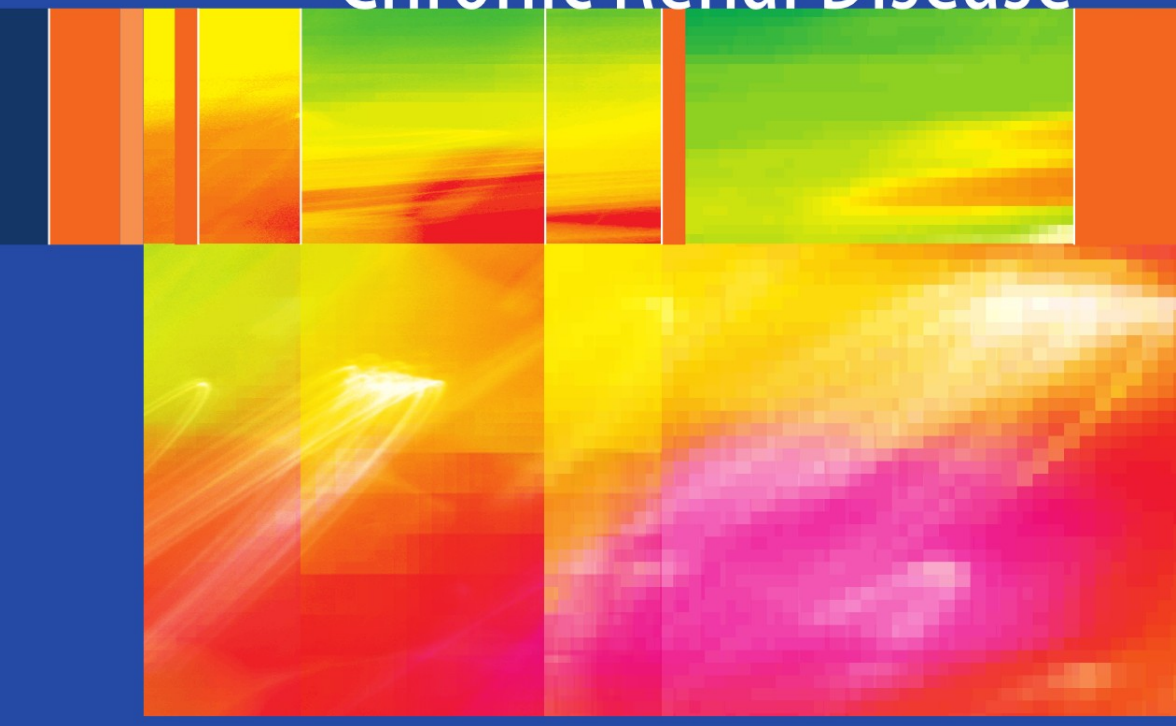
Chen H. Hsu *Editor*

Calcium and Phosphate Metabolism Management in Chronic Renal Disease



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Edited by

Chen Hsing Hsu, MD

Professor of Internal Medicine
The University of Michigan
Medical Center
Ann Arbor, Michigan

Chen Hsing Hsu, MD
Professor of Internal Medicine
The University of Michigan
Medical Center
Ann Arbor, Michigan
USA

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Contents

Contributors

1. Melissa A. Cadnapaphornchai, MD
   University of Colorado Health Science Center, Division of Renal Disease & Hypertension, Denver, CO
2. Pravit Cadnapaphornchai, MD
   Wayne State University, School of Medicine, Department of Medicine, Division of Nephrology, Detroit, MI
3. Griet Glorieux, MD
   Nephrology Unit, Department of Internal Medicine, University Hospital, Gent University, Belgium
4. Chen Hsing Hsu, MD
   Department of Internal Medicine, Nephrology Division, University of Michigan, Ann Arbor, MI
5. Sharon M. Moe, MD
   Department of Internal Medicine, Nephrology Division, Indiana University School of Medicine, Indianapolis, IN
6. Krishna R. Polu, MD
   Internal Medicine, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA
7. Eva Schepers, MD
   Nephrology Unit, Department of Internal Medicine, University Hospital, Gent University, Belgium
8. Ajay K. Singh, MB, MRCP
   Internal Medicine, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA
9. Raymond Camille Vanholder, MD
   Nephrology Unit, Department of Internal Medicine, University Hospital, Gent University, Belgium
10. Eric W. Young, MD
    Department of Internal Medicine, Nephrology Division, University of Michigan, Ann Arbor, MI

Preface

The kidney is one of the most complex organs. Once it fails, it can no longer carry out the complicated tasks performed by the normal kidney. The ensuing disorders of calcium (Ca) and phosphate (P) metabolism are common and difficult problems in chronic kidney disease (CKD). This book is intended for medical students, house officers, and general nephrologists. As such it tries to address issues involved in Ca and P metabolism in a concise and yet comprehensive way.

Normal Ca and P metabolism as well as problems frequently encountered in patients with CKD are discussed in the first two chapters. Unfortunately, information on normal Ca metabolism in persons older than 35 years of age is not available; therefore, many problems of management of Ca in older age groups of CKD patients could not be addressed in this chapter. The third chapter discusses secondary hyperparathyroidism. In 1966 Briker suggested that calcium is the main factor responsible for secondary hyperparathyroidism. Thereafter, calcium was used as a main drug to suppress parathyroid hormone (PTH). Recent studies have indicated that phosphate is the main factor in regulating PTH in chronic renal diseases. Chapter 3 addresses treatment of secondary hyperparathyroidism with calcitriol and its derivatives. Although use of these products is controversial, one should realize that they are effective in suppressing PTH. However, these products will increase Ca and P absorption in end-stage renal disease patients who do not have a route for excreting Ca and P. Further, plasma Ca and P do not reflect total Ca and P in the body. Uremic toxins are discussed in Chapter 4. A large number of toxins can affect Ca and P as well as calcitriol (vitamin D end-product) metabolism. Only a few toxins have been determined to affect Ca, P, and calcitriol metabolism. Many other toxins need to be studied for their effect on Ca, P, and calcitriol metabolism. Abnormal function of calcitriol in patients with renal failure has been discussed to some extent in the past. The toxins may exert effects on other hormones, for example, thyroid, estrogen, and other related hormones. Current knowledge of the metabolism of calcitriol and its action in CKD is discussed in Chapter 5. Renal osteodystrophy is discussed in Chapter 6. Bone diseases are a common incidence in patients with renal failure, and the chapter

discusses normal bone physiology and abnormal bone disorder in renal failure patients. Nephrolithiasis is discussed in Chapter 7. The human kidney often forms kidney stones, although this issue is not entirely caused by abnormal kidney function. Abnormal Ca and P metabolism could be one of the main factors.

Express deep appreciation to all of the authors, who contributed to this book in a short time and who are experts in their own fields. My colleague and friend Eric Young especially helped me enormously in editing this book, which hope I will help readers to manage disorders of Ca and P metabolism. I dedicate this book to my deceased parents: Yen and Jane Hsu, who supported me through medical school and sent me to the United States to pursue a career in medicine. What I have and have accomplished today, I owe in large measure to them.

Chen Hsing Hsu
Ann Arbor, Michigan

Chapter 1

Historical Perspective of Calcium Management in Patients with Chronic Renal Diseases

Chen Hsing Hsu

I. Introduction

The trade-off hypothesis emphasized the importance of calcium in the regulation of secondary hyperparathyroidism in chronic renal disease (CRD).[1] Subsequently, Slatopolsky et al.[2] in 1971 proved that restriction of phosphate (P) controls secondary hyperparathyroidism. Although the duration of the experiment was 1 year, restriction of phosphorus did control elevation of parathyroid hormone (PTH), because subjects maintained calcium (Ca) levels within the normal range. For the past 30 years, physicians have managed secondary hyperparathyroidism by increasing exogenous calcium intake[3] and dialysate Ca concentrations. Soft tissue calcification was well recognized in 1977 by Kuzela et al.[4] Because of increased exogenous Ca intake, tissue calcification occurs frequently in dialysis and in CRD patients.[5,6] In 1995 Slatopolsky[7] and others,[8] discovered that phosphorus was the major factor in controlling PTH. Because dietary restriction of P is very difficult to achieve, P binding agents (e.g., aluminum and magnesium compounds) were used to regulate P metabolism until these agents were discontinued because they were neurotoxic and caused bone damage. A recent study indicated that lanthanum carbonate deposits in tissues, for example, brain, liver, kidney, skeletal muscle myocardium, and lung, and in five of six nephrectomized rats after taking this compound for 4 weeks.[9] Therefore, lanthanum carbonate should be used with caution.

II. Calcium Metabolism in Normal Subjects

To understand how to manage Ca metabolism in CRD, in normal subjects who ingest 1000 mg of dietary calcium/day, approximately 800 mg of calcium

is recovered in the feces. The intestine absorbs approximately 330 mg of ingested calcium and reabsorbs 70 mg of calcium secreted by the intestine (intestinal secretion is approximately 200 mg and 130 mg of the secreted amount is lost in the feces).[10] A balance study has shown that a low-calcium diet increases and a high-calcium diet decreases fractional calcium absorption that occurs primarily in the ileum.[11] Further, we need to have insight into Ca

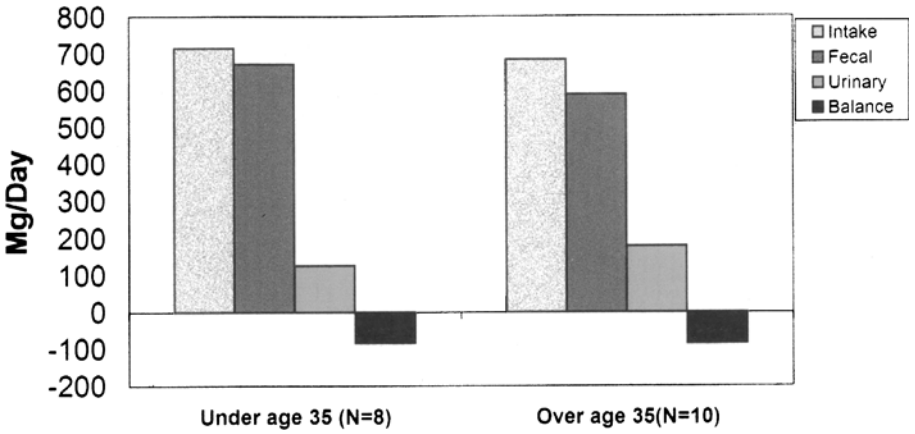

Figure 1-1. Calcium balance of women consuming a self-selected diet. (Reproduced with permission of Am J Clin Nutr 1984;40:1368.)

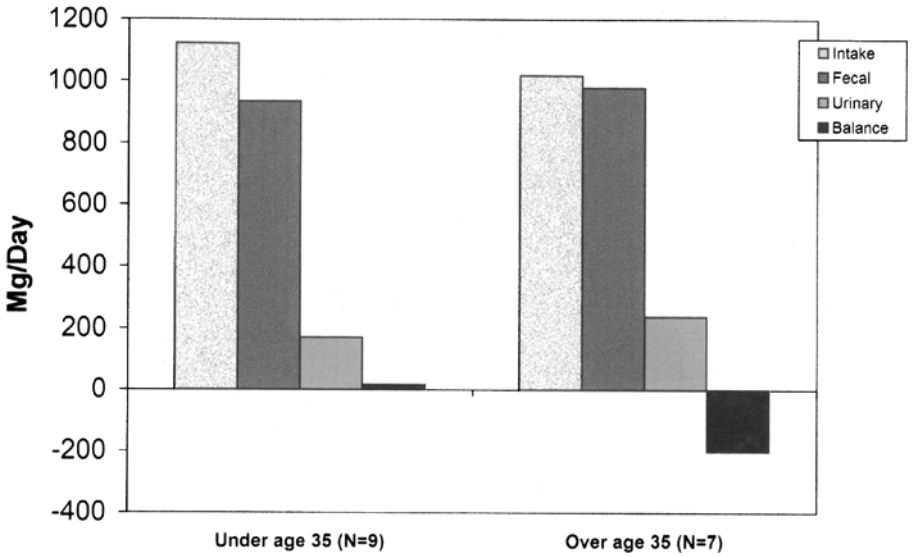

Figure 1-2. Calcium balance of men consuming a self-selected diet. (Reproduced with permission of Am J Clin Nutr 1984;40:1368.)

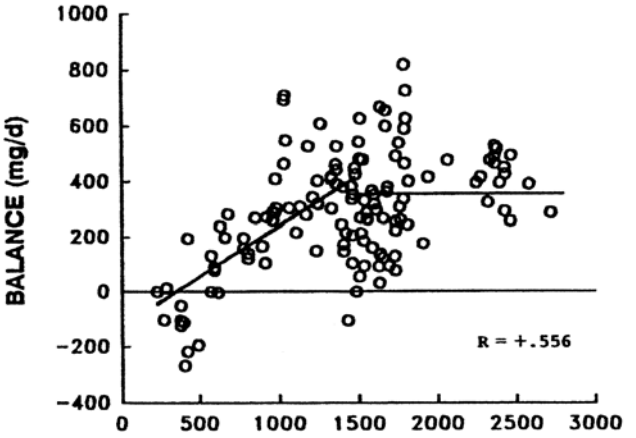

Figure 1-3. Two-component regression of calcium balance intake in young adult. (Reproduded with permission of Am Soc Clin Nutr 1992;55:992–6).

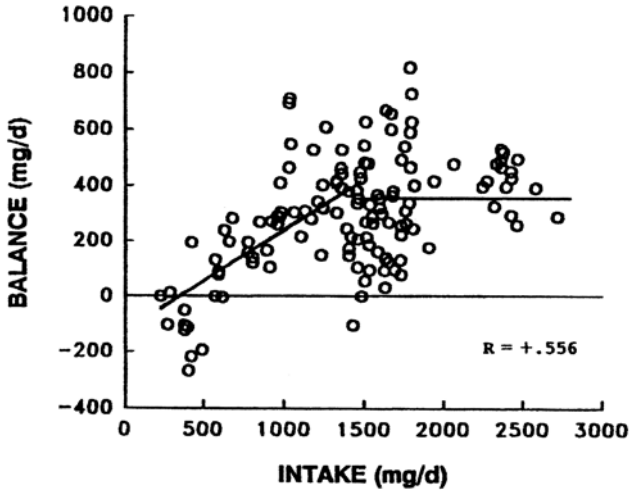

Figure 1-4. Two-component regression of calcium balance intake in adolescents. (Reproduced with permission of Am J Clin Nutr 55:992–6.)

metabolism in young adults (18 to 30 years age): intake threshold of Ca is 957 and the threshold balance is $+114 \pm 133$ mg/day.[12] The intestine of normal adults consuming self-selected diets absorbs approximately 330 mg; slightly negative in female subjects aged 20 to 53 years and male subjects older than 35 years (Fig. 1-1),[13] whereas it is slightly positive in male subjects younger than 35 years (Fig. 1-2).[14] In another study, Matkovic and Heaney[12] collected 34 published records of young adults 18 to 30 yeras of age in whom Ca reached 1500 mg/day, and the threshold balance reached approximately 400 mg/day (Fig.1-3). In young adults (age 31 to 35), with Ca intake of 1000 mg/day, the

intake threshold reached nearly 200 mg (Fig. 1-4). In young adults 19 to 25 years of age the optimal intake of calcium balance also depends on dietary protein intake. Unfortunately, we have no information on Ca metabolic intake and output for normal subjects older than 35 years of age.

III. Calcium Metabolism in Patients with CRD

A. Calcium Absorption in CRD Patients

Intestinal calcium absorption appears to be decreased in CRD.[15] Fractional absorption of calcium is inversely related to plasma concentration of blood urea nitrogen.[15] In patients with advanced CRD (serum creatinine > 2.5 mg/dl) consuming an average of Ca/day, fractional absorption of calcium is 17% compared with 25% in normal subjects with an average dietary calcium intake of 795 mg/day.[16,17] Patients with CRD tend to ingest less calcium in their diets than normal subjects. Accordingly, net calcium absorption is reduced in CRD as a consequence of both decreased fractional absorption and intake of calcium. In fact, investigators from one institution found that their subjects' dietary calcium intake was less than 20 mg/kg.[18,19] Conversely, most studies report that patients with CRD have positive net calcium absorption at lower dietary levels of calcium.[20–22] The fractional calcium absorption of dialysis patients was approximately 19% on an average diet of calcium 500 mg.[17] It should be noted that patients with CRD are capable of adsorbing Ca compounds.[6,23] Thus, addition of calcium compounds in CRD, in which the patients are unable to properly excrete exogenous calcium, further aggravates calcium retention and metastatic calcification of vital organs.[24] Similar findings were observed by Japanese researchers, although in a much larger age group (age mean 53 ± 14),[25] as well as in another large study group.[26]

B. Management of Calcium in Renal Dialysis Patients

Because threshold calcium balance varies with age, using one concentration of calcium dialysate is not an appropriate treatment modality for all end-stage renal disease (ESRD) patients. The current use of 2.5 and 3.5 mEq/L of Ca hemodialysis dialysate or 3.5 mEq/L of Ca (personal observation in Taiwan) continuous ambulatory peritoneal dialysis (CAPD) dialysate provides adequate calcium for patients younger than 20 years of age. These concentrations, however, provide excess calcium load to ESRD patients older than 35 years[27,28] (Tables 1-1 and 1-2). Calcification complications are frequent among long-term dialysis patients. A recent study[29] of 192 patients (mean age, 55 ± 12 years) on CAPD (mean $\pm$ SD duration of dialysis, 39 ± 31 months) screened for calcification of the aortic valve, mitral valve, or both. Valvular calcification

was present in 62 patients. Overall 1-year survival was 70% and 93% for patients with and without valvular calcification ($p < 0.0001$, log-rank test). Cardiovascular mortality was 22% and 3% for patients with and without valvular calcification, respectively ($p < 0.0001$). Cardiovascular mortality was 22% and 3% for patients with and without valvular calcification ($p < 0.0001$). Thus, cardiac valve calcification is a predictor of mortality and cardiovascular deaths in long-term dialysis patients. Valvular calcification by itself has similar prognostic importance as the presence of atherosclerotic vascular disease. Its coexistence with other atherosclerotic complications indicates more severe disease and has the worst outcome.

As stated previously, normal subjects eventually achieve zero or even negative calcium balance if they are older than 35 years of age.[13] Consequently, the excess calcium retention would not necessarily accumulate in the bones of older ESRD patients. Investigators found that using low-calcium dialysate concentrations of 1.5 mEq/L for hemodialysis[27] and 2.5 mEq/L for CAPD patients[28,30] can remove calcium during dialysis, although this treatment regimen still cannot achieve a negative or zero calcium balance in older ($>$ 35 years) ESRD patients (Tables 1-1 and 1-2). It is important to use 3 mEq/L of ionized Ca for the dialysate, because this concentration affects serum and extracellular Ca concentration and thus influences all bodily organ functions. Further studies are needed to fine tune the level of dialysate calcium concentration that can safely achieve a negative calcium balance. It should be cautioned that low ionized calcium dialysate (e.g., $<$ 2.08 mEq/L) might

Table 1-1. Estimated Calcium Balance in Hemodialysis Patients.

Calcium balance using 3.5 mEq/L of Ca dialysate
Positive Ca flux $\sim$ 896 mg/4 hours dialysis or +2688 mg/week (384 mg/day)[a]
Dietary intake of Ca $\sim$ 800 mg/day
Fractional absorption $\sim$ 152 mg/day (19%)
Total Ca balance $\sim$ +536 mg/day
Calcium balance using 2.5 mEq/L of Ca dialysate
Positive Ca flux $\sim$ +150 mg/4 hours dialysis or +450 mg/week (64 mg/day)
Dietary intake of Ca $\sim$ 800 mg/day
Fractional absorption $\sim$ 152 mg/day (19%)
Total Ca balance $\sim$ +216 mg/day
Calcium balance using 1.5 mEq/L of Ca dialysate
Negative Ca flux $\sim$ −230 mg/4 hours dialysis or −690 mg/week ($\sim$ −100 mg/day)
Dietary intake of Ca $\sim$ 800 mg/day
Fractional absorption $\sim$ 152 mg/day (19%)
Total Ca balance $\sim$ +52 mg/day

[a]Reproduced with permission of Am J Kidney Dis 29:641–9.

Table 1-2. Estimated Calcium Balance in Peritoneal Dialysis Patients.

Calcium balance using 3.5 mEq/L of Ca and 1.5% dextrose dialysate
Positive Ca flux ~ +14 mg/exchange or +56 mg/day
Dietary intake of Ca ~ 800 mg/day
Fractional absorption ~ 152 mg/day (19%)
Total calcium balance ~ +208 mg/day
Calcium balance using 2.5 mEq/L of Ca and 1.5% dextrose dialysate
Negative Ca flux ~ −18 mg/exchange or −72 mg/day
Dietary intake of Ca ~ 800 mg/day
Fractional absorption ~ 152 mg/day (19%)
Total Ca balance ~ +80 mg/day
Calcium balance using 2.5 mEq/L of Ca and 4.25% dextrose dialysate
Negative Ca flux ~ −30 mg/exchange or −120 mg/day
Dietary intake of Ca ~ 800 mg/day
Fractional absorption ~ 152 mg/day (19%)
Total Ca balance ~ 32 mg/day

[a]Reproduced with permission of Am J Kidney Dis 29:641–9.

precipitate hypotension and decrease myocardial contractility.[31] Recent studies also suggest that low-calcium dialysate (2.5 mEq/L) may aggravate secondary hyperparathyroidism.[32] Adequate phosphate control may be necessary before one can reduce dialysate calcium concentration.

Despite decreased calcium intake and intestinal absorption, calcium balance studies indicate that patients with CRD have only slightly negative balances.[18–20] This is presumably because of decreased renal excretion and the resultant whole body calcium retention in patients with CRD.[20] Hence, patients with CRD can maintain a positive calcium balance if they are provided with a normal or high-calcium diet.[23] Most of the patients with ESRD have positive calcium balances because they have no route of calcium excretion. For example (Table 1-1), excluding dietary calcium absorption in ESRD patients, one can expect positive calcium fluxes of on average +896 mg/4 hours or +384 mg/day and +150 mg/4 hours or 64 mg/day thrice-weekly hemodialysis, respectively, when using 3.5 mEq/L, and 2.5 mEq/L calcium dialysate.[27] Similarly, peritoneal dialysate using 3.5 mEq/L and 1.5% dextrose provides calcium fluxes of an average of 14 mg/per exchange or approximately 56 mg/day in normocalcemic patients (Table 1-2).[28,30] In a metabolic balance of CAPD patients using 3.5 mEq/L calcium, and 4.25% and 1.5% dextrose dialysate, the average net calcium balance was +112 mg (1 g/kg of protein) to 198 mg (1.4 g/kg of protein diet) per day.[33] Assuming the ESRD patients consume 800 mg/day dietary calcium and an estimated fractional absorption of Ca of 19%,[34] the calculated daily calcium balances for the adult ESRD patients would exceed the average normal calcium threshold balance of 114 mg/day for individuals

ages 18 to 30 estimated by Matkovic and Heaney (Figs. 1-3 and 1-4).[35] Addition of calcium carbonate as a phosphate binding agent or calcitriol for the treatment of renal osteodystrophy or secondary hyperparathyroidism in older patients would further increase calcium absorption and retention.[36] Therefore, current treatment regimens provide excess calcium to ESRD patients who have no route to dispose the surplus of this mineral. Therefore, it is not surprising that calcification occurs in coronary artery calcification in young adults (age 20 to 30 years), because their calcium intakes exceed the intake threshold of this age group (6456 ± 4278).[37] A recent study indicated a high mortality related to calcification with common carotid artery, aorta, and femoral artery in ESRD patients on calcium supplement as a P binder.[38] Similar findings were observed by London et al.[39]

Patients with CRD on dialysis have two- to fivefold more coronary artery calcification than age-matched individuals with angiographically proven coronary artery disease.[40] In addition to increased traditional risk factors, CRD patients also have a number of nontraditional cardiovascular risk factors that may play a prominent role in the pathogenesis of arterial calcification, including duration of dialysis and disorders of mineral metabolism.[40]

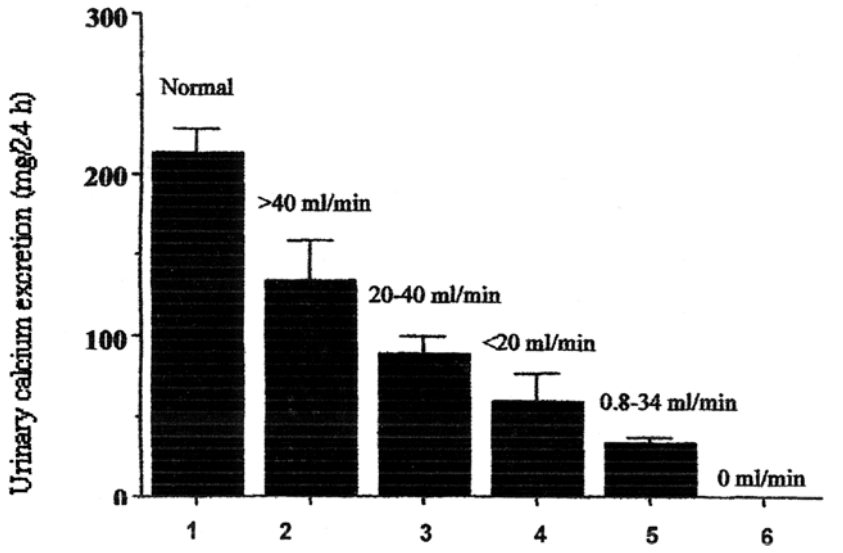

Figure 1-5. Urinary excretion of calcium in chronic renal failure. (Reproduced with permission of Am J of Kidney Dis 29:641–64eier M, Nowack R, et al. Renal function reserve in healthy elderly subjects. J Am Soc Nephrol 1993;3:1371–7.)

C. Urinary Excretion of Calcium in CRD

The kidney is the primary site for calcium excretion, although intestinal secretion of calcium also could account for a small loss of calcium in the feces.[41] Urinary excretion of calcium is decreased starting from the early stages of decreased renal function and fall in proportion to the decrease in glomerular filtration rate (GFR)[33,42] (Fig. 1-5). The potential reasons for decreased calcium excretion include decreased filter loads, increased PTH, or decreased intestinal absorptions. However, balance studies indicate that despite ingesting high-calcium diets, in patients with CRD, urinary excretion of calcium remains very low even when intestinal absorption is increased.[19,20] Thus, decreased calcium excretion is attributable to decreased GFR and not to decreased intestinal absorption (Fig. 1-5). In 254 normal subjects, the mean creatinine clearance decreased was 0.75 ml/min per day (age 22 to 97).[42]

In older normal subjects, the creatinine clearance decreased from an average of 1.2 ml/min per year and it declined from 52 ml/min at age 75 to 27 ml/min at age 95.[43]

D. Calcium Metabolic Problems in Renal Transplant Patients

Thirty-eight patients undergoing a transplant had a baseline computed tomography (CT) scan. Twenty-three underwent a follow-up scan. Unfortunately, 15 patients were not available for follow-up study. The coronary artery calcification change/score/days was not significant. In contrast, there is a trend toward an increase in aortic calcification.[44] I believe if large number of patients are included, they may show more calcification in major arteries.

E. Management of Calcium Metabolism in CRD Patients

We can achieve negative calcium fluxes by ultrafiltration during dialysis using dialysates with concentrations of calcium identical to those of plasma ionized calcium. Assuming a diffusible plasma calcium concentration of 5 mg/dl, 4 L of ultrafiltration per dialysis will remove approximately 200 mg of calcium. Another method of solving ESRD patients' calcium intake is to observe their weekly dietary calcium intake. It will help to use cellulose sodium phosphate. One gram of this compound will bind 1.8 mmol of calcium; sodium will exchange for calcium and becomes cellulose calcium phosphate and is excreted in the feces (Mission Pharmaceutical Co.). If we can maintain zero balance of calcium in ESRD patients, we may at least achieve further unnecessary metastatic calcification in the important organs.

F. Conclusion

Unfortunately there are no renal calcium excretion data for individuals older age than 35. Though two studies indicated that a group of 254 normal subjects,

creatinine clearance decreased 0.75 ml/min per year.[45] Similar findings were observed by Fliser et al.[46] We believe that before CRD patients reach ESRD, their calcium intake should follow that shown in Fig. 1-5. If their Ca dietary intakes are greater than their kidney can excrete, they could take the necessary amounts of cellulose sodium phosphate[47] as stated previously. One gram of cellulose sodium phosphate binds 36 mg of Ca. For example, if a person's creatinine clearance is 20 to 40 ml/min, he or she should take approximately 3.5 g of cellulose sodium phosphate daily. This dose could bind approximately 120 mg/day of calcium. In a recent study Merx et al.[48] stated that atherosclerosis in dialysis patients is characterized by severe vascular calcification involving arterial media, cardiac valves and the myocardium. Feturin-A/a2-Heremans-Schmid glycoprotein is as abundant serum protein with a strong calcification inhibitory factor; unfortunately, this protein is decreased in uremic patients.

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Chapter 2

Disorders of Phosphorous Homeostasis in CKD

Sharon M. Moe

I. Introduction

Inorganic phosphorus is critical for numerous normal physiologic functions including skeletal development, mineral metabolism, cell membrane phospholipid content and function, cell signaling, platelet aggregation, and energy transfer through mitochondrial metabolism. Because of its importance, normal homeostasis maintains serum phosphate concentrations between 2.5 and 4.5 mg/dl (0.81 to 1.45 mmol/L). Levels are highest in infants and decrease throughout growth, reaching adult levels in the late teens. The total adult body store of phosphorus is approximately 700 g, of which 85% is contained in bone in the form of hydroxyapatite [$(Ca)_{10}(PO4)_6(OH)_2$]. Of the remaining, 14% is intracellular, and only 1% is extracellular. Of the extracellular phosphorus, 70% is organic (phosphate) and contained within phospholipids and 30% is inorganic, 15% is protein bound, and the remaining 85% is either complexed with sodium, magnesium, or calcium or circulates as the free monohydrogen or dihydrogen forms. It is this latter 0.15% of total body phosphorus (15% of extracellular phosphorus) that is freely circulating and measured (Fig. 2-1). At pH 7.4, it is in a ratio of about 4:1 HPO_4^{-2} to H_2PO^{-1}. For this reason, P_i is usually expressed in millimoles rather than milliegvivalents per liter. Thus, serum measurements reflect only a minor fraction of total body phosphorus, and therefore do not accurately reflect total body stores in the setting of abnormal homeostasis such as in chronic kidney disease (CKD). The terms phosphorus and phosphate are often used interchangeably, but strictly speaking, the term phosphate means the inorganic freely available form (HPO_4^{-2} to H_2PO^{-1}). However, most laboratories report this measurable, inorganic component of total body phosphorus as "phosphorus." In the remainder of the chapter we use the abbreviation P_i to represent phosphate and/or phosphorus.

The average Western diet contains approximately 1000 to 1400 mg of P_i per day, while the recommended daily allowance (RDA) is 800 mg/day.

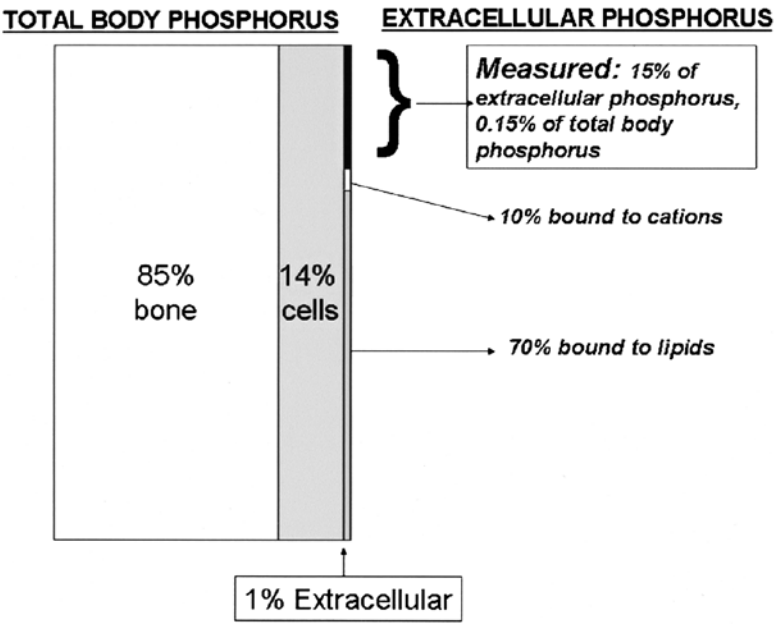

Figure 2-1. Distribution of total body phosphorus.

Table 2-1. Phosphorus Content of Food.

High (> 200 mg P per 100 g)
Milk products
Meats
Fish
Dry fruits
Chocolate
Medium (> 100 but < 200 mg P per 100 g)
Cereals
Legumes
Low (< 100 g P per 100 g)
Vegetables
Fruits

Approximately two thirds of the ingested P_i is excreted in the urine, and the remaining one third in stool. Thus, as detailed later, patients with advanced CKD will retain P_i on a typical American diet. Many prepackaged, fast food, and dark (cola) beverages contain extra P_i as a preservative and it is difficult to accurately predict P_i intake based on the food type alone. In general, foods high in protein and dairy products contain the most P_i, whereas fruits and

vegetables contain the least (Table 2-1). In addition, some protein foods have increased protein:P_i ratio and thus would be ideal for a CKD patient who has increased protein needs, whereas others have a low protein:P_i ratio and are not recommended.[1] This complexity and the abundance of P_i in foods make it difficult for patients with CKD to adhere to a phosphate-restricted diet while simultaneously increasing protein intake.

II. Normal Phosphorus Homeostasis

Three organs are involved in P_i homeostasis (regulation of extracellular and intracellular P_i levels): intestine, kidney, and bone. The major hormones controlling P_i levels are vitamin D and parathyroid hormone (PTH). More recently, there is increasing evidence for an important role of a group of circulating factors called phosphatonins in the regulation of serum P_i.

III. Intestinal Absorption

Between 60% and 70% of dietary P_i is absorbed by the gastrointestinal tract, in all intestinal segments.[2] P_i absorption is dependent on both passive transport related to the concentration in the intestinal lumen (i.e., increased after a meal) and active transport stimulated by 1,25-$(OH)_2$D (calcitriol), the active metabolite of vitamin D (see Chapter 5). Passive absorption (dependent on luminal P_i concentration) occurs via the epithelial bush border sodium phosphate cotransporter (NPT2b) utilizing energy from the basolateral sodium–potassium ATPase transporter. The NPT2b is in the terminal web, just below the brush border in "ready to use" vesicles that are then transported to the brush border in response to acute and chronic changes in P_i concentration.[3] Medications or foods that bind intestinal P_i (antacids, phosphate binders, calcium) can decrease the net amount of P_i absorbed by decreasing the free phosphate for absorption. Calcitriol can upregulate the sodium–phosphate cotransporter and therefore actively increase P_i absorption.[4] However, in contrast to calcium, the active vitamin D-mediated absorption is a minor component of total absorption, supported by data that there is near normal intestinal absorption in the absence of vitamin D. However, similar to calcium, the kidneys play a critical role in the maintenance of normal homeostasis.

IV. Renal Handling

Most inorganic P_i is freely filtered by the glomerulus. Approximately 70% to 80% of the filtered load of P_i is reabsorbed in the proximal tubule, which

serves as the primary regulated site of the kidney. The remaining approximately 20% to 30% is reabsorbed in the distal tubule. Factors that increase P_i excretion are primarily an increased plasma P_i concentration and PTH. Conversely, acute or chronic P_i depletion will decrease excretion. Renal P_i excretion is also increased, although to a lesser extent, by volume expansion, metabolic acidosis, glucocorticoids, and calcitonin. Additional factors that may decrease P_i excretion include growth hormone and thyroid hormone. The majority of this regulation occurs in the proximal tubule via the sodium–phosphate cotransporter.[3] Similar to the intestine, the sodium–phosphate cotransporter rests in the terminal web, and can be acutely moved to the brush border in the presence of acute or chronic phosphate depletion. Alternatively, after a phosphate load or in the presence of PTH, the exchanger is removed from the brush border and catabolized.[5] The ability of the kidneys to control P_i becomes impaired because of decreased renal mass at glomerular filtration rates (GFR) of approximately 50 ml/min. However, the subtle elevations in serum P_i (due to decreased kidney function) stimulate PTH, which in turns increases P_i excretion to maintain normal serum P_i levels. This compensatory mechanism is why frank hyperphosphatemia is seen only in patients with very advanced kidney disease.

V. Bone Remodeling

The majority of the total body stores of calcium and P_i are located in bone in the form of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. Trabecular (cancellous) bone is located predominately in the epiphyses of the long bones, which is 15% to 25% calcified, and serves a metabolic function with a relatively short turnover rate. In contrast, cortical (compact) bone is in the shafts of long bones, and is 80% to 90% calcified. This bone serves primarily a protective and mechanical function, and has a turnover rate of months. Bone consists principally (90%) of highly organized crosslinked fibers of type I collagen; the remainder consists of proteoglycans and "noncollagen" proteins such as osteopontin, osteocalcin, osteonectin, and alkaline phosphatase. Osteoclasts are the bone-resorbing cells and derive from circulating hematopoietic cells, and osteoblasts are the bone-forming cells that derive from the marrow.

Bone is a dynamic organ and remodels or turns over in response to hormones, cytokines, and changes in mechanical forces. The control of bone remodeling is highly complex, but appears to occur in very distinct phases: (1) osteoclast resorption, (2) reversal, (3) preosteoblast migration and differentiation, (4) osteoblast matrix (osteoid or unmineralized bone) formation, (5) mineralization, and (6) quiescent stage. At any one time, less than 15% to 20% of the bone surface is undergoing remodeling, and this process in a single bone

remodeling unit can take 3 to 6 months.[6] How a certain piece of bone is committed to undergo a remodeling cycle, and how the osteoclasts and osteoblasts signal each other, is not completely clear but is likely mediated through the interaction of osteoprotegerin (OPG) and receptor activator of nuclear-factor κB (RANK). This important control system is regulated by nearly every cytokine and hormone thought important in bone remodeling, including PTH, 1,25-$(OH)_2$D, estrogen, glucocorticoids, interleukins, prostaglandins, and members of the transforming growth factor-beta (TGF-β) superfamily of cytokines.[7–9] Thus, all of these factors, by inducing bone remodeling, can affect P_i homeostasis. However, of these, PTH is most clinically relevant, especially in CKD.

