supersaturated, or unstable with respect to calcium oxalate or the stone-forming calcium phosphate crystals (brushite, octacalcium phosphate, hydroxyapatite, and apatite). However, most of the time, it is metastably supersaturated and, particularly for brushite, close to the formation product. [61]
Homogeneous Versus Heterogeneous Nucleation:

Nucleation, the initial precipitating event in stone formation, may be homogeneous or heterogeneous. Much higher levels of supersaturation are required to produce homogeneous nucleation than heterogeneous nucleation. In an unstable solution, crystals form spontaneously by homogeneous nucleation. Heterogeneous nucleation occurs in metastably supersaturated urine as certain macromolecules, or other crystals that can act as nuclei, stimulate precipitation. Hence, when urine contains a number of macromolecular and cellular degradation products, crystallization is most often heterogeneous. The efficiency of heterogeneous nucleation depends on the similarity between the spacing of charged sites on the preformed surface and the spacing in the lattice of the crystal that is to grow on that surface. This matching is referred to as epitaxis, and its extent is usually referred to as a good or poor epitaxial relationship. A number of urine crystals have good epitaxial matching and behave toward one another as heterogeneous nuclei. Indeed, monosodium urate and uric acid are excellent heterogeneous nuclei for calcium oxalate. Heterogeneous nucleation is thought to play a role in linking hyperuricosuria to calcium oxalate stones. Epitaxial overgrowth of calcium oxalate on a surface of uric acid has been experimentally documented. At a pH above 6.9, brushite may transform to hydroxyapatite, which can serve as a nucleus for calcium oxalate. Based on observations that calcium phosphate is the most common crystal in human urine, is ubiquitous in human urinary stones, and is often seen at the center of mixed calcium oxalate/calcium phosphate urinary stones, nucleation of calcium oxalate crystals is proposed to be induced by calcium phosphate. In addition, both apatite and brushite crystals induce crystallization of calcium oxalate in vitro from metastable solutions of calcium oxalate. [61]
**Crystal Growth:**

Once present, crystal nuclei grow if suspended in urine with an activity product ratio above 1. Crystal growth is critical to stone disease, as microscopic nuclei are too small to cause obstruction. Crystals are regular lattices, composed of repeating subunits, and they grow by the incorporation of calcium and oxalate or phosphate, into new subunits on their surfaces. In metastable solutions at 37°C, growth rates of calcium oxalate and the stone-forming calcium phosphate crystals are rapid. However, appreciable changes in macroscopic dimensions occur over hours to days. Growth rate increases with the extent of supersaturation and tends to be most rapid in urine having the highest APR. [61]

**Factors Influencing Crystal Growth:**

In the urine sample, the upper limit of metastability is higher and crystal growth rates lower than in a salt solution with the same APR. In fact, the nature of the materials that confer crystal growth rate inhibition on urine is not completely known. Crystal growth inhibitors for calcium phosphate crystals may not be the same as the substances that affect calcium oxalate crystal growth. [61]

Evidently, inorganic pyrophosphate increases the FPs of calcium phosphate and calcium oxalate in salt solutions and by adsorbing their surfaces, retards the growth of hydroxyapatite and calcium oxalate crystals. Urinary pyrophosphate concentrations range from 20 to 40 μM in adults. This concentration is sufficient to inhibit crystal growth. Fleisch and Bisaz suggest that urine raises the FP for calcium phosphate above the level expected from the pyrophosphate it contains. They suggest that other inhibitors accounted for approximately 50% of the total inhibition of calcium
phosphate crystal growth. Smith and colleagues have produced similar estimates. Bisaz suggests that pyrophosphate, citrate, and magnesium ions contribute about 77% of the total calcium phosphate. However, pyrophosphate contributes insignificantly to calcium oxalate crystal growth inhibition as well. [104]

**Calcium Phosphate Precipitates Chemistry:**

CaHPO₄ precipitates are formed when the ion product for ionized calcium and divalent phosphate (HPO₄²⁻) exceeds its solubility product constant (Ksp) and its ability to remain dissolved in a supersaturated solution (table 7). Only one sixth of the urine phosphate is in the form of HPO₄²⁻ when the urine pH is 6.1 whereas half of the urine phosphate will be in its HPO₄²⁻ form at a urine pH of 6.8 (the pK for this buffer system in urine, Table 7); hence, a urine pH of 6.8 increases the potential risk for precipitation of CaHPO₄ by 3-fold. On the other hand, there is only a little extra risk when the urine pH rises from 7.1 to 7.5. [10]

**Randall’s plaque Formation:**

Blood itself harbors no supersaturation or precipitate formation in normal healthy humans. Consequently, glomerular ultrafiltrate and proximal tubule fluid generally should have no supersaturation. Calcium reabsorption in the proximal tubule proceeds in parallel with water extraction, tubule fluid-to-plasma inulin concentration ratio (TF/P_{inulin}) calcium values lie close to 1.0, given extraction of at most 70% of filtered water. [101]