VI. Phosphatonins

This is a group of proteins that have been identified in patients with renal phosphate wasting. Fibroblast growth factor 23 (FGF-23) is produced by tumors from patients with tumor-induced osteomalacia, and corresponding genetic defects were identified in autosomal dominant hypophosphatemic rickets (ADHR). FGF-23 is made in bone cells, and directly affects the conversion of

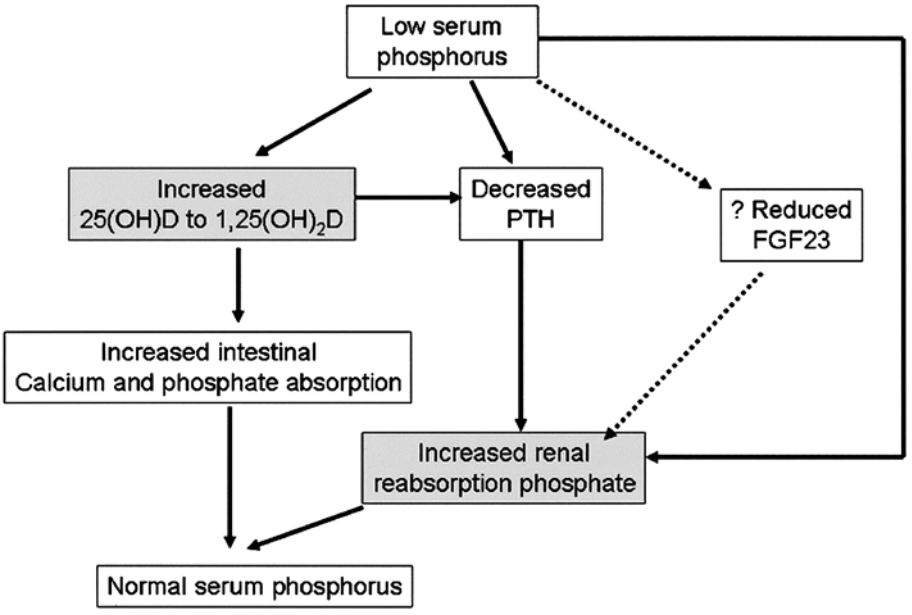

Figure 2-2. Normal homeostatic response to hypophosphatemia. The solid lines represent known pathways. The dotted line represents an important pathway in disease, but of unclear importance in normal physiology.

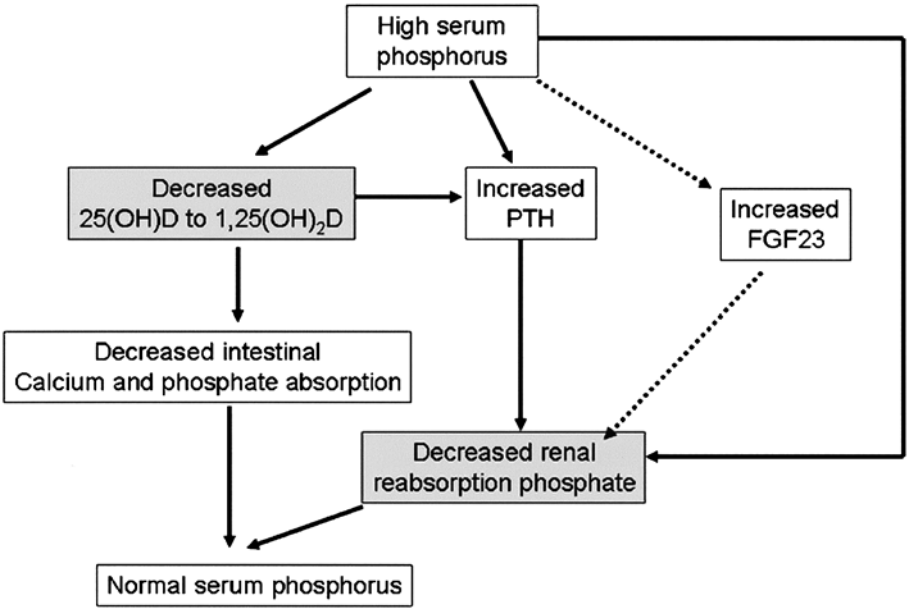

Figure 2-3. Normal homeostatic response to hyperphosphatemia. The solid lines represent known pathways. The dotted line represents an important pathway in disease, but of unclear importance in normal physiology.

25- to 1,25-$(OH)_2D$ by inhibition of the 1α-hydroxylase enzyme in the renal tubules. Levels of FGF-23 are very elevated in patients with CKD, presumably because of net phosphate retention.[10,11] Another factor, secreted frizzled-related protein 4 (FRP-4), also can induce renal phosphate wasting.[10,11] Thus, these factors are important in non-PTH-mediated urinary phosphate handling, although their precise role in normal homeostasis is not yet clear.

In summary, homeostasis, or control of serum homeostasis is controlled primarily by the serum level of P_i/dietary intake, PTH, and possibly phosphatonins. This regulation occurs primarily at the level of the kidney, although the intestine and bone are also involved (Figs. 2-2 and 2-3).

VII. Disorders of P_i in Non-CKD Patients

A. Hypophosphatemia

Hypophosphatemia can occur when there is decreased P_i intake (decreased intestinal absorption or increased gastrointestinal losses), or excess renal wasting from renal tubular defects or hyperparathyroidism. In addition, low serum P_i levels may also occur in the setting of extracellular to intracellular shifts. In the case of cellular shifts, total body P_i may not be depleted. By convention,

Table 2-2. Cause of Hypophosphatemia.

Decreased intestinal absorption: Antacid abuse, malabsorption, chronic diarrhea, vitamin D deficiency, starvation, anorexia, alcoholism
Increased urinary losses: Primary hyperparathyroidism, postrenal transplant, extracellular fluid volume expansion, glucosuria (after treating DKA), post obstructive or resolving ATN diuresis, acetazolamide, Fanconi's syndrome, X-linked and vitamin D-dependent rickets, oncogenic osteomalacia
Redistribution: Respiratory alkalosis, alcohol withdrawal, severe burns, TPN, recovery from malnutrition when inadequate phosph is provided (post-feeding syndrome), leukemic blast crisis

hypophosphatemia is often graded as mild (< 3.5 mg/dl), moderate (< 2.5 mg/dl), or severe (< 1.0 mg/dl). Moderate and severe hypophosphatemia will generally occur only when there are multiple problems. The causes of hypophosphatemia are listed in Table 2-2.

Hypophosphatemia is a common finding; it is seen in 3% of all hospitalized patients, 10% of hospitalized alcoholic patients, and more than 50% of ventilated ICU patients.[12] Symptoms of hypophosphatemia are usually seen only in patients with moderate or severe hypophosphatemia and include muscle weakness (and difficulty weaning from a ventilator), hemolysis, impaired platelet and white blood cell function, rhabdomyolysis, and in rare cases neurologic disorders. Hypophosphatemia is probably overtreated in the ICU, where the "difficult to wean" patient is given P_i, when the low levels are actually caused by cellular shifts from respiratory alkalosis due to difficulty weaning. A careful review of the trend in serum P_i with arterial blood pH can help discern which patients need to be treated.

The treatment of hypophosphatemia in the non-CKD patient is based on the underlying cause. The differential diagnosis and treatment approach will be based on the cause and site of P_i loss. Usually the cause is clinically apparent, but if not, the simplest test is to measure a 24-hour urine P_i. In the setting of hypophosphatemia, the kidney should be reabsorbing all P_i. If the urinary excretion is < 100 mg/24 hours, then the kidney has an appropriate response, and there are gastrointestinal losses or extracellular to intracellular shifts. P_i can be replaced by increased intake, but is usually necessary only in patients with moderate to severe hypophosphatemia. Oral intake is preferable, as the acute intravenous administration of phosphate can complex calcium and lead to extraskeletal calcification. Oral P_i supplementation can be given with skim milk (1000 mg/quart), whole milk (850 mg/quart), Neutraphosph K capsules® (250 mg/capsule; maximum dose is 3 tablets every 6 hours), or Neutraphosph® solution (128 mg/ml solution). Milk is much better tolerated (and less expensive!). Intravenously, P_i can be replaced as K phosphate (3

mmol/ml of phosphate, 4.4 mEq/ml of K) or Na phosphate (3 mmol/ml of phosphate, 4.0 mEq/ml of Na).

B. Hyperphosphatemia

Because elevated serum P_i levels can stimulate rapid renal P_i excretion, persistent hyperphosphatemia for more than a few hours occurs almost exclusively in the setting of acute or chronic kidney disease. It is important to emphasize that serum creatinine is not a sufficient indicator of abnormal kidney function and formulas for predicting GFR should be used. These include either the Cockcroft–Gault formula that utilizes creatinine, age, gender, and weight,[13] or the modified diet in renal disease (MDRD)[14] that utilizes creatinine, age, gender, and race (black or non-black). The latter can be downloaded from the National Kidney Disease Education Program Web Page into a PDA.

The causes of hyperphosphatemia are listed in Table 2-3. As indicated previously, hyperphosphatemia can occur from increased intestinal absorption or rapid intracellular to extracellular shifts. However, persistent hyperphosphatemia requires kidney dysfunction as any increase in serum P_i will quickly lead to increased renal excretion and a compensatory rise in serum PTH, with normalization of serum P_i. Demonstration of this rapid physiologic response is shown in Fig. 2-4. The graphs represent data from patients with normal kidney function who underwent bowel preparation with oral sodium phosphate solution for a colonoscopy. As shown in Fig. 2-4, after administration of 45 ml of oral sodium phosphate cleansing preparation (arrows), there is an acute rise in serum P_i (top panel), and an almost immediate increase in urine P_i excretion (bottom panel). PTH also increases modestly. After a second dose the serum and urinary P_i levels again increase.[15] This study illustrates the importance of the kidneys in the maintenance of serum P_i levels. It also illustrates that the administration of P_i by oral or intravenous routes can increase the serum level acutely, which can lead to precipitation of calcium and P_i in some situations. Thus P_i repletion should be judiciously used in the treatment of hypophosphatemia.

Table 2-3. Causes of Hyperphosphatemia.

Increased intake: Phosphate-containing solutions (oral or enema sodium phosphate, intravenous phosphate), vitamin D overdose
Decreased renal excretion: Kidney disease (acute or chronic), thyrotoxicosis, acromegaly
Intracellular to extracellular shift: Tumor lysis syndrome, rhabdomyolysis, hemolysis, hyperthermia, profound catabolic stress, acute leukemia

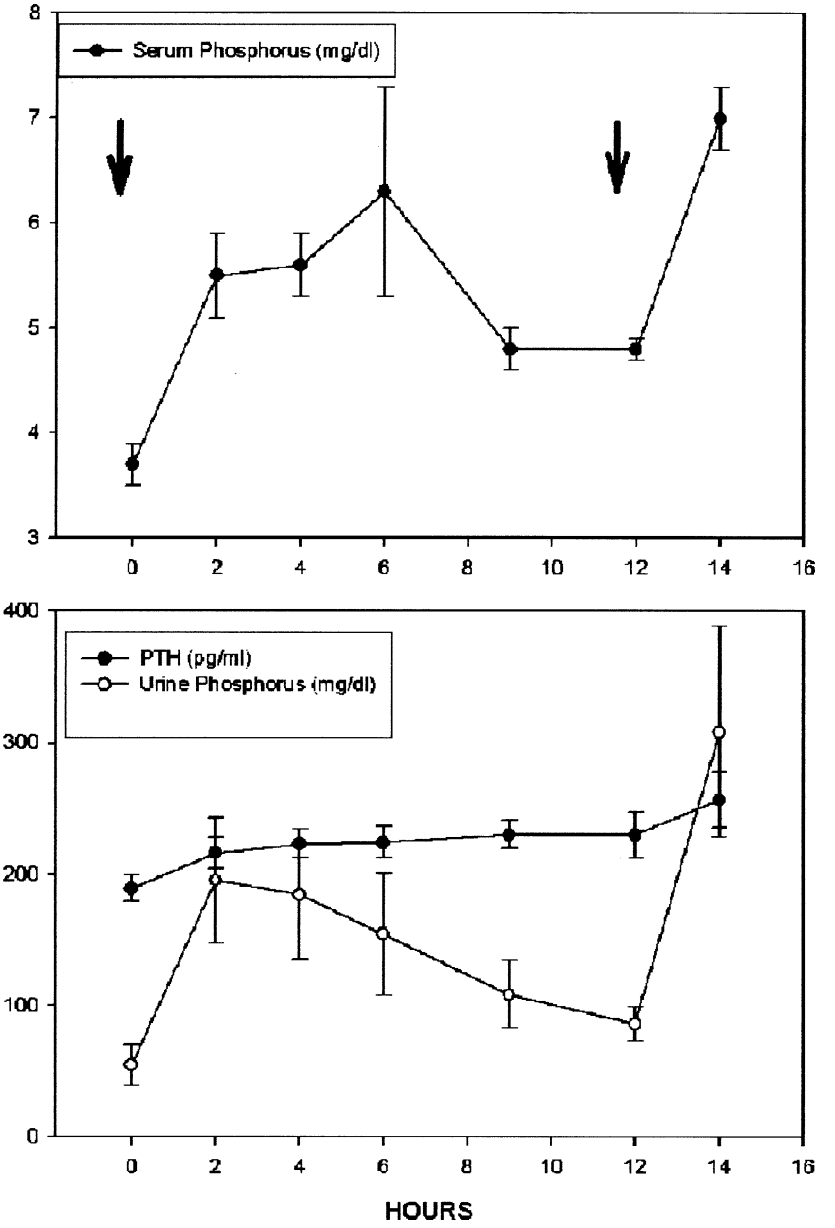

Figure 2-4. Adaptive response to phosphorus intake. Rapid response to phosphate load in the form of sodium phosphate bowel enema administered 12 hours apart (arrows, upper panel). The serum P_i increases (upper panel), and there is a very rapid increase in urinary P_i excretion (lower panel). In addition, there is a later, more modest increase in parathyroid hormone (PTH; lower panel). Thus, changes in serum P_i induce a rapid change in P_i excretion. (Adapted from ref. 15.)

Acute hyperphosphatemia generally does not cause symptoms unless there is precipitation of calcium × P_i, although chronic hyperphosphatemia seen in CKD is associated with adverse sequelae (see later). Treatment of acute hyperphosphatemia includes volume expansion, dialysis, and phosphate binders, although in the setting of normal, or even mild to moderate kidney disease it is usually self-resolving owing to the continued ability of the kidney to excrete a P_i load.

VIII. Disorders of P_i In CKD Patients

As should be apparent from the preceding discussion, hyperphosphatemia is a common problem in patients with CKD. Levels of P_i are above the normal range in most subjects once the GFR is less than 15 to 30 ml/min.[16] The reason that serum levels are often maintained within the normal range at abnormal GFR is because of a compensatory secondary hyperparathyroidism, which then increases the urinary P_i excretion. This will lead to normal serum levels until the maximum excretion per nephron is reached AND there is a decrease in the number of functioning nephrons. This rise in serum PTH at the expense of maintaining normal serum P_i is believed to be a major mechanism by which secondary hyperparathyroidism develops in patients with CKD (Fig. 2-5)[17] and is often referred to as the "trade-off hypothesis."[18] Support for this hypothesis is generated by animal studies in which P_i restriction can slow or halt the progression of secondary hyperparathyroidism in CKD.[19] In addition, despite the maintenance of serum P_i in the normal range in patients with CKD stage 3 (GFR 60–30 ml/min), there is a gradual increase in the serum levels,[20,21] indicating a new "steady state" of slightly higher serum P_i and increased PTH. For example, in a recent national cross-sectional study, the mean serum P_i level

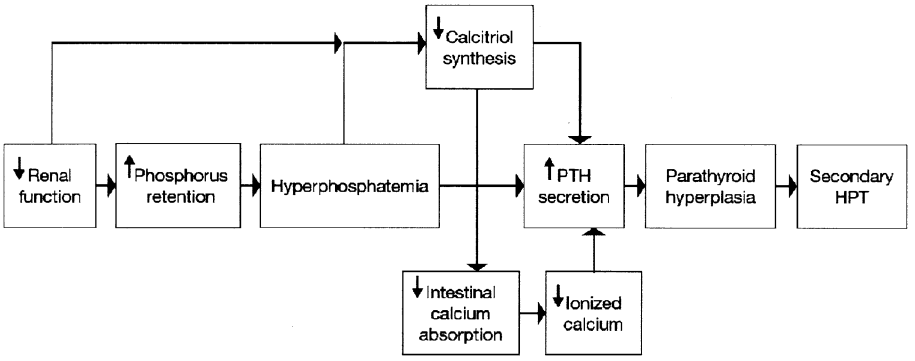

Figure 2-5. The pathogenesis of secondary hyperparathyroidism. (Reprinted from ref. 17 with permission.)

in patients with CKD stage 3 was 3.5 ±0.5 mg/dl ($n = 65$); in stage 4, was 4.1 ±1.1 mg/dl ($n = 113$); and in stage 5 not on dialysis 4.4 ± 1.1 ($n = 22$).[21]

P_i has a direct effect on the synthesis of PTH. In addition, P_i retention will inhibit the conversion of 25-(OH) vitamin D to 1,25-$(OH)_2$D by inhibiting the 1α-hydroxylase enzyme in the kidney, and will lower the ionized calcium by complexing with free calcium. Together these factors contribute to the pathogenesis of secondary hyperparathyroidism in CKD (Fig. 2-5). The therapeutic approach to treat secondary hyperparathyroidism includes control of P_i load through the use of dietary restriction, phosphate binders, administration of vitamin D, and normalizing serum calcium levels. These options are further discussed in Chapter 3.

IX. Dialytic Removal of Phosphate

Unfortunately, standard dialysis is ineffective in clearing P_i, owing to only a small percentage of total body P_i present in the extracellular space. The extracellular serum levels decrease during a single hemodialysis treatment, but immediately after a hemodialysis treatment there is a rapid equilibration of intracellular P_i to the extracellular space resulting in a "rebound" of the serum levels to near predialysis values within 2 to 4 hours after the treatment.[22] Increasing the blood flow or using larger dialyzers has little effect on overall clearance of P_i during hemodialysis.[23] Similarly, peritoneal dialysis can remove some P_i, but not enough to keep up with normal daily intake.[24] As a result, more than 90% of patients on standard thrice weekly hemodialysis or peritoneal dialysis require phosphate binders. Unfortunately, patients have a difficult time remaining compliant with phosphate binders owing to the large number of pills required and the frequency of administration (with each meal).[25] However, reducing the dietary intake of P_i from 1500 mg to 900 mg per day can reduce the number of binders needed by nearly one half. Thus, a combination of dietary restriction and phosphate binders are needed with standard dialysis regimens.

The overall clearance of P_i is increased with more frequent dialysis or longer sessions. Continuous forms of dialysis such as slow nocturnal hemodialysis, in which patients are dialyzed over an 8- to 10-hour period each night is highly effective in removing P_i such that patients no longer require phosphate binders and some even require P_i supplementation.[26] Similarly, patients undergoing short daily dialysis also have significant reductions in phosphate binder requirements.[27] Further supporting that increased frequency of dialysis is important in P_i removal are data showing that serum P_i levels are highest after the longest interdialysis interval (i.e., Mondays for patients dialyzed on Monday, Wednesday, and Friday).[28] Given the significant morbidity and

mortality associated with hyperphosphatemia, these new dialytic therapies may improve P_i control in the future if they become more utilized.

X. Consequences of Hyperphosphatemia

Cross-sectional studies have demonstrated that hyperphosphatemia is associated with all cause and cardiovascular mortality in one study of patients with CKD stages 3 and 4 (GFR 15 to 60 ml/min),[29] and in several studies in patients on hemodialysis in the United States[30–32] and throughout the world[33,34] (Fig. 2-6). The level of P_i at which there is an increased mortality depends on the study design and the reference range set. The first study used a "normal" range of 5 to 7 mg/dl and found levels greater than those that were associated with increased mortality when adjusted for multiple confounding factors.[30] Subsequent studies have used even lower levels as the reference

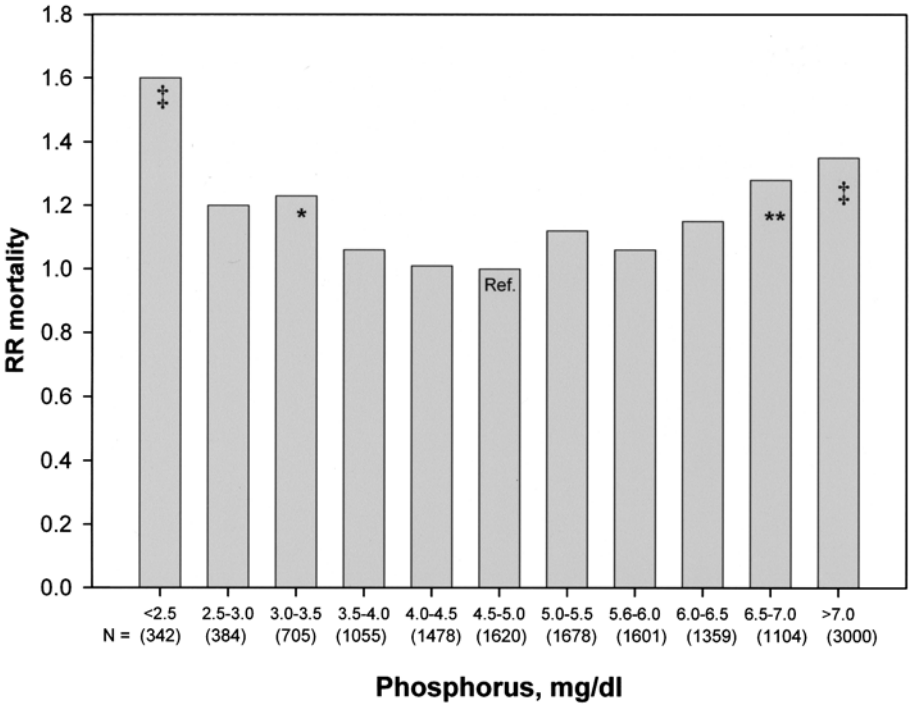

Figure 2-6. Association between all-cause mortality and serum phosphorus concentration, stratified by country and adjusted for serum concentrations of calcium and PTH, dialysate calcium concentration, age, gender, race, duration of ESRD, hemoglobin, albumin, Kt/V, and 14 summary comorbid conditions. *$p < 0.05$; **$p < 0.01$; †$p < 0.001$; ‡$p < 0.0001$. (Reprinted with permission from ref. 34.)

range (5.0 mg/dl) and have found levels above that increase the risk of all cause and cardiovascular mortality.[31] A study by Block et al.[31] found a 4% increase in mortality per 1 mg/dl (0.32 mmol/L). Another study in Canada, after adjustment for demographic variables, dialysis type and adequacy, hemoglobin, and albumin, found that serum P_i independently predicted mortality with a relative risk of 1.56 per 1 mmol/L.[35] Thus, the target P_i level in CKD patients should be less than 5.0 mg/dl, and preferably in the normal range for the general population. However, very low levels of P_i (< 2.5 mg/dl) are associated with osteomalacia and bone disease, and can even induce rhabdomyolysis and therefore should be avoided. However, whether low levels of P_i are associated with increased mortality or are only a marker for malnutrition is controversial.[31,34] Although these data are compelling, it is important to emphasize that the cross-sectional nature of these studies cannot confer a true cause-and-effect relationship. There are no prospective trials demonstrating that reduction in serum P_i is associated with increased survival. However, observational data confirm that P_i is a modifiable risk factor, control of which predicts long-term survival.[36]

What are the potential mechanisms by which hyperphosphatemia can cause cardiovascular disease and death? P_i is an important component of cell membranes, and its intracellular:extracellular ratio is tightly controlled. Hyperphosphatemia may hasten the decline in kidney disease based on animal models,[37] presumably owing to deposition in the renal tubules or calcification of the arteries causing glomerular ischemia. High levels of P_i, but in the range observed in many dialysis patients, can induce vascular smooth muscle cells to transform, or dedifferentiate to osteoblast (bone-like) cells in culture.[38] These differentiated cells express the factor Cbfa1 (core binding factor alpha 1), an important transcription factor that directs a pluripotent mesenchymal stem cell to become an osteoblast. Once transformed, these cells can lay down a matrix of collagen and noncollagenous bone proteins that can become calcified.[39] High levels of both calcium and P_i can induce vascular smooth muscle cells to mineralize in vitro, and the effects of both are additive.[40,41] This transformation is further accelerated by other uremic toxins.[42] There are additional data that in humans with CKD, hyperphosphatemia is associated with vascular calcification, and vascular calcification is associated with increased cardiovascular and all cause mortality.[39,43] The risk of vascular calcification increases with advancing age, duration of dialysis, and in patients with diabetes mellitus,[39] all risk factors for poor survival. Thus, there is biologic plausibility based on in vitro data and some human studies that hyperphosphatemia may lead to increased cardiovascular mortality by inducing vascular calcification.

In addition to the effects on vascular calcification, hyperphosphatemia is a major factor in the pathogenesis of secondary hyperparathyroidism. Elevated levels of PTH are also associated with increased all cause mortality and

cardiovascular mortality,[31,33,34] as well as increased hospitalizations,[31] hip fractures,[44] and cardiac dysfunction.[45] PTH directly affects the intracellular calcium concentration of nearly all cells, and can thereby adversely affect many cellular functions.[46]

XI. Conclusions

P_i is a key ion in the body, with important diverse functions. The maintenance of serum P_i levels is dependent on normal kidney function. As a result, patients with kidney disease are often hyperphosphatemic. Elevations in serum P_i are associated with increased morbidity and mortality in patients with CKD, may hasten loss of residual renal function, and can cause secondary hyperparathyroidism. Unfortunately, current removal of P_i with thrice weekly hemodialysis or daily peritoneal dialysis is not adequate for normal dietary intake. As a result, phosphate binders are a mainstay of therapy in patients with CKD.

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Chapter 3

Pathogenesis and Management of Secondary Hyperparathyroidism

Krishna R. Polu and Ajay K. Singh

I. Introduction

Secondary hyperparathyroidism is a ubiquitous complication of advanced chronic kidney disease that has been recognized since the 1930s.[1] The "trade-off hypothesis" in the late 1960s cemented the notion that secondary hyperparathyroidism represents a complex interplay of various hormonal and biochemical factors leading to excess synthesis and secretion of parathyroid hormone (PTH).[2] Hyperphosphatemia, hypocalcemia, and vitamin D deficiency all play a role in the development of secondary hyperparathyroidism (SHPT), and more recent data suggest that the calcium-sensing receptors on the parathyroid gland also contribute to the etiology of secondary hyperparathyroidism in patients with chronic kidney disease (CKD).

SHPT is a significant cause of morbidity and mortality in patients with advanced CKD and controlling PTH and defects in mineral metabolism is crucial in improving patients' quality and duration of life. However, managing SHPT can be difficult and is a challenge that all nephrologists face in the treatment of patients with CKD. Although the management of SHPT is a clear focus in the care of patients on dialysis, detection and management of this problem in early stages of CKD is performed with less frequency and rigor. Early recognition and diagnosis of SHPT are made difficult by the lack of laboratory derangements of mineral metabolism early in the course of disease. Further, the complexity of addressing this problem involves balancing the suppression of parathyroid hormone secretion with supplementation of vitamin D and the appropriate control of serum levels of calcium and phosphorus. Traditional therapies with vitamin D sterols and phosphorus binders remain the mainstay of therapy; however, these treatments can be complicated by elevated serum levels of calcium and higher calcium/phosphorus products, which have been associated with a greater risk for vascular calcifications.

Novel therapies have been introduced recently that target the calcium-sensing receptor of the parathyroid gland that may have the additional benefit of avoiding these pitfalls. However, in extreme and resistant cases of secondary hyperparathyroidism, parathyroidectomy may be indicated. Fortunately, new insights into the pathogenesis of SHPT may offer additional targets in the management of this complex disease. The appropriate management of this syndrome has implications for the prevention of bone disease, soft tissue and vascular calcification, and ultimately, reduction of cardiovascular morbidity and mortality.

II. Pathogenesis

The pathogenesis of secondary hyperparathyroidism is multifactorial and begins early in CKD (Fig. 3-1). The central abnormality is an excess secretion of PTH by the parathyroid glands. PTH secretion is influenced by extracellular calcium and phosphorus balance and the prevailing density and activation of vitamin D (VDR) and calcium-sensing receptors (CaR) on the parathyroid gland. In the early stages of CKD, reduced kidney function results in attenuated 1α-hydroxylase activity and decreased levels of calcitriol (1,25-dihydroxyvitamin D). Because of this deficiency in calcitriol there is reduced absorption of calcium in the gut and reduced mobilization of calcium.[3,4] In addition, excess extracellular phosphorus binds to calcium and further drives down the ionized plasma calcium concentration. Collectively, mild to modest hypocalcemia

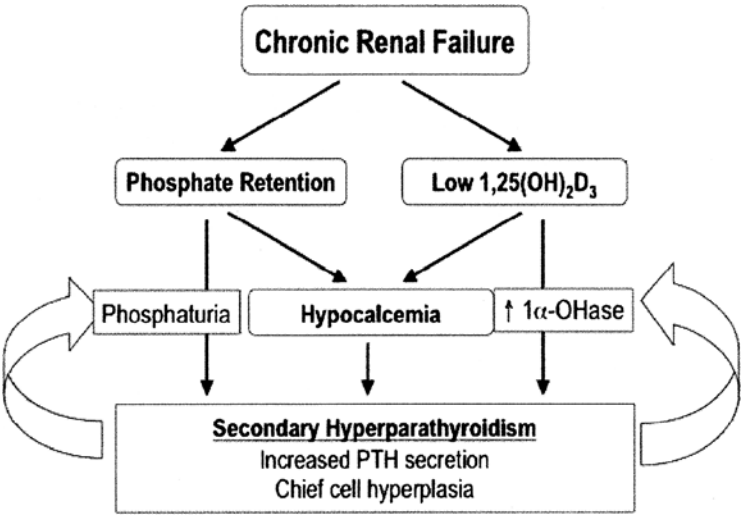

Figure 3-1. Pathogenesis of secondary hyperparathyroidism.

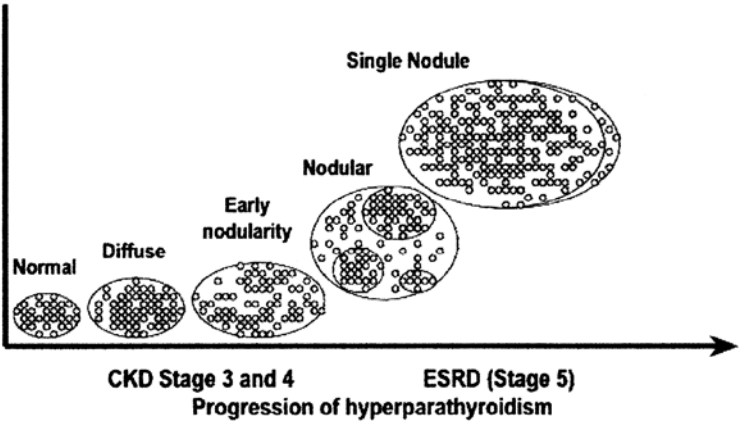

Figure 3-2. Progression of parathyroid hyperplasia. (From Tominaga and Takagi, 1996.)

ensues. Hypocalcemia, in turn, stimulates the synthesis and secretion of PTH. As kidney disease progresses there is a decrease in the number of VDR and CaR on the parathyroid gland.[5,6] Reduction in VDR and CaR density causes resistance of the parathyroid glands to both calcitriol and calcium.[7–10] In parallel, excess extracellular phosphorus induces hyperplasia of the parathyroid glands independent of calcium and calcitriol. Further, elevated phosphorus by a posttranscriptional mechanism increases PTH synthesis and secretion. The initial hyperplasia of the parathyroid gland is followed in later stages by nodularity (Fig. 3-2), with evidence at a cellular level of monoclonal cellular expansion.[11,12]

Several studies have demonstrated that circulating calcitriol levels begin to fall when the glomerular filtration rate is less than 40 ml/min. By the time patients have progressed to end-stage renal disease (ESRD) the calcitriol level is markedly reduced.[13] The removal of the normal suppressive effect of calcitriol on the parathyroid glands results in PTH secretion. The lack of calcitriol is thought to cause downregulation of vitamin D receptors, which then promotes parathyroid chief cell hyperplasia and nodule formation. The resistance of parathyroid cells to calcitriol appears to serve as a second stimulus for PTH secretion in patients with advanced CKD. Reduction of calcitriol receptor density in parathyroid glands is currently considered the mechanism responsible for the resistance to fairly robust doses of vitamin D in chronic renal failure. As renal failure progresses, this disturbance may form a vicious cycle of further reducing calcitriol receptor density leading to progressive resistance to calcitriol.

A. Role of Phosphate

As mentioned previously, phosphate homeostasis plays a key role in the development of secondary hyperparathyroidism in patients with CKD. As glomerular filtration rate (GFR) declines with advancing renal disease, renal phosphate excretion is reduced and serum levels of phosphate rise. Circulating levels of phosphate are also influenced by a variety of other factors; dietary phosphorus intake, intestinal absorption, and exchange with bone reservoirs. The major hormones that regulate phosphate homeostasis through these mechanisms are 1,25-$(OH)_2D_3$ and PTH. PTH inhibits renal phosphate absorption and stimulates 1α-hydroxylase activity. This is turn increases the synthesis of active vitamin D (calcitriol), which has a direct effect on phosphate reabsorption in the gut. As renal disease progresses, rising serum phosphate levels and decreased 1,25-$(OH)_2D_3$ synthesis contribute to increase PTH production and the development of secondary hyperparathyroidism.

Regulation of phosphate homeostasis in the kidney occurs primarily in the proximal tubule. Approximately 80% of the filtered load of phosphorus is reabsorbed here along with sodium via a NaP_i IIa contransporter.[14] Reabsorption is regulated by controlling the numbers of NaP_i IIa cotransporters on the brush border of the proximal tubule cells. Regulation of the density of these cotransporters is achieved either through mobilization from intracellular stores during times of reduced dietary phosphate acutely or through transcription-dependent methods in periods of more chronic changes in dietary phosphate intake.[15] Both PTH and calcitriol modulate NaP_i IIa expression in the kidney to control serum phosphate levels. Whereas PTH serves to decrease phosphate reabsorption by downregulating expression of the NaP_i II cotranporter, vitamin D has the opposite effect to enhance phosphate retention.[16,17] Calcitriol further regulates the expression of the NaP_i IIB channel in the jejunum to mediate active intestinal phosphate transport into the blood.[18] In addition to vitamin D and PTH, an emerging class of phosphate-regulating proteins called phosphatonins may also play an important role in modulating phosphate reabsorption and 1,25-$(OH)_2D_3$ production.

B. Phosphotonins

Phosphatonins are a group of proteins that were first discovered in the characterization of a group of pathologic conditions characterized by phosphate wasting. Importantly, these diseases were not associated with changes in serum calcium, PTH, and calcitriol. These disorders included X-linked hypophosphatemic rickets (XLH), autosomal dominant hypophosphatemic rickets (ADHR), and tumor-induced osteomalacia (TIO) and were characterized by hypophosphatemia, hyperphosphaturia, and defective bone mineralization.[14] Activating mutations in the genes encoding phosphatonins are a common

feature of these syndromes and early studies have identified three proteins associated with these syndromes: fibroblast growth factor 23 (FGF-23), frizzled-related protein 4 (FRP-4), and matrix extracellular phosphoglycoprotein (MEPE).

Among the phosphotonin proteins described, activating mutations in the gene encoding FGF-23 were found to be common among TIP, XLH, and ADHR. FGF-23 directly contributes to hypophosphatemia and phosphaturia by directly suppressing 1,25-$(OH)_2D_3$ formation through inhibition of 1α-hydroxylase activity.[14] In addition, FGF-23 has been shown to reduce expression of NaP_i-IIa cotransporter expression in the proximal tubule cells and through this mechanism also contributes to urinary phosphate loss.[19,20]

The role of phosphotonins in phosphate wasting syndromes has led to additional investigation of their contribution to phosphate homeostasis. Of the initial phosphotonin proteins reported, FGF-23 has probably been the most extensively studies in this regard. In a study of normal subjects, FGF-23 levels varied appropriately with changes in dietary phosphate intake and were found to be inversely related to renal P_i transport and serum calcitriol levels.[21]

Understanding the expression of FGF-23 and other phosphatonin proteins such as FRP-4 and MEPE may help further explain the early development of SHPT. Indeed, studies have shown alterations in FGF-23 expression with increasing levels as renal function declines.[22,23] In an analysis of 62 predialysis patients, serum FGF-23 levels increased with a decrease in creatinine clearance. Further, a negative correlation was found between FGF-23 and serum 1,25-$(OH)_2D_3$ levels.[23] Gutierrez et al. further evaluated the role of FGF-23 in advancing kidney disease and disorders of mineral metabolism. FGF-23, iPTH, 25-$(OH)D_3$, calcitriol, calcium, phosphate, and urinary fractional excretion of phosphate [Fe(PO_4)] were measured in 80 CKD patients not on dialysis. Both increased FGF-23 and PTH were independently associated with increased Fe (PO_4). Both FGF-23 and decreased 25-$(OH)D_3$ levels were independently associated with decreased calcitriol levels. The authors concluded that increased FGF-23 may contribute to maintaining normal phosphate levels early in CKD through its phosphaturic effect, which may explain the lack of laboratory phosphate derangement seen early in SHPTH. More importantly, elevated FGF-23 may contribute to worsening 1,25-$(OH)D_3$ deficiency and be a central factor in the early pathogenesis of SHPT.[24]

In dialysis patients, elevated levels of phosphate and intact PTH may be potent regulators of FGF-23 activity. Measuring FGF-23 levels may have more immediate clinical relevance in the dialysis population and potentially offer prognostic information in the treatment of secondary hyperparathyroidism. At the time of initiation of dialysis, FGF-23 levels may be extremely elevated.[22] In a regression analysis of 158 male hemodialysis patients, plasma FGF-23 levels demonstrated a significant and positive correlation with serum inorganic

phosphate, intact PTH, corrected calcium, and duration of dialysis.[25] Nakanishi and colleagues evaluated 103 nondiabetic dialysis patients with SHPT. After a 2-year follow-up, they determined that elevated levels of FGF-23 ($\geqslant$ 7500 ng/L) were predictive of refractory secondary hyperparathyroidism.[26] Kazama et al. evaluated 62 dialysis patients with secondary hyperparathyroidism (iPTH $>$ 300 pg/ml) before vitamin D therapy. Lower pretreatment levels of FGF-23 ($\leqslant$ 9860 ng/L) were associated with greater success (88.2%) as opposed to dialysis patients with elevated levels ($>$ 9860 ng/L) who had much lower success with vitamin D therapies (4.2%).[27]

The pathogenesis of secondary hyperparathyroidism is a complex process that begins early in CKD. The modulation of phosphate, calcium, active vitamin D, and potentially phosphotonin proteins play a role in the development of SHPT and offer targets for therapy.

III. Clinical Manifestations

The clinical manifestations of secondary hyperparathyroidism involve several target organs in patients with chronic kidney disease and contribute to significant morbidity and mortality. Renal osteodsytrophy is the oldest and best described complication in patients with SHPT and consists of both high and low bone turnover diseases. Defects in osteoid mineralization resulting in osteomalacia also comprise part of the spectrum of bone disease seen in patients with CKD. During the past 20 years the nonskeletal complications of secondary hyperparathyroidism have become better recognized. Soft tissue calcification, particularly vascular calcification, is common and potentially a devastating complication of hyperparathyroidism.[28] The pathogenesis and management of vascular calcification are particularly complex, as some of the therapies used to manage SPTH and hyperphosphatemia may contribute to progression. Examples of extraosseous calcification include the cardiovascular system, skin and subcutaneous tissue, cornea and conjunctiva, muscle, lung, and gastrointestinal tract.[28–32] Cardiac calcification can involve the myocardium, the electrical conduction system, and cardiac valves.[33–36] Both an elevated serum phosphorus level and an elevated calcium–phosphorus product have been demonstrated to correlate with soft tissue and vascular calcification.[33,34,37] More recently, a strong correlation of vascular calcification with mortality has been reported on the basis of observational studies.[38–41] Other important nonskeletal complications include neurologic manifestations, pruritus, hypertension, and anemia; all are associated with uncontrolled secondary hyperparathyroidism and improve with either medical or surgical treatment of hyperparathyroidism.[42,43]

A. Bone Disease

Bone disease may be found in up to 60% of patients with advanced CKD and consists of both high and low turnover bone disease.[44] As CKD progresses, bone weakens and mineral density declines incrementally.[45] These changes correlate with elevations in serum concentrations of PTH and phosphorus and decrements in serum calcium and calcitriol levels.[45] By the time patients reach ESRD and dialysis, their risk for fracture, particularly femoral neck fractures, is significantly higher.[46,47] Renal osteodystrophy is a significant problem in patients with SHPT and is discussed briefly later. A more extensive and detailed discussion on the pathogenesis and manifestations of renal osteodystrophy is provided in Chapter 6.

In patients with long-standing secondary hyperparathyroidism, bone mass and structure are adversely affected and characterized by the classic lesion osteitis fibrosa.[48] Clinically, patients with osteitis fibrosa present with bone pain and fractures.[35,48] Osteitis fibrosa is characterized by high bone turnover, increased remodeling (both formation and resorption), and peritrabecular fibrosis.[49] In the majority of patients with CKD, secondary hyperparathyroidism-associated bone disease (high-turnover bone disease) is only a part of the abnormal picture in bone metabolism and management is more complex (Table 3-1). Usually, renal bone disease is characterized by a mixture of high and low bone turnover lesions.[50] Low-turnover uremic osteodystrophy consists of adynamic bone disease which can result from aggressive correction of serum PTH concentrations, PTH resistance, and

Table 3-1. Spectrum of Bone Disease in CKD Patients.

Osteitis fibrosa
PTH-mediated high bone turnover
Treat by suppressing PTH
Adynamic bone
Low bone turnover
Pathologically the same as osteoporosis
Usually due to low PTH
Treat by:
Avoiding calcium binders
Avoiding active vitamin D
Using low-calcium bath
Osteomalacia
Low bone turnover with large amounts of unmineralized osteoid
Usually due to vitamin D deficiency
In the past commonly due to aluminum
Suspect in dialysis patients with low bone mass and frequent fractures
Treat with ergocalciferol with or without active vitamin D

downregulation of osteoblast function.[51] Mixed uremic osteodystrophy is characterized by mild to moderate hyperparathyroidism in patients with evidence of osteomalacia and defective mineralization.

The skeletal actions of PTH are important in the pathophysiology of renal osteodystrophy in patients with advanced CKD.[52] Ample evidence indicates that PTH is the major regulator of bone remodeling and skeletal turnover in patients with CKD.[53–55] Although PTH promotes recruitment and differentiation of osteoclasts it does this indirectly. This is because osteoclasts do not have PTH receptors. PTH works through various cytokines and growth factors to stimulate bone resorption.[53–55] In addition, extrinsic factors such as an inflammatory state may further modulate PTH action because of the secretion of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1). Since osteoblasts abundantly express PTH receptors, excess PTH promotes osteoblast number and activity. Osteoblasts synthesize type I collagen and are responsible for its subsequent extrusion into the extracellular space to form a collagenous bone matrix.[53] Thus, excess PTH impacts on both osteoblast and osteoclast activity as well as influencing the growth factor and cytokine milieu.[56] Collectively, the direct and indirect actions of PTH on bone cause a reduction in cortical bone mineral density, particularly of the appendicular skeleton, but tend to spare cancellous bone, especially at cortical sites such as the distal forearm.[50] Intermediate reduction is found at sites in which both trabecular and cortical bone are represented, such as the hip, and relative preservation is observed at sites of predominantly trabecular bone, such as the vertebrae. Severe secondary hyperparathyroidism may result in marrow fibrosis (osteitis fibrosa cystica). Fibrous tissue accumulation within the marrow space can displace normal blood-forming elements and aggravate the anemia associated with CKD.[50]

B. Calciphylaxis

Soft tissue calcification, calciphylaxis, or calcific uremic arteriolopathy (CUA) is a poorly understood and highly morbid condition that occurs in CKD patients with secondary hyperparathyroidism, particularly in those on dialysis. It is associated with hypercalcemia, hyperphosphatemia, and elevated levels of PTH. Despite the frequency of these abnormalities in ESRD, calciphylaxis remains rare. However, there is a concern that the incidence may be increasing as a result of the widespread use of parenteral vitamin D in the treatment of SHPT.

Clinically, calciphylaxis is characterized by painful red nodules or plaques that tend to distribute themselves on the lower extremities, thighs, abdomen, and buttocks. These lesions are painful and progression can be quick, leading to ulceration, infection, sepsis, and amputations. Biopsy and histologic examination of these ulcerations demonstrate calcifications in the media of arterioles

of the subcutaneous fat and dermis of the skin. The mortality associated with calciphylaxis is significant and approaches 65%.[57]

The risk factors associated with CUA are not limited to hyperparathyroidism, hyperphosphatemia, and an elevated Ca × P product. Female gender, obesity, hypoalbuminemia, and the use of coumadin have also been associated with the risk of development of soft tissue calcification.[58,59] Higher doses of iron and erythropoietin do not seem to be independent predictors of calciphylaxis.[59] Managing patients with calciphylaxis is mainly supportive, including pain control and aggressive wound care and débridement to minimize the risk of infection and sepsis. Avoidance of calcium-based phosphate binders, utilization of low-calcium dialysis baths, and intensification of dialysis should be followed. Managing serum phosphate is also critical and may be the most important factor in reducing the risk of calciphylaxis. In a study of dialysis patients by Mazhar et al., there was a 3.51-fold increase in the risk of calciphylaxis associated with each mg/dl increase in the mean serum phosphate.[59] However, in patients with existing soft tissue calcification, controlling PTH and serum phosphorus may not be sufficient and subtotal parathyroidectomy may be indicated in severe cases.

C. Vascular Calcification

Cardiovascular mortality is the leading cause of death in patients with chronic kidney disease and is markedly higher than in the general population when stratified by age, race, and gender.[60] The high rate of mortality from cardiovascular causes has been attributed to the burden of diabetes and hypertension in this population. However, a portion of cardiovascular disease (CVD) in the ESRD population cannot be explained by these and other traditional risk factors alone.[60] Defects in mineral metabolism, particularly hyperphosphatemia and secondary hyperparathyroidism, increase the risk of CVD in patients on dialysis.[38,46] Abnormalities in mineral metabolism contribute to the development of CVD, likely through the process of extraossesous calcification which affects not only the coronary arteries but also the myocardium, conducting system, and cardiac valves.[58]

The burden of vascular calcification in the dialysis population is reflected by the higher prevalence and severity of coronary artery calcification when compared to age-matched healthy controls.[58] In addition to abnormalities in serum calcium and phosphorus, risk factors for the development for vascular calcifications also include increased age, longer duration of dialysis, inflammation, hypertension, dyslipidemia, and calcium-based phosphate binders. The latter was demonstrated in the Treat to Goal study which compared Ca-based phosphate binders to the non-Ca-based phosphate binder sevelamer.[61] Despite similar goals achieved (serum P, Ca × P, and PTH), coronary artery and aorta

calcification increased in the group that used Ca-based binders. It is unclear if the differences between groups were the result of an increased risk from the calcium load or a decreased risk associated with the lipid attenuating effect of sevelamer.

The process of vascular calcification is a complex process, and attributing the pathogenesis to the precipitation of calcium and phosphate in the walls of arteries oversimplifies the problem. Rather, calcification in the arteries of dialysis patients likely results from numerous insults, which when combined lead to vascular disease. Inflammation, uremia, hyperphosphatemia, hypertension, hyperlipidemia, and hypercalcemia all could be expected to play a role in this process. The complexity and severity of this process is confirmed in autopsy studies of patients who die of CVD; heavier plaque calcification is seen in the coronary arteries of those patients with ESRD.[62]

Before the deposition of calcium, the pathogenesis of vascular calcification may begin with the deposition of certain bone related proteins in the vascular smooth muscle.[63] Transcription factor Cbfa1 (core binding factor alpha 1) turns stems cells into osteoblasts in fetal development and has been shown to be upregulated in vascular smooth muscle cells (VSMCs) when exposed to phosphorus. Biopsy studies of inferior epigastric arteries obtained at the time of kidney transplantation also show evidence of Cbfa1 expression in VSMC adjacent to intimal and medial calcifications.[64] Multiple insults probably induce the osteoblast phenotype in vascular smooth muscle cells, which lay the foundation for calcium deposition.[65] These osteoblast-like vascular smooth muscle cells likely secrete collagen and noncollagenous proteins into the intima or media of the artery wall, forming a matrix that increases wall stiffness.[66] This matrix eventually is mineralized with calcium and phosphate, lending the vessel wall susceptible to further injury, vascular dysfunction, and ischemic events. Independent of serum calcium and phosphorus, there may be unidentified uremic toxins that accelerate the process of calcification as demonstrated in invitro studies of bovine smooth muscle cells.[67]

Interestingly, some dialysis patients, despite the uremic environment and hyperphosphatemia, do not develop vascular calcifications.[68] Although young age may be partially protective, inhibitors of vascular calcification may also play a role. Matrix gla protein (MGP), a locally produced inhibitor, has been associated with protection against arterial calcification in mice.[69] Additional inhibitors, such as osteoprotegerin and Fetuin-A, have been shown in animal studies to be protective of calcification as well.[70] The exact mechanism by which these proteins are protective in humans has yet to be defined and warrants further study.

Reducing the risk of vascular calcifications has been aimed at controlling serum phosphorus, calcium, and PTH. As with calciphylaxis, in severe cases of coronary and peripheral vascular disease, parathyroidectomy may be indicated

and can lower the rate of long-term mortality associated with secondary hyperparathyroidism.[71] Before that, traditional control of serum phosphorus, however, may have the biggest impact in reducing the risk of progression of extraosseous calcification.[72] Use of calcium-based phosphate binders and the nonabsorbable polymer sevelamer both have roles in controlling serum phosphorus; however, the calcium load imposed by high doses of calcium salts may increase the risk of vascular calcification. A randomized study of 200 dialysis patients compared the use of traditional calcium-based phosphorus binders and sevelamer on the development of calcifications of the coronary arteries and thoracic aorta.[61] Although equivalent control of phosphorus was found in both groups, the group treated with calcium salts experienced more problems with hypercalcemia (16% vs. 5%, $p = 0.04$). Interestingly, PTH was better controlled in the calcium-treated group. However, when calcification scores were measured using electron beam tomography, median calcium scores increased in the coronary arteries and aorta in the calcium-treated group when compared to the sevelamer group.

When comparing calcium salts, there appears to be no difference between calcium carbonate and calcium acetate in the risk of development of vascular calcifications; both appear to increase progression of vascular calcification.[73] Independent of phosphorus and PTH, using non-calcium-based binders may have an advantage in minimizing vascular calcification. Sevelamer is less likely to cause progressive coronary and aortic calcifications, and this effect is likely mediated through a combination of lower levels of serum calcium and additional effects on serum lipids. Reduction in low-density lipoprotein (LDL) and elevations in high-density lipoprotein (HDL) have been demonstrated and may contribute to the decrease risk of vascular disease.[74] The addition and increasing use of calcimimetics to treat SPHT also offers promise in the prevention of vascular calcification. The role of long-term use of these agents in prevention of vascular and soft tissue calcification has yet to be fully defined.

IV. Management of Secondary Hyperparathyroidism

The central tenets of treatment of hyperparathyroidism in chronic dialysis patients are ameliorating hypocalcemia, reducing hyperphosphatemia, and maintaining physiological concentration of 1,25-dihydroxyvitamin D (calcitriol) (Fig. 3-3). However, despite routine use of phosphate binders and oral active vitamin D sterols, it is still difficult to control PTH secretion in some patients. Such patients appear to respond to either supraphysiological concentrations of vitamin D sterols or to the adjunctive use of cinacalcet. In patients with milder impairment in renal function, therapy is aimed largely at

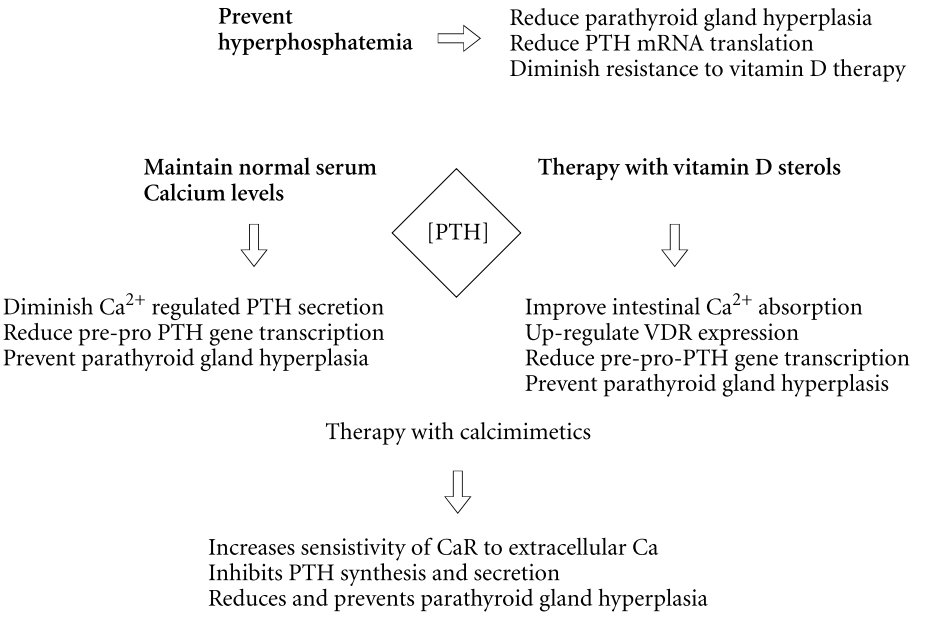

Figure 3-3. Management of SHPT.

correcting mild hypocalcemia and hyperphosphatemia and by supplementing vitamin D.

An improved understanding of the pathophysiologic basis of hyperparathyroidism has heralded several new therapeutic agents and treatment paradigms.[75–77] New agents include non-calcium-containing phosphate binders,[78–80] calcimimetics,[81–83] and less-calcemic vitamin D analogues.[84,85] Treatment paradigms have centered on the adoption of K-DOQI guidelines that have been developed by the National Kidney Foundation and are now widely accepted.[86] These guidelines have been developed for both pre-ESRD CKD patients and ESRD patients on dialysis (Figs. 3-4 and 3-5).

A. Phosphorus Control

Hyperphosphatemia underlies the development of hyperparathyroidism, renal osteodystrophy, and soft tissue and vascular calcifications, and ultimately plays a role in cardiovascular morbidity and mortality. Importantly, phosphorus restriction reverses secondary hyperparathyroidism independent of changes in calcium and calcitriol.[87]

Positive phosphate balance is a function of reduced renal elimination, increases intestinal absorption of dietary phosphorus, and ultimately increases phosphate efflux from bone as parathyroid hormone secretion rises. In patients on dialysis, renal replacement therapy alone is ineffective in removing serum

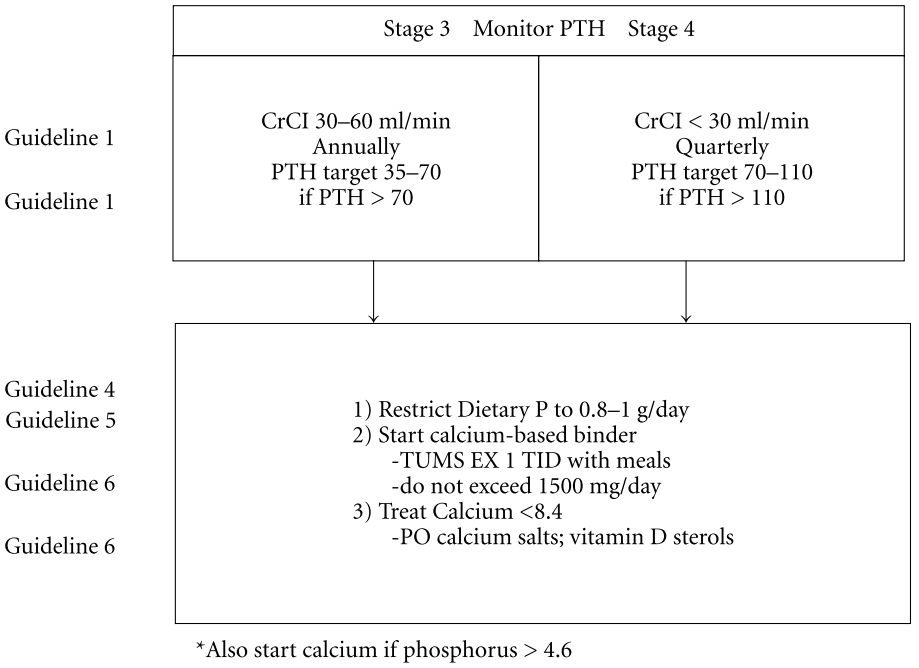

Figure 3-4. National Kidney Foundation K-DOQI Guidelines summary for PTH management in CKD patients.

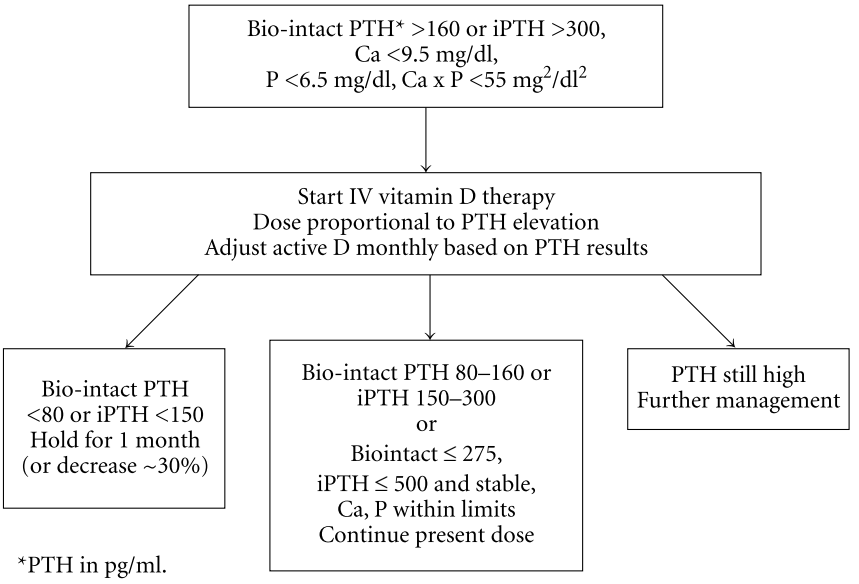

Figure 3-5. K-DOQI Guidelines summary for management of high PTH ESRD patients on dialysis.

phosphorus. Typically about 1000 mg of phosphate is removed with each dialysis session or 3000 mg/week. With an average of 1000 mg of dietary phosphate absorbed per day, a net positive phosphorus balance of 4000 mg/week would result.[88] Therefore, controlling serum phosphate through dietary measures and phosphate binders remains a critical component of therapy in patients with chronic kidney disease.

Unfortunately, current control of phosphorus in the dialysis population may be inadequate and underscores the need for a multifactorial approach by nephrologists and additional novel therapies. In an analysis of two large national, random, cross-sectional samples of hemodialysis patients receiving dialysis for at least 1 year, Block and colleagues found that 10% of patients had serum phosphate levels greater than 9 mg/dl and 30% had serum phosphate concentrations greater than 7 mg/dl. The risk of death associated with levels higher than 6.5 mg/dl was 1.27 relative to those with levels between 2.4 and 6.5 mg/dl and this risk was found to be independent of PTH levels. A multivariate analysis of these data demonstrated that younger age at onset of ESRD, female sex, white race, diabetes, active smoking, and higher serum creatinine levels were found to be significant predictors of hyperphosphatemia (levels > 6.5 mg/dl). Those with elevated Ca $\times$ P products (> 72) were also found to have a higher risk of mortality (OR 1.32), when compared to those with Ca $\times$ P products between 42 and 52. Hypercalcemia was not found to correlate with relative risk of death in this study.[89] The mechanisms for higher rates of morbidity and mortality are not clearly known; however, better management of serum phosphorus may help to reduce the risk in patients with chronic kidney disease.

B. Dietary Phosphorus Restriction

Phosphate retention plays a major role in the development of secondary hyperparathyroidism, and dietary phosphate restriction is an important element in the conservative management of patients with chronic kidney disease and in the control of SHPT. For the average American, daily dietary intake of phosphorus is approximately 1550 mg for males and 1000 mg for females. In general, foods that are high in protein are also high in phosphorus. Daily intake of phosphorus may be on the rise among individuals on a Western diet as phosphates are currently being added to a large number of processed foods including meats, cheeses, dressings, beverages, and bakery products. These additives may increase the phosphorus intake by as a much as 1 g/day. Moreover, the addition of phosphates to these processed foods is not accounted for in nutrient composition tables, which results in an underestimate of the dietary intake of phosphorus in our patients.[90]

The benefit of phosphate restriction in renal disease is mediated both through primary and secondary effects on PTH production. In the early stages

of renal failure, limiting dietary phosphate increases 1,25-hydroxy vitamin D levels which in turn downregulates PTH production.[91] In the later stages of renal disease when hydroxylation of 25-OH-vitamin D is limited and vitamin D receptor density on the parathyroid gland is reduced, the benefit of phosphate restriction may be mediated more significantly through primary feedback on PTH synthesis. Hyperphosphatemia affects the production of growth factors, which alters both parathryroid glandular cell proliferation and posttranscriptional production of PTH.[92–94] High dietary phosphate increases parathyroid expression of transforming growth factor-alpha (TGF-α), which promotes glandular cell growth, whereas phosphate restriction induces the cyclin-dependent kinase inhibitor p21, leading to growth arrest. These effects are specific for the parathyroid and changes in these growth factors are not seen in other organs.[95] The advantage of dietary phosphate restriction on parathyroid gland hyperplasia and alterations in these growth factors has been demonstrated in animal models. Uremic rats fed high-phosphorus diets show elevations in TGF-α and epidermal growth factor receptor, known enhancers of parathyroid glandular cell growth. In the same study, uremic rats fed low-phosphorus diets did not develop secondary hyperparathyroidism or parathyroid glandular hyperplasia, emphasizing the important role of dietary phosphate in the pathogenesis of SHPT.[93]

In humans, dietary restriction of phosphorus in patients with stage III and IV CKD has been shown to be effective in reducing PTH levels.[96–98] Dietary measures may be most effective when combined with calcium supplementation in order to reduce the stimulus for PTH secretion.[99] Adhering to a low-phosphorus diet in the early stages of kidney disease prevents the development of parathyroid gland hyperplasia through cellular mechanisms; however its ability to induce regression of glandular hyperplasia, once established, may be limited.[95,100]

Further, low-phosphorus diets may have the additional benefits of slowing the progression of renal failure and reduce cardiovascular morbidity and mortality. Dietary phosphate restriction has been shown to be effective in preventing progression of renal disease in animal models; however, its ability to do the same in human renal disease is controversial and has not been fully established.[101] Ibels, Alfrey, and colleagues initially demonstrated in the remnant kidney model in rats, that phosphate restriction changed the course of renal failure and prevented proteinuria, renal calcification, histologic changes, functional deterioration, and death. In the rats with unrestricted phosphate diets, histologic examination demonstrated calcium phosphate deposits in the cortical tubular cells, basement membranes, and interstitium. This pattern is similar to that seen in the end-stage kidney suggesting the role serum phosphate levels have in the progression of chronic kidney disease.[102]

Small studies have demonstrated conflicting results in humans. In a small trial of 40 patients with stage IV CKD, those placed on low-protein, low-phosphorus diets (7 mg/kg and 0.5 mg/kg) had improvement in renal function as measured by creatinine clearance when compared to those on higher phosphorus and higher protein diets (12 mg/kg and 0.8 mg/kg).[103] The study was confounded by the higher intake of dietary protein in the group with renal deterioration. In addition, severe restriction of dietary protein may have impacted lean body weight and thereby limited the ability to use creatinine clearance as a measure of renal function. Conversely, the lack of benefit of phosphate restriction in slowing the progression of renal disease was demonstrated in another small randomized study with limited follow-up. In this study, no difference was found between groups with low-phosphate diets and in those with no phosphate restriction, when controlled for protein intake.[104]

Dietary phosphorus restriction is an important element in the plan to reduce serum phosphate levels in patients with chronic kidney disease. As kidney disease advances, this alone may be inadequate to control hyperphosphatemia. Particularly at the time of initiation of dialysis, adjunctive agents to bind dietary phosphate in the gut may be needed to control serum phosphate.

C. Phosphorus Binders

The treatment of hyperphosphatemia in patients with chronic kidney disease has relied heavily on phosphate binders. The selection of phosphate binders should be based on patient characteristics, including serum phosphate, serum calcium, intact parathyroid hormone concentrations, and patient tolerability. Oral phosphate binders have been around since the 1970s and the first generation of binders to be used was aluminum salts. Use of these agents fell out of favor in the 1980s with evidence of significant toxicity in dialysis patients.[105,106] In the 1980s, the second generation of binders, primarily calcium salts (calcium carbonate and then later in the 90s calcium acetate) became the standard in treating patients and were shown to be safe and effective in controlling both serum phosphate and PTH. However, uses of calcium-based binding agents could be limited, especially in patients with ESRD, by the resulting positive calcium balance leading to hypercalcemia. These limitations led to the development of non-calcium-based agents. The polymer-based binding resin sevelamer hydrochloride, a rare earth element salt, and lanthanum carbonate, comprise the newest and the third generation of phosphorus binders. This third generation of phosphorus binders offers nephrologists new strategies to control dietary phosphate absorption and may prove to be more effective in controlling secondary hyperparathyroidism. Historically, other alternatives have included magnesium and iron salts. Magnesium salts are not particularly effective and are limited by hypermaganesemia and diarrhea. Iron salts are not currently approved for use as phosphorus binders and are not generally used.[107]

The effectiveness and choice of these agents can be better understood by recognizing their dependence or independence on gastric pH. Hydroxide, carbonate, and acetate salts of the metal ions aluminum, calcium, and lanthanum are pH dependent for dissolution of the salt and then subsequent binding of the metal ion to phosphate. Acidic environments are best to dissolve and ionize the salt. This solubility may be best achieved at a pH between 1 and 3, as seen in the environment of the stomach. However, binding to phosphate requires a different pH and is optimal in more alkaline environments at a pH above 5.[107] The pH-dependent effect is particularly important in understanding the utility of calcium-based salts, which are more pH dependent than the alkaline aluminum salts. Whereas the effectiveness of calcium carbonate is seen over a narrow range of gastric pH, calcium acetate is effective over a wider range, which may improve its utility as a phosphorus binder. Hypo- or achlorhydria, antacids, H_2 blockers, and proton pump inhibitors can increase gastric pH and further alter the effectiveness of these calcium-based binders. The non-calcium-, non-aluminum-based binder sevelamer hydrochloride is effective in both acidic and alkaline pH, which may offer an advantage over more traditional phosphate binders.[108]

1. Aluminum

The problem of hyperphosphatemia and kidney disease has been known for more than 80 years.[107] Aluminum hydroxide was first used to bind phosphorus and treat bone disease in the early 1940s. However, it was not until the 1970s, when hyperphosphatemia was described as a contributing factor in the development of hyperparathyroidism, did the utility of aluminum salts gain widespread popularity.[2] Aluminum salts, previously used as antacids, which included the aluminum hydroxide gel (Amphogel®) and aluminum carbonate gel (Basaljel®), were also found to be potent phosphorus binders. In in vivo studies on normal subjects, aluminum carbonate reduced gastrointestinal absorption of phosphorus from 66% to 18%.[109] In dialysis patients, they have been demonstrated to be less, but still effective in reducing dietary phosphate resorption (70% to 35% to 49%).[110]

Their ultimate utility was, however, limited by poor tolerability, large doses, and long-term side effects. Patient complaints of constipation and taste limited compliance. In addition, awareness of toxicity also became apparent in the same decade in which these medicines gained popularity.[111] High doses of dietary aluminum required to bind phosphorus and the renal dependent excretion led to an accumulation in various tissues and organs in patients with kidney disease. Toxicity was seen in patients with chronic use and resulted in hypochromic microcytic anemia, osteomalacia, adynamic bone disease, myopathy, and progressive dementia. A landmark study by Allen Alfrey and colleagues at the University of Colorado demonstrated that the risk of these

adverse events was increased in dialysis patients at centers where the municipal water supply was contaminated by high levels of aluminum.[105] Importantly the same problem was also seen in predialysis CKD patients who used the phosphate binder calcium citrate, which was found to increase the absorption of aluminum.[112]

Today, chronic use of aluminum salts as phosphorus binders is less prevalent. However, aluminum-based binders remain one of the most effective dietary binders available. They continue to be used acutely to reduce dangerous levels of hyperphosphatemia in patients with chronic kidney disease.

2. Calcium Salts

Treatment of hyperphosphatemia and secondary hyperparathyroidism with calcium salts is currently the mainstay of therapy in patients with chronic kidney disease, particularly in those prior to reaching dialysis. Use of these binders with meals, in addition to dietary phosphate restriction, can be effective in control serum phosphate. Calcium carbonate and calcium acetate (PhosLo®) are the most utilized calcium-based binders in the United States. Calcium citrate (Citracal®) is a less utilized and a less effective phosphorus binder because the citrate anion competes with phosphorus for binding to calcium. In addition its use has also been limited by the augmentation of absorption of aluminum increasing the risk for systemic toxicity.[112]

Calcium carbonate, the first calcium-based binder, became an alternative to the more toxic aluminum salts. Soluble in acidic environments and insoluble at neutral and alkaline pH, calcium carbonate is most effective in binding dietary phosphorus in the stomach. In patients with mild to moderate renal insufficiency, up to 3 g daily of calcium carbonate may be needed to effectively control serum phosphate and PTH.[113] With advancing disease, larger doses may be required and up to 10 g per day were needed to effectively control serum phosphate in one study of patients on dialysis.[114] As a result, large doses of calcium carbonate led to higher calcium loads, a positive calcium balance, and a greater risk of hypercalcemia.[114] The difficulty in taking high numbers of tablets with each meal to bind dietary phosphorus may further limit the effectiveness of these binders.

The development of calcium acetate (PhosLo®) by Nabi Pharamaceuticals arose in response to the limitations of calcium carbonate to effectively bind dietary phosphorus. Calcium acetate is significantly more water soluble than calcium carbonate, binds phosphorus twice as effectively, results in less dietary calcium being absorbed, and is more effective in lowering serum phosphate and PTH.[109,115–117] However, there is controversy of whether the lower doses of calcium acetate used compared to calcium carbonate actually translates into lower frequency of hypercalcemia; some studies show less hypercalcemia,

while others show no difference.[116,118–120] The discrepancy may reflect additional variables such as skeletal release of calcium in hyperparathyroidism, bone turnover rate, and vitamin D dose and utilization which may be important determinants in serum calcium concentrations.[107]

Concerns regarding hypercalcemia and a potential association with vascular calcifications may hinder the prescription of adequate doses to control serum phosphate. Hyperphosphatemia and high calcium–phosphorus products are associated with cardiovascular mortality, presumably through a higher burden of vascular calcifications.[89,121] However, there is no strong evidence to suggest that hypercalcemia is independently associated with morbidity and mortality in patients with chronic kidney disease.

3. Sevelamer Hydrochloride

Sevelamer (Renagel®, Genzyme, Inc) is a non-calcium, non-aluminum binding gel of crosslinked poly allylamine hydrochloride that is not degraded in the digestive tract or absorbed by the gut. Sevelamer hydrochloride binds phosphorus most effectively in alkaline environments at pH between 5 and 7.[122] Currently licensed to Genzyme, sevelamer has been used as an alternative to calcium salts to bind dietary phosphorus in patients with advanced chronic kidney disease and is as effective in controlling serum phosphorus when compared to calcium carbonate and acetate.[123] While controlling serum phosphorus and the calcium $\times$ phosphorus product, it does not have the unwanted complication of increasing serum calcium.[123,124] In addition, sevelamer has an additional benefit of decreasing LDL and total cholesterol by binding bile salts in the gut.[123] In a long-term study of dialysis patients treated with sevelamer, LDL cholesterol levels decreased by 30%, while HDL cholesterol levels rose by 18% after 46 weeks of treatment.[124]

Proponents of non-calcium binders cite the advantage of its use in later stages of ESRD when hypercalcemia often limits the use of appropriate doses of calcium-based therapies. What about superiority in controlling serum phosphorus? The CARE study sought to answer the question of whether there was a difference in efficacy in these two therapies. It compared the efficacy of calcium acetate (Phoslo®) and Sevelamer (Renagel®) in achieving control of phosphorus ($\leqslant$ 5.5 mg/dl) and Ca $\times$ P product ($\leqslant$ 55 mg^2/dl^2) in a randomized, double-blind study of 100 dialysis patients over 8 weeks.[125] The study demonstrated that calcium acetate was superior in reducing serum phosphorus levels, in achieving the target phosphorus level, and reaching the Ca $\times$ P goal. Mean serum phosphorus levels between weeks 5 and 8 were 5.5 mg/dl compared to 6.4 mg/dl in the calcium acetate and sevelamer group, respectively ($p = 0.038$). The mean Ca $\times$ P product over this time period was not statistically significant between the groups. Hypercalcemia, however, was noted to be more severe in those receiving calcium acetate. Hypocalcemia

was more of a problem with the sevelamer group (6.7%) than in the calcium acetate treated patients (2.2%). In addition, patients treated with sevelamer required more tablets on average to control serum phosphorus; 17 versus 11 in the calcium acetate and sevelamer group, respectively. The differences in outcomes could not be accounted for by compliance with the study regimen or baseline use of IV vitamin D therapies or iPTH levels. Importantly, there was no difference in the iPTH levels at the conclusion of the 8-week study. Critics of the study have noted the relative short-term follow-up of 8 weeks; however, the results have significantly challenged the notion of the superiority of sevelamer in controlling serum phosphorus and SHPT.

However, the higher incidence of hypercalcemia in the group using calcium acetate and the known lipid lowering benefit of sevelamer cannot be overlooked. The short-term follow-up of CARE and the ability of calcium salts to protect CKD patients from cardiovascular complications were challenged by the Treat to Goal working group. In a cross-sectional study of 200 hemodialysis patients, equivalent phosphorus control was seen in patients receiving both sevelamer and calcium-based phosphorus binders. The use of sevelamer was associated with less hypercalcemia (16% vs. 5%), but more importantly it was associated with lower calcium scores in the coronary arteries and aorta as measured by electron beam tomography.[61] The conclusions by the authors suggest the advantage of sevelamer in limiting progressive coronary and aortic calcifications in hemodialysis patients. This study had its own limitations, including un-blinding of the study drug, confounding effects of LDL lowering with Renagel, and large patient dropout. Improved randomized blinded studies are needed to test the hypothesis that sevelamer indeed does limit vascular and valvular calcifications and that this translates into improved cardiovascular outcomes.

The benefit of sevelamer in the dialysis population may extend beyond control of phosphorus and lower serum calcium concentration. As mentioned earlier, sevelamer is a bile acid sequestrant, and when given in large doses, has been shown to lower LDL and raise HDL cholesterol levels.[124] The benefit of controlling serum cholesterol levels in dialysis patients is still in question as the ESRD population has been largely excluded from large clinical trials evaluating 5-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors. Also, although the benefit has been assumed from retrospective observational studies, this has been recently challenged by a large multicenter randomized trial evaluating the benefit in dialysis patients.[126–128]

The long-term benefit of sevelamer was recently evaluated in a large outcomes study (DCOR-Dialysis Clinical Outcomes Revisited). The study of 2100 dialysis patients over 3 years compared the difference of mortality and morbidity outcomes in dialysis patients receiving Renagel® with those receiving calcium-based phosphate binders. The results of the DCOR trial were

recently released and discussed at the 2005 American Society of Nephrology meeting in Philadelphia. The study failed to demonstrate a mortality benefit in the sevelamer treatment group (primary end-point) at 3 years. However, in a subanalysis of patients treated for longer than 2 years and who were older than 65 years of age, the group who used sevelamer experienced a 9% reduced risk of death from all causes relative to patients using calcium-based binders. In addition, patients treated with sevelamer were less likely to be hospitalized and were hospitalized for a shorter period of time. The results of the study have not been published at the time of this submission.

Despite its advantages, the use of Renagel may be most limited by cost. The projected cost for treatment with calcium acetate would be $732 compared to $4383 for sevelamer.[129] Without strong evidence to support an advantage in phosphorus control, long-term cardiovascular benefits, and overall mortality, justification for this more expensive therapy in the absence of hypercalcemia may not be warranted. Another limitation may be the acid load imposed by sevelamer hydrochloride in predialysis patients who have less renal buffering capacity. The acidosis imparted is reflected in the lower serum bicarbonate levels when compared to individuals treated with calcium carbonate and calcium acetate. The acidosis may increase muscle metabolism and accelerate bone loss.[130,131]

4. Lanthanum Carbonate

Lanthanum carbonate (LC) (Fosrenol®, Shire Pharmaceuticals) is a novel non-calcium-based therapy used in binding dietary phosphate in the treatment of hyperphosphatemia and secondary hyperparathyroidism in patients on dialysis. Lanthanum, a rare earth element, is a trivalent cation that binds phosphorus in the gut to form lanthanum phosphate. There is minimum absorption of lanthanum by the gastrointestinal tract and no excretion by the kidneys; absorbed lanthanum is excreted by the biliary system in contrast to aluminum, which is excreted mostly by the kidney. LC has a strong affinity for phosphate (> 97%) and has been shown to be effective in the treatment of hyperphosphatemia in patients on dialysis, while avoiding complications of hypercalcemia as seen in traditional calcium-based phosphate binders (6% vs. 49%).[132–134]

Two randomized trials have supported the safety and efficacy of LC. In a 6-month randomized trial of 800 patients, randomized to receive either LC or calcium carbonate (CC), both groups showed similar phosphate control while the lanthanum group had substantially less hypercalcemia (0.4%) when compared to the CC group (20.2%).[133] No differences in adverse events were seen between the two groups. In another randomized placebo-controlled trial, patients receiving LC showed a dose-dependent reduction in phosphate with escalating doses of lanthanum—675, 1350, and 2250 mg.[135] Adverse events

were mainly gastrointestinal and no significant difference was seen with the placebo arm. Importantly, the mode of dialysis does not seem to alter efficacy; patients on hemodialysis and peritoneal dialysis both were able to achieve reductions in phosphorus and Ca × P products in one short-term placebo-controlled study.[136]

Because of the physiochemical similarity to calcium and aluminum, the deposition in bone and effect on bone histology by LC has been studied. In a rat model of chronic kidney disease with secondary hyperparathyroidism, a dose-dependent reduction in bone formation rate and increase in osteoid area as measured by bone histomorphometry was seen in those rats loaded with LC (1000 mg/kg per day for 12 weeks). These changes were not believed to be secondary to the toxic effects of LC on osteoblasts, but rather the result of phosphate depletion from a combination of lanthanum therapy in conjunction with decreased 25-vitamin D_3 levels in the uremic rat.[137]

In humans the results in the treatment of renal osteodystrophy have been more encouraging. Therapy with LC has been shown to be more effective than CC in the treatment of renal osteodystrophy. A study of 68 patients who received either LC or CC evaluated baseline and changes in renal osteodsytrophy as determined by bone biopsies after treatment for 1 year.[134] At the conclusion of 1 year, in the LC group, five of seven (71%) patients with low bone turnover disease (either adynamic bone disease or osteomalacia) and four of five (80%) with baseline hyperparathyroidism normalized their bone turnover as compared to three of seven (42%) and three of six (50%) in the CC group. In addition, those given LC showed a decrease in adynamic bone disease, osteomalacia, or hyperparathyroidism from 12 (36%) to 6 (18%) at 1-year follow-up. In contrast, in the calcium-treated group, the number of patients with renal osteodystrophy increased from 13 (43%) to 16 (53%). There were no baseline differences in vitamin D usage in either group and both groups showed well-controlled phosphorus levels throughout the study. The maximum dose of LC was 1250 mg/day with median dose of 370 mg/day. The plasma lanthanum levels ranged from 0.51 to 1.08 μg/L, did not depend on dose, and reached a plateau after 12 weeks. The concentration in bone of lanthanum was 1.8 μg/g with the highest value reaching 5.5 μg/g. It is unclear what the long-term effects on bone and other organ systems would be with persistent elevations in levels of lanthanum carbonate.

Overall the use of lanthanum appears to be well tolerated.[134,138] Nausea (26%), peripheral edema (23.4%), and myalgias (20.8%) were the most commonly reported side effects in a short-term extension study to look at safety at 1 year. No serious adverse events occurred in this follow-up study.[139] Despite its tolerability and short-term efficacy, many have raised concerns about the potential for accumulation of LC in the bone and other tissues. There are limited data to demonstrate that accumulation in bone and other tissues will

not occur similar to aluminum. Long-term follow-up is not available in patients using this phosphate-chelating agent to answer the question. However, animal studies have supported the concerns about lanthanum deposition in tissues. In one study, control and uremic rats exposed to dietary LC experienced a 10-fold increase in LC in some organs, including the liver, kidney, and lung. This effect was further enhanced in the uremic rats, supporting concerns about the long-term safety of this rare earth element used to bind dietary phosphorus.[140] Although LC was effective in controlling serum phosphorus, additional studies need to be performed to determine its long-term safety.

D. Vitamin D Therapy

Declining kidney function leads to a progressive deficiency of activated vitamin D or calcitriol (1,25-$[OH]_2D_3$). The deficiency is a function of reduced 1α-hydroxylase activity and subsequent conversion of 25-vitamin D by the proximal tubular cells in the kidney.[141,142] Reduced levels of activated forms of vitamin D (1,25-$[OH]_2D_3$) occur in significant numbers of patients with early stages of CKD and in almost all individuals with more advanced CKD. Reduced levels of active vitamin D in addition to disorders of mineral metabolism are important in the pathogenesis of SPTH. Further, deficient 25-hydroxyvitamin D stores, from poor nutrition or limited sun exposure, may also aggravate the development of SHPT in later stages of CKD.[143] Replacing vitamin D can treat secondary hyperparathyroidism in patients with advanced renal disease. In addition, maintaining adequate vitamin D levels may have additional benefits to other target organ systems and is discussed later.

1. 25-Vitamin D

Identifying patients with 25-vitamin D deficiency is an important first step in the evaluation of SHPT and a common problem in patients with renal insufficiency.[142,144] Identifying low 25-vitamin D stores may be particularly important in early stages of CKD when 1,25-vitamin D production is highly dependent on the supply of its precursor 25-vitamin D.[144] This is different from normal individuals, including those with vitamin D deficiency, in whom 1,25-vitamin D levels are not dependent on 25-vitamin D levels. In addition to CKD, advancing age and an African-American ethnic background have all been shown to be risk factors for 25-vitamin D deficiency.[145–148] K/DOQI guidelines recommend that vitamin D levels be measured in patients with stage III, IV CKD with elevations in iPTH. If 25-vitamin D levels are less than 30 ng/ml, then vitamin D replacement is indicated. Replacing 25-vitamin D stores is not only important in the treatment of SHPT, but has also been demonstrated to be important in bone formation and reduction of fracture risk.[149,150]

2. 1,25-Vitamin D

Replacement of calcitriol plays a central role in the modulation of parathyroid gland growth, PTH production, and regulation of bone formation. Calcitriol inhibits parathyroid hyperplasia by inhibiting cell growth and differentiation and has a direct inhibitory effect on PTH gene transcription through activation of the vitamin D receptor. Stimulation of the VDR decreases PTH production over a period of days to weeks in patients with CKD.[151] Calcitriol regulates both bone formation and bone resorption in the skeleton. In children, it also has a role in conjunction with calcium in regulating development of the cartilaginous growth plate.[149] In the United States, three commercially available preparations of 1,25-vitamin D are available to prevent and treat SHPT; calcitriol (1α-, 25-$[OH]_2D_3$), and the two less calcemic vitamin D_2 analogues, paricalcitol (19-nor-1α-, 25 $[OH]_2D_2$)(Zemplar®, Abbott Laboratories), and doxercalciferol (1α-$(OH)D_2$)(Hecterol®, Genzyme). In Asia, 22-oxacalcitrol or maxacalcitol, another nonhypercalcemic vitamin D analog, is also used to treat SHPT.

Calcitriol and paricalcitol exhibit similar pharmacokinetic profiles with half-lives of each drug ranging from 15 to 30 hours in patients on hemodialysis. When given intravenously, both drugs produce superphysiologic levels of activated vitamin D within minutes. However, serum levels rapidly decline and by 24 hours are virtually undetectable.[152] Doxercaliferol is a prohormone that requires hepatic transformation for activation. As a result, its pharmokinetics are different than those of calcitriol and paricalcitol. Peak levels of active metabolite occur at 8 to 12 hours. There does not appear to be any difference in peak serum levels by route—intravenous or oral. The half-life is approximately 45 hours in dialysis patients and results in less interdose fluctuation when compared to paricalcitol and calcitriol. All three agents are highly protein bound and are not significantly removed in dialysis. It is unclear if the differences in pharmacokinetic profiles seen between the vitamin D sterols have an effect on the long-term safety and efficacy of these agents in the treatment of SHPT.[152]

Calcitriol remains the mostly widely used vitamin D preparation in the treatment of secondary hyperparathyroidism. In patients with moderate renal failure, daily treatment with oral calcitriol (0.5 mcg/day) can reverse hypocalcemia, reduce PTH levels, and improve bone disease associated with hyperparathyroidism.[153,154] Higher doses or oral calcitriol may be required in patients who reach dialysis but can be effective in patients both on hemodialysis and peritoneal dialysis.[155,156] However, oral doses of calcitriol may be insufficient in patients on dialysis to achieve adequate serum concentrations of 1,25-vitamin D and control SHPT. In these cases, IV calcitriol can enhance the therapeutic efficacy and achieve reductions in serum PTH levels when administered at very high doses three times a week.[157,158] In peritoneal

dialysis patients, intraperitoneal (IP) administration of calcitriol can also be successful in lowering PTH.[159] Use of IV calcitriol can maintain reductions in serum PTH; however, this may come at the expense of hyperphosphatemia which can limit its long-term effectiveness.[160] In addition, therapy in dialysis patients may be further complicated by hypercalcemia.[161] In severe cases of hyperparathyroidism, intravenous calcitriol may be inadequate warranting parathryoidectomy.[162]

Paricalcitol, like calcitriol, is used in the treatment of secondary hyperparathyroidism by targeting the vitamin D receptor on the parathyroid gland. Similarly it suppresses PTH gene transcription and secretion. The development of paricalcitol heralded a new generation of vitamin D analogues, including doxercalciferol and 22-oxacalcitriol, which limit the calcemic response seen with calcitriol. The benefit of paricalcitol in suppressing PTH without altering serum calcium levels has been shown in both animal models and clinical trials in patients with CKD.[163,164] In an open-label study of 164 hemodialysis patients treated with paricalcitol, PTH levels decreased rapidly during the first 4 months of therapy and reached the target range by 5 months (mean 295.3 ± 25.69 pg/ml). During the course of the study, the mean calcium level was maintained well within the normal range (9.44 to 9.94 mg/dl) and serum phosphorus did not increase from baseline.[165]

In head to head studies, paricalcitol appears to be more effective than calcitriol. A large observational study demonstrated less hypercalcemia in dialysis patients treated with paricalcitol when compared to calcitriol. At 12 months, calcium and phosphorus levels had increased by 6.7% and 11.9% respectively, in the paricalcitol group, as compared with 8.2% and 13.9% respectively, in the calcitriol group ($p < 0.001$).[166] In addition, in a prospective randomized trial, paricalcitol was shown to be superior to calcitriol in controlling PTH. The study randomized 263 hemodialysis patients with SHPT (PTH > 300) to receive either agent for 32 weeks. The fraction of patients showing a $\geqslant 50\%$ reduction of PTH was 62% in the paricalcitol group versus 54% in the calcitriol group ($p =$ NS). When long-term side effects were looked at, including hyperacalcemia (> 11.5 mg/dl) and elevations in serum Ca $\times$ P product $\geqslant 75$, the results favored the paricalcitol group, with 18% of patients meting these criteria versus 33% in the calcitriol group.[167]

Paricalcitol may have additional advantages in the treatment of SHPT. In cases in which calcitriol has been ineffective in controlling hyperparathyroidism, paricalcitol can be used as salvage therapy. A study of 37 dialysis patients treated with calcitriol with resistant secondary hyperparathyroidism (PTH $\geqslant 600$ pg/ml) was converted to paricalcitol at a dose ratio of 1:4 and 1:3. Mean PTH levels (901 ± 58 pg/ml) decreased rapidly over 2 months and were 165 ± 24 pg/ml at 16 months. Mean calcium and phosphorus levels did not change appreciably over the 16 months.[85] Furthermore, there

is some evidence that less frequent dosing with paricalcitol may be applicable and effective. In a small study of stable hemodialysis patients (PTH 100 to 500 pg/ml) receiving paricalcitol, once weekly dosing was an effective option in controlling PTH. No appreciable differences were seen in baseline PTH, serum calcium, phosphorus, and Ca × P product after conversion to once-weekly dosing.[168] Less frequent dosing may be more convenient, use fewer resources, and offer an additional option in other dialysis modalities.

In addition to treatment of SPHT, the use of paricalcitol may have other long-term benefits. In a retrospective analysis of 11,433 hemodialysis patients, those receiving paricalcitol versus calcitriol had fewer all-cause hospitalizations, fewer hospitalizations per year, and fewer hospital days per year. In addition, the paricalcitol group had fewer PTH-related hospitalizations.[169] Epidemiological evidence also suggests a survival advantage for hemodialysis patients receiving paricalcitol. Mortality was 16% lower in dialysis patients receiving paricalcitol as the vitamin D preparation compared to calcitriol. In addition, there seemed to be a survival benefit in the cohort of individuals who switched to calcitriol to paricalcitol as compared to those who switched from paricalcitol to calcitriol.[166] However, prospective randomized trials need to be carried out to confirm these finding and eliminate potential biases that can be seen in epidemiologic studies.

Doxercalciferol (1α-hydroxyvitamin D_2), a 1,25-vitamin D prohormone, has also been shown to be safe and effective in controlling SHPT in patients with chronic kidney disease with minimal increase in serum calcium and phosphorus.[170] In an open label, randomized, placebo-controlled trial of 138 hemodialysis patients receiving daily oral doxercalciferol, target levels of PTH were achieved in 83% of patients.[170] Both oral and IV preparations of doxercalciferol are effective in lowering PTH in patients on dialysis without significantly affecting serum calcium. This was demonstrated in a safety and efficacy study of hemodialysis patients treated with both preparations of doxercalciferol. Hypercalcemia was limited in these patients and the prevalence of serum calcium levels greater than 11.2 mg/dl during oral and intravenous treatment were 3.62% and 0.86% of calcium measurements, respectively ($p < 0.001$).[171] In addition to treating SHPT, doxercalciferol has also been demonstrated to increase bone density in dialysis patients. In a 16-week safety and efficacy study of hemodialysis patients treated with doxercalciferol, bone mineral density increased in all areas measured: total skeleton (+6.5%), lumbar spine (+6.9%), and total femur (+4.3%).[172]

The use of vitamin D sterols is an important component in the treatment of secondary hyperparathyroidism. Newer analogues appear to be as effective and in some cases more effective in treating SHPT without inducing hypercalcemia and hyperphosphatemia. Long-term benefits of vitamin D therapy may be recognized by fewer hospitalizations and improved mortality as demonstrated

in observational studies. Importantly, additional benefits of vitamin D therapy may warrant more aggressive use in earlier stages of CKD.

3. Additional Benefits of Vitamin D

Supplementing vitamin D may have significant implications in the health and normal function of several target organ systems in patients with CKD. In addition to treatment and prevention of renal osteodystrophy, use of vitamin D may have therapeutic applications in immunologic function and cardiovascular disease prevention.[166,173] The ubiquitous nature of the vitamin D receptor in several organ systems underscores the important role of vitamin D replacement in patients with CKD beyond treatment of SHPT (Table 3-2). In particular, the role of vitamin D therapies on cardiovascular outcomes in patients with CKD has been suggested in several studies.[166,174,175]

Vitamin D may mediate its benefits to the cardiovascular system through a variety of mechanisms. Its effect may be mediated through cardiac remodeling and interruption of the renin–angiotensin system. Calcitriol has been shown to be a potent inhibitor of renin expression in the kidney, and, like angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), vitamin D sterols and its analogues may interrupt the renin–angiotensin cascade.[176,177] In a preliminary clinical trial, treatment with calcitriol resulted in regression of left ventricular hypertrophy, and significant reductions in vasoactive hormones renin, angiotensin II, and atrial natriuretic peptide were seen.[178] In patients on dialysis, these benefits may be minimized by exacerbation of hypercalcemia and hyperphosphatemia and their contri-

Table 3-2. Tissue Distribution of the Vitamin D Receptor (VDR).

System	Tissue and Cell
Gastrointestinal	Esophagus, stomach, small intestine, large intestine, colon
Hepatic	Liver parenchyma cells
Renal	Proximal and distal tubules, collecting ducts
Endocrine	Parathyroid, pancreatic β-cells, thyroid C cells
Exocrine	Parotid gland, sebaceous gland
Reproductive	Testis, ovary, placenta, uterus, endometrium, yolk sac
Immune	Thymus, bone marrow, B and T cells
Respiratory	Lung alveolar cells
Musculoskeletal	Osteoblasts, osteocytes, chondrocytes, striated muscle
Epidermis/appendage	Skin, breast, hair follicles
Central nervous	Brain neurons
Connective tissue	Fibroblasts, stroma
Cardiovascular	Myocytes, smooth muscle, and endothelial cells

[a]Adapted from Brown et al. Am J Physiol 1999.

bution to vascular calcifications. Therefore, utilizing vitamin D sterols that minimize hypercalcemia may be better suited to achieve the cardiovascular benefits.

Vitamin D may also contribute to modulation of the immune system. For patients with CKD, especially those on dialysis, maintaining the integrity of the immune system can be critical in limiting morbidity and mortality from acute and chronic infections. The active form of vitamin D has been shown to play an integral role as a regulator of both natural and acquired immunity through both genomic-dependent as well as nongenomic cell surface receptor-mediated pathways.[179] The role of vitamin D in the immune system is supported by the presence of the VDR found on immune calls, namely activated T cells and antigen presenting cells (APCs).[151] In addition, the role of activated vitamin D has been associated with a reduced risk of certain immunologic diseases such as type 1 diabetes and thyroiditis.[180,181] Further, in patients with renal transplants, supplementation with vitamin D can improve tolerance and prolonged graft survival in the allograft.[182] The role of vitamin D therapies in modulating immune function has not been studied prospectively in larger clinical trials and warrants further research. Because vitamin D and its analogues affect gene transcription, it is unclear if structural difference between these compounds would modulate the immune system in different ways. Understanding these differences may provide additional insight into the advantages of vitamin D therapy in patients with CKD.

E. Calcimimetics

Traditional treatment with vitamin D sterols and calcium may be ineffective in the management of secondary hyperparathyroidism in patients with CKD. Progressive renal failure and advancing parathyroid hyperplasia lead to resistance to vitamin D therapy from downregulation of VDRs. Hyperphosphatemia and advancing uremia may also inhibit the transcriptional activation of vitamin D responsive genes from the VDR receptor, further enhancing PTH synthesis and secretion.[183] As CKD progresses, nodular hyperplasia develops in the parathyroid gland, and the number of calcium sensing receptors declines. Thus, higher calcium concentrations are required to suppress PTH secretion.[184] Traditional therapies for SHPT can lead to worsening hypercalcemia and hyperphosphatemia, limiting their utility in the treatment of secondary hyperparathyroidism. Calcimimetic agents offer a new therapeutic modality that may avoid these pitfalls while effectively lowering serum PTH levels in patients with CKD. Cinacalcet (Sensipar®, Amgen) is the first calcimimetic approved by the FDA for the treatment of SHPT in patients with CKD.

Parathyroid cells express cell surface calcium receptors that respond to the concentration of ionized extracellular calcium and were first cloned in

1993 from bovine parathyroid.[185] In addition to the parathyroid gland, these receptors are also expressed in the kidney and thyroidal C cells. A member of the G-protein-coupled receptor superfamily, the calcium sensing receptor is the primary mechanism by which the parathyroid gland regulates hormone secretion and achieves calcium homeostasis in the body.[186] Mutations in the gene encoding the calcium sensing receptor were also found to be responsible for familial hypocalciuric hypercalcemia.[187] Calcimimetic agents target the calcium sensing receptor to mimic or potentiate the effect of extracellular calcium on the calcium sensing receptor.[188] These agents thereby effectively lower serum PTH levels, and in higher doses, can also increase the secretion of calcitonin. The additional effect on calcitonin secretion plus urinary calcium excretion explains the hypocalcemia that can occur at higher doses.[186]

Early trials in animal models of secondary hyperparathyroidism and in humans with primary hyperparathyroidism demonstrated the ability of first generation (R-568) calcimimetics to lower serum levels of PTH.[189] However, R-568 was abandoned largely because of limited bioavailability and inconsistent pharmacokinetic profile. Newer second-generation compounds were then introduced (AMG 073) that effectively lowered plasma PTH levels without increasing serum calcium and phosphorus levels. Quarles and colleagues evaluated the safety and efficacy of AMG 073 in 71 hemodialysis patients with uncontrolled secondary hyperparathyroidism who failed to respond to conventional therapy. In an 18-week oral dose titration study administering up to 100 mg a day, the authors found a mean decrease of 33% of serum PTH in the AMG 073-treated patients compared with an increase of 3% in the placebo group ($p = 0.001$). In addition, 44% of patients were able to achieve a mean PTH level < 250 pg/ml in the AMG 073 group compared to 20% in the placebo arm. Further, 53% achieved a PTH reduction of $\geqslant 30\%$ compared to 23% in placebo patients. Calcium phosphorus product was better controlled in the treatment group, with a reduction by 7.9% in the AMG 073 arm versus an increase of 11.3% in the placebo group. Overall, the calcimimetic agent was well tolerated, with vomiting being the most common side effect, and proved to be an effective agent in reducing serum PTH and Ca $\times$ P product in patients with ESRD.[190]

A larger double-blind randomized placebo-controlled clinical trial further evaluated the safety and efficacy of the second-generation calcimimetic, cinacalcet, in patients with inadequately controlled SHPT on hemodialysis. Block and colleagues evaluated 371 patients on cinacalcet and 370 patients on placebo over a 26-week period. Doses were titrated from 30 mg to 180 mg a day to achieve a target PTH level of $\leqslant 250$ pg/ml. Forty-three percent of the cinacalcet group reached the primary endpoint compared to 5% in the placebo arm. In addition, mean PTH levels decreased by 43% in those receiving the study medication versus only 9% in the placebo group. Cinacalcet

had the additional benefit of lowering the Ca × P product by 15%. There was no change in Ca × P product for patients receiving placebo. Baseline characteristics of both groups indicated an equal number of individuals on phosphate binders and vitamin D sterols and equivalent disease severity in both groups. In general, the drug was well tolerated and nausea was the most commonly noted side effect and occurred in 32% of those receiving cinacalcet versus 19% in placebo. Nausea was unrelated to the dose; however, vomiting did appear more frequently at higher dose ranges. Hypocalcemia (< 7.5 mg/dl) occurred in 5% of patients receiving the calcimimetic. The study demonstrated that cinacalcet is an effective therapy in lowering PTH in patients on hemodialysis.[81] Similarly, cinacalcet has been shown to be safe and efficacious in treating SHPT in patients on peritoneal dialysis.[191]

The initial studies of calcimimetics agents tested their efficacy in patients on dialysis. More recently, studies have demonstrated their value in treating SHPT in patients with chronic kidney disease not receiving dialysis and in patients with renal transplants.[192,193] The results in kidney transplantation are less robust, and in a small series of 14 kidney transplants patients prescribed low doses of cinacaclet (30 mg), there was little effect in reducing PTH, but it was effective in lowering serum calcium levels.[194] The latter may reflect modulation of the calcium sensing receptor in other tissues, such as urinary calcium excretion in the kidney and calcitonin production by C-cells in the thyroid. In addition, the use of cinacalcet may also put patients with more residual renal function at higher risk of hypercalcemia. The lack of efficacy in this study of renal transplant patients may reflect the lack of dose titration rather a treatment failure. Additional large prospective studies need to be carried out in recipients of renal allografts to determine the value of treatment of persistent SHPT with cinacalcet. Furthermore, the effect on bone remodeling, long-term side effects, and potential benefits in reducing cardiovascular endpoints have yet to be evaluated in patients on dialysis receiving cinacalcet and warrant additional study.

F. Parathryoidectomy

In severe cases of secondary hyperparathyroidism, medical therapy with vitamin D sterols and calcimimetics may be ineffective. In addition, treatment with traditional therapies may result in repeated bouts of hypercalcemia, which may also prompt surgical intervention. In these cases parathyroidectomy (PTX) may be indicated. In an analysis of Medicare payment information from 1992 to 2002, factors associated with parathyroidectomy included younger age, female gender, white race, absence of diabetes, longer duration of hemodialysis, previous renal transplantation, and use of intravenous vitamin D.[195] In this study, the annual incidence reported of PTX in 1992 was 11.6 per 1000 patient-years, which then fell to a nadir in 1998 of 6.8 per 1000 patient-years. Since

then the incidence of parathyroidectomy has been on the rise, and in 2002 the incidence had climbed back to 11.8 per 1000 patient-years. Its unclear what prompted the rise in incidence and may reflect changes in therapy for SHPT.

When PTX in indicated, there is disagreement about whether total or subtotal parathyroidectomy should be performed. The current K/DOQI guidelines recommend subtotal parathyroidectomy or total parathyroidectomy with autotransplantation. In cases of autotransplantation, forearm grafts may be preferred because of the ease of removal in cases of recurrence rather than neck reexploration.[196,197] Others have also had success with four-gland removal without autotransplantation with good biochemical and clinical results at 2-year follow-up. Still, even with four-gland removal, postoperative hyperparathyroidism recurred in 15% of patients.[198] Subtotal PTX can also achieve good results with normalization of PTH levels within 3 weeks.[199,200]

Regardless of the method chosen, ectopic sites of parathyroid tissue should be considered and may be a cause of surgical failure.[200] In a study of 1156 patients, 4.2% had persistent hyperparathyroidism and reoperation was required in approximately 40% of these cases. In more than half of these, the missed glands removed at reoperation were supernumerary or mediastinal. The authors stressed the need to resect all parathyroid glands to prevent persistent hyperaparathyroidism.[201]

After parathyroidectomy, the most common complication is hypocalcemia or hungry bone syndrome and can occur in up to 76% of patients.[198] Return to normocalcemia may require judicious use of calcium supplementation in the immediate postoperative period and after discharge. In one study of dialysis patients who received subtotal parathyroidectomies, serum calcium was not normalized until the end of the third week.[199]

Parathyroidectomy is associated with higher short-term mortality and lower long-term mortality in patients on dialysis. In a cohort study of 4558 dialysis patients undergoing their first PTX, the 30-day postoperative mortality rate was 3.1%. When these individuals were matched with dialysis patients not undergoing PTX, the long term relative risk of death was 10% to 15% lower in the group having surgery.[71] These results underscore the risk of death associated with uncontrolled secondary hyperparathyroidism in patients with CKD and the ability of surgery to improve outcomes in these patients. Overall, parathyroidectomy is a safe and effective treatment for secondary hyperparathyroidism.[202] In cases of repeated bouts of hypercalcemia and hyperphosphatemia with traditional therapies, medical interventions should not be prolonged and surgical interventions should be considered.

V. Conclusion

Secondary hyperparathyroidism is a universal complication in patients with CKD and occurs early in the development of renal failure. As GFR declines, reduction in serum calcitriol levels, moderate decreases in ionized calcium, and reduced excretion of serum phosphorus contribute to the development of SHPT. Traditional approaches in the treatment of SHPT have focused on phosphorus control, through dietary phosphate restriction, calcium and non-calcium-based phosphate binders, and vitamin D sterols. In cases of severe hyperaparathyroidism resistant to traditional therapies, parathyroidectomy was the treatment of choice. However, now with the introduction of the calcimimetic agent cinacalcet, additional medical therapy is available as an alternative to surgery. The importance of controlling PTH, serum phosphorus, and calcium goes beyond treatment of bone disease and may have a significant impact on other organ systems and ultimately cardiovascular morbidity and mortality. Prospective, randomized trials to test the value of vitamin D therapies and calcimimetics in reducing cardiovascular and all-cause mortality need to be pursued.

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Chapter 4

Uremic Toxins in Chronic Renal Failure

Griet Glorieux, Eva Schepers, and Raymond Camille Vanholder

I. Introduction

The uremic syndrome is a complex of biological and biochemical alterations that result in a host of failing organs and disturbing symptoms. It originates from the retention of solutes, which under normal conditions are cleared by the kidneys into the normal urine, although derangements of hormonal, metabolic, and enzymatic axes also play a role. The impact of retention is underscored by the clinical improvement resulting from dialysis and kidney transplantation.

The uremic syndrome is characterized by a deterioration of biochemical and physiologic functions (Table 4-1), in parallel with the progression of renal failure. This results in a variable number of symptoms, which mimic the picture of exogenous poisoning. Although the link between clinical deterioration and uremia was recognized decades ago, and although the number of new pathophysiologic elements provided in this area has risen exponentially over the last few years, our knowledge about the responsible factors remains incomplete.

In this chapter, current knowledge about uremic solute retention and its clinical and biological effects is reviewed.

II. Uremic Solute Retention

A. General Classification of Uremic Solutes

A gradual retention of a large number of organic metabolites of proteins, fatty acids, and carbohydrates characterizes the progression of renal failure, whereby partial metabolization and elimination via routes other than renal pathways may compensate for the loss of renal clearance. Some of the retained compounds are proven toxins. Toxicity is not a simple monofactorial process whereby only one or a few toxins affect many different metabolic processes at

Table 4-1. The Uremic Syndrome: Main Clinical Alterations.

Cardiovascular system	*Bone disease*
Atheromatosis	Adynamic bone disease
Arteriosclerosis	Amyloidosis (β_2-microglobulin)
Cardiomyopathy	Defective calcitriol metabolism
Decreased diastolic compliance	Osteitis fibrosa
Hyper/hypotension	Osteomalacia
Pericarditis	Osteoporosis
Nervous system	*Skin*
Concentration disturbances	Melanosis
Cramps	Pruritus
Dementia	Uremic frost
Depression	
Fatigue	*Gastrointestinal system*
Headache	Anorexia
Motor weakness	Dyspepsia
Polyneuritis	Gastrointestinal ulcers
Reduced sociability	Hiccup
Restless legs	Nausea, vomiting
Sleep disorders	Pancreatitis
Stupor, coma	
Hematological system / coagulation	*Pulmonary system*
Anemia	Pleuritis
Bleeding	Pulmonary edema
Hypercoagulability	Sleep apnea syndrome
Immunological system	*Miscellaneous*
Inadequate antibody formation	Hypothermia
Stimulation of inflammation (baseline)	Thirst
Susceptibility to cancer	Uremic foetor
Susceptibility to infection	Weight loss
Endocrinology	
Dyslipidemia	
Glucose intolerance	
Growth retardation	
Hyperparathyroidism	
Hypogonadism	
Impotence, diminished libido	

a time. Other retained substances are nontoxic but can be used as markers of retention.

A recent survey of the literature revealed the retention in uremia of at least 90 compounds, for which the concentration had been reported.[1] It is very likely

that this is only the tip of the iceberg. Whereas in the aforementioned survey approximately 25 middle molecular weight peptides had been described, a recent study using highly sophisticated proteome analysis revealed the presence of at least 1000 such compounds in ultrafiltrate from dialyzed patients.[2]

Under normal conditions, the glomerular filter clears molecules with a molecular mass up to $\pm 58{,}000$ daltons. All these substances are assumed to be retained in renal failure. An additional role should be attributed to changes in tubular secretion, reabsorption, and metabolic breakdown, all of which altered when renal mass decreases. The molecules metabolized by the kidneys may have a higher molecular mass ($> 58{,}000$ daltons) than those cleared. Renal and nonrenal metabolization of solutes and nonrenal clearance may in turn be inhibited after uremic retention.

Uremic retention products are arbitrarily subdivided according to their molecular weight.[3,4] Low molecular weight molecules are characterized by a molecular weight (MW) up to 500 [e.g., urea (MW 60), creatinine (MW 113)]. They can further be subdivided in protein-bound and non-protein-bound molecules. Substances with a molecular weight range above 500 are called middle molecules [e.g., parathyroid hormone (PTH, MW 9,424), β_2-microglobulin (β_2-M, MW 11,818)]. Several clinical, metabolic, and/or biochemical disturbances such as food intake, apolipoprotein (apo) A-I secretion, osteoblast mitogenesis, cell growth, lymphocyte proliferation, and interleukin production are caused by uremic compounds that conform to the middle molecular weight range.[5–10] Several of the recently defined uremic compounds, for example, β_2-M, various peptides, some of the AGE, as well as PTH, conform to the definition of the middle molecules (MM) (cfr. B2; B11; B1 and B10 respectively).

Dialysis membranes with the capacity to remove MM (high-flux membranes) have been related to lower mortality,[11–15] as well as a slower loss of residual renal function,[16] less preponderant dyslipidemia,[17] improvement of polyneuropathy,[18] and a lower prevalence of carpal tunnel syndrome.[19] However, these highly efficient membranes are often at the same time less complement activating than unmodified cellulose, and in many studies their counterpart. Hence, the relative importance of the removal of MM versus biocompatibility-related events is not always clear. Two studies, however, point to an independent benefit of large molecule removal.[20,21]

In the prospective randomized HEMO study, however, on primary analysis no significant impact on mortality was found for high-flux dialyzers although a trend was evident.[22] On secondary analysis, a benefit was found for large-pore membranes with regard to cardiovascular events.[22] Patients who had been treated with dialysis for a long time obtained an additional benefit.[23]

Removal of larger molecules is more efficient when the high-flux membranes are used in a convective mode;[24] no data are available regarding whether

this affects mortality. Convective treatment modalities have a positive impact on the development of carpal tunnel syndrome.[19] On-line hemodiafiltration with large convective volumes results in a rise of erythrocyte counts and a decrease of erythropoietin needs.[25] Even if highly efficient dialysis is clinically superior, its cost-effectiveness still needs to be demonstrated.

During dialysis, small protein-bound compounds such as hippuric acid or indoxyl sulfate behave like MM, owing to their high protein binding. Their removal by classical hemodialysis systems, even with large-pore membranes, remains disappointingly low,[26] which may be attributed to the complex distribution and intradialysis kinetics of these compounds. Therefore, removal strategies alternative to the classical ones should be considered, such as adsorption, changes in time frames, use of protein leaking membranes, and/or stimulation of metabolic pathways. Even small water-soluble compounds, which in principle should show the same characteristics as urea, quite often show different kinetics, as has been demonstrated recently for the guanidines.[27]

Peritoneal dialysate is a much richer source of protein-bound compounds than hemodialysate,[28] because peritoneal pore size allows the transfer of substantial quantities of albumin together with its bound moieties, which is not the case for even the most open hemodialyzer membranes. In addition, the continuous time frame might enhance the removal of these compounds.[29]

Until recently, no data had confirmed a potential clinical impact of protein-bound molecules. Recently, at least two studies pointed into that direction.[30,31]

B. Main Uremic Retention Products

Several uremic retention solutes influence biological functions. Other compounds have no proven direct toxicity, but may be useful markers of uremic retention. An overview of the pathologically most relevant uremic retention solutes with their molecular weights is given in Table 4-2. It should be noted that inorganic compounds such as water and potassium exert toxicity as well. In what follows, we concentrate on the organic retention compounds.

1. Advanced Glycation End Products (AGE)

As first described by Maillard, glucose and other reducing sugars react nonenzymatically with free amino groups to form reversible Schiff base adducts (in days) and stable Amadori products (in weeks), which are then converted into AGE through chemical rearrangements and degradation reactions.[32] Several AGE are peptide-linked degradation products[33] (MW 2,000 to 6,000), although the baseline AGE such as pentosidine, 2-(2-fuoryl)-4(5)-(2-furanyl)-1H-imidazole (FFI), imidazolone, 3-deoxyglucosone, pyrrole aldehyde, and N^{ε}-(carboxymethyl)lysine have a substantially lower MW (Table 4-2).

AGE are retained not only in renal failure, but also in diabetes mellitus and aging,[34] in which they are responsible for tissue damage and func-

Table 4-2. Major Uremic Retention Solutes and Their Molecular Weight.

Compound	MW	Compound	MW
ADMA/SDMA	202	Adrenomedullin	5729
ANF	3080	Benzylalcohol	108
β-Endorphin	3465	β-Guanidinopropionic acid	131
β_2-Microglobulin	11,818	CGRP	3789
Cholecystokinin	3866	CIP	8500
Clara cell protein	15,800	CML	188
CMPF	240	Complement factor D	23,750
Creatine	131	Creatinine	113
Cystatin C	13,300	Cytidine	234
DIP I	14,400	DIP II	24,000
3-Deoxyglucosone	162	Dimethylarginine	202
Endothelin	4283	γ-guanidinobutyric acid	145
Glomerulopressin	500	GIP I	28,000
GIP II	25,000	Guanidine	59
Guanidinoacetic acid	117	Guanidinosuccinic acid	175
Hippuric acid	179	Homoarginine	188
Homocysteine	135	Hyaluronic acid	25,000
Hypoxanthine	136	Imidazolone	203
Indole-3-acetic acid	175	Indoxyl sulfate	251
Leptin	16,000	Melatonin	126
Methylguanidine	73	Myoinositol	180
Neuropeptide Y	4272	Orotic acid	156
Orotidine	288	o-OH-hippuric acid	195
Oxalate	90	p-Cresol	108
p-OH-hippuric acid	195	Parathyroid hormone	9225
Pentosidine	135	Phenylacetylglutamine	264
Phenol	94	Phosphate	96
Pseudouridine	244	Putrescine	88
Retinol binding protein	21,200	Spermine	202
Spermidine	145	Thymine	126
Trichloromethane	119	Tryptophan	202
Urea	60	Uric acid	168
Uridine	244	Xanthine	152

tional disturbances. In the uremic population, the level of glucose-modified proteins is higher than in diabetic individuals without renal failure,[35] and AGE concentration does not depend on the glycemic status.[36,37] Production of AGE in end-stage renal disease (ESRD) has been related to oxidative and carbonyl stress, rather than to reactions with glucose.[38] Not all AGE generation is oxidative, however. AGE provoke monocyte activation[39], as well as the induction of interleukin-6, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) generation.[40] AGE-modified β_2-M may play a role in the

generation of dialysis-associated amyloidosis[41] (cfr. B2). Serum pentosidine levels are higher in patients with dialysis-related amyloidosis, compared to their amyloid-free counterpart.[42] AGE can react with and chemically inactivate nitric oxide (NO),[43] a potent endothelium-derived vasodilator, antiaggregant, and antiproliferative factor. AGE are also related to oxidative protein modification.[44] 3-Deoxyglucosone inactivates glutathione peroxidase, a key enzyme in the neutralization of hydrogen peroxide.[45] AGE accumulate in atheromatous plaque of the aortic wall of subjects with ESRD, where they may contribute to a more rapid progression of atherosclerosis.[42] To our knowledge, however, there is no observational study in uremia linking AGE directly to atherogenesis.

Late glycation products increase polymorphonuclear leukocyte (PMNL) chemotaxis.[46] Other recent data suggest that whereas AGE increase baseline leukocyte response, an activated response to infectious stimuli is blunted.[47] This suggests a dual response, related at the clinical level both to atherogenesis and susceptibility to infection.[48]

Most of the biological actions of AGE documented to date, however, have not been obtained with AGE recovered from uremic or diabetic serum, but with AGE artificially prepared in the laboratorium.[48] Recent data underscore as well the immune enhancing effect of genuine AGE, as they are found in renal failure.[49]

Concentrations in ESRD patients might be attributed to increased uptake, production, and/or retention. During industrial food processing, cooking, and storage of foods, food proteins are modified by carbohydrates,[50–52] and those are absorbed via the gastrointestinal tract.[36] Healthy kidneys are responsible not only for glomerular filtration but also for tubular reabsorption and degradation of AGE.[53,54] Specific receptors for AGE have been identified (RAGE) and their expression is enhanced during uremia.[55] AGE binding to RAGE has been shown to stimulate mesothelial cell activity, and results in overexpression of vascular cell adhesion molecule (VCAM-1), which activates human peritoneal cells and promotes local inflammation, implicating the development of tubular injury.[56]

In spite of continuous contact with glucose via the dialysate, continuous ambulatory peritoneal dialysis (CAPD) patients do not have higher serum AGE levels than hemodialysis patients.[33] Nevertheless, protein glycation has been demonstrated in the peritoneal membrane.[57] Heat sterilization of glucose-containing peritoneal dialysate induces the formation of glucose degradation products (GDP), which are precursors of AGE.[58] GDPs inhibit leukocyte response, and this effect is attenuated when heat sterilization is replaced by other procedures (e.g., filter sterilization).[59]

Removal of AGE is significantly more important with high-flux hemodialysis than with conventional dialysis with low-flux membranes.[60] AGE show a

marked heterogeneity in removal pattern, even during high-flux dialysis.[51,61] It is unclear which compounds could be defined by their removal pattern in a way that they could serve as a marker for the overall group of AGE.

2. β_2-Microglobulin (β_2-M)

β_2-M (MW approximately 12,000) is a component of the major histocompatibility antigen. Uremia-related amyloid is to a large extent composed of β_2-M, and is essentially found in the osteoarticular system and in the carpal tunnel, although deposition can be systemic as well.[62] Uremia-related amyloidosis becomes clinically apparent most often after several years of chronic renal failure and/or in the aged.[63] According to the most recent studies, its prevalence tends to decrease,[64] probably as a result of modifications in dialysis strategies.

AGE-modified β_2-M has been identified in amyloid of hemodialyzed patients[65] and enhances monocytic migration and cytokine secretion,[66] suggesting that foci containing AGE-β_2-M may initiate inflammatory response, leading to bone and joint destruction. The lack of a higher clinical incidence of β_2-M-amyloidosis in diabetic dialysis patients,[67] who generate large quantities of AGE in the presence of hyperglycemia, casts a doubt on the pathophysiologic role of AGE in amyloid formation. Possibly, the AGE transformation plays a more important role in the inflammation surrounding β_2-M-amyloid than in its generation.

Long-term hemodialysis with large-pore membranes results in a progressive decrease of predialysis β_2-M concentrations; the levels remain, however, far above normal, even after intensive removal therapy.[68,69] Long-term dialysis with large-pore dialyzers results in a lower prevalence of dialysis-related amyloidosis and/or carpal tunnel syndrome.[11,19,70,71] Whether this benefit is attributable to a better removal of β_2-M, to lower complement and leukocyte activating capacity, or to protection against the transfer of dialysate impurities into the blood stream (e.g., lipopolysaccharides)[64] is not evident, because most of the dialyzers associated with a lower incidence of amyloidosis have all three above-mentioned properties.

Because β_2-M is removed only by dialyzers with a large pore size, its kinetic behavior might be representative for other large molecules. Behavior of β_2-M during dialysis, however, is not necessarily representative of that of other MM. Discrepancies in behavior in the long run have been demonstrated in relation to other MM, such as complement factor D.[72]

Recently, several devices with strong adsorptive capacity for β_2-M have been developed.[73]

The clinical expression of dialysis-related amyloidosis disappears after kidney transplantation, but the underlying pathologic processes such as bone

cysts and tissular β_2-M remain preserved.[74] Possibly, immunosuppressive therapy plays a role in the regression of the symptomatology.

3. Creatinine

Creatinine belongs to the large group of guanidines (cfr. B6). Because of the specific value of creatinine as a marker of renal function, this compound is discussed separately.

The rise in serum creatinine during renal failure is not linearly related to the decrease in glomerular filtration rate (GFR), which may decrease by more than 50% without marked changes in serum creatinine. Changes become more prominent in the lower range of filtration. In spite of the extensive use of creatinine as a marker of uremic toxin retention, it has been considered responsible for only a few uremic side effects, such as chloride channel blocking,[75,76] and the reduction of the contractility of cultured myocardial cells,[77] however, at concentrations exceeding those encountered in ESRD. Creatinine is also a precursor of the toxic compound methylguanidine.[78,79] It interferes with some of the central neurological functions.[76,80]

Serum creatinine concentration is the result not only of uremic retention but also of muscular breakdown; therefore, a high serum creatinine may be the consequence of high muscular mass, and hence an indicator of metabolic well-being. Morbidity and mortality in hemodialyzed patients are positively correlated with serum creatinine.[78]

4. Cytokines

In view of the strong associations between atherosclerosis, malnutrition, and inflammation,[81] it may be speculated that factors associated with malnutrition and inflammation may contribute to the excess prevalence of cardiovascular disease. The causes of inflammation in ESRD patients are probably multifactorial. All available evidence suggests that the pro-inflammatory cytokine system activity is elevated in ESRD patients.[82] It has been hypothesized that epoetin resistance is due to enhanced levels of immune activation because chronic inflammation can modify the process of erythropoiesis. The accumulation of TNF-α may contribute to the development of neurologic and hematologic complications in uremia. Several lines of evidence suggest that decreased renal clearance might play an important role.[83] However, as the half-life of various cytokines is short and local tissue cytokine inactivation may be the most important pathway of cytokine degradation, more research is needed to determine the relative importance of the kidney in cytokine clearance.

5. Dinucleoside Polyphosphates

Dinucleoside polyphosphates are a group of substances considered to be involved in the direct regulation of the vascular tone as well as growth of vascular smooth muscle cells[84] and mesangial cells.[85] Specific members of this

group, the diadenosine polyphosphates, were detected in hepatocytes, human plasma,[86] and platelets. In addition, concentrations of diadenosine polyphosphates were shown to be increased in platelets[87] from hemodialysis patients.[88] Recently, uridine adenosine tetraphosphate (Up4A) was isolated and identified as a novel endothelium-derived vasoconstrictive factor. Its vasoconstrictive effects, plasma concentration, and release on endothelial stimulation strongly suggest that Up4A has a functional vasoregulatory role.[89]

6. Guanidines

Guanidines are structural metabolites of arginine. Among them are well known uremic retention solutes, such as creatinine and guanidine, and moieties such as asymmetric and symmetric dimethylarginine (ADMA and SDMA).

Guanidine levels have been determined in serum, urine, cerebrospinal fluid, and brains of uremic patients.[90,91] Four compounds—creatinine, guanidine, guanidinosuccinic acid (GSA) and methylguanidine (MG)—are highly increased.

Several of the guanidino compounds modify key biological functions. GSA inhibits the production by 1α-hydroxylase of the active vitamin D metabolite, $1,25(OH)_2VitD_3$ (calcitriol),[92] and interferes with activation of ADP-induced platelet factor 3[93] at concentrations currently found in hemodialyzed uremics.[94,95] A mixture of guanidino compounds suppresses the natural killer cell response to interleukin-2[96] and free radical production by neutrophils.[97] In recent studies, guanidine compounds have been shown to enhance baseline immune function, related to vascular damage.[98] In addition, they also have been related to a decreased protein binding of homocysteine, another compound with vessel damaging potential.[99]

γ-Guanidinobutyric acid (GSA), methylguanidine, homoarginine, and creatine induce seizures after systemic and/or cerebroventricular administration to animals.[75,76] GSA plays an important role in the hyperexcitability of the uremic brain.[100] GSA probably also acts as a selective agonist at the *N*-methyl-D-aspartate (NMDA) receptor.[101,102] GSA displays in vivo and in vitro neuroexcitatory effects that are mediated by ligand- and voltage-gated Ca^{2+} channels, suggesting an involvement of the guanidines in the central nervous complications of uremia.[103]

Arginine enhances NO production. Some of the other guanidines, such as arginine analogues, are strong inhibitors of NO synthase. The inhibition of NO synthesis results in saphenous[104] and mesenteric vasoconstriction,[105] hypertension,[106] ischemic glomerular injury,[107] immune dysfunction,[108] and neurological changes.[109] ADMA is the most specific endogenous compound that inhibits NO synthesis. ADMA accumulates in the body during the development of renal failure,[110,111] related to decreased renal excretion but possibly also to suppressed enzymatic degradation by dimethylarginine

dimethylaminohydrolase.[112] The increase in SDMA is more pronounced, but this compound is biologically less active. In the brain, ADMA causes vasoconstriction and inhibition of acetylcholine-induced vasorelaxation.[113] Also in thoracic and radial vessels, ADMA induces contractions.[114] Recently, estrogen has been shown to alter the metabolism of ADMA reducing the circulating concentration in vivo.[115] Methylguanidine, another endogenous guanidine, also shows a certain inhibitory activity on cytokine- and endotoxin-inducible NO synthesis, although to a limited extent.[116]

In contradiction to the hypothesis of inhibition of NO synthesis in uremia, Noris et al. described an enhanced NO production in patients susceptible to uremic bleeding tendency.[117] Possibly, this effect is limited to a subgroup of the uremic population.

In the renal proximal convoluted tubule of rats with renal failure, the generation of guanidinoacetic acid and creatine from arginine is depressed,[118] whereas the synthesis of GSA, guanidine, and methylguanidine is markedly increased, owing to urea recycling.

Dialytic removal of guanidino compounds is subjected to a substantial variability.[95] Possibly, tissular distribution or protein binding play a role. In spite of a low molecular weight, removal by hemodialysis of ADMA is only in the range of 20% to 30%.[111] Several of the guanidines have a substantially larger distribution volume than the standard marker urea, resulting in a decreased dialytic effective removal and substantial post-dialysis rebound.[27]

7. Homocysteine

Homocysteine (Hcy), a sulfur-containing amino acid, is produced by the demethylation of dietary methionine. Retention results in the cellular accumulation of *S*-adenosyl homocysteine (AdoHcy), an extremely toxic compound, which competes with *S*-adenosylmethionine (AdoMet) and inhibits methyltransferase.[119] Moderate hyperhomocysteinemia, caused by a heterozygous deficiency of Hcy breakdown or by vitamin B_6, B_{12}, or folate deficiency, is an independent risk factor for cardiovascular disease in the general population.[120,121] Reduced and oxidized forms of Hcy are present in the plasma, and total fasting levels are a reflection of intracellular metabolism and cellular excretion of Hcy.[122]

Hcy increases the proliferation of vascular smooth muscle cells, one of the most prominent hallmarks of atherosclerosis.[123] Moderate hyperhomocysteinemia may involve endothelial dysfunction and generate reactive oxygen species.[124] The administration of excessive quantities of the Hcy precursor methionine to rats induces atherosclerosis-like alterations in the aorta.[125] Hcy also disrupts several anticoagulant functions in the vessel wall, which results in enhanced thrombogenicity.[126] Guanidines have been related to release

of homocysteine from its protein binding sites, by induction of structural modifications of albumin.[99]

Patients with chronic renal failure have total serum Hcy levels two- to fourfold above normal. The serum concentration depends not only on the degree of kidney failure, but also on nutritional intake (e.g., of methionine),[127] vitamin status (e.g., of folate),[128,129] genetic factors,[130–132] and decreased renal metabolization.[119] Almost all filtered Hcy is reabsorbed in the tubular system so that urinary excretion is minimal.[133] Detoxification by remethylation of homocysteine to methionine is inhibited in hemodialysis patients.[134,135]

Hyperhomocysteinemia is the most prevalent cardiovascular risk factor in ESRD.[132,136] Plasma homocysteine and cardiac mass correlate to each other.[137] In a study by Suliman et al., however, total plasma Hcy was lower in hemodialysis patients with cardiovascular disease than in those without.[127] In this study, a correlation was found between total Hcy and serum albumin, pointing to a negative impact of malnutrition on Hcy concentrations. Also more recent data point to an inverse relationship between homocysteine levels and mortality.[138] Hcy is partly bound to albumin, which hampers removal by hemodialysis. Hyperhomocysteinemia is more pronounced in hemodialysis patients than in peritoneal dialysis.[129] In hemodialyzed patients, homocysteine levels correlate with plasma folate,[128,129] and with the activity of enzymes that are at play in Hcy metabolism. Even with peritoneal dialysis, it is impossible to reduce total Hcy plasma levels to normal.[139]

Dialysis with extremely leaky hemodialyzer membranes with large pore size (so-called super-flux membranes) results in a progressive decline of predialysis plasma homocysteine concentrations.[140] This effect has at least in part been attributed to changes in homocysteine metabolism, induced by enhanced middle molecule removal through these highly efficient membranes.

Hcy levels can be reduced by folic acid, vitamin B_6, and vitamin B_{12}.[141] The population with ESRD might require high quantities of vitamins.[142]

Possibly, the disappointing efficiency of folic acid might be related to an impairment of the metabolization of folic acid to 5-methyltetrahydrofolate (MTHF), which is the active compound in the remethylation pathway.[143] In an attempt to obviate such a deficiency, Bostom et al. directly administered oral MTHF (17 mg/day) to hemodialyzed patients.[144] No benefit was found, however. Touam et al. on the other hand, could reduce total Hcy to normal in approximately 80% of the studied population, by the administration of folinic acid, a precursor of MTHF.[143] Since the supplementation with folate is inexpensive and relatively harmless, there is no formal objection against its therapeutic use.

Direct clinical proof of the benefit of a lower Hcy concentration in uremia is, to our knowledge, not available. Even when it was possible to decrease Hcy levels therapeutically, carotid artery stiffness was not altered.[145]

8. *Indoxyl Sulfate*

Indoxyl sulfate is metabolized by the liver from indole, which is produced by the intestinal flora as a metabolite of tryptophan. It enhances drug toxicity by competition with acidic drugs at the protein binding sites,[146] inhibits the active tubular secretion of these compounds,[147] and inhibits deiodination of thyroxin 4 by cultured hepatocytes.[148]

The oral administration of indole or indoxyl sulfate to uremic rats causes a faster progression of glomerular sclerosis and of renal failure.[149] This effect is possibly mediated by the renal gene expression of transforming growth factor-beta (TGF-β), tissue inhibitor of metalloproteinase-1 (TIMP-1) and pro-alpha1(I) collagen.[150] In animals, progression of renal failure is restrained by adsorbant administration, together with a diminished expression of the above mentioned factors.[150] Indoxyl sulfate restrains endothelial repair upon trauma.[151]

Reduction of serum indoxyl sulfate concentration, by intraintestinal absorption of the precursor indole, reduces uremic itching.[152] AST-120 retards the development of acquired renal cystic disease and aortic calcification,[153] and ameliorates tubulo-interstitial injury by reducing the expression in the kidneys of ICAM-1, osteopontin, TGF-β1, and clusterin in uninephrectomized rats.[154]

Because of protein binding (approximately 100% in normal subjects and 90% in uremics), the intradialytic behavior of indoxyl sulfate diverges from that of other small compounds such as creatinine. Removal by CAPD is more effective.[155] High-flux hemodialysis does not enhance removal.[26] Alternative extracorporeal removal procedures such as hemoperfusion might be considered. Dialysis against albumin-containing dialysate removes albumin-bound uremic toxins such as indoxyl sulfate more efficiently than conventional dialysis and may be useful for reducing these compounds.[156]

9. *Oxidation Products*

Oxidative capacity is increased in uremia[157–159] both before and after the start of dialysis.[44] Uremic patients also show an impaired antioxidant response, partly related to plasma glutathione deficiency.[160]

Oxidatively modified proteins act as mediators of oxidative stress and monocyte respiratory burst.[44] Albumin seems to be one of the target proteins of these oxidative reactions.[44,161] Structural modification of albumin may alter its binding capacity for drugs and other solutes.[162] Modification of hemoglobin to glutathionylhemoglobin has been proposed as another marker of oxidative stress.[163]

Low-density lipoprotein (LDL) from uremic patients is more susceptible to oxidation than that from control subjects[164] (oxidized LDL [oxLDL]). This chemically modified LDL is more readily accumulated in macrophages, which results in the development of foam cells, an early event in atherogenesis. LDL

autoantibodies against oxLDL have been demonstrated in ESRD, especially in hemodialyzed patients.[165] Oxidative modification of the protein moiety of LDL is a trigger of macrophage respiratory burst.[166,167]

Malondialdehyde levels are increased in ESRD.[168] The capacity of malondialdehyde to form DNA adducts[169] may play a pathophysiologic role in carcinogenesis. Low-dose IV folinic acid given to dialysis patients reduced the levels of serum malondialdehyde and thus improved the cardiovascular risk profile.[170]

Several small molecular weight compounds might also be modified by oxidation. Organic chloramines are generated by the chemical binding of hypochlorite, a free radical produced by activated leukocytes, to retained organic compounds.[171]

10. Parathyroid Hormone (PTH)

PTH, a MM with a molecular weight of ± 9000, is generally recognized as a major uremic toxin, although its increased concentration during ESRD is attributable merely to enhanced glandular secretion, rather than to decreased removal by the kidneys. Excess PTH gives rise to an increase in intracellular calcium, which results in functional disturbances of virtually every organ system, including bone mineralization, pancreatic response to glucose, erythropoiesis, cardiovascular, immune, and liver function.[172–177] PTH is one of the few substances that have been causally linked to uremic neuropathy.[178] It also plays a role in fibroblast activation,[172] and has been related to uremic pruritus.

Paradoxically, moderate hyperparathyroidism (intact PTH 60 to 200 ng/ml—normal range up to 60 ng/ml) has been demonstrated to improve the osseous response of uremic patients. If PTH remains in the lower range, patients may suffer from relative hypoparathyroidism, which results in aplastic bone, inadequate calcium handling, and redistribution of body calcium stores leading to metastatic tissue calcification.[179] The current test methods for the determination of PTH levels overestimate true concentrations, as they react as well with intact PTH as with functionally inactive fragments.[180] As a consequence, it has been suggested that to have a normal bone turnover, PTH levels measured by classical methods should be two to three times above the upper normal limit.[175] At present, new test methods have been developed that estimate only intact PTH.[180]

Hyperparathyroidism results from phosphate retention, decreased production of calcitriol (1,25 $(OH)_2$ vitamin D_3) and/or hypocalcemia. In HD patients, however, correction of metabolic acidosis reduced intact PTH levels in the presence of secondary hyperparathyroidism.[181]

Therapy with calcitriol alone or one of its analogues lowers serum PTH levels.[182] Uremia is characterized not only by a depressed production of calcitriol, but also by resistance to this hormone; this resistance is induced by

uremic biological fluids, such as ultrafiltrate and chromatographic fractions of this ultrafiltrate.[183]

Only dialysis membranes with a large pore size remove PTH.[184] Differences in concentration at the end of the dialysis session are, however, clinically irrelevant. Increased removal will probably be compensated by enhanced endocrine production (trade-off). A more efficient way to correct PTH hypersecretion is correction of plasma calcium, calcitriol, and phosphorus levels.[185] If these interventions remain ineffective, parathyroidectomy is the ultimate therapeutic resource. A pharmacologic option for the future are the calcimimetics.[186,187] Apart from hypocalcemia, side effects are very rare.[188] A calcium-free phosphate binder (Renagel®) is now commercially available with promising results.[189] Another calcium-free phosphate binder that became available recently is lanthanum carbonate. This compound is a trace element but it seems possible to administer it safely without its deposition in bone. New vitamin D analogues that have less calcemic and phosphatemic effects are under development.[190] All these newly developed measures should help in combating hyperparathyroidism without increasing circulating calcium levels. In contrast, traditional therapeutic options such as classical vitamin D analogues and calcium salts easily induce hypercalcemia, hence increasing the risk of calcium deposition in the tissues and vascular damage.

11. Peptides

Peptides constitute a heterogeneous group of molecules. In general, peptides can be considered as typical MM. β_2-M and PTH have been discussed previously.

Granulocyte inhibiting protein I (GIP I—28 kDa), recovered from uremic sera or ultrafiltrate, suppresses the killing of invading bacteria by polymorphonuclear cells.[191] The compound has structural analogy with the variable part of kappa light chains. Another peptide with granulocyte inhibitory effect (GIP II—9.5 kDa) is partially homologous with β_2-M, and inhibits granulocyte glucose uptake and respiratory burst activity.[192] A degranulation inhibiting protein (DIP—24 kDa), identical to angiogenin, was isolated from plasma ultrafiltrate of uremic patients.[193] The structure responsible for the inhibition of degranulation is different from the sites that are responsible for the angiogenic or ribonucleic activity of angiogenin. A structural variant of ubiquitin inhibits polymorphonuclear chemotaxis (chemotaxis inhibiting protein [CIP]—8.5 kDa).[194]

Atrial natriuretic peptide (ANP—3.1 kDa) and endothelin (3.5 kDa) are elevated in dialysis patients, and may play a role in the regulation of blood pressure.[195] ANP levels correlate with left atrial size, fluid overload, and decreased systemic clearance.[196] Endothelin causes peripheral insulin resistance,

even at concentrations that induce no blood flow changes,[197] and may play a role in uremic hypertension.[198]

The opioid peptides β-endorphin (3.5 kDa), methionine-enkephalin (0.6 kDa), and β-lipotropin (1.9 kDa) are elevated in dialyzed patients.[199] Delta sleep-inducing peptide (0.9 kDa) may modulate sleep–wakefulness.[200]

Neuropeptide Y (NPY—4.3 kDa) is increased in uremia,[201] and tends to increase further during hemodialysis.[202] It is a 36-amino-acid peptide with renal vasoconstrictive activity.[203] Recently, plasma NPY was found to predict incident cardiovascular complications in ESRD.[204] NPY also acts as an orexigen.[205] Uremic patients with anorexia have lower NPY levels.[205,206] The concentration of the anorexigen cholecystokinin (CCK) is increased in most patients with chronic renal failure.[205]

Adrenomedullin, a 52-amino-acid and potent hypotensive peptide, is found at markedly increased concentrations in chronic renal failure patients,[207] and activates inducible nitric oxide synthase.[208]

Cystatin C (13.3 kDa), Clara cell protein (CC16) (15.8 kDa), and retinol binding protein (RBP) (21.2 kDa) are elevated in renal failure.[209] Cystatin C is an inhibitor of proteinases and cathepsins.[210] CC16 is an immunosuppressive α-microprotein.[211]

Leptin, a 16-kDa plasma protein, decreases appetite of uremic patients.[212] The rise in serum leptin is attributed mostly to decreased renal elimination,[213] and is almost entirely limited to the free fraction.[213] Increased leptin is associated with low protein intake and loss of lean tissue in chronic renal failure.[212] Recent data suggest an inverted correlation between leptin and nutritional status,[214] and a direct correlation with C-reactive protein (CRP).[215] In CAPD patients, serum leptin showed a progressive rise only in individuals with body weight loss.[216] Erythropoietin treatment results in a decline of leptinemia and an improvement of nutritional status.[217]

However, leptin levels are also elevated in obese people and are hence not necessarily related to reduced appetite. Body fat and serum leptin also correlate in uremia.[215] Female gender and obesity are important factors that affect serum leptin in ESRD patients as well.[218] Don et al. suggest that in ESRD patients, leptin may be depressed during inflammation, and may actually act as a negative acute phase reactant.[219] Therefore, the biochemical role of leptin in renal failure remains inadequately defined.

Ghrelin is a recently described polypeptide hormone produced mainly in the stomach but also synthesized in various tissues including the kidney.[220] Ghrelin has been shown to stimulate a variety of nutrition-related effects, such as growth hormone (GH) release from the pituitary gland,[221] increase in food intake,[222] fat accumulation, and body weight gain.[223] A recent study described that plasma ghrelin was significantly increased in chronic kidney disease patients compared with those in patients with normal renal function,

and that plasma ghrelin was significantly correlated with both serum creatinine and GH. Moreover, heminephrectomy in mice caused a marked increase in the plasma ghrelin without significant changes in ghrelin mRNA levels in the stomach, suggesting that the kidneys are important in ghrelin clearance.[220]

The status of ghrelin as a uremic toxin can be doubted. Recent data point to the favorable effects of subcutaneously administered ghrelin on the nutritional condition of malnourished patients on peritoneal dialysis[224] and on the vascular status of rats in vivo and in vitro.[225]

12. Phenols

Phenol depresses various functional parameters of enzymatic activity in polymorphonuclear leukocytes.[226] A depressive effect was demonstrated on the 3′,5′-cyclic monophosphate response of the neostriatum to dopamine.[227] This effect was abolished after conjugation of phenol to phenylglucuronide. These findings may be relevant to hepatic and uremic coma. In vitro, phenol prevents the inhibition of parathyroid cell proliferation induced by calcitriol.[228]

Phenols are lipophilic and protein-bound, and their removal by hemodialysis is markedly less than that of urea and creatinine.[26] Daily hemodialysis results in lower predialysis serum levels compared to conventional alternate-day dialysis.[229] In a hemodialysis setting, the removal of *p*-cresol and that of urea and creatinine are not correlated,[26] demonstrating that the latter markers are not representative for the intradialysis behavior of protein-bound compounds. Levels are markedly lower in peritoneal dialysis compared to hemodialysis.[230]

Hypoalbuminemia and a rise in total concentration are correlated to an increase of the free active fraction.[30] A correlation between free *p*-cresol and hospitalization rate was demonstrated.[30] Patients hospitalized for infection also had a higher free *p*-cresol.[30] *p*-Cresol also correlates with clinical uremic symptoms.[231]

13. Phosphate

High phosphate levels are associated with pruritus and hyperparathyroidism.[232] They affect PTH levels indirectly by decreasing Ca^{2+} and calcitriol,[233] but also by direct stimulation of PTH secretion.[234] Low dietary phosphate prevents parathyroid hyperplasia in early uremia, whereas a high dietary phosphate enhances the production of tumor growth factor-alpha (TGF-α) which functions as an autocrine signal to stimulate growth further.[235]

Hyperphosphatemia is not only a cause of hyperparathyroidism[236] but is also the result of the action of PTH on bone. The administration of calcitriol in an attempt to control PTH produces hyperphosphatemia as well.[237]

The blood phosphorus concentration is the result of protein catabolism and protein intake as well as of the ingestion of other sources (e.g., Coca-Cola®). Restriction of oral intake increases the risk of protein malnutrition,[232] which can be avoided by the administration of oral phosphate binders.[238] Until

recently, these consisted mainly of aluminium or calcium salts. The effect of the latter, however, is often insufficient, especially in subjects with a high intake. New phosphate binders such as lanthanum carbonate and sevelamer hydrochloride offer the advantage that they contain no calcium.[239] Sevelamer hydrochloride has a lipid lowering effect[240] and reduces cardiovascular calcification.[241] Lanthanum is a cationic trace element.[242]

Phosphorus is a small water-soluble molecule, but with a retention and removal pattern that hardly mimics that of any other molecule. Cellular clearance during hemodialysis is markedly lower than that of urea,[243] resulting in a substantial post-dialysis rebound.[244] Removal seems to be effective only during the initial phase of a hemodialysis session, after which transfer from the intracellular compartment becomes the rate-limiting step.[245] Alternative dialytic strategies such as daily dialysis,[246,247] slow prolonged dialysis sessions,[247] or hemodiafiltration[248] all might improve phosphate removal. The application of daily dialysis even results in a decreased intake of peroral phosphate binders.[246]

Currently, 60% of hemodialysis patients in the United States have serum phosphate levels higher than 5.5 mg/dl.[249] Such high phosphate levels are directly correlated to mortality,[250] which appears to be linked to a high Ca $\times$ P product, and an enhanced tissular deposition of Ca-containing complexes, e.g., in vessel walls.[250]

14. Phenylacetic Acid (PAA)

PAA is a degradation product of the amino acid phenylalanine. Plasma concentrations of PAA in patients with ESRD strongly exceed those in healthy controls. PAA was shown to inhibit iNOS expression and consequently, NO production[251] and was identified as an inhibitor of Ca^{2+} ATPase activity in ESRD.[252]

15. Purines

Uric acid, xanthine, and hypoxanthine are the most important purines retained in uremia. The purines disturb calcitriol production and metabolization.[253] Administration of purines to animals results in a net decrease of serum calcitriol,[253] and a decrease of uric acid in response to allopurinol administration results in a rise of plasma calcitriol levels.[254] Purines are involved in the resistance to calcitriol of immune competent cells.[255] Xanthine and hypoxanthine have been implicated as modulators of neurotransmission, poor appetite, and weight loss.[256] Both xanthine and hypoxanthine induce vasoconstriction[257] and disturb endothelial barriers.[258]

Uric acid is a small water-soluble compound that is removed by hemodialysis from the plasma in a similar way as urea,[259] but removal from the intracellular compartment is by far not as efficient.[260] Dialytic removal of xanthine and hypoxanthine shows no correlation with that of urea and creatinine.[259]

16. Urea

For the extensive number of toxicity studies to which urea has been submitted, the number in which a well-defined adverse biochemical or physiologic impact has been reported at concentrations currently encountered in uremia is relatively low. Interestingly, in a classical study by Johnson et al., long-lasting dialysis against dialysate containing high urea concentrations had no consistent impact on uremic clinical symptoms.[261] More recently, two large controlled clinical studies, the ADEMEX and the HEMO-study, could not demonstrate an impact of enhanced urea removal on survival outcome.[22,262]

Nevertheless, urea is the precursor of some of the guanidines, such as guanidinosuccinic acid (see earlier); those guanidino compounds induce by themselves biochemical alterations. As the uremic retention solute with the highest net concentration, urea may also be involved in dialysis disequilibrium, if the decrease in plasma concentration during dialysis occurs too rapidly. Urea may also be a source of generation of cyanate and isocyanic acid, and these might be at the origin of carbamoylation, resulting in structural and functional changes of amino acids and proteins.[263–267] Serum urea is the most consistent predictor of carbamylated hemoglobin in uremia.[264]

Urea is unequivocally used as a marker of solute retention and removal in dialysed patients. However, dynamic urea kinetic parameters, reflecting dialytic removal (total clearance normalized for distribution volume—Kt/V) are more valuable indices of dialysis adequacy than static parameters (e.g., predialysis urea concentrations). High blood concentrations of urea do not necessarily relate to poor outcome if removal is sufficient.[268] The reason for this apparent paradox is that urea concentration is influenced not only by dialytic removal but also by protein intake, which is actually a factor related to a good metabolic status.

One might question the validity and representativeness of urea as a marker for the retention and the removal of other solutes. Biochemical systems are at least in part affected by compounds with a kinetic behavior that largely differs from that of urea (e.g., MM, protein-bound solutes). Even if dialytic removal from the plasma is similar, as is the case for other small, water soluble, non-protein-bound compounds such as creatinine or uric acid,[259] the shift from intracellular to the plasma might occur at a different rate,[260] again resulting in divergent kinetics.

III. Conclusion

The uremic syndrome is the result of a complex set of biochemical and pathophysiologic disturbances, emanating in a state of generalized malaise and dysfunction. This condition is related to the retention of a host of compounds;

many of them exert a negative impact on key functions of the body; those molecules have consequently been identified as uremic toxins. Up to now, the toxic action of single solutes has repeatedly been studied, but the intermutual interference between compounds has rarely been considered. Although solute retention is one of the major pathophysiologic events, deficiencies are functionally important as well.

Removal and generation of many compounds with proven biological or biochemical impact, especially toxins that are hydrophobic and/or not generated from protein breakdown, can hardly be predicted by the intradialysis behavior of urea, a current marker but a small water-soluble compound generated from protein, with relatively little biological impact.

Solute clearance eventually reaches a plateau as dialyzer blood flow and/or dialysate flow are increased; this plateau is reached much sooner for molecules with a higher molecular weight. As a result, clearance of MM stricto sensu is relatively blood and dialysate flow independent. Only an increase of dialysis time, dialyzer surface area, ultrafiltration rates and/or dialyzer pore size can enhance their removal.

Removal of solutes that behave like larger molecules owing to their protein binding, multicompartmental distribution, and/or lipophilicity will be less affected by the use of high flux dialyzers and/or dialyzers with a larger pore size. To improve the clearances of these "new definition MM," it may be necessary to develop renal replacement systems with different characteristics, e.g., specific adsorption systems and/or procedures that allow a slower exchange of solutes.

Earlier concepts of adsorption, eventually largely abandoned, should perhaps be reconsidered, especially for the removal of organic acids. More specific and/or more efficient adsorptive systems may be needed, however. As an alternative, adsorption of toxins or of their precursors may be pursued at the intestinal level.

The next step is to pursue more specific removal. However, before this can be realized, we will need to know more about the toxic compounds responsible for these disturbances.

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Chapter 5

Calcitriol Metabolism and Action in Chronic Renal Disease

Chen Hsing Hsu

I. Introduction

Abnormal calcitriol [1,25$(OH)_2$ vitamin D] metabolism plays a major role in the pathophysiology of renal osteodystrophy and other alterations of mineral metabolism associated with chronic renal failure. Growing knowledge of the protean biologic actions of calcitriol suggests that abnormal calcitriol metabolism may also play a role in other homeostatic perturbations associated with renal failure such as abnormal immune function,[1–5] impaired growth and development,[6] and abnormal cardiac and skeletal muscle function.[7–9] In view of the central and growing role of calcitriol in the pathophysiology of uremic syndrome, it is important to understand the nature of altered calcitriol metabolism in renal failure. Emerging evidence has focused on primary areas of altered calcitriol metabolism in renal failure: diminished production of calcitriol, decreased concentration of the calcitriol receptor, and altered DNA binding properties of the receptor–hormone complex. The effects of calcitriol on multiple organs are also described in this chapter. These alterations result in attenuated end-organ responsiveness to calcitriol and the consequent abnormalities of mineral metabolism and other functions.[10–14]

The genomic action of calcitriol is mediated through the interaction of the calcitriol receptor (VDR) with vitamin D response elements (VDREs) of the target genes. We have shown that the interaction of VDRs with VDREs is inhibited by uremic toxins. We hypothesize that uremic toxins form Schiff bases with the lysine residues of the VDR DNA binding domain and inhibit VDR interaction with VDRE (Fig. 5-1). In this study, pyridoxal 5′-phosphate was used as a probe to test Schiff base formation as the inhibitory mechanism because it forms Schiff bases with steroid receptors. Pyridoxal 5′-phosphate inhibited VDR binding to VDREs and chemically modified the DNA binding domain of the VDR in vitro. The inhibition was reversed when pyridoxal 5′-phosphate

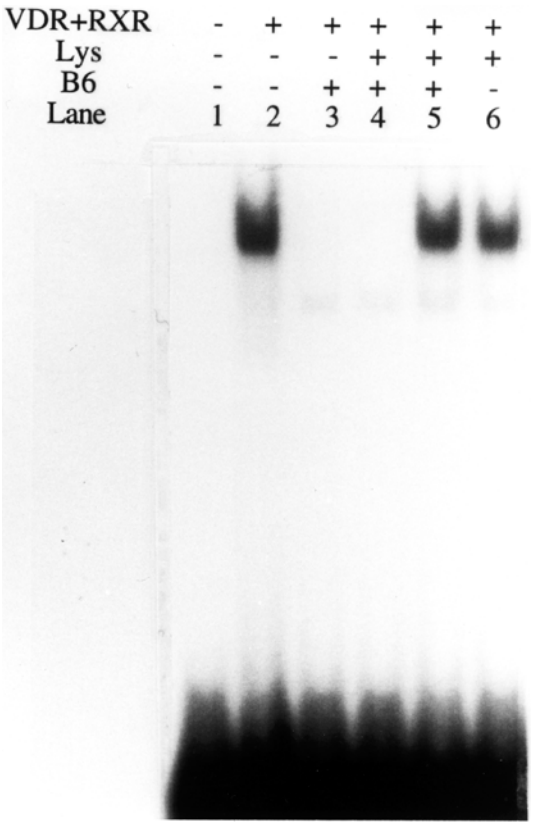

Figure 5-1. Inhibitory effect of pyridoxal 5′-phsophate on VDR-RXR-VDRE complex is reversed by L-lysine. *Lane 1*, free radiolabled osteocalcin VDRE; *lane 2*, intestinal VDR-VDRE complex; *lane 3*, addition of 5 mM pyridoxal 5′-phsophate abolished the VDR-VDRE complex; *lane 4*, addition of 5 mM pyridoxal 5′-phsophate preincubated in water at 37°C for 12 days also abolished the VDR-VDRE complex; *lane 5*, addition of 5 mM pyridoxal 5′-phsophate preincubated with 20 mM L-lysine for 12 days failed to inhibit VDR binding to VDRE; *lane 6*, addition of 20 mM L-lysine preincubated at 37°C for 12 days did not inhibit VDR-VDRE complex formation. The above study was conducted by electrophoretic mobility shift assay. (From Kidney Int 50:1539–45. Reproduced with permission.)

was preincubated with lysine. Further, this chemical agent also blocked the production of the enzyme chloramphenicol acetyltransferase (CAT) induced by calcitriol in cells transfected with a constructed VDRE attached to a CAT reporter gene. This finding is consistent with the hypothesis that pyridoxal 5′-phosphate could interact with the VDR and impair its DNA binding within cells. Because induction of 24-hydroxylase synthesis is a receptor-mediated process, we studied the effect of pyridoxal 5′-phosphate on the synthesis of renal 24-hydroxylase in rats. When pyridoxal 5′-phosphate was infused into

rats, renal 24-hydroxylase activity was suppressed; consequently, degradation of calcitriol was also reduced in these animals. Thus, chemicals capable of Schiff base formation potentially could alter the physiological function of VDR and calcitriol.[15] The nature of the alterations in calcitriol metabolism is reviewed in detail in this chapter.

II. Effect of Calcitriol on Various Organs

A. Effect of Calcitriol on the Nervous System

VDR distribution has been demonstrated in the brains of normal rats.[16] VDR is distributed in cerebellar and hippocampal granular cells and in pyramidal neurons of hippocampus, in cerebellar Purkinje cells, and in ganglia. The observation of the presence of VDR and the presence of activity of its metabolic enzymes in the nervous system provide further support that calcitriol plays a major role in the brain. In avian intestinal nerves from vitamin D deficient and replete chickens, vitamin D deficiency was associated with intestinal conduction velocity.[17] VDR-like sites in neurons are present in a wide range of specific brain nuclei that regulate a number of systems—sensory, motor, autonomic, and endocrine—as well as in spinal cord and sensory ganglia.[18] Cultured rat cardiac muscle revealed radiolabled calcitriol binding sites.[19]

In ischemic cerebral injuries a cascade of degenerative mechanisms occur,[20] all of which participate in the development of oxidative stress and influence the condition of the tissue. The survival of viable tissue affected by secondary injury depends largely on the balance between endogenous protective mechanisms and the ongoing degenerative processes. The inducible enzyme heme oxygenase-1 metabolizes and thus detoxifies free heme to the powerful endogenous antioxidants biliverdin and bilirubin, thereby enhancing neuroprotection. Calcitriol is a modulator of the immune system and also exhibits a strong potential for neuroprotection, as recently shown in the middle cerebral artery occlusion (MCAO) model of cerebral ischemia. They studied the effects of calcitriol treatment on heme oxygenase-1 expression after focal cortical ischemia elicited by photothrombosis. Postlesion treatment with calcitriol (4 μg/kg body weight) resulted in a transient but significant upregulation of glial heme oxygenase-1 immunoreactivity concomitant with a reduction in glial fibrillary acidic protein immunoreactivity in remote cortical regions affected by a secondary spread of injury. The size of the lesion's core remained unaffected, however. Calcitriol did not produce a temporal shift or extension of injury-related heme oxygenase-1 responses, which indicates that calcitriol did not prolong ischemia-related heme oxygenase-1 responses. In contrast to glial heme oxygenase-1 upregulation, glial fibrillary acidic protein, a sensitive marker for reactive gliosis, was significantly reduced. These findings support an

additional protective action of calcitriol at the cellular level in regions affected by secondary injury-related responses.

B. Effect of Calcitriol on Cardiac Function

Experimental and clinical studies demonstrated that calcitriol could improve cardiac contractility. Further, in chronic renal disease (CRD) and end-stage renal disease (ESRD) patients, calcitriol improved left ventricular function.[21] However; calcitriol will increase calcification of important organs including heart and should be used cautiously. However, others had demonstrated that vitamin D deficiency results in increased cardiac contractility.[22]

C. Effect of Calcitriol on the Parathyroid Gland

Suda et al.[23] demonstrated that calcitriol plays an important role in the terminal differentiation of promyelocytes to monocytes which are precursors of the giant osteoclasts. These cells differentiated to a functional cell line, and growth ceased. The function did not involve Ca and P and was related to vitamin D-induced production of osteoclasts through the receptor activator nuclear factor-κB (RANKL) system.[23] VDR has been found in the parathyroid gland.[24] In treatment of renal osteodystrophy with calcitriol and its analogues, the essential site for the therapy is the VDR in the parathyroid gland.[24] Calcitriol induced production of osteoclasts through the RANKL system.[23] Another important function of VDR is to keep the production of the preparathyroid gene under control and reasonably suppressed.[23] The recent study of Suda et al. indicated that ESRD patients who received vitamin D injection (37,173 patients) had longer survival than those (13,864) who did not receive vitamin D injection.[25] However, the study was conducted for only 2 years, and a long-term study may produce a different result because vitamin D injection increases total body calcification.

D. Effect of Calcitriol on Colon Carcinogenesis

Many epidemiological studies have been conducted on the relationship between calcium and vitamin D and the incidence of colon cancer. Recently the Calcium Prevention Study demonstrated that calcium supplementation can reduce the recurrence of colon cancer, but the effect depends on serum vitamin D levels. Another study indicated that Ca supplementation can prevent colon polyp. Harris and Go used the Apc (min) mouse model of intestinal cancer to investigate the effects of vitamin D treatment and calcium intake. They found that calcitriol is potent in inhibiting tumor load; however, the dose used to achieve this antiproliferative effect led to deleterious effects on serum calcium homeostasis. These effects were minimized by the use of a synthetic analogue with reduced toxicity. In addition, they tested the effect of a modified-calcium

diet in Apc(min) mice but did not find a protective effect, perhaps because of a reduction in circulating levels of 25-hydroxycholecaliferol with increasing levels of dietary calcium. A number of other studies that use rodent models with vitamin D supplementation or deficiency illustrate the efficacy of vitamin D in colon cancer prevention. The mechanism of the direct action of vitamin D on colonic epithelium includes regulation of growth factor and cytokine synthesis and signaling, as well as modulation of the cell cycle, apoptosis, and differentiation. Because of the apparent synergistic effect of vitamin D and calcium, supplementation of both nutrients in cancer prevention programs may be advised.[26] One can use calcitriol derivatives to manage this issue, although they all could increase Ca absorption. Unfortunately, elevation of Ca does not reflect total body Ca content; therefore, if renal function is normal this will not be a problem, because Ca can be excreted through normal renal function. But nephrolithiasis could develop as a complication.

E. Effect of Calcitriol on Prostate Cancer

A number of hormonal ligands and/or the nuclear receptors that mediate their actions have been targeted for prostate cancer therapy.[27] Androgens, the ligands for the androgen receptor (AR), are critical for the growth of prostate cancer. Inhibition of androgen production has been the mainstay of treatment for advanced prostate cancer for decades. Other more recently tested targets include retinoid receptors (RARs and RXRs), glucocorticoid receptors (GRs), estrogen receptors (ERs) and peroxisome proliferator-activated receptors (PPAR). Calcitriol, acting through the vitamin D receptor (VDR), has many tumor suppressive activities in the prostate, including inhibition of proliferation, induction of apoptosis and/or differentiation, and reduction of cellular invasion. Because of these properties, calcitriol and its less hypercalcemic analogues are being evaluated as agents to prevent or treat prostate cancer. Androgens, retinoids, glucocorticoids, estrogens, and agonists of PPAR directly or indirectly impact vitamin D signaling pathways, and vice versa. To design the most effective strategies to use calcitriol to prevent or treat prostate cancer, the interactions of other nuclear receptors and their ligands with the vitamin D signaling pathway need to be considered. This was further confirmed in a study by Beer et al.[28]

F. Effect of Calcitriol on Pulmonary Cancer

Calcitriol, the major regulator of calcium homeostasis, has potent antiproliferative and antiinvasive properties in vitro in cancer cells. In vivo studies demonstrated that calcitriol slows the progression of breast, prostate, and other carcinomas. A key question is whether calcitriol exerts its anticarcinogenic

effects in vivo by a mechanism that is dependent on its capacity to limit the proliferation and invasiveness of cancer cells in vitro. It has not been clear whether the calcemic activity and regulation of the host defenses by calcitriol contribute to the effect on cancer cells. Nagakawa et al.[29] have focused on the influence of calcitriol on the metastasis of lung cancer, without involvement of the calcemic activity and other effects of calcitriol in the host. They used metastatic Lewis lung carcinoma cells expressing green fluorescent protein (LLC-GFP cells) and examined metastatic activity in vitamin D receptor (VDR) null mutant [VDR(−/−)] mice and their wild-type counterparts [VDR(+/+) mice]. VDR(−/−) mice exhibit hypocalcemia and extremely high serum levels of calcitriol. They expected that serum calcitriol would act in vivo to directly inhibit the metastatic growth of VDR-positive LLC-GFP cells in VDR(−/−) mice. The metastatic activities of LLC-GFP cells were remarkably reduced in VDR (−/−) mice compared with VDR (+/+) mice. To test the hypothesis that serum calcitriol is an intrinsic factor that inhibits metastatic growth of lung cancer cells, they corrected hypocalcemia and/or hypervitaminosis D in VDR (−/−) mice by dietary manipulation. The metastatic growth of LLC-GFP cells was remarkably reduced in response to serum levels of calcitriol, but not to serum calcium levels. Furthermore, they found that VDR (+/+) mice fed the manipulated diets displayed an apparent inverse relationship between the physiological levels of serum calcitriol (8 to 15 pg/ml) and tumorigenesis. They show that calcitriol inhibits the metastatic growth of lung cancer cells in a defined animal model.[29]

G. Immunoregulatory Function of Calcitriol

Conditions such as tuberculosis and sarcoidosis are characterized by chronic granulomatous inflammation. During the inflammatory process, alveolar macrophages acquire 1α-hydroxylase activity and the ability to metabolize 25-hydroxyvitamin D_3 (25-D_3) to the active metabolite,[30] calcitriol. Calcitriol is a potent differentiation agent that modulates mononuclear phagocyte activation and effector functions. The mediators that induce macrophage 1α-hydroxylase activity are not well delineated. Further, it is unclear whether calcitriol is produced by terminally differentiated macrophages only or whether less mature mononuclear phagocytes can produce it as well. The ability of newly recruited monocytes to produce calcitriol as an autocrine differentiation agent is particularly important in inflammation, as it may substantially expand the functional repertoire of these cells. To assess the effect of cytokines on 1α-hydroxylase activity, blood monocytes were cultured in the presence and absence of human recombinant tumor necrosis factor-alpha (TNF-α), interferon (IFN-r), and interleukins 1 and 2 and then incubated with 25-D_3 substrate. The conditioned media were assayed for calcitriol by high-performance liquid chromatography and a competitive receptor binding assay.

No detectable calcitriol was produced by unstimulated monocytes. However, all the cytokines markedly increased monocyte calcitriol production (range 133 to 151 pg/mg of protein; in all cases $p < .001$). We then determined whether calcitriol production was suppressed by preincubation with either dexamethasone or the putative uremic toxin guanidinosuccinic acid (GSA). Dexamethasone pretreatment significantly inhibited subsequent cytokine-induced calcitriol production by monocytes, as did GSA (average 69% and 63% of control, respectively).[31] Calcitriol exerts profound effects on the immune system.[32] They provide an overview of the metabolism and molecular and cellular action of calcitriol with particular regard to its immunomodulatory function. Effects of calcitriol on the immune system are manifold and include suppression of T-cell activation, shaping of cytokine secretion patterns, induction of regulatory T cells, modulation of proliferation, and interference with apoptosis. Calcitriol further influences maturation, differentiation, and migration of antigen presenting cells. Altogether, its immunomodulatory potency is comparable to that of other established immunosuppressants without sharing their typical adverse effects. This profile makes calcitriol a potential drug for the treatment of immune-mediated diseases. Yet, the major obstacle for its clinical use, its potent calcemic activity, has been not overcome to date. The identification or generation of novel vitamin D derivatives with dissociated calcemic and immunomodulatory properties is therefore a major task. Achievement of this goal might eventually lead to promising drugs for future therapeutic exploitation of a wide array of immune diseases, such as psoriasis, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and others.

III. Calcitriol Production in Chronic Renal Disease

The plasma concentration of calcitriol is determined by its synthesis and degradation rate. However, the plasma concentration of calcitriol will begin to decrease when its synthesis is markedly reduced regardless of the degradation rate. In general, the plasma concentration of calcitriol is decreased in renal failure owing to decreased production of calcitriol. Calcitriol production is controlled by many factors. Accordingly, multiple factors contribute to decreased calcitriol production in renal failure.

A. Decreased Renal Tissue

The plasma concentration of calcitriol is regulated in normal subjects[33] and decreased in patients with chronic renal failure[34] and in the renal ablation model of renal failure.[35] Several studies suggest that the plasma calcitriol concentration may be perceptibly decreased in the early phases of renal failure.[36,37] The fall in serum calcitriol concentration in renal failure is attributable mainly to decreased renal production of calcitriol. As calcitriol production

decreases further owing to reduced renal mass, the plasma levels decrease to below normal levels. Decreased renal mass has long been considered to be a major factor responsible for the decreased total production rate and serum concentration of calcitriol in chronic renal failure. Ordinarily, the proximal tubular cell of the kidney is the site of conversion of 25-$(OH)D_3$ to the much more biologically active calcitriol.[38] Prince et al.[39] have shown that calcitriol production can be stimulated in patients with mild renal failure, suggesting that factors other than decreased renal mass account for decreased production.

B. Hyperphosphatemia

Inorganic phosphorus is known to suppress 1α-hydroxylase activity. Dietary phosphorus restriction in rodents[40] and in humans[41] has been shown to increase plasma levels or synthesis of calcitriol. Intracellular phosphorus concentration or transcellular influx of phosphorus could play a role in regulating 1α-hydroxylase activity. Hypophosphatemia may regulate calcitriol synthesis through somatomedins, because hypophysectomy eliminates the effect of phosphorus on 1α-hydroxylase activity. Moreover, injection of growth hormone into hypophysectomized rats restores the regulatory role of phosphorus.[42]

C. Metabolic Acidosis

Metabolic acidosis occurs frequently with chronic renal failure and has been shown to suppress calcitriol production.[43] However, the issue remains controversial because other studies have not found that chronic acidosis decreases plasma calcitriol.[44,45] Acidosis may suppress 1α-hydroxylase activity directly by increasing the ionized calcium concentration in serum[46,47] and in proximal tubule mitochondria.[48]

D. Extrarenal Production of Calcitriol

ESRD patients can produce calcitriol if given a pharmacological dose of 25-OH-D_3.[49] It appears that the source of calcitriol synthesis in ESRD patients is the monocyte. Peripheral blood monocytes are recruited to sites of inflammation where they differentiate and become activated. Despite extensive investigation, the precise signals that modulate these changes remain unclear. We propose that the elaboration of calcitriol is an autocrine mechanism by which mononuclear phagocytes regulate differentiation and activation in response to inflammatory mediators.[31] The generation of calcitriol at inflammatory sites may be of particular benefit in the defense against certain infections such as tuberculosis. In vitro studies demonstrated that calcitriol can substantially increase intracellular killing of *Mycobacterium* (M) *tuberculosis* by macrophages and is synergistic with pyrazinamide.[31]

E. Uremic Toxins

Several lines of evidence indicate a potentially important role for uremic toxins in the suppression of calcitriol synthesis. For example, there is an additional fall in calcitriol production when partially nephrectomized rats are placed on a high-protein diet.[50] A high-protein diet increases the generation of uremic toxins that further suppress calcitriol production beyond what occurs with renal failure alone. Further, when urine from which phosphorus had been removed was infused into normal rats, there was a decrease in calcitriol production.[50] Infusion of uremic plasma ultrafiltrate to normal rats in an amount that did not raise plasma concentrations of urea nitrogen and creatinine also suppressed the calcitriol production rate and renal 1α-hydroxylase activity.[51] Enzyme activity was also inhibited in kidney homogenates incubated with uremic ultrafiltrate.[52]

In an effort to better characterize the toxins, plasma ultrafiltrates of normal subjects and hemodialysis patients were divided by semipreparative high-performance liquid chromatography (HPLC) into 13 distinct fractions. We found at least two groups of chemically distinguishable compounds that profoundly inhibited calcitriol production.[51] One inhibition region localized to fractions 6 to 13. Several chemical substances coeluted in these fractions including hippuric acid, indole-3-acetic acid, tryptophan, and indoxyl sulfate.[51] None of these compounds have been tested for their effects on calcitriol production. The other region of inhibition was eluted in fraction 4, which coeluted with the purine compounds uric acid, xanthine, hypoxanthine, and guanidinosuccinic acid (GSA).

GSA, a low molecular weight toxin that is a putative uremic toxin, was also found to suppress calcitriol production.[52] Infusion of a small quantity of GSA (1.5 mg/dl, 20 ml) to normal rats reduced the calcitriol production rate by more than 40%. Although the levels of GSA were not measured in these animals, the expected plasma concentration was lower than in patients with moderate chronic renal failure (0.35 ± 0.03 mg/dl; serum creatinine, 4 to 6 mg/dl)[53] and in hemodialysis patients (2.3 ± 1.4 mg/dl).[54] Thus, suppression of calcitriol synthesis by GSA could occur in mild renal failure. Enzyme kinetic analysis indicated that GSA inhibition was noncompetitive.[55] Preliminary experiments with cultured proximal tubule cells also found that GSA (2 mg/dl) suppressed calcitriol production by nearly 50%.

F. Purine Derivatives

As noted in the preceding text, the fraction of uremic ultrafiltrate that contained purine compounds was empirically found to suppress calcitriol production. Therefore, we studied the effect of purines and related substances (e.g., uric acid, xanthine, and theophylline) on the production rate of calcitriol in normal rats. Infusion of uric acid for 20 hours into normal rats at a rate

sufficient to raise the plasma urate concentration from 1.1 to 4.2 mg/dl resulted in a 42% reduction in the calcitriol production rate and suppression of renal 1α-hydroxylase activity.[34] Similarly, calcitriol production by kidney homogenate was reduced by incubating with xanthine for 3 hours.[34] The effect of uric acid on the plasma concentration of calcitriol was investigated further in nine patients with stable chronic renal failure.[56] Plasma calcitriol levels were measured before and 1 week after administration of allopurinol, 300 mg daily. The plasma uric acid concentration decreased (7.3 ± 0.4 mg/dl to 4.0 ± 0.4 mg/dl, $N = 9$, $p < 0.01$) and the calcitriol concentration increased in each patient after ingestion of allopurinol (plasma calcitriol, $30.8 + 2.7$ to $38.2 + 4.8$ pg/ml, $p < 0.01$).[56] Allopurinol itself, tested in normal rats, had no effect on renal 1α-hydroxylae and calcitriol synthesis.[34] Theophylline, a purine compound, also markedly inhibited 1α-hydroxylase activity and calcitriol synthesis in normal rats. Thus, uric acid and purine related compounds appear to be endogenous inhibitors of 1α-hydroxylase activity, similar to phosphorus (Figs. 5-2, 5-3, and 5-4). Finally, Barrett-Connor et al. found that drinking coffee is associated with osteoporosis (Fig. 5-5).[57] Coffee large amounts of caffeine and the structure of caffeine is similar to that other purine metabolites; they all contain a carbonyl group that can binds to VDRE of calcitriol and eliminate its effect. This could explain the mechanism of coffee-induced osteoporosis.

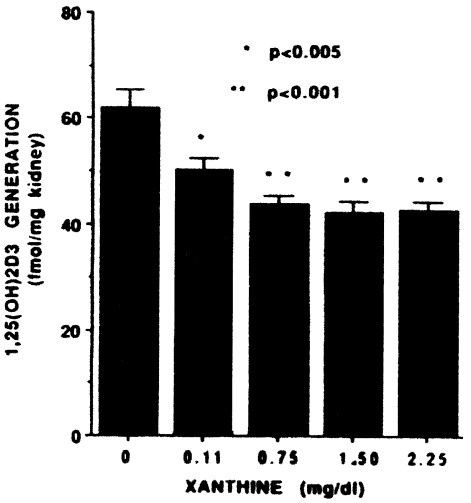

Figure 5-2. Generation of calcitriol measured at 20 min after addition of 25(OH)D_3 to kidney homogenates preincubated for 3 h with buffer solutions containing xanthine in concentration of 0, 0.11, 0.75, 1.50, and 2.25 mg/dl (N = 4). (From Am J Physiol 29:F596–601, 1991. Reproduced with permission.)

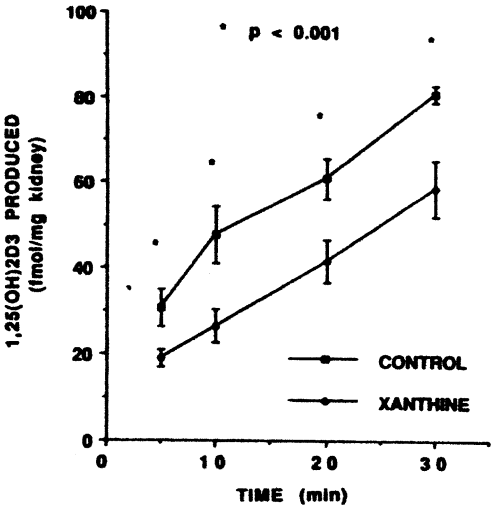

Figure 5-3. Generation of calcitriol measured at 5, 10, 20, and 30 min after addition of 25(OH)D_3 to kidney homogenates preincubated for 3 h at 37°C with 0 mg/dl xanthine or without xanthine (N = 4). (From Am J Physiol 29:F596–601, 1991. Reproduced with permission.)

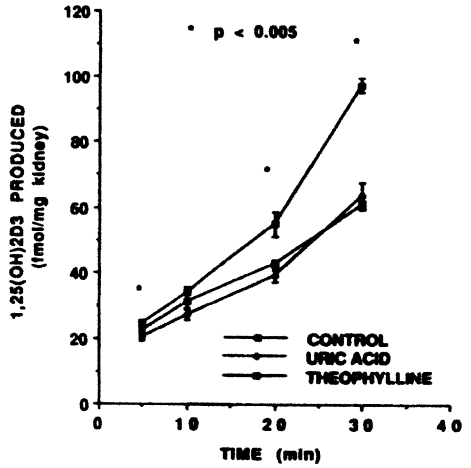

Figure 5-4. Generation of calcitriol measured at 5, 10, 20, and 30 min after addition of 25(OH)D_3 to kidney homogenates obtained from rats infused with saline, theophyline, or uric acid. Each point represents the mean ± SE of five rats. (From Am J Physiol 29:F596–601, 1991. Reproduced with permission.)

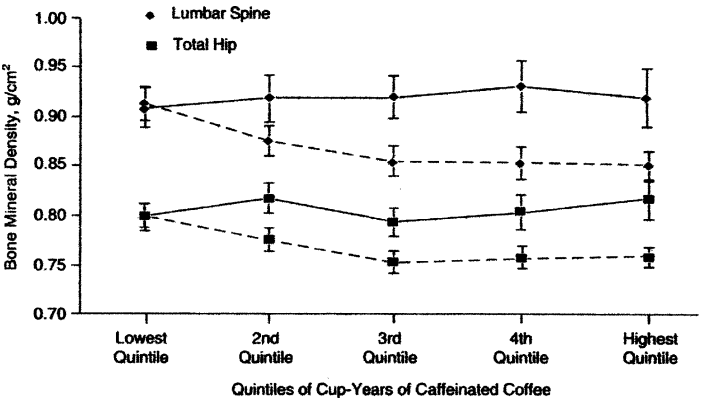

Figure 5-5. Lumbar spine and total hip bone mineral density by milk consumption in adults ages 20 to 50 years. (The solid line indicates one or more glasses of milk per day; the dashed line indicates less than one glass of milk per day.). Models were adjusted for age, body mass index, years since menopause, cigarette smoking, alcohol use, thiazide use, exercise, and estrogen replacement therapy. (From N Engl J Med 271:280–3. Reproduced with permission.)

G. Parathyroid Hormone (PTH)

PTH has been shown to stimulate 1α-hydroxylase activity. Parathyroidectomy decreased the conversion of 25-(OH)D_3 to calcitriol in vitamin D-depleted rats[58] and decreased 1α-hydroxylase activity in vitamin D replete[59] or vitamin D-depleted chicks.[60] Infusion of PTH hormone into normal subjects raised the plasma concentration of calcitriol.[61] Further, the plasma concentration of calcitriol is increased in patients with primary hyperparathyroidism and decreased in patients with hypoparathyroidism.[6]

Although decreased calcitriol synthesis underlies various pathophysiological abnormalities of renal failure, it may be an important adaptation that is crucial to the survival of patients with renal failure. If decreased calcium excretion in renal failure were accompanied by normal production of calcitriol and normal absorption of intestinal calcium, patients would face the danger of excessive calcium retention and soft tissue calcification. Thus, chronic replacement of calcitriol in renal failure, though it effectively suppresses PTH and prevents renal osteodystrophy,[14] may lead to excessive calcium retention.

H. Effect of Glucose on the Function of the Calcitriol Receptor and Vitamin D Metabolism

The genomic action of calcitriol is mediated through the interaction of the VDR with VDREs of the target genes. It has been proposed that chemicals capable of Schiff base formation with the VDR potentially could alter the

physiological function of VDR and calcitriol metabolism.[15] Because glucose-6-phosphate has been shown to form Schiff bases with proteins, we tested the hypothesis that glucose could influence the function of VDR and thereby alter calcitriol metabolism. Glucose 6-phosphate inhibited VDR binding to the osteocalcin VDRE and chemically modified the DNA binding domain or the dimerization domain of the VDR in vitro. Further, glucose also blocked the production of the enzyme chloramphenicol acetyltransferase (CAT) induced by calcitriol in cells transfected with a constructed VDRE attached to a CAT reporter gene.[62] Hyperglycemia induced by glucose infusion or by induced streptozotocin in normal rats significantly reduced intestinal 1,25-dihydroxyvitamin D-24-hydroxylase activity. Taken together, these findings are consistent with the hypothesis that glucose could interact with the VDR to impair its DNA binding and function within cells.[63]

IV. Metabolic Degradation of Calcitriol in Renal Failure

Recent studies have demonstrated that the fall in calcitriol production rate (PR) in renal failure is partially offset by a concomitant decrease in the metabolic clearance rate (MCR) of calcitriol.[35,61] The observed reduction in the metabolic clearance of calcitriol appears to minimize the fall in serum calcitriol concentration in renal failure. While there may be several reasons for the decreased MCR of calcitriol in renal failure,[64] our initial studies have led to the conclusion that uremic substances play a major role in the suppression of calcitriol degradation.[35,50,65,66] Preliminary data suggest that the molecular mass of the toxins suppressing calcitriol metabolism is less than 10,000 Da.[50,65]

Degradation of calcitriol occurs in the intestine, liver, bone, and kidney.[67] Calcitriol is converted to $1,24,25(OH)_3D_3$ and $1,25,26(OH)_3D_3$ which are further metabolized to calcitroic acid[68] and $23(S),25(R)$-$1,25(OH)_2D_3$-26,23-lactone,[69] respectively. There are several reasons to suggest that 24-hydroxylase activity is decreased in renal failure: (1) Decreased calcitriol production in renal failure could lower 24-hydroxylase activity.[70] (2) Decreased vitamin D receptor concentration in renal failure[71] may decrease the biological response to calcitriol and reduce the synthesis of 24-hydroxylase.[72] (3) In renal failure, plasma contains factors that inhibit 24-hydroxylase activity.[73] Induction of 24-hydroxylase synthesis by calcitriol is dependent on changes in gene transcription and protein synthesis.[72] It is believed that the calcitriol-occupied receptor complex binds to DNA and activates genes coding for the synthesis of 24-hydroxylase.[74] In renal failure, uremic toxins could decrease genomic synthesis of the degradation enzyme (e.g., 24-hydroxylase) by reducing the binding affinity of the hormone–receptor complex for DNA.[62] Decreased synthesis of degradation enzymes will result in lower MCR of calcitriol.

Alternatively, uremic toxins could directly inhibit the activity of calcitriol degradation enzymes, thereby reducing the MCR.[73]

In summary, decreased calcitriol production could reduce 24-hydroxylase synthesis in renal failure. Chronic replacement of calcitriol in renal failure increases the metabolic degradation and the enzymatic activity of 24-hydroxylase.[75] Decreased receptor number in renal failure diminishes the biological response to calcitriol and reduces the synthesis of 24-hydroxylase.

V. Reduced Calcitriol Receptor Concentration in Chronic Renal Disease

A considerable body of evidence suggests that the calcitriol receptor concentration is decreased in renal failure. Calcitriol-induced receptor synthesis is believed to be a transcriptional event, although this issue remains controversial. Strom et al.[76] have demonstrated that calcitriol increases the concentration of receptor as well as receptor mRNA in vitamin D-depleted rats. However, others were unable to demonstrate an association between increased receptor concentration with transcription of receptor mRNA[77,78] or a consistent increase in receptor mRNA after calcitriol treatment.[79,80] In tissue culture systems, calcitriol increased both receptor concentration and receptor mRNA levels in ROS 17/2.8 cells and mouse 3T6 fibroblasts.[81,82] On the other hand, Brown et al. reported that calcitriol fails to upregulate calcitriol receptor in cultured bovine parathyroid cells.[83]

Regulation of calcitriol receptor concentration is an important mechanism for modulating cellular responsiveness to calcitriol,[72,84] as the biological response to calcitriol is directly related to receptor number[84] and occupancy.[85] Decreased calcitriol receptor concentration could diminish the biological response to calcitriol.[86] In chronic renal failure, the concentration of the calcitriol receptor has been reported to be decreased[86–88] although a recent study[89] reported no decrease in calcitriol receptor in uremic rats. These investigators attributed the low receptor concentrations reported in previous studies[86–88] to proteolytic degradation of the receptor during preparation (without addition of protease inhibitors). However, we have demonstrated decreased intestinal calcitriol receptor concentration in rats with chronic renal insufficiency[71] even when adequate amounts of protease inhibitors were used throughout the preparative process. Further studies are needed to clarify this discrepancy, although we believe the preponderance of available evidence suggests that the calcitriol receptor concentration is low in chronic renal failure.[62]

Assuming that the receptor concentration is truly decreased in renal failure, three mechanisms have been suggested: (1) Because calcitriol is known to upregulate its own receptor,[90] the low plasma calcitriol concentration in renal

failure could downregulate the calcitriol receptor. (2) The high plasma PTH concentration in renal failure could decrease and the concentration of the calcitriol receptor. PTH downregulates the receptor and receptor mRNA in ROS 17/2.7 cells. PTH also blocks calcitriol-induced upregulation of receptor in normal rats.[91] Further, elevation of PTH secondary to calcium deficiency is associated with a significant downregulation of kidney calcitriol receptor even in the presence of a high concentration of plasma calcitriol.[92] (3) A previous report has indicated that the concentration of receptor (N_{max}) is positively correlated with the glomerular filtration rate,[86] suggesting that accumulation of uremic toxins is responsible for the lower concentration of calcitriol receptor in renal failure.

We tested the hypothesis that uremic toxins could be responsible for decreased receptor synthesis. Infusion of uremic ultrafiltrate (30 ml for 20 hours) to normal rats reduced the receptor concentration by 23% compared to rats infused with normal ultrafiltrate. Calcitriol-induced upregulation of receptor was also blocked by uremic ultrafiltrate, suggesting that uremic rats require a higher dose of calcitriol in order to achieve receptor levels similar to those of normal rats.[71] In summary, the combination of low concentration of plasma calcitriol, high plasma PTH concentration, and uremic toxins could explain decreased calcitriol receptor concentrations in renal failure.

VI. Hormone–Receptor Interaction with Nuclear Chromatin Is Decreased in Chronic Renal Failure

The calcitriol receptor has three major regions (I–III) of conserved amino acids.[93] Region I include the DNA binding domain and contain a highly conserved 66 amino acid sequence. Regions II and III are located within the C-terminal or hormone binding domain of the receptor. The DNA binding domain has two zinc fingers that contain eight cysteine residues and two zinc molecules. There are three variable and two conserved amino acid residues situated at the end of the first finger. This region (P box) serves to recognize specifically the primary nucleotide sequence of the half sites.[94] Another region, located at the beginning of the second finger (D box), contains five amino acid residues[95] and is critical for determining the half-site spacing.[94]

The DNA binding domain of nuclear receptors is sensitive to sulfhydryl blocking agents. For example, *p*-chloromercuribenzoate and iodoacetamide inhibit the binding of the calcitriol receptor to DNA-cellulose[96] and pyridoxal 5′-phosphate inhibits the binding of steroid hormone (glucocorticoid, estrogen, progesterone, and androgen)–receptor complexes to DNA-cellulose.[97–100] The latter compound reacts with the amino group of lysine residues in the DNA binding domain by forming a Schiff base and thereby interfering with receptor

binding to DNA.[97–100] An interesting possibility is that uremic toxins could also diminish the biological response to steroid hormones by chemical modification of the DNA binding domain.

The genomic action of calcitriol appears to be mediated through a hormone–receptor complex interacting to regulate gene expression in a manner analogous to other steroid hormones. The hormone–receptor complex is thought to interact with specific DNA sequences regulating the transcription rate of target genes. The resultant mRNA species are then translated into proteins that, after being processed, are ultimately responsible for the biological activity of calcitriol.[73,101,102] A defect in receptor function could lead to diminished biological activity of the hormone. The consequences of a functional defect of the calcitriol receptor are best illustrated by type II vitamin D-dependent rickets, a syndrome characterized by rickets, osteomalacia, hypocalcemia, secondary hyperparathyroidism, and high plasma concentrations of calcitriol. Genomic DNA analysis of affected family members has identified a single nucleotide mutation in the DNA binding domain encoding the receptor protein.[103–106] This mutation produces a defective calcitriol receptor that is unable to interact with DNA-cellulose in a normal fashion.[105,107] Failure to interact with vitamin D response elements diminishes the biological action of calcitriol as reflected by decreased production of bioactive proteins such as 24-hydroxylase.[108]

Calcitriol receptor function is altered in renal failure. DNA-cellulose chromatography was used to model the interaction of the calcitriol receptor with DNA. Normal intestinal cytosolic receptor was incubated in buffer solution containing tritiated calcitriol and a 20% solution of either normal or uremic ultrafiltrate. Uremic ultrafiltrate significantly reduced the hormone–receptor interaction with DNA-cellulose.[73] The soluble hormone–receptor complex eluted as a single peak at an ionic concentration 154 mM in the presence of uremic ultrafiltrate whereas the complex eluted as a discrete peak at 185 mM ionic concentration in the presence of normal ultrafiltrate. Recently, we found that one of the HPLC fractions of uremic ultrafiltrate markedly inhibited the interaction of calcitriol receptor with DNA-cellulose in proportion to the concentration of the uremic fraction.[71] Receptors incubated with uremic ultrafiltrate eluted as a single peak at a lower ionic strength than receptors incubated with normal ultrafiltrate. Increasing the concentration of uremic ultrafiltrate (5% to 40%) progressively weakened the interaction of the receptors with DNA-cellulose. In contrast, the receptors incubated with an increasing concentration of normal ultrafiltrate had stable binding properties except at a concentration of 40% normal ultrafiltrate, at which the receptor had a slightly decreased interaction with DNA.[71] The in vitro effect of uremic toxins to inhibit the interaction of receptor with nuclear chromatin could block the biological action of calcitriol.

The DNA-cellulose binding characteristics of intestinal receptors isolated from rats with chronic renal failure also differ from those of receptor isolated

from normal animals.[71] Receptors from normal rats eluted as a single peak at 0.22 M KCl on a linear salt gradient whereas receptors from chronic renal failure rats eluted as two distinct peaks, one of normal activity at 0.22 M KCl and the other of weak activity at 0.12 M KCl. Infusion of uremic ultrafiltrate to normal rats for 20 hours resulted in receptor binding characteristics identical to those of rats with renal failure, suggesting that uremic toxins produce the abnormal binding properties of the receptor.[71]

Studies with radiolabeled recombinant VDR were performed to exclude the possibility that the effect of uremic ultrafiltrate on DNA binding was due to proteolytic degradation of the VDR. [^{35}S]VDR was incubated with 100% normal ultrafiltrate (N-UF), 100% uremic ultrafiltrate (U-UF), or buffer alone for 1 hour at room temperature and then analyzed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The pattern of translation products was identical for all incubations and was identical to that of freshly translated [^{35}S]VDR. The major translation product was approximately 45 kDa and several minor translation products also were observed. The results exclude proteolytic activity as an explanation for the impaired VDR–VDRE interaction after incubation of VDR with uremic ultrafiltrate.

We have further confirmed that HPLC-fractionated uremic ultrafiltrate indeed inhibited the calcitriol receptor binding to the osteocalcin gene. Using uremic ultrafiltrate concentrations of 30%, 60%, and 100%, the inhibition appeared to be dose-dependent. By contrast, normal ultrafiltrate did not inhibit receptor binding to the osteocalcin gene even at an ultrafiltrate concentration of 100%. Further, binding of osteocalcin gene by receptors isolated from chronic renal failure was also decreased.[62] Scatchard analysis was employed to quantify [^{32}P]VDRE specific binding. VDR obtained from renal failure rats had significantly lower binding capacity for the VDRE ($N_{max} = 295 \pm 78$ fmol/pmol [^{3}H]calcitriol binding protein, $N = 5$) when compared to that of control rats (543 ± 55 fmol/pmol [^{3}H]calcitriol binding protein, $N = 5$, $p < 0.05$). However, the K_d was not different between the two groups of animals (control, 4.07 ± 0.54 nM vs. renal failure, 4.75 ± 1.41 nM, $N = 5$ for both, $p > 0.05$). Immunoblot studies of the VDR extracted from control and renal failure rats showed that they had identical molecular masses of approximately 44 kDa. These studies indicate that the VDR from rats with renal failure has a decreased binding capacity for the osteocalcin VDRE (despite a normal calcitriol binding capacity). These results are equivalent to the effect of incubation of normal VDR with uremic ultrafiltrate in vitro, as described previously. It should be noted that these observations were made in vitro; the relevance of these interactions in vivo remains to be determined.

Using reconstituted intestinal nuclei,[109,110] we studied the effect of HPLC-fractionated uremic plasma ultrafiltrate on in vitro nuclear uptake of calcitriol–receptor complex.[111] Nuclear uptake of the labeled calcitriol receptor was

significantly decreased within 15 minutes of incubation with uremic ultrafiltrate. The nuclear uptake of labeled calcitriol receptor obtained from rats with renal failure or from rats infused with uremic ultrafiltrate was also significantly lower than that from normal rats or from rats infused with normal ultrafiltrate, respectively. The impaired nuclear uptake of calcitriol receptor may be a consequence of decreased DNA binding. While the magnitude of the effect observed in this reconstituted nuclei model is modest, it is possible that diminished nuclear uptake of the calcitriol–receptor complex in the intact cell accounts for part of the calcitriol resistance seen in renal failure.

There are several possible explanations for the observed changes in receptor binding properties. Functional activation of receptor requires calcitriol-induced phosphorylation.[112,113] Uremic toxins could reduce calcitriol binding to the receptor and inhibit receptor phosphorylation. This mechanism is unlikely, as we have found that uremic ultrafiltrate did not reduce hormonal binding to the receptor.[111] Alternatively, the uremic toxins could directly inhibit phosphorylation at the DNA binding domain and hinder a conformational change of the receptor. Recent studies have suggested that the interaction of receptor with a specific VDRE requires a mammalian cell nuclear accessory factor.[102,114] The formation of a heterodimer made of receptor with nuclear accessory factor is critical for high-affinity DNA binding. In the absence of this nuclear factor, receptor interacts weakly with the vitamin D responsive element.[102,115] To examine the effect of UUF on retinoid X receptor (RXR) or VDR, we preincubated[62] recombinant VDR or retinoid X receptor (RXR) separately with N-UF or U-UF and then analyzed these in an electrophoretic mobility shift assay (EMSA) with the other receptor and the osteopontin VDRE as the probe.[62] Preincubation of VDR with U-UF inhibited formation of the VDR–RXR–DNA complex in a dose-dependent manner, whereas N-UF had no effect. In contrast, preincubation of RXR with U-UF failed to impair formation of the VDR–RXR–VDRE complex. These results indicate that the ability of U-UF to impair the formation of a VDR–RXR–DNA complex is due to an effect on the VDR. Alternatively, decreased synthesis of this nuclear accessory factor in chronic renal failure (not yet demonstrated) could reduce calcitriol receptor interaction with DNA.

VII. Secondary Hyperparathyroidism and Calcitriol

High PTH levels can precipitate Ca and P in tissues.[116] Calcitriol can inhibit elevation of PTH; therefore, in ESRD patients, physicians frequently use calcitriol to prevent secondary hyperparathyroidism. It should be cautioned that in addition of calcitriol to inhibit elevation of PTH,[117] calcitriol can enhance Ca and P absorption in ESRD patients.[118] Further study is needed to consider how

to manage secondary hyperparathyroidism. My personal opinion is to treat the basic problem of secondary hyperparathyroidism. Because P elevation is the main problem, control of P is still the most important treatment of elevated PTH.

VIII. Effect of Uremia on Other Members of the Steroid Hormone Receptor Superfamily

The steroid receptor superfamily includes glucocorticoid, mineralcorticoid, androgen, estrogen, progesterone, calcitriol (thyroid and retinoids receptors—not steroid receptors). All these receptors have three major regions (I–III) of conserved amino acids.[119] Region I include the DNA binding domain and contain a highly conserved 66-amino-acid sequence. Regions II and III are located within the C-terminal or hormone binding domain of the receptor. The DNA binding domain has two zinc fingers that contain eight cysteine residues and two zinc molecules. There are three variables and two conserved amino acid residues situated at the end of the first finger. This region (P box) serves to recognize specifically the primary nucleotide sequence of the half-sites. For example, synthesis of a chimeric receptor by altering the variable amino acids of the P box of a steroid receptor would render the chimeric receptor to bind to other steroid nucleotide sequence recognized by the altered P box amino acids.[2,51] Another region, located at the beginning of the second finger (D box), contains five amino acid residues and is critical for determining the half-site spacing.[51] The half-site spacing plays a critical role in determining the hormonal response to steroids and retinoic acid.[52] For example, a direct repeat of A (adenine), G (glycine), G (glycine), T (threonine), C (cysteine), A (adenine) separated by three, four, or five nucleotides corresponds to the vitamin D, thyroid, or retinoic acid receptor response elements, respectively. All these findings indicate the structural resemblance of the steroid receptor superfamily. Therefore, an important possibility is that Schiff base formation of lysine residues of the steroid receptor superfamily with uremic toxins could also diminish the biological response to other steroid hormones and account for multiple endocrinopathy in renal failure.

Emerging evidence suggests that uremic toxins inhibit the binding of VDR to the VDREs of target genes. The inhibitory mechanism perhaps involves Schiff base formation of reactive aldehydes accumulated in uremia with lysine residues of the DNA binding domain. Decreased VDR binding to the VDREs and a lower concentration of VDR could underlie the end-organ resistance to calcitriol in renal failure.

IX. Conclusion

Renal failure is accompanied by multiple alterations of calcitriol metabolism and action. These alterations play a key role in the pathogenesis of renal osteodystrophy as well as other manifestations of renal disease.

As described previously, calcitriol action is reduced in renal failure for multiple reasons including decreased production, decreased receptor concentration, and altered hormone–receptor interaction with nuclear chromatin. The resultant attenuation of calcitriol action contributes to altered mineral metabolism. In particular, intestinal calcium absorption is decreased and PTH secretion is stimulated. These alterations contribute to bone disease associated with kidney failure. Altered mineral metabolism contributes directly or indirectly to other clinical consequences. Mineralization of organs and tissues can induce wide-ranging functional compromise. In addition, direct toxicity has been ascribed to PTH excess and calcitriol deficiency. Consequently, a wide range of uremic problems are linked to altered mineral metabolism including pruritus, anemia, neuropathy, impotence, cardiac arrhythmias, arthritis, and muscle weakness. Decreased calcitriol action, based on multiple lesions, contributes to many aspects of the uremic syndrome. Understanding the reasons for diminished calcitriol action will guide efforts to improve treatment of renal osteodystrophy in particular and the uremic syndrome in general.

In addition to its complex relationship with mineral and bone metabolism, calcitriol appears to have other important actions. The best described actions involve the cell-mediated host defense response to certain infections and inflammatory states. Diminished host defense responses and increased infection risk have been described in patients with renal disease. The current evidence suggests that calcitriol deficiency (broadly defined as decreased availability and action) may contribute to this aspect of the uremic syndrome. Further research is needed in this area as it could influence the way that vitamin D replacement therapy is used in these patients.

Finally, the described alterations in the properties of the calcitriol hormone receptor raise many intriguing questions about other hormone receptors that are part of the same highly homologous, coevolved superfamily of receptors. Circumstantial and some clinical evidence support the concept of attenuated action of other important hormones in the uremic state including cortisol, testosterone, estrogen, and thyroid hormone. Much more needs to be learned about the nature, implications, and correction of hormone receptor function in renal failure. Calcitriol provides a useful and relevant springboard for this avenue of inquiry into improved understanding of the uremic state.

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Chapter 6

Renal Osteodystrophy

Eric W. Young

I. Introduction

Renal osteodystrophy refers to bone disease that occurs in patients with kidney disease. Bone disease occurs across the full spectrum of kidney disease, ranging from early renal insufficiency to established chronic kidney disease and end-stage renal disease (ESRD). The severity of bone disease tends to be associated with the severity and duration of kidney disease. As a practical matter, the most clinically important bone disease occurs among patients with ESRD. Renal osteodystrophy presents with a characteristic spectrum of clinical, biochemical, and histologic abnormalities. Patients and physicians have access to a growing array of preventive and active treatments.

II. Classification of Bone Disease

Renal bone disease is usually classified according to the histologic appearance. Although definitive diagnosis in an individual patient requires a bone biopsy, much information about bone disease can be inferred from clinical and laboratory findings. Accordingly, most practitioners do not perform bone biopsies on a routine basis. Even in the absence of routine bone biopsies, knowledge of the histologic classification provides a useful framework for diagnosis and treatment.

Osteitis fibrosa cystica is a high-turnover bone disease that is most prominently associated with hyperparathyroidism. Adynamic bone disease is a low bone turnover that is predominantly associated with low parathyroid hormone (PTH) activity. Osteomalacia is a low-turnover condition that is strongly associated with aluminum toxicity. Mixed uremic bone disease is characterized by the simultaneous appearance of multiple histologic patterns. In addition,

patients with kidney disease may develop several other syndromes that may affect bone such as β_2 microglobulin disease (renal amyloidosis).

Each histologic pattern is associated with characteristic clinical and biochemical features. A presumptive diagnosis is often possible, even without a bone biopsy. Different treatment approaches are indicated for each histologic pattern. It is likely that metabolic acidosis contributes to all forms of renal bone disease because of loss of bone calcium in the physiologic process of buffering the extracellular concentration of hydrogen.[1]

III. Clinical Manifestations

Overt bone symptoms are absent in most patients with kidney disease. However, clinical findings can be discerned in a significant number of patients. The prevalence and severity of clinical manifestations increase with duration of ESRD. Some patients experience bone pain, which may be vague and elusive. Renal osteodystrophy contributes to growth retardation in children with kidney disease, although other important factors are involved, including protein malnutrition. Patients with kidney disease have a demonstrated increased incidence of fractures, presumably related to altered bone architecture and strength.[2,3]

A variety of clinical manifestations occur that are largely related to abnormal mineral metabolism rather than bone disease per se. Soft tissue manifestation may include spontaneous tendon rupture and soft tissue precipitation of calcium phosphate deposits. Calcium phosphate precipitation in skin triggers pruritus that may be severe and incapacitating in some patients. Conjunctival mineral deposition leads to local irritation, injection, and characteristic band keratopathy. Ocular findings provide a useful window to systemic mineral deposition.

Although most studies of altered mineral metabolism in patients with kidney disease have focused on bone disease, there has been increasing recognition of an important relationship with mortality and cardiovascular disease. Patient mortality is strongly associated with the serum concentrations of calcium, phosphorus, and the calcium-phosphorus product.[4,5] Cardiovascular mortality is even more strongly associated with these mineral metabolism markers.[5] In addition, several studies have found that the prevalence of vascular calcification is associated with serum calcium and phosphorus levels.[6] These observations have contributed to the hypothesis that altered mineral metabolism in renal failure promotes vascular injury and premature atherosclerosis with all the attendant cardiovascular complications. Complications can affect all major vascular beds including the coronary, cerebral, and peripheral circulations. The hypothesis is supported by a growing body of clinical evidence. As such, addressing abnormal mineral metabolism associated with renal failure

is increasingly seen as prevention and treatment of cardiovascular as well as bone complications.

IV. Osteitis Fibrosa Cystica

Osteitis fibrosa cystica is the best understood bone disease associated with kidney failure. Once the most common form of bone disease, its prevalence has declined with the emergence of effective treatments.[7] Osteitis fibrosa cystica is strongly linked with hyperparathyroidism. Patients with renal disease develop hyperparathyroidism secondary to calcitriol deficiency, hypocalcemia, and hyperphosphatemia. Each of these factors stimulates production and secretion of PTH from the parathyroid glands.

Calcitriol deficiency develops because of decreased activity of the 1α-hydroxylase enzyme in the proximal tubule of the kidney. Renal 1α-hydroxylase is required to convert the vitamin D metabolite, 25-hydroxyvitamin D, into 1,25-dihydroxyvitamin D (calcitriol). Renal 1α-hydroxylase activity is reduced in part because of parenchymal cellular injury associated with the underlying process that produced kidney disease (e.g., diabetes, glomerulonephritis). In addition, hyperphosphatemia (see later) independently suppresses renal calcitriol production. The biologic actions of calcitriol include promotion of active intestinal calcium absorption and suppression of PTH secretion from the parathyroid glands. Calcitriol deficiency results in diminished calcium absorption and hypersecretion of PTH. In addition, kidney failure is associated with relative insensitivity to calcitriol at the level of the parathyroid calcitriol receptor.[8] The combination of decreased calcitriol production and calcitriol receptor insensitivity means that the normal suppressive action of calcitriol on PTH secretion is lost.

Hypocalcemia occurs largely as a consequence of calcitriol deficiency. As noted previously, calcitriol normally stimulates active intestinal absorption of calcium. Low levels of serum calcium normally stimulate renal production of calcitriol in a classic physiologic feedback mechanism. However, calcitriol production is suppressed in patients with kidney disease. In addition, a small decrease in serum calcium concentration can be attributed to hyperphosphatemia. As the serum concentration of phosphate increases, precipitation and soft tissue deposition of calcium phosphate occurs, leading to a small decrement in the serum calcium concentration. The serum calcium concentration directly influences PTH secretion through the parathyroid gland calcium receptor. Calcium suppresses PTH secretion; PTH secretion increases as the serum calcium falls.

Hyperphosphatemia is directly related to the loss of glomerular filtration, which is the hallmark of kidney disease. Phosphate is contained in many normal

food items such as protein sources and dairy products. Dietary phosphate is absorbed in the intestine through both passive and active processes. Absorbed phosphate is normally eliminated by the kidney under the control of PTH in a process that ordinarily maintains a normal serum phosphate concentration. However, the blood phosphate concentration increases as glomerular filtration rate (GFR) declines in patients with kidney disease. Hyperphosphatemia contributes to hyperparathyroidism in two ways. First, phosphate directly suppresses calcitriol production. Second, hyperphosphatemia directly stimulates PTH secretion.[9,10] In fact, animal studies have shown that hyperparathyroidism of kidney disease can be prevented through dietary restriction of phosphorus and avoidance of hyperhosphatemia.[10]

Over the short run, hyperparathyroidism is an appropriate adaptive mechanism to the mineral abnormalities that occur with kidney failure.[11] High PTH promotes renal excretion of phosphorus and production of calcitriol, which in turn increases intestinal calcium absorption. To a point, the secondary hyperparathyroidism of renal failure will resist the factors responsible for PTH secretion. However, the underlying abnormalities persist in patients with chronic kidney disease and the underlying problems (decreased calcitriol production, hypocalcimia, hyperphosphatemia) cannot be reversed.

Hyperparathyroidism persists with its attendant long-term complications. High PTH has also been implicated as a major uremic toxin that contributes to a number of clinical manifestations of kidney disease such as anemia, neuromuscular symptoms, sexual dysfunction, and bone disease.

Osteitis fibrosa cystica is the bone disease associated with hyperparathyroidism, regardless of the etiology of the excess PTH. Similar bone abnormalities are seen in patients with primary hyperparathyroidism due to a parathyroid adenoma (or hyperplasia) but no underlying kidney disease. Long-term exposure of bone to high levels of PTH results in increased bone turnover. Histologic examination reveals increased numbers of osteoblasts and osteoclasts. The rate of new bone formation is determined by administration of tetracycline at two distinct times before bone biopsy. Special stains are then used to visualize the incorporation of tetracycline into the bone front, giving an indication of bone formation. In osteitis fibrosa cystica, two distinct and well separated tetracycline lines are seen indicative of an increased rate of bone formation under PTH stimulation. Wide osteoid seams are seen indicating that bone is turning over faster than it can be mineralized. Foci of marrow fibrosis are characteristically seen. The resultant bone architecture is distinctly abnormal and intrinsically weaker and less durable than normal bone. As a result, patients with osteitis fibrosa cystica may develop bone pain and fractures.

In general, osteitis fibrosa cystica is associated with high levels of intact PTH (often greater than 450 pg/ml). Newer generation PTH assays have been developed but are not yet sufficiently well characterized to alter the existing

clinical guidelines.[12] The blood concentration of alkaline phosphatase (both nonspecific and bone-specific) is elevated, reflecting the increased rate of bone cell activity, remodeling, metabolism, and turnover. Hypocalcemia and hyperphosphatemia are also characteristic, as described previously. However, prior values over an extended period of time are more important than isolated readings, which can be modified by diet and treatment much faster than the bone disease. Also, patients occasionally develop hypercalcemia as a result of very longstanding hyperparathyroidism and parathyroid gland hyperplasia (a condition sometimes referred to as "tertiary hyperparathyroidism"). These biochemical features are characteristic of osteitis fibrosa cystica but not diagnositic. Definitive diagnosis requires a bone biopsy. However, specialized equipment and personnel are required for proper processing and interpretation of bone tissue. Many clinicians feel they are able to satisfactorily manage most patients without a bone biopsy.

Prevention and treatment of osteitis fibrosa cystica are focused on the hyperparathyroidism. A low-phosphate diet should be prescribed. Phosphate binders should be used regularly to control hyperphosphatemia. Frequent and close follow-up is required. Direct suppression of PTH can be achieved with vitamin D analogues and calcimimetic compounds that emulate the action of calcium on the parathyroid calcium receptor. Available vitamin D compounds include calcitriol, paricalcitol, and doxercalciferol in both oral and intravenous forms. Large variations in vitamin D therapy exist among dialysis facilities and countries.[5] Differential patient survival has been found based on the type and route of vitamin D therapy,[13,14] although this finding has not been tested in clinical trials. Cinacalcet is an oral calcimimetic compound that effectively suppresses PTH. Cinacalcet can be used alone or in combination with a vitamin D compound.[15] Applied early, these approaches should prevent osteitis fibrosa cystica. In advanced cases, parathyroidectomy may be indicated to reduce PTH levels. For patients with active bone disease, these treatments can lower PTH and promote more physiologic bone remodeling with correction of abnormal bone architecture and, presumably, reduction in fracture risk.

V. Adynamic Bone Disease

Adynamic bone disease has emerged in recent years as the most common histologic form of renal osteodystrophy.[7] The growth in adynamic bone disease no doubt relates to improvements in treatment that have decreased the incidence of osteitis fibrosa cystica and osteomalacia. Adynamic bone disease is poorly understood at present. However, one common theme is relative suppression of PTH which, in turn, leads to decreased bone turnover. Low PTH levels are

often attributable to the suppressive effects of vitamin D therapy, calcium-containing phosphate binders, and perhaps relatively high concentrations of dialysate calcium. Approximately 50% of hemodialysis patients have an intact PTH concentration below the current K/DOQI lower bound guideline of 150 pg/ml.[5] Low bone turnover, like the high bone turnover seen in osteitis fibrosa cystica, leads to disruption of the normal bone architecture. The result is weakened bone that is potentially susceptible to fracture. An elevated risk of hip fractures was found in patients with low PTH concentrations and a presumed disposition to adymamic bone disease.[3]

Adynamic bone disease is characterized by low bone turnover and mineralization rate, as determined by tetracycline labeling. Osteoclast and osteoblast activity is low. Specialized stains reveal thin osteoid seams and low osteoid mass.

As noted, the major biochemical feature of adynamic bone disease is a low PTH concentration. Current guidelines call for an intact PTH concentration of 150 to 300 pg/ml[16] although there is controversy about the preferred range and assay type. Future guidelines may move toward higher PTH concentrations and new assay methods.[17]

At present, treatment consists of attempting to desuppress PTH through withdrawal of vitamin D therapy, conversion to calcium-free phosphate binders, and use of lower dialysate calcium concentrations. Even with these measures, PTH remains inexplicably low in some patients.[5]

VI. Osteomalacia

Osteomalcia in patients with kidney disease is almost always attributable to aluminum intoxication. The usual cause of osteomalacia in patients without kidney failure is vitamin D deficiency. However, vitamin D deficiency in patients with renal disease is much more likely to be associated with osteitis fibrosa cystica as described earlier. These different patterns of bone disease associated with vitamin D deficiency probably reflect the complicated multifactorial and interactive nature of metabolic bone disease.

Aluminum intoxication arises from contaminated dialysate fluid and oral aluminum-containing antacids administered for the purpose of binding dietary phosphate. Both sources introduced small amounts of aluminum into patients, either directly through the blood route (dialysate contamination) or indirectly through intestinal absorption (aluminum antacids). However, significant toxicity developed in many patients after years of low-level exposure in patients who lack normal renal routes of aluminum excretion. Removal of aluminum through dialysis is relatively inefficient.

Dialysate contamination occurs when dialysate fluid is prepared from water sources that have not been adequately purified. Many municipal water sources contain relatively high concentrations of aluminum. Water preparation procedures have been tightened since the recognition of the serious adverse impact of aluminum and other impurities. Aluminum antacids were used as phosphate binders for many years under the assumption of negligible and clinically insignificant intestinal absorption of aluminum. However, as noted, low-level exposure over many years in the absence of a route of excretion led to aluminum accumulation and toxicity. Aluminum gels have largely been replaced by alternative compounds. Initially, calcium salts were used. Several non-calcium compounds have recently been introduced as phosphate binding agents including sevelamer hydrochloride and lanthanum carbonate. These compounds do not increase the calcium load and may offer clinical advantages.[18–21]

Widespread aluminum deposition has been reported in dialysis patients exposed to aluminum through dialysate and aluminum-containing antacids. Aluminum toxicity is a multisystem syndrome. Deposition in the central nervous system produced rapid and debilitating dementia ("dialysis dementia"). Bone marrow deposition resulted in microcytic anemia that failed to respond to iron or erythropoietin therapy. Aluminum deposition in emerging bone fronts during normal bone remodeling process led to the osteomalacia form of renal osteodystrophy. Thankfully, all the aluminum toxicity syndromes are now uncommon as a result of concerted efforts to minimize aluminum exposure.

Osteomalcia due to aluminum is characterized by low bone turnover and mineralization. Tetracycline labeling generally reveals a single, poorly stained bone front instead of two distinct bands seen with normal bone remodeling. Osteoblast and osteoclast activity is low, presumably owing to direct cellular toxicity of aluminum. Wide unmineralized osteoid seams are found. Special stains reveal the presence of aluminum.

Aluminum also deposits in parathyroid tissue and suppresses PTH production, resulting in characteristically low intact PTH blood levels. Blood alkaline phosphatase activity is generally not elevated, given the relative lack of bone turnover and metabolic activity. Patients generally exhibit characteristic hyperphosphatemia and hypocalcemia. However, hypercalcemia may emerge in later phases.

Aluminum toxicity should be suspected in patients with a consistent clinical presentation and a plausible history of exposure over an extended period of time. An elevated blood aluminum level is supportive. A large increment in serum aluminum concentration after desferoximine (DFO) challenge strongly points to aluminum toxicity. Bone biopsy provides a definitive diagnosis but may not be necessary in all cases.

Treatment consists of a prolonged course of aluminum chelation with DFO. DFO treatment improves the bone disease and anemia but is less effective for dementia. The best treatment is prevention through use of purified water for the dialysate and avoidance of aluminum-containing phosphate binders.

VII. Mixed Bone Disease

Mixed bone disease describes the combination of osteitis fibrosa cystica and defective bone mineralization. This insufficiently understood entity illustrates the complexity and poor understanding of renal osteodystrophy.

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Chapter 7

Nephrolithiasis

Melissa A. Cadnapaphornchai and Pravit Cadnapaphornchai

I. Introduction

Nephrolithiasis develops in all age groups. The incidence of kidney stones has risen during the past 20 years. It causes pain and suffering, loss of work time, and occasional loss of kidney function due to urinary tract obstruction and infection. It is also associated with chronic kidney disease and accounts for 2% to 3% of end-stage renal disease (ESRD). In most patients, the causes of kidney stones are metabolic and when found, they can be effectively treated and recurrence can be prevented. The previously recommended low-calcium diet for patients with calcium stones should be abandoned. It is now clear that a low-calcium diet is associated with increased risk of nephrolithiasis. This chapter reviews several aspects of nephrolithiasis, both in children and in adults, and details the pathophysiologic aspects of several metabolic disorders associated with kidney stones.

II. Epidemiology

It is estimated that 12% of men and 5% of women will have at least one episode of kidney stones during their lifetimes.[1] Once kidney stones develop, the recurrence rate is estimated to be 14% at 1 year, 35% at 5 years, and 52% at 10 years. The incidence in the general population is about 1 in 1000 adults per year. In the United States, the incidence of kidney stones is greater in the northeast and southeast regions. Over the last 20 years, the incidence of kidney stones has increased by 37% in the United States.[2] Several potential factors have contributed to this rising incidence, including the increased frequency of obesity, diabetes mellitus, insulin resistance, and high-protein diets. In addition, technologic advancements in imaging studies have led to an increase in the detection of patients with asymptomatic stones. Kidney stones are more

Table 7-1. Risk Factors Associated with Nephrolithiasis.

Positive risk factors	Negative risk factors
Positive family history	High urine volume > 2000 ml/day
Living in southeastern United States	Increased urine excretion of citrate
Overweight, obesity, or increased BMI[a]	Normal dietary calcium intake of 1–1.2 g/day
Low urine volume < 1500 ml/day	
High dietary animal protein intake	
Low dietary calcium intake	
Increased ingestion of grapefruit juice	
Increased urine excretion of calcium oxalate, uric acid, cystine	
Low urine pH	
Urinary tract structural abnormalities leading to stasis of urine flow	

[a] BMI, body mass index.

common in men than in women. The prevalence of kidney stones varies by ethnicity; they occur most commonly in non-Hispanic Caucasians and only rarely in African Americans, with intermediate frequencies in those of Hispanic or Asian descent.

Risk factors for nephrolithiasis are shown in Table 7-1. A positive family history can frequently be identified. Obesity is a well established risk factor for stone formation.[3,4] Body mass index (BMI) is known to correlate positively with urine excretion of uric acid, sodium, ammonium, and phosphate, and negatively with urine pH.[4,5] Maalouf et al.[5] have confirmed an inverse correlation between urine pH and body weight. Thus, obese individuals are at increased risk to develop uric acid nephrolithiasis. Other risk factors for nephrolithiasis include low urine volume; low urine pH; and increased urine supersaturation of calcium, oxalate, uric acid, and cystine. As noted earlier, a low-calcium diet is now considered a risk factor for kidney stones.

The prevalence of nephrolithiasis in North American children varies widely by geographic region, with increased occurrence in the southeastern United States, similar to the figure for adults. Nephrolithiasis affects boys and girls equally and accounts for 1 in 1000 to 1 in 7600 hospital admissions, a rate approximately one tenth of that observed in adults.[6] Kidney stones affect children of all ages with relatively equal frequency. As in adults, kidney stones are much more common in Caucasian than in African American children. The rate of recurrent stones in childhood has been reported to be as high as 50%, with a mean interval to recurrence of 3 to 6 years.[7,8] As might be anticipated, children with an underlying metabolic disorder are nearly fivefold more likely

Table 7-2. Types of Kidney Stones.

Calcium oxalate
Combined calcium oxalate and calcium phosphate (hydroxyapatite)
Calcium phosphate (brushite)
Calcium carbonate (carbonate apatite)
Uric acid
Cystine
Magnesium ammonium phosphate (struvite)
Others
Xanthine
Silica
Derived from medications or natural products

Table 7-3. Metabolic Derangements Associated with Nephrolithiasis.

Decreased urine volume
Hypercalciuria
Hyperoxaluria
Hypocitraturia
Hyperuricosuria
Hypomagnesiuria
Cystinuria
Low urine pH
High urine pH

to have recurrent nephrolithiasis than children with no identifiable metabolic disorder.[9]

Common types of kidney stones observed in both children and adults in Western societies are listed in Table 7-2. Calcium oxalate and combined calcium oxalate and calcium phosphate stones make up 70% to 80% of all kidney stones. Struvite constitutes approximately 5% to 10% while uric acid and cystine stones account for an additional 5% to 10%.

Clinically, certain chemical abnormalities of the urine are associated with altered risk of nephrolithiasis (Table 7-3).

III. Pathogenesis of Nephrolithiasis

The pathogenesis of nephrolithiasis is complex and involves the interaction of several physicochemical and anatomic factors. Urine flow rate, urine solute excretion rate, ion supersaturation, urinary ionic strength, urine pH, and urinary tract anatomy all affect one's risk of urinary crystal formation. Supersaturation

of ions such as calcium, oxalate, and phosphate in the urine results in crystal formation with subsequent growth and aggregation. These crystals may be excreted or they may adhere to the renal tubular epithelial cells. Internalization of crystals can also occur. In patients with calcium oxalate stones, calcium phosphate plaques can form in the basement membrane of cells in the thin limb of Henle's loop, with subsequent extension into the papillary interstitium or into the basement membrane of medullary collecting duct cells. Such plaques may erode the papillary urothelium, resulting in deposition of calcium oxalate at the papillary tip with associated stone generation. In patients with anatomic or functional small intestinal bypass, interstitial plaques do not occur. Instead, crystals have been shown to attach to the collecting duct cells. Both interstitial plaque and collecting duct plaque are seen with calcium phosphate (brushite) stones.[10]

The pathogenesis of uric acid stones is largely pH dependent, with an abnormally low urine pH resulting in urinary uric acid precipitation. This increased urine acidity is likely due to decreased urine ammonium excretion. In the hyperuricosuric calcium oxalate stone former, the presence of high levels of uric acid enhances the urinary supersaturation of calcium oxalate, leading to stone formation. Patients with hyperuricosuria and calcium oxalate stones have higher urine pH than those with gouty diathesis or uric acid stones. Cystine stones tend to develop when the content of cystine is elevated and the urine pH is reduced.

The effect of organic and inorganic inhibitors of stone formation, including magnesium, citrate, pyrophosphate, and several high molecular weight compounds in low quantities, must also be considered. These inhibitors prevent crystallization by modulating the nucleation, growth, aggregation, and adhesion of crystals to renal epithelial cells. Inhibitors such as magnesium and citrate have thus been used to prevent renal stones. However, the beneficial effects of these supplements for stone prevention have been well documented only for citrate.

Specific solutes contributing to stone formation are discussed in further detail in the paragraphs that follow.

IV. Clinical Presentation

Adults with nephrolithiasis may be entirely asymptomatic, with stones discovered incidentally on imaging studies obtained for other purposes. Acute clinical manifestations of nephrolithiasis include abdominal or flank pain, hematuria, gastrointestinal symptoms other than pain, and urinary tract infection. Abdominal and/or flank pain with or without radiation to the groin or genital area is the most frequent presentation. Two types of pain are observed

in patients with nephrolithiasis. One is a dull aching flank pain associated with kidney swelling due to urinary tract obstruction, while the other is a colicky pain associated with ureteral contraction. Hematuria, which can be microscopic or gross in nature, is a frequent finding. However, hematuria is absent in 10% of patients with acute renal colic. Other symptoms can include nausea, vomiting, ileus, and urinary tract infection, especially with recurrent stones or urinary tract obstruction. Elevations in blood urea nitrogen and creatinine are rare because the obstruction is generally unilateral; an exception is the obstructed or infected solitary kidney. Chronic manifestations of recurrent stones are dependent on the type of metabolic abnormalities that contribute to stone formation. Such manifestations can include decreased bone mineral density in idiopathic hypercalciuria or musculoskeletal pain and nephrocalcinosis in distal renal tubular acidosis.

In older children and adolescents, the clinical manifestations of renal stone disease are similar to those in adults. Specifically, abdominal, flank, or pelvic pain occurs as the initial symptom in up to 50% of children with nephrolithiasis. Hematuria, either gross or microscopic, leads to the diagnosis of childhood nephrolithiasis in approximately one third of cases, while kidney stones are diagnosed as an incidental radiographic finding in 15% or in association with infection in 11% of cases.[11] Up to half of children manifest urinary symptoms such as urgency, frequency, or dysuria. Renal stones may be more difficult to clinically diagnose in young children due to nonspecific symptoms. Among children 5 years of age and younger, urinary tract infection and incidental radiographic findings lead to the diagnosis in 43% of cases.[11]

V. Clinical Evaluation

The clinical evaluation of risk factors for nephrolithiasis is an office procedure and should never be performed during an acute stone event or in a hospital. The optimal time of evaluation is 6 to 8 weeks following the acute stone event, when the patient has returned to his or her normal diet. Appropriate evaluation includes history, physical examination, stone analysis if possible, serum and urine chemistries, and imaging studies.

A. History and examination

A thorough history and complete physical examination are indicated in the evaluation of both children and adults with nephrolithiasis. The history should focus on the identification of physicochemical, anatomic, and genetic factors that predispose to nephrolithiasis.

A detailed review of dietary and fluid intake is an essential component in the evaluation of nephrolithiasis. The diet is very rarely the cause of

nephrolithiasis; however, certain dietary patterns can increase one's risk of stone disease. Decreased fluid intake with resultant decreased urine output is associated with increased risk of stone formation and vice versa.[12,13] High fluid intake decreases stone recurrence rate and prolongs the interval of recurrence. Whether specific types of fluid are more beneficial in reducing stone risk remains controversial. In the Health Professional Follow-up Study involving men, intake of coffee, tea, beer, and wine was associated with reduced stone risk. Apple juice and grapefruit juice intake was associated with increased stone risk.[14] In the Nurses' Health Study, decaffeinated and caffeinated coffee, wine, and tea were associated with decreased stone risk while grapefruit juice was associated with increased stone risk.[15] In one small study, ingestion of large quantities of grapefruit juice has been shown to result in increased urine oxalate and citrate excretion with no change in supersaturation of calcium oxalate, calcium phosphate, or uric acid.[16] Cranberry juice ingestion is associated with decreased urine oxalate and increased urine citrate excretion and is probably beneficial for stone formers.[17] Again, it is not clear if it is the quantity or the type of fluid ingested that mediates stone risk.

High animal protein intake is associated with an increased incidence of upper urinary tract stones.[18] Increased animal protein intake is associated with increased urinary excretion of calcium, oxalate, and uric acid; decreased urinary excretion of citrate; and decreased urine pH. Urine sodium, potassium, magnesium, and inorganic phosphate excretion are also increased. High sodium intake has been shown to increase urine calcium excretion. One could argue that because magnesium is an inhibitor of stone formation, high urinary magnesium excretion may be beneficial in the prevention of stones. However, this potential effect may be offset by the aforementioned risk factors.

In adults, normal calcium intake is between 800 and 1500 mg per day. In normal individuals, even with a daily intake of 2000 mg, urine calcium excretion seldom exceeds 280 mg/day. Urine calcium changes by an average of only 6% despite marked variability in calcium intake. However, patients with a history of calcium nephrolithiasis demonstrate increased urine calcium excretion as compared to non-stone formers at any level of calcium intake. It is now clear that low calcium intake increases risk for nephrolithiasis.[19,20] Because calcium binds oxalate in the gastrointestinal tract, a low-calcium diet can lead to hyperoxaluria. Increased urinary oxalate excretion has been shown to be a more critical factor than increased urinary calcium excretion in stone formation,[21] although on a molar basis, more calcium than oxalate is present in urine.

Only 10% to 15% of dietary oxalate is absorbed by the gastrointestinal tract, with the remainder degraded by enteric bacteria or excreted in the stool as calcium oxalate. However, it has been estimated that dietary oxalate intake contributes to 40% of urinary oxalate excretion. As noted previously, oxalate

absorption appears to vary inversely with intestinal calcium and magnesium content. Spinach and rhubarb are high in oxalate content.

Elimination of fruits and vegetables from the diet is associated with decreased urinary excretion of potassium, magnesium, oxalate, and citrate, but increased excretion of urinary calcium and increased supersaturation of calcium oxalate and calcium phosphate.[22]

In the evaluation of nephrolithiasis, systemic diseases associated with increased risk of nephrolithiasis should be excluded. The incidence of nephrolithiasis in cystic fibrosis is estimated to be 3%, which is increased over age-matched controls.[23,24] Calcium oxalate stones are seen most frequently in this population, and patients with cystic fibrosis have been shown to have hyperoxaluria and hypocitraturia. A lack of *Oxalobacter formigenes*, intestinal bacteria that contribute to the degradation of oxalate, may contribute to hyperoxaluria in these patients who receive chronic antibiotic therapy. The prevalence of kidney stones in autosomal dominant polycystic kidney disease (ADPKD) is higher than in the general population. ADPKD patients with nephrolithiasis appear to have more and larger cysts than those without stones. ADPKD patients with stones also demonstrate lower creatinine clearance and decreased urinary excretion of magnesium, potassium, and citrate.[25,26] Medullary sponge kidney (MSK) is frequently associated with nephrolithiasis. In a large population of patients with recurrent calcium nephrolithiasis, the prevalence of MSK was 12%. Such patients do not demonstrate hypercalciuria but instead have decreased urinary excretion of citrate, magnesium, calcium, and uric acid when compared with non-MSK patients.[27] Parks et al. observed a similar prevalence of renal stone disease in women with MSK.[28] Kidney stones affect 50% of patients with active Cushing's disease compared with 27% of those whose Cushing's disease has been cured and 5% of control subjects.[29] Patients with active disease demonstrate increased urinary excretion of calcium and uric acid and decreased urinary excretion of citrate. Systemic diseases that result in hypercalciuria, including granulomatous disease such as sarcoidosis, hypervitaminosis D, renal tubular acidosis, and primary hyperparathyroidism, should also be considered.

Prior history of urinary tract infection should be documented. For patients with recurrent stones, a review of the past medical history should include frequency of recurrent stones, previous documentation of stone analysis, history of urologic interventions, and previous medical treatment including response to and compliance with the prescribed treatment. The use of medications known to cause nephrolithiasis should be reviewed.

The family history of nephrolithiasis should be reviewed. A positive family history is seen in 15% to 30% of patients with nephrolithiasis.[30–32] It is unclear whether genetic or environmental factors or both are responsible. Individuals in the same family are likely to have similar dietary patterns and to be exposed

to the same environment. A family history of early-onset nephrolithiasis should alert the physician to inherited disorders such as cystinuria, primary hyperoxaluria, Dent's disease, hereditary hypophosphatemic rickets, and distal renal tubular acidosis. However, hypercalciuric nephrolithiasis is also common in children.

There are few physical findings specific for stone disease. An assessment of body weight and BMI is important given the association between obesity and nephrolithiasis. Failure to thrive in young children may be suggestive of associated renal tubular acidosis.

B. Stone Analysis

When a stone is available, stone analysis must be performed. Specific treatments can be offered for nephrolithiasis due to cystine, uric acid, or struvite. Uric acid stones in particular can be dissolved with bicarbonate or citrate treatment. Renal tubular acidosis, primary hyperparathyroidism, and milk-alkali syndrome are commonly associated with calcium phosphate stones. Staghorn calculi that conform to the configuration of the renal calices and pelvis are typically composed of struvite, cystine, or uric acid. Calcium oxalate and calcium phosphate are unlikely to form staghorn calculi. Unfortunately, the finding of calcium oxalate, the most common type of nephrolithiasis, is not particularly helpful.

C. Laboratory Evaluation

In addition to stone analysis, urine chemistries should be assessed because information can be obtained that can aid in the diagnosis and prevention of nephrolithiasis. Normal values for urine chemistries are provided in Table 7-4. Urine analysis for crystals (Fig. 7-1) can be diagnostic for cystinuria or indinavir administration. The presence of triple phosphate crystals suggests struvite nephrolithiasis. Urine with numerous uric acid crystals together with low urine volume and low urine pH favors the diagnosis of uric acid stones. Morning urine pH is usually below 5.5. Persistently elevated urine pH may suggest distal renal tubular acidosis. Spot urine pH should not be done shortly following a meal because of alkaline tide with associated increased pH.

It is preferable to obtain a timed (e.g., 24-hour) urine collection for assessment of volume, osmolality, creatinine, urea nitrogen, calcium, sodium, potassium, phosphorus, oxalate, uric acid, cystine, citrate, and magnesium. It is important to determine the adequacy of urine collection. This can be done by measurement of urine creatinine excretion. The 24-hour urine creatinine excretion should be between 20 and 25 mg/kg ideal body weight in men, 15 to 20 mg/kg ideal body weight in women, and 15 to 25 mg/kg body weight in children; this calculation assumes that the patient has normal muscle

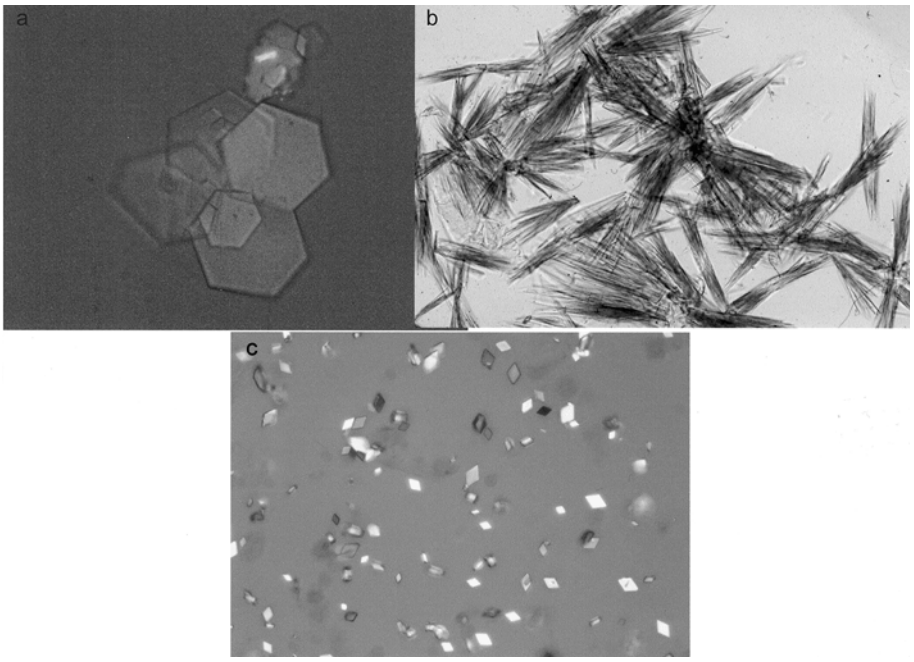

Figure 7-1. Urine crystals. Cystine (a), indinavir (b) and uric acid (c).

Table 7-4. A. Normal Values for Urinary Solute Excretion in Timed Urine Collections.

Calcium	< 4 mg/kg/day or 300 mg/day in men and 250 mg/day in women	< 0.1 mmol/kg/day or 7.5 mmol/day in men and 6.2 mmol/day in women
Oxalate	< 50 mg/1.73 m^2/day or 40 mg/day	< 0.57 mmol/1.73 m^2/day or 0.45 mmol/day
Uric acid	< 815 mg/1.73 m^2/day or 800 mg/day in men and 700 mg/day in women	< 4.85 mmol/1.73 m^2/day or 4.8 mmol/day in men and 4.4 mmol/day in women
Cystine	< 60 mg/1.73 m^2/day	< 0.25 mmol/1.73 m^2/day
Phosphorus	< 1000 mg/day	< 32 mmol/day
Citrate	> 180 mg/g creatinine in males	> 108 μmol/mmol creatinine in males
	> 300 mg/g creatinine in females	> 180 μmol/mmol creatinine in females
	> 320 mg/day in adults	> 193 μmol/day in adults
Magnesium	> 1.6 $\pm$ 0.8 mg/kg/day or > 50 mg/day	> 0.07 $\pm$ 0.03 mmol/kg/day or > 2.1 mmol/day
Creatinine	15–25 mg/kg/day in children	133–221 μmol/kg/day in children
	20–25 mg/kg/day in men	177–221 μmol/kg/day in men
	15–20 mg/kg/day in women	133–177 μmol/kg/day in women

Table 7-4. B. Normal Values for Urinary Solute to Creatinine Ratios in Random Urine Samples.

	Age	mg/mg	Age	mmol/mmol
Calcium/creatinine	0–6 months	< 0.8	0–6 months	< 2.24
	6–12 months	< 0.6	6–12 months	< 1.68
	> 2 years	< 0.2	> 2 years	< 0.56
Oxalate/creatinine	< 6 months	< 0.29	< 6 months	< 0.37
	6 months to 2 years	< 0.20	6 months to 2 years	< 0.26
	2–5 years	< 0.11	2–5 years	< 0.14
	6–12 years	< 0.06	6–12 years	< 0.08
Cystine/creatinine	All ages	< 0.02	All ages	< 0.01
Citrate/creatinine	All ages	> 0.51	Male, < 1 year	> 1.9
			Female, < 1 year	> 0.63
			Male, 1–12 years	> 0.27
			Female, 1–12 years	> 0.33
			Male, > 13 years	> 0.32
			Female, > 13 years	> 0.28
Uric acid/GFR	$\geqslant$ 3 years	< 0.56	$\geqslant$ 3 years	< 0.03

[a]GFR, glomerular filtration rate. Uric acid/GFR is calculated as (urine uric acid $\times$ serum creatinine) $\div$ (urine creatinine) with all values in same units.

mass and it is not reliable in patients with significant wasting or underlying muscle disease. When the actual urine creatinine is markedly above or below the calculated one, over- or under-collection of urine, respectively, should be considered. Another indirect way to estimate urine output is the use of urine osmolality. In normal adults, the osmotic waste product excreted per day is approximately 600 mOsmol. If 24-hour urine osmolality is 600 mOsmol/kg of H_2O, the urine volume is likely to be 1 L/day. If the urine osmolality is 300 mOsmol/kg H_2O, the urine volume is likely to be 2 L/day. Finally, if the urine osmolality is 900 mOsmol/kg H_2O, the urine volume is likely to be 750 ml/day.

It is important to consider the effect of bacterial overgrowth on timed urine collection. Specifically, bacteria will consume citrate. As a consequence, when bacterial overgrowth is not prevented, urine citrate concentration may be erroneously low. In addition, hypokalemia, renal failure, and systemic acidosis decrease citrate excretion. These should be taken into account in interpretation of laboratory results.

Determination of urine urea nitrogen can provide a rough estimate of protein intake by the following formula:

$$[\text{Urine urea nitrogen (g/24 hours)}] \times 6.25 + 4 = \text{daily protein intake}$$

For example, if the urine urea nitrogen is 15 g, the protein intake is likely to be $(15 \times 6.25) + 4 = 98$ g. If the person weighs 70 kg, the appropriate protein intake of 0.8 to 1 g/kg should be 70 g/day.

In young children, because of difficulty in obtaining 24-hour urine collection, a random urine sample can be collected and the ratios of urine solute to creatinine used instead (Table 7-4).

Blood chemistries should include serum potassium, total CO_2 content, calcium, magnesium, phosphorus, and uric acid. Determination of parathyroid hormone (PTH) when hypercalcemia is present and 1,25-dihydroxyvitamin D_3 (calcitriol) when there is clinical evidence of granulomatous disease is indicated. These conditions are rare in childhood.

D. Radiographic Imaging

The utility of radiographic imaging in stone disease is to diagnose nephrolithiasis, to detect recurrent or residual stones, to detect and monitor nephrocalcinosis, to exclude urinary tract obstruction associated with nephrolithiasis, and to exclude underlying renal or urologic anomalies that can predispose to stone formation. KUB or plain abdominal radiographs are simple to perform for the detection of radioopaque stones. Radiolucent stones caused by uric acid, xanthine, or triamterene will not be detected. Cystine stones are quite dense and may be apparent on KUB. Because 90% of the stones are radioopaque, KUB should be the first imaging study performed for evaluation of stone disease in adults. Unfortunately, sensitivity (57%) and specificity (71%) are poor, making it unpopular for the initial imaging study in acute renal colic.[33,34]

Intravenous urography (IVU) remains one of the best imaging studies available for acute renal colic in adults. It provides visualization of the entire excretory system, can detect both radioopaque and radiolucent stones, and can detect urinary tract obstruction. However, intravenous urography is not suitable for patients with impaired kidney function or contrast allergy and is rarely used in children. Its sensitivity and specificity are 87% and 97%, respectively.[34,33] However, it is the best imaging study for medullary sponge kidney.

Renal ultrasonography is noninvasive and can detect both radiolucent and -opaque stones. In addition, it provides information regarding urinary tract obstruction when hydronephrosis or hydroureter is present. Unfortunately, renal ultrasound utility is confined to the kidney, pelvis, and early proximal ureter with poor imaging of the distal ureter. In addition, this modality may not detect stones less than 3 mm in size. However, sensitivity (87%) and specificity (94%) are improved as compared to KUB.[34] This modality is also easy to perform in children.

The best imaging study available currently is nonenhanced helical computed tomography (CT). The sensitivity (96%) and specificity (100%) are excellent.[35]

This modality has the advantage of being able to detect ureteral stones in addition to providing information regarding urinary tract obstruction. It can also detect abnormalities outside the urinary tract that may be a source of abdominal pain. The disadvantage is exposure to relatively high-dose radiation as compared with KUB and IVU. In addition, young children may require sedation to obtain adequate images. Recently, helical CT with reduced radiation dose has been shown to provide results comparable to those of conventional helical CT in adults weighing less than 200 lbs.[36]

Imaging studies that reveal nephrocalcinosis rather than discrete calculi should raise concern regarding underlying distal renal tubular acidosis, medullary sponge kidney, sarcoidosis, primary hyperoxaluria, and primary hyperparathyroidism.

VI. Etiology of Nephrolithiasis

As noted previously, approximately 70% to 80% of all kidney stones in adults are comprised of calcium oxalate or combined calcium oxalate/phosphate, while struvite and cystine each make up 5% to 10% of kidney stones in this population. Similarly, more than 75% of kidney stones in children are composed of calcium oxalate or calcium phosphate.[11] Specific metabolic abnormalities contributing to stone formation are discussed in detail below.

A. Calcium (Ca MW 40)

1. Calcium Metabolism

Serum and urine calcium are maintained by the combined effects of PTH and 1,25-dihydroxyvitamin D3 (calcitriol) on intestinal absorption, bone resorption, and renal excretion. Calcium absorption occurs in the small intestine, largely in the duodenum. Two mechanisms are responsible for intestinal transport, including transcellular calcitriol-dependent and paracellular calcitriol-independent pathways. Transport of calcium across intestinal apical membrane occurs via TRPV6 (CaT1/ECaC) with exit across the basolateral membrane via PMCA1b (Ca-ATPase) and Na–Ca exchanger (NCX1). Active transport occurs in the duodenum and early jejunum whereas paracellular transport occurs throughout the whole intestine. Of normal adult calcium intake of 800 to 1200 mg/day, net absorption is 150 mg, with 150 mg excreted by the kidney, thus maintaining calcium homeostasis.

Serum calcium exists in three forms, including ionized (50%), protein-bound (40%, predominantly bound to albumin), and complexed with sulfate, phosphate, and citrate (10%). Only non-protein-bound calcium is available for filtration at the glomerulus. Once filtered, 70% is reabsorbed by the proximal

tubule, 20% by the thick ascending limb, and the remaining amount by the distal tubule. Transport occurs predominantly via the paracellular pathway in the proximal tubule and the ascending limb, via the concentration gradient in the proximal tubule, and via positive voltage in the thick ascending limb. In the distal tubule, calcium is transported transcellularly through the epithelial calcium channel TRPV5/TRPV6 (ECaC) in the apical membrane and exits across the basolateral membrane via Ca-ATPase and Na–Ca exchanger. Distal tubular transport of calcium is mediated predominantly by PTH and the calcium sensing receptor (CaSR), which have opposing effects. PTH stimulates calcium reabsorption by activating ECaC. In contrast, activation of CaSR results in decreased calcium absorption with calciuria via suppression of a calcium-sensitive potassium channel.

2. Causes of Hypercalciuria-associated Nephrolithiasis (Table 7-5)

Hypercalciuria is a common cause of nephrolithiasis in children and adults and results from increased intestinal absorption, increased bone resorption, and/or decreased renal calcium reabsorption. Causes of hypercalciuria are listed in Table 7-5. Macrophages in granulomata possess 1α-hydroxylase which converts 25-hydroxyvitamin D_3 to active calcitriol, which is responsible for increased intestinal calcium absorption and hypercalciuria. PTH is

Table 7-5. Causes of Hypercalciuria-Associated Nephrolithiasis in Children and Adults.

Idiopathic hypercalciuria
Increased intestinal calcium absorption
Granulomatous disease, e.g., sarcoidosis
Hypervitaminosis D
Milk alkali syndrome
Increased bone resorption
Immobilization, e.g., paraplegia and quadriplegia
High animal protein diet
Systemic acidosis, e.g., distal renal tubular acidosis
Adrenocorticotrophic hormone or cortisol excess
Primary hyperparathyroidism
Decreased renal calcium reabsorption
Bartter's syndrome
Chronic loop diuretic administration
X-linked hypercalciuric nephrolithiasis
Familial hypocalcemic hypercalciuria
Systemic diseases associated with chronic hypercalcemia
Williams syndrome
Primary hyperparathyroidism

suppressed in this condition. Milk-alkali syndrome, first described by Cope in 1936[37] and subsequently confirmed by Burnett et al. in 1949,[38] is characterized by metabolic alkalosis, renal dysfunction, hypercalcemia, and nephrocalcinosis after taking milk and sodium bicarbonate for peptic ulcer disease. Kapsner et al. in 1986[39] reported 56 post-cardiac transplant patients who developed the syndrome after taking 2 to 3 g of calcium carbonate four times a day. In 14 immobilized patients with paraplegia or quadriplegia from spinal cord injury, fasting urine calcium was increased during dietary calcium restriction (400 mg/day), with suppression of calcitriol and PTH concentrations, suggesting increased bone resorption of calcium.[40] In addition, many of these patients developed chronic recurrent urinary tract infections with urease-producing organisms. This combination of hypercalciuria and urinary tract infection with high urine pH contributed to the formation of struvite stones. Renal tubular acidosis is associated with hypercalciuria. In normal healthy individuals, administration of ammonium chloride to induce acidosis resulted in increased urinary excretion of calcium, phosphate, and hydroxyproline.[41,42] Acidosis is also known to reduce citrate excretion; citrate is an inhibitor of stone formation. Thus, combined hypercalciuria and hypocitraturia appear to be responsible for calcium nephrolithiasis in renal tubular acidosis. X-linked hypercalciuric nephrolithiasis consists of four different clinical syndromes: X-linked recessive nephrolithiasis,[43] Dent's disease,[44] X-linked recessive hypophosphatemic rickets,[45] and low molecular weight proteinuria with hypercalciuria and nephrocalcinosis.[46] These clinical syndromes are characterized by Fanconi syndrome, hypercalciuria, low molecular weight proteinuria, nephrocalcinosis, and nephrolithiasis.[47] These syndromes are due to mutations of the chloride channel *CLC5* gene.[47–49] It is unclear how mutations of *CLC5* cause the clinical syndrome as observed. Williams syndrome is due to a sporadic defect in the elastin gene and is associated with specific facial features including elfin facies, mild mental retardation, and cardiac lesions such as supravalvular aortic stenosis, peripheral pulmonary stenosis, aortic hypoplasia, coronary artery stenosis, and atrial or ventricular septal defects. Affected patients have been shown to have delayed excretion of a calcium load with evidence of increased production of 25-hydroxyvitamin D_3.

3. Idiopathic Hypercalciuria (IHC)

IHC is defined as increased urine calcium excretion in the absence of hypercalcemia or systemic diseases that induce hypercalciuria. IHC is the most common cause of nephrolithiasis; as many as 40% to 50% of children and adults with kidney stones have IHC. Increased intestinal calcium absorption has been repeatedly demonstrated in such patients. For any amount of calcium intake, IHC patients absorb and excrete more calcium than non-stone formers. Increased sensitivity to vitamin D has been suggested to be responsible

for this condition. However, more recently it has been suggested that this increased intestinal absorption may be secondary to increased bone resorption of calcium. When patients with IHC are placed on a calcium-restricted diet, urine calcium excretion remains high, leading to negative calcium balance. Thus, bone resorption may be the primary event, leading to hypercalciuria with secondary increased intestinal absorption through transient elevation of PTH and vitamin D levels. Support for increased bone resorption in IHC derives from the finding of decreased bone mineral density in patients with IHC.[50–53] It has been suggested that the increased bone resorption is attributable to increased monocyte cytokine production. Cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1alpha (IL-1α), IL-1β, IL-6, and granulocyte macrophage colony-stimulating factor (GM-CSF) have been shown to be elevated.[53–55] In addition, IL-1 correlates with hydroxyproline, a marker of bone resorption. These cytokines stimulate osteoclastic bone resorption.[56] The increased bone resorption and negative calcium balance when subjects are on calcium-restricted diets argues against prescribing dietary calcium restriction for this population. Others have suggested a role for the Western diet or high protein intake in the increased urine calcium excretion and increased bone resorption. Neutralization of the Western diet by replacing bicarbonate for chloride has been shown to result in decreased metabolic acidosis, decreased cortisol concentration, and decreased markers of bone resorption.[57]

The gene for CaSR is located on chromosome 3q13.3–q21. Mutation activation of the gene mimics hypoparathyroidism and is characterized by hypocalcemia, normal PTH, and hypercalciuria. It is an autosomal dominant syndrome.[58]

Risk factors for calcium stones include high animal protein intake, low calcium and magnesium intake, low urine volume, low urine citrate excretion, and increased urine excretion of uric acid (hyperuricosuric calcium nephrolithiasis) and oxalate.

4. Treatment of Hypercalciuria (Table 7-6)

The underlying primary disorder should be evaluated and treated appropriately. Then, the primary objective in the management of hypercalciuria is to reduce urine calcium excretion. This can be accomplished through increased fluid intake, a low-sodium diet,[59] limitation of animal protein intake, maintenance of normal dietary calcium intake, and the use of thiazide diuretics. The mechanisms of hypocalciuria with thiazide diuretics include enhanced proximal paracellular calcium reabsorption as a consequence of volume depletion and stimulation of transcellular calcium transport across distal convoluted tubule cells. This latter mechanism likely has less clinical significance because decreased expression of calcium transport proteins has been documented in thiazide-induced hypocalciuria.[60] Thiazides can be administered as long-acting

Table 7-6. Drug Therapy for Nephrolithiasis.

Conditions	Treatment
Idiopathic hypercalciuria	HCTZ plus K citrate or potassium-sparing diuretic[a] Adults: HCTZ 25–50 mg/day plus K citrate 30–60 mEq/day or amiloride 5–10 mg/day Children: HCTZ 2–2.2 mg/kg/day plus K citrate 2–3 mEq/kg/day or amiloride or spironolactone Alendronate for decreased bone mineral density?
Hyperoxaluria	Vitamin B_6
Enteric hyperoxaluria	Ca and/or Mg citrate and/or K citrate Cholestyramine
Hypocitraturia	K citrate 2–3 mEq/kg/day or 30–60 mEq/day
Normocalciuria CaOx or CaP	K citrate 2–3 mEq/kg/day or 30–60 mEq/day
Hyperuricosuric CaOx stone	Allopurinol 100–300 mg/day
Uric acid stone	K citrate 2–3 mEq/kg/day or 30–60 mEq/day Allopurinol if urine uric acid is elevated
Cystine stone	K citrate to increase urine pH to 7–7.5 Tiopronin Adult: 800 mg in 3 divided doses 1 h before or 2 h after meal. Children > 9 years: 15 mg/kg/d in 3 divided doses ? Captopril 25–50 mg TID
Struvite stone	Acetohydroxamic acid 250 mg tid
Renal tubular acidosis	K citrate 2–3 mEq/kg/day or 30–60 mEq/day

[a]Combination products of HCTZ and amiloride or HCTZ and spironolactone are commercially available in the United States.

drugs such as chlorthalidone and trichloromethazide or as chlorothiazide or hydrochlorothiazide. Dosing guidelines for children are readily available for both chlorothiazide and hydrochlorothiazide. Alternatively, amiloride or combined amiloride/hydrochlorothiazide or spironolactone/hydrochlorothiazide can be used. Hypokalemia results in hypocitraturia, and therefore the administration of a potassium-sparing drug in conjunction with a thiazide or supplementation with potassium citrate may be clinically indicated. Potassium bicarbonate but not sodium bicarbonate can also be used.[61] Potassium bicarbonate has been shown to be superior to potassium chloride in reducing urine calcium excretion.[62] In adults with hypercalciuria associated with bone resorption and decreased bone mineral density, alendronate should be considered, although no large-scale controlled studies have been performed. Alendronate has been shown to reduce urine calcium excretion and supersaturation in genetic hyper-

calciuric rats.[63] In Dent's disease, chlorthalidone is effective in reducing urine calcium excretion.[64]

B. Oxalate (-$C_2O_4^{-2}$, MW 88)

1. *Oxalate Metabolism*

Oxalate is a metabolic waste product in humans. Oxalic acid is a dicarboxylic acid with two p*K*a, 1.23 and 4.19. Serum oxalate comes from two sources. Endogenous oxalate comes from glyoxylate metabolism in the liver while exogenous oxalate derives from dietary intake and intestinal absorption.

Endogenous Production of Oxalate (Fig. 7-2). Endogenous oxalate derives from several sources. Glyoxylate is the major precursor for hepatic production of oxalate and accounts for 50% to 70% of endogenous oxalate production. Glyoxylate is produced either through the oxidation of glycolate by glycolate oxidase (GO) or through the oxidative deamination of glycine by D-amino acid oxidase. The major route of metabolism of glyoxylate is via transamination to glycine through the enzyme alanine-glyoxylate aminotransferase (AGT). Glyoxylate is also converted to glycolate by glyoxylate reductase (GR). These metabolic pathways regulate the amount of glyoxylate available for conversion to oxalate. The second source of oxalate is ascorbic acid, which contributes

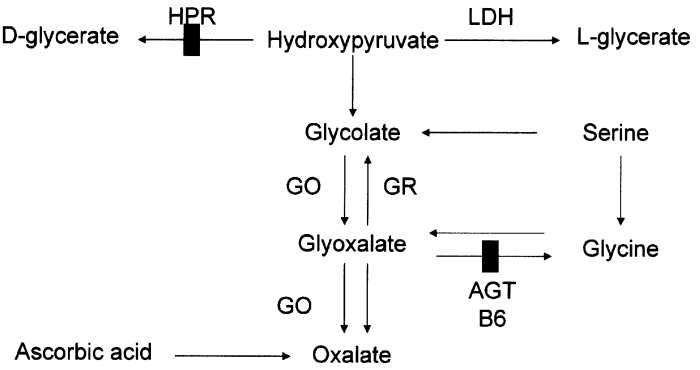

Figure 7-2. Oxalate metabolism.

up to 30% to 50% of endogenous oxalate. Ascorbic acid turnover to oxalate is saturated at 200 mg/day.[65] The impact of ascorbic acid supplementation on endogenous oxalate production remains controversial owing to difficulties in measuring urinary oxalate in the presence of significant amounts of ascorbate. This is reviewed in further detail later.

2. Intestinal Handling of Oxalate

In normal individuals, dietary oxalate contributes significantly to urine oxalate. Holmes et al. have shown that on a 10 mg/day oxalate diet, 24% of urinary oxalate can be attributed to intake of oxalate.[66] On a 250 mg/day oxalate diet, as much as 42% of urine oxalate is derived from intake. In normal individuals, only 10% to 20% of dietary oxalate is absorbed in the gastrointestinal tract. The rest is degraded by enteric bacteria or excreted unchanged in the stool. However, in stone formers, oxalate absorption is increased when compared to non-stone formers, and it appears that ascorbic acid enhances oxalate absorption.[67] Oxalate absorption in the gastrointestinal tract occurs largely in the small intestine. Intestinal absorption is dependent on the amount of free oxalate present. When calcium or magnesium is also present in the intestinal lumen, oxalate complexes to these cations, with less free oxalate available for absorption.[68] Specifically, with calcium intake of 200 mg/day, intestinal oxalate absorption is 17%; however, with calcium intake of 1200 mg/day, intestinal oxalate absorption is only 2.6%.[69] Intestinal oxalate absorption is also increased in patients with malabsorption of fat and bile acids, as occurs with Crohn's disease or jejuno–ileal bypass.

Renal Handling of Oxalate. Oxalate is excreted primarily through the kidney. It is freely filtered at the glomerulus. Both reabsorption and secretion occur in the proximal tubule via exchange with sulfate and chloride, respectively, at the apical membrane. On the basolateral side, oxalate is exchanged for sulfate or cotransported with sodium. As noted previously, it appears that renal secretion of oxalate can be enhanced in hyperoxaluric subjects.

3. Causes of Hyperoxaluria

The causes of hyperoxaluria are listed in Table 7-7. The primary hyperoxalurias (PHs) are caused by autosomal recessive defects resulting in overproduction of hepatic oxalate (Fig. 7-2). The prevalence of these conditions is estimated to be between 1.04 and 2 per million.[70] PH accounts for approximately 0.5% of children with end-stage renal disease reported in the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) (Talley L, Stablein DM. NAPRTCS Annual Report, 2004). As noted earlier, the hepatic peroxisomal enzyme AGT is responsible for conversion of glyoxylate to glycine. In primary hyperoxaluria type 1 (PH1), a reduction or absence of AGT activity leads to increased glyoxylate levels with increased conversion

Table 7-7. Causes of Hyperoxaluria.

Increased endogenous oxalate load
Primary hyperoxaluria
Type I Hepatic alanine:glyoxylate aminotransferase deficiency
Type II Hepatic glyoxylate reductase/hydroxypyruvate reductase deficiency
Ascorbic acid
Vitamin B_6 (pyridoxine) deficiency
Increased exogenous oxalate load
Increased intestinal absorption
High oxalate intake
Low calcium or magnesium intake
Enteric hyperoxaluria
Lack of colonic oxalate-degrading bacteria (*Oxalobacter formigenes*)
Idiopathic

to oxalate in hepatic peroxisomes via glycolate oxidase and in cytosol via lactate dehydrogenase. Administration of pyridoxine, a cofactor for AGT, can enhance residual AGT activity in some affected patients. Excess glyoxylate is also converted to glycolate by glyoxylate reductase; thus increased urine glycolate excretion has been utilized as a diagnostic marker in PH1. More than 50 mutations of the AGT gene on chromosome 2q37.3 have been shown to cause PH1.[71,72] PH1 can be definitively diagnosed by liver biopsy with evaluation of AGT activity and immunoreactivity. Such testing is performed only at selected centers.

Primary hyperoxaluria type 2 (PH2) is characterized by hyperoxaluria with increased *L*-glycerate excretion and normal glycolate excretion. In this condition, patients lack glyoxylate reductase and hydroxypyruvate reductase activity, resulting in decreased conversion of hydroxypyruvate to *D*-glycerate. The excess hydroxypyruvate is converted to *L*-glycerate and excreted in the urine. Defective conversion of glyoxylate to glycolate results in increased production of oxalate with subsequent clinical features of hyperoxaluria. Mutations in glyoxylate reductase/hydroxypyruvate reductase (GRHPR) have been localized to chromosome 9.[73] To date, 14 mutations of the GRHPR gene have been identified.

The clinical course of the primary hyperoxalurias is characterized by calcium oxalate stone formation or nephrocalcinosis, often with onset in childhood. As noted earlier, both PH1 and PH2 are autosomal recessive. Nearly half of patients with PH manifest symptoms by 5 years of age. However, the spectrum of disease is variable. Moreover, there is poor correlation between disease severity and either genotype or liver biopsy, even within families.[74] With normal renal clearance, excess oxalate is excreted in the urine. However,

with declining renal function to less than 50% of normal due to calcium oxalate deposition, plasma oxalate levels begin to rise and calcium oxalate deposits in multiple organs. Systemic oxalosis can result in metabolic bone disease, anemia, cardiomyopathy, cardiac conduction defects, and skin ulcerations. Long-term renal prognosis is generally poor, with a median age at end-stage renal failure of 25 years.[75] PH1 appears to be more common and more severe than PH2,[76] although patients with both types may progress to end-stage renal disease (ESRD).[77]

The diagnosis of PH depends on the demonstration of increased urine oxalate excretion, which typically exceeds 100 mg/day and ranges up to 400 mg/day. In the absence of a known family history of PH, a detailed history and physical examination should help to exclude patients with enteric hyperoxaluria. Reduction of dietary oxalate with documentation of a reduction in urine oxalate excretion can be helpful in this regard. Additional urine testing may reveal increased urinary glycolate excretion in patients with PH1 or increased urinary glycerate excretion in patients with PH2. However, neither test is 100% sensitive; urine glycolate is increased in only two thirds of patients with PH1 while increased urine glycerate has been found in most patients with known PH2. Recent reports have found that screening for the three most common mutations of AGT (c.508G>A, c.33_34insC, c.731T>C) and the most common mutation of GRHPR (c.103delG) could identify 34% of PH1 and 25% of PH2 cases previously confirmed by liver biopsy. Thus, DNA analysis might prove to be a useful tool in the future evaluation of some patients with suspected PH. At this time, however, liver biopsy with enzymatic studies remains the definitive test for diagnosis.[78]

Therapy of PH is aimed at reducing urine oxalate excretion and increasing the solubility of calcium oxalate in the urine. General guidelines including increased fluid intake and avoidance of high-oxalate foods should be prescribed. The only pharmacologic intervention known to reduce oxalate production in patients with PH1 is pyridoxine supplementation. As noted previously, pyridoxine is a cofactor for AGT-mediated conversion of glyoxylate to glycine. Approximately one third of patients with PH1 will demonstrate a significant reduction in urine oxalate excretion with pyridoxine treatment.[79,80] The dose is 1 to 10 mg/kg per day, keeping in mind that high-dose pyridoxine can result in peripheral neuropathy. Potassium citrate supplementation has also been shown to reduce calcium oxalate supersaturation in a short-term study of five children with PH.[81] However, long-term studies of potassium citrate treatment in PH are not available. Milliner et al.[80] have advocated the use of orthophosphate for treatment of PH. This drug is known to (1) reduce intestinal calcium absorption via suppression of calcitriol production with resultant decreased urine calcium excretion; (2) increase excretion of pyrophosphate, which is an inhibitor of calcium crystallization[82]; and (3) decrease calcium oxalate supersaturation by

increasing the amount of urinary phosphate available to bind to calcium. Such therapy has been proposed to delay progression to ESRD in PH.[80]

The definitive treatment for PH1 is liver transplantation. Although the kidney is the organ most damaged in this condition, the liver is the source of excess oxalate production. The donor liver provides functional enzyme and thus oxalate metabolism is normalized. The native liver must be removed at the time of transplant. Isolated renal transplantation addresses the end-stage renal disease, but with continued hepatic overproduction of oxalate, the renal transplant is at risk for progressive loss of function. Survival of the renal transplant in this setting has been reported to vary from 20% after 3 years[83] to 50% at 6 years.[84] It has been suggested that isolated renal transplantation is most appropriate for those patients with pyridoxine-responsive PH1 while combined liver–kidney transplantation should be considered for pyridoxine-unresponsive patients.[85,86] Patients with ESRD undergoing liver–kidney or kidney transplantation require extensive hemodialysis to reduce the renal oxalate burden perioperatively. Studies are in progress to evaluate the feasibility of both transfection of AGT into hepatocytes and hepatocyte transplantation.[87,88]

4. Enteric Hyperoxaluria

As noted previously, hyperoxaluria and increased oxalate stones are seen in patients with inflammatory bowel diseases, small bowel resection, and small bowel bypass. It is now clear that small bowel bypass is associated with the most extreme hyperoxaluria. In contrast, small bowel resection is associated with modest increases in urine oxalate.[89] In small bowel resection, the length of bowel removed appears to be important. When the total resection exceeds 30 cm, hyperoxaluria occurs.[90,91] Colon surgery alone is associated with decreased urine volume and pH, factors that increase risk for uric acid nephrolithiasis. Several mechanisms have been proposed for hyperoxaluria in patients with small bowel bypass and small bowel resection. First is the decreased availability of intestinal calcium and magnesium. When steatorrhea is present, dietary calcium is bound by free fatty acids and bile salts in the intestinal lumen, leaving less calcium available to bind oxalate. Under this circumstance, intestinal absorption of oxalate is increased, resulting in hyperoxaluria. Second, with small intestine malabsorption, increased delivery of bile acids and free fatty acids to the colon injures colonic mucosa, thus enhancing oxalate permeability and absorption in the colon.[92,93] Third, such patients often have decreased colonization with oxalate-degrading bacteria, resulting in an increase in bioavailable oxalate.[94] These findings support the concept that an intact colon is essential for hyperoxaluria to develop in patients with small intestine resection or bypass.

The effect of vitamin C on urine oxalate excretion is controversial. In normal individuals, administration of vitamin C has been reported to be

associated with increases in urine oxalate of 20% to 56%[95,96] or with no change despite supplementation with ascorbic acid 2 to 4 g/day.[97,98] In seven healthy volunteers, ascorbic acid 1000 mg/day was associated with increases in both urine oxalate and uric acid excretion.[95] In contrast, a prospective study of a large cohort of women with no history of nephrolithiasis receiving more than 1500 mg/day ascorbic acid showed no increased stone risk.[99] In stone formers, urine oxalate excretion is reported to increase by 20% to 60% with vitamin C supplementation.[96,100] Some of these discrepancies may be explained by difficulties with the chemical assay for oxalate in the presence of large amounts of ascorbate. Specifically, ascorbate is believed to undergo nonenzymatic conversion to oxalate in vitro during timed urine collection.[101] This conversion appears to be pH-dependent. At a urine pH of 10, 40% of ascorbate is converted to oxalate while at a urine pH of 8, 20% is converted.[102] Despite these conflicting results, the current consensus is that ascorbic acid supplementation may increase urine oxalate, but a dose not exceeding 1 g/day is probably acceptable.[95] Mega-dose ascorbic acid has also been shown to increase urine uric acid excretion,[103] with 4 g/day ingestion of ascorbic acid leading to an increase in uric acid clearance of 200%. At this time, it is not clear whether this observation is associated with uric acid nephrolithiasis.

Treatment of enteric hyperoxaluria (Table 7-6) should focus on minimizing dietary intake of oxalate-rich foods such as rhubarb, spinach, soy products, tofu, nuts, etc., and increasing fluid intake. Although the effect of excessive protein intake on urine oxalate excretion requires further study, it is reasonable to restrict animal protein intake to normal amounts in patients with enteric hyperoxaluria. In addition, these patients have low urine calcium, magnesium, and citrate excretion. Therefore, supplementation with calcium citrate, magnesium citrate, and/or potassium citrate is appropriate. Low-calcium diets have been associated with increased urine oxalate excretion[66,104] and thus should be avoided. As noted previously, this has been attributed to decreased availability of calcium to bind to free oxalate in the intestine. Decreased magnesium intake contributes to enteric hyperoxaluria by the same mechanism. Cholestyramine, an anion-exchange resin, can also be used to bind oxalate. Patients with chronic diarrhea may demonstrate chronic hypocitraturia and low urine pH and may benefit from citrate supplementation.

C. Uric Acid ($C_5H_4N_4O_3$, MW 168)

Uric acid is the end product of purine metabolism in many mammals including humans. In birds and reptiles, uric acid is oxidized further to allantoin in the liver by the enzyme uricase, which is not present in humans. Allantoin is a more soluble product. Uricase is now available for clinical use and has been used successfully to prevent uric acid nephropathy in patients with tumor

lysis syndrome. Uric acid from exogenous and endogenous sources is excreted by the gastrointestinal tract and the kidney. The renal fractional excretion of uric acid is less than 10%. It is unclear why a metabolic waste product is 90%-retained by the kidney. Uric acid is only 5% bound, so most of the plasma uric acid is filtered at the glomerulus. Thus, secretion of uric acid is anticipated to be of small magnitude. Both reabsorption and secretion of uric acid take place in the proximal tubule with no further tubular handling of uric acid beyond this site. Recently, the urate transporter URAT1 has been identified in the luminal membrane of the proximal tubule. URAT1 acts as an antiporter, exchanging urate for intracellular anions such as chloride ion. Mutations in URAT1 are associated with idiopathic hypouricemia syndrome, with a marked increase in uric acid excretion and exercise-induced acute renal failure, presumably owing to tubular deposition of uric acid. The luminal presence of probenecid, phenylbutazone, salicylic acid, indomethacin, or losartan inhibits urate transport by URAT1, resulting in increased urine uric acid excretion. The solubility of uric acid in the urine is volume- and pH-dependent. At a pH of less than 5.5, 2 L of urine are required to dissolve 600 mg of uric acid while at a pH of 6.5, only 1 L is required to dissolve the same amount of uric acid.

Several factors are known to contribute to uric acid nephrolithiasis via increased urine uric acid excretion and/or decreased solubility of uric acid in urine (Table 7-8). Clinically, uric acid nephrolithiasis is seen in patients with gout associated with uric acid overproduction, chronic diarrhea, high animal protein intake, and occasional patients with very low urine pH. In chronic diarrhea, the combination of volume depletion, systemic acidosis with low urine pH, and low citrate excretion is responsible for uric acid stone formation. In patients with high animal protein intake, the increased urine uric acid content

Table 7-8. Factors Contributing to Uric Acid Nephrolithiasis.

Decreased urine solubility of uric acid
Excessive acidity of urine (pH < 5)
Increased urine uric acid content
Overproduction of uric acid
Enzyme deficiency or overactivity
Hypoxanthine guanine phosphoribosyl transferase deficiency
Phosphoribosyl pyrophosphate synthetase overactivity
Glucose-6-phosphatase deficiency
Myeloproliferative diseases
Primary gout (10%)
Decreased tubular reabsorption of uric acid
Isolated hypouricemia
Fanconi syndrome

from increased exogenous purine intake and decreased urine citrate excretion are likely responsible.

The mechanism of low acidity in some patients with uric acid stones is not clear. In normal individuals, urine ammonium acts as a buffer to increase urine pH. Thus, an ammonium defect may contribute to the very acidic urine. Ammonium defects may result from decreased ammoniagenesis by the proximal tubule, decreased transport across the medullary thick ascending limb, or decreased dissociation of NH_4 into NH_3 and H^+ in the medullary interstitium, so that less NH_3 is available to bind H^+. In a study of 14 patients with uric acid nephrolithiasis and a low urine pH less than 5.5, 10 of the patients had a low rate of ammonium excretion with normal citrate excretion.[105] The authors postulated that an alkaline proximal tubular cell pH may be responsible. Decreased ammonium excretion in patients with uric acid stones was confirmed by Sakhaee et al.[106] In addition, they found that more than half of patients have either diabetes mellitus or glucose intolerance. Insulin has been shown to stimulate ammoniagenesis in canine renal tubular cells incubated with glutamine at pH 7.5.[107] Insulin has also been shown to stimulate the sodium–hydrogen exchanger NHE3, which is responsible for ammonium transport across the proximal tubular cell where NH_4 replaces H^+.[108] Decreased insulin sensitivity or increased insulin resistance could result in decreased ammoniagenesis and transport across the proximal tubular cells. In addition, a defect in sodium-bicarbonate cotransport on the basolateral membrane may result in an alkaline pH of the proximal tubular cells.

Prevention of uric acid stones is based on the pathogenetic mechanism (Table 7-6). If it is caused by overproduction of uric acid with subsequent increased urine uric acid excretion, allopurinol is effective. A dose of 100 to 300 mg/day is usually sufficient in adults. If it is due to decreased urine volume, increased fluid intake to produce 2 to 2.5 L of urine per day is effective. Finally, if it is due to acidic urine, administration of oral bicarbonate or citrate to maintain a urine pH of 6 to 6.5 is indicated. In patients who consume high quantities of animal protein, reduction of protein intake to one meat meal 5 to 6 times per week is effective. For existing stones, alkalinization of the urine is highly effective in dissolving uric acid stones. Even if the stone is causing ureteral obstruction, placement of a ureteral stent followed by alkalinization has been successful.

D. Hyperuricosuric Calcium Nephrolithiasis (HUCN)

In patients with hyperuricosuria, defined as urine uric acid greater than 700 mg per day in women or greater than 800 mg per day in men, two types of renal stones are commonly seen, including calcium oxalate stones and uric acid stones. Patients who form calcium stones differ from those who form uric acid

stones. The former group (HUCN) has normal serum uric acid concentration, higher urine uric acid excretion, and higher urine pH compared to the latter group.[109] Calcium oxalate stone formers may also demonstrate hypercalciuria. In HUCN, increased urine uric acid excretion raises the "threshold" for calcium oxalate supersaturation and is the mechanism responsible for calcium oxalate stone formation.[110,111] Such patients are likely to have high protein and purine intake which contributes to the increase in urine uric acid excretion. In contrast, low urine pH is responsible for those who form uric acid stones. HUCN patients can be treated by reduction of protein and purine intake and administration of allopurinol and/or potassium citrate. In those forming uric acid stones, treatment with potassium citrate to increase urine pH is highly effective.

E. Cystine ($C_6H_{12}N_2O_4S_2$, MW 240)

Cystinuria is an autosomal recessive disorder of renal tubular amino acid transport and accounts for approximately 10% of nephrolithiasis in children. The condition is characterized by impaired transport of the amino acids cystine, ornithine, lysine, and arginine in the proximal tubule because of a defective subunit of the transporter molecule. At least three specific genetic mutations have been identified on chromosomes 2 and 19. Of these amino acids, only cystine results in clinical disease, due to its poor solubility at urine pH between 5 and 7. The disease is characterized by onset of nephrolithiasis early in life, with more than 80% of patients developing stones within the first two decades of life.[112] Affected males appear to have more severe disease with earlier onset of nephrolithiasis and increased stone recurrence compared to females.[113] The reasons for this are unclear. Recurrent nephrolithiasis with its complications is the only clinical manifestation of cystinuria.

Hexagonal cystine crystals in the urine are diagnostic for this condition. However, such crystals are detected in only 20% to 25% of urine samples of affected patients. Thus, timed urine collection for cystine excretion is the preferred diagnostic test, with homozygotes excreting more than 1300 μmol/g creatinine (150 μmol/mmol) cystine.[114] If timed urine collection is not possible or practical, the random urine cystine/creatinine ratio can be utilized to distinguish homozygous or compound heterozygous cystinuric patients from heterozygous carriers.[115] Cystine stones are poorly radioopaque and thus may be difficult to diagnose by plain radiograph. However, the high frequency (60%) of recurrent stones makes stone analysis a helpful component of clinical evaluation.

The goal of treatment of cystinuria is to increase the solubility of urinary cystine and thus prevent further stone formation and its associated complications. General measures should be instituted, including increased fluid intake, a low-salt diet, and appropriate urinary alkalinization. Therapy goals with

increased fluid intake include a urinary flow rate greater than 1.5 L/m^2 per day and a urinary cystine concentration less than 250 mg/dl. This frequently translates to fluid intake of approximately 3 L/day for children and 4 to 5 L/day for adults. Fluid intake should be distributed throughout the day and night, as urine cystine concentration is maximal during the night. Affected patients should be encouraged to drink before going to bed, as well as during the night following micturition.[116,117] Reduction of dietary intake of methionine, which is metabolized to cystine, may be mildly helpful in older patients. However, strict protein restriction is not recommended in children for whom adequate protein intake is necessary for normal growth. A low-salt diet (e.g., 2 g/day in adolescents and adults) has been shown to reduce urine cystine concentration in both children and adults.[118,119]

Urine alkalinization to pH 7.5 or greater is known to improve the urinary solubility of cystine and is an important component of the management of affected patients. Potassium citrate should be provided in two to three doses per day to maintain urine pH 7.5 to 8. Potassium citrate is preferable to sodium citrate or bicarbonate treatment, as the sodium load associated with the latter contributes to increased urinary cystine excretion. Citrate also interferes with calcium oxalate crystallization, which may occur in some patients. Excessive urine alkalinization is to be avoided, however, as it increases the risk of calcium phosphate stone formation.

Should these measures fail to control recurrent nephrolithiasis in cystinuric patients, treatment with *D*-penicillamine or α-mercaptoproprionyl glycine (MPG) (Tiopronin) should be considered.[120] Both drugs cleave the disulfide bond of cystine into cysteine, which is 50 times more soluble than cystine. However, both drugs are associated with frequent side effects. Specifically, side effects occur in up to 50% of patients receiving *D*-penicillamine, including fever, rash, nephrosis, pancytopenia, hypoguesia, epidermolysis, and pyridoxine deficiency. Side effects of MPG include rash, arthralgia, pemphigus, thrombocytopenia, polymyositis, proteinuria, and nephritic syndrome. Side effects of MPG are seen in nearly 75% of treated patients but appear to be less severe than those associated with *D*-penicillamine.

Administration of captopril, a first-generation angiotensin converting enzyme inhibitor that contains free sulfhydryl groups, has been proposed to enhance urinary cystine solubility via formation of captopril–cystine complexes. However, conflicting results have been observed in clinical studies.[121–123] Nonetheless, the use of this drug in combination with MPG may be considered in refractory cases.

Most patients with cystinuria will require multiple urologic interventions for stone removal. Shock wave lithotripsy is a relatively noninvasive treatment that is often effective for patients with upper tract cystine stones measuring less than 1.5 cm. This technique appears to be particularly useful in children

with cystinuria.[124,125] However, it should be noted that, in contrast to adults, most children with cystinuria will require general anesthesia for this procedure. Surgical options for the management of cystinuria include ureteroscopy, percutaneous nephrolithotomy, and open surgery. Ureteroscopy is of particular value for stones located in the distal ureter or for fragments refractory to prior shock wave lithotripsy treatment. Percutaneous lithotomy is recommended for stones exceeding 1.5 to 2 cm in size including some staghorn calculi. Open surgery is rarely indicated in adults but may have a role in the management of stone disease in children with cystinuria, particularly in children with complex staghorn stones or abnormal renal anatomy.[126]

F. Infection-Related Stones

Infection-related stones form as a consequence of urinary tract infection associated with urea splitting organisms and frequently occur in the setting of urinary tract obstruction or stasis. Such calculi are composed of magnesium ammonium phosphate (struvite) and carbonate apatite. Occasionally, ammonium urate stones also occur. The term "struvite" derives from the name of Russian diplomat and naturalist von Struve and was coined by the Swedish urologist Ulex. As mentioned previously, risk factors associated with infection stones include urinary tract obstruction, neurogenic bladder, diabetes mellitus, multiple sclerosis, and urinary diversion. The