It has long been appreciated that the osmolality of tubular fluid increases progressively between the corticomedullary junction and the papillary tip, due to either active secretion of
solute or passive absorption of water along the descending thin limb. According to traditional description, if water extraction continues downstream from the proximal tubule in the thin descending segment of the loop of Henle and it is not accompanied by calcium or bicarbonate reabsorption, at the bend of the loop, one must expect an increase in both pH and calcium concentration. Even with novel concentrating mechanisms explained by Halperin et al this will be correct. We have reasoned that this could create calcium phosphate supersaturations at the bend of the loop. Such a local supersaturation could create calcium phosphate nuclei that could, in turn, foster calcium oxalate monohydrate nucleation in the collecting duct.

The most immediately available hypothesis is that when water conservation is increased, tubule fluid in the thin limbs of Henle’s loop becomes maximally concentrated. This fluid is known to have high concentrations of calcium and phosphorus, as well as a pH near that of blood.

Although epithelial permeabilities are low for calcium and phosphorus, time is not a limiting variable because tubule fluid is always continually passing by. What matters is the net resultant of ion movement into the interstitium, opposed by removal by the vasa recta. Because the latter tend to accumulate materials in the papilla via countercurrent exchange, interstitial concentration in the vicinity of the thin limbs could rise proportional to water conservation. Idiopathic hypercalciuria, the usual cause of high calcium excretion in stone formers, is known to arise via increased vitamin D activity. In particular, since intestinal calcium absorption is elevated, after eating a meal, one would predict pulses of increased filtered load of calcium that deliver high calcium fluxes into the thin limbs. The same mechanism is true, potentially, for phosphorus.

Low et al study have shown that Randall’s plaque as assessed by endoscopic inspection was more prevalent among stone formers than in those undergoing endoscopy for conditions other than
stones. Interstitial plaque deposits arise from driving forces that are reflected in local supersaturation at every single time. Plaque coverage, expressed as a percentage of total papillary area, varies directly with urine solubility product.

![Figure 51. Randall’s Plaques](image)

**Figure 51. Randall’s Plaques** Arrowheads: sites of Randall plaque, Asterisk yellowish crystalline deposit at the openings of Bellini ducts, Arrow: Bellini ducts occasionally enlarged & filled with a crystalline material protruded from the duct that might serve as a site for stone attachment, B: Arrow deposits in the lumens of an inner medullary collecting duct and in nearby Henle loops are shown. The crystal deposits greatly expanded the lumen of these tubules, and cell necrosis. reprinted with permission from the *Journal of clinical Investigation and Kidney International*.

Given that plaque forms on the basolateral membrane of thin ascending limb of Henle’s loop, one is not surprised that its abundance increases with interstitial calcium phosphate solubility product at any given time. This is an unexplored area of research. In producing acid urine, inner medullary epithelial cells could concomitantly increase interstitial fluid pH. Since apatite crystals form preferentially in alkaline media, this might be an explanation for the link between urine pH and plaque abundance. Until an animal model of plaque can be obtained, this matter cannot be fully explored.
Characteristics of interstitial plaque (Randall’s plaque)

Alexander Randall developed a significant hypothesis about the development of calcium nephrolithiasis based on his detailed examination of autopsy kidneys, in which he noted the presence of interstitial papillary deposits which he referred to as plaque. [103]

When these deposits become exposed to the urinary space following erosion of the overlying urothelium, Randall thought that they would become nidi for the formation of calcium stones. [103] Randall’s plaque coverage fraction as it has been measured correlates strongly with urine measurements made at an entirely different point in time, thus confirming that the urinary milieu has important pathophysiologic significance in the case of calcium nephrolithiasis. [94]

The earliest and most minimal deposits of plaque are in the basement membrane of the thin loops of Henle (Figure 52a). This location is invariably involved with plaque and is the only location where plaque can be found in isolation. In many regions, plaque becomes very dense around thin loops and appears to spread from the loops into the surrounding interstitial space (Figure 52b). Often, as in this illustration, one finds plaque migrating down the papillary tip towards the urothelium. Plaque is never found in the lumens of tubules or vessels, or within epithelial cells; it is uniquely located in the basement membranes and the interstitium. Therefore, as expected, the renal epithelial cells invariably appear normal, as does the cellular interstitium. Within the basement membranes, plaque consists of individual laminated particles in which zones of crystal and organic matrix overlay each other in a tree-ring pattern (Figure 52c and d). In the interstitium, the particles fuse to form a syncytium of crystal islands in an organic sea (Figure 52e and f). At no time have we found crystals not coated by an organic layer. This means it is most
unlikely that calcium oxalate stones grow over plaque crystals themselves. Rather, calcium oxalate must grow over the organic material that invariably coats plaque crystals. [102]

**Relationship of plaque to the urothelium:**

Not only does the organic layer intervene between calcium oxalate and plaque crystals, but also the urothelium is present as well. Consequently, plaque without stones appears at surgery almost always covered by a shiny, intact urothelial layer. Moreover, the urothelial layer is coated by a complex glycoprotein mixture that includes a variety of glycosaminoglycans. This means calcium oxalate must somehow localize itself to plaque areas despite at least two intervening barriers, urothelium and its organic coating. This implies some complex biology, perhaps induced by plaque and affecting urothelium, about which nothing is presently known. In essence, the events involved in the transition from plaque to plaque with stone remain, at this time, unclear. [102]

**Nature of the plaque crystal:**

Using high-resolution Fourier Transform Infrared Microspectroscopy (FTIR) and electron diffraction, the crystal component of plaque particles is calcium phosphate in the form of apatite. The apatite is seemingly identical to that found in bone. Given that the apatite microparticles are forming in the type IV collagen of basement membranes and type I in the interstitial space, the process is most closely analogous to that of bone formation. Whether this analogy will be fruitful in terms of new research remains to be tested. An example of what may be a similar process is coronary artery calcification, in which cell transformation to osteoblast character has been documented. Although calcium oxalate crystals can be found in tissues of animals induced to form stones via oxalate loading, that crystal is never present in interstitial plaque. In fact, thus far, in the
studies of a range of human stone formers, calcium oxalate was never found in the renal interstitium. [102]

Figure 52. Histologic images showing sites of Randall's plaque and its progression. (a) The initial site and size of calcium deposits in the papillary tissue of a CaOx patient as seen by light microscopy, while (c) and (d) show these same structures by transmission electron microscopy. Sites of crystalline material (arrows) are first noted in the basement membranes of the thin loops of Henle. The individual deposits are as small as (c) 50 nm and (d) grow into multi-laminated spheres of alternating light and electron dense rings. Extensive accumulation of crystalline deposits occur around the loops of Henle and spread into the nearby interstitial space (b) extending to the urothelial lining of the urinary space. Individual deposits accumulate in the interstitium forming an island of mineral encased in a matrix material (e). These islands can completely surround individual tubules (f). Original magnification, (a) ×900; (b) ×500; (c) ×25 000; (d) ×70 000; (e) ×13 000; (f) ×10 000. Panels (c and e) reprinted with permission from the *Kidney International*. [137]
Nature of plaque matrix:

Osteopontin have been identified among the constituents of matrix. The osteopontin appears to localize preferentially at the matrix–apatite crystal interface. This is not surprising given the well-known affinity of osteopontin for calcium crystal surfaces. The role of osteopontin in plaque formation is unknown. Osteopontin can inhibit nucleation, growth, and aggregation of calcium phosphate crystals, and if anchored to collagen or crystal surfaces could also serve as a nucleating site for new crystals. Obviously, there are many other molecules in the organic matrix beside osteopontin that remain to be discovered. This is an important area of potential new research.

Calcium Oxalate Growth over Randall’s Plaques:

Many of calcium oxalate stones are attached to the renal papillae, over a whitish deposit which is Randall’s plaque. In a recent study of idiopathic calcium stone former (ICSF) patients, plaques were found in 100% of cases and stones were found attached to areas of Randall’s plaque in 48%. If indeed calcium oxalate stones require plaque to anchor them and permit development into clinically relevant stones, we should expect that patients with more stones would have a higher fraction of their papillae covered by plaque. This is indeed the case. The relationship holds even after an adjustment for the number of years of stone formation. This kind of correlation does not tell us whether plaque fosters stones, or the converse, but given our histopathology and gross findings, the former is overwhelmingly the more reasonable alternative.
SUGGESTIONS FOR FUTURE DIRECTIONS:

Short periods' supersaturation analyses as a better reflector of precipitate formation, should be studied in stone former patients. These analyses would likely provide a better risk stratification and therefore, better preventative recommendations in recurrent nephrolithiasis patients. Besides, cost implications as well as practical implications need to be studied to establish an economic and convenient approach.

Furthermore, corresponding physiologic protective mechanisms in overnight periods, need to be examined during fasting periods, which is another example of trough level of urine flow rate. Since, our ancestors survived throughout many periods of fasting and thirst, it is very possible that normal physiologic mechanisms during fasting when urine flow rate approaches its nadir, avoid supersaturation and therefore, precipitate formation.

Finally, the sufficiency of increased fluid ingestion should be studied in recurrent stone formers by using short collections to see whether it really will reduce supersaturation. Applying the same logic concluded from the third objective of our study in healthy subjects, it can be anticipated that an increase in fluid intake will provide intermittent periods of water retention followed by periods of brisk water diuresis. Intermittent elevated urine flow rate interspersed by long periods of low flow rate would not avert supersaturation and therefore precipitate formation.
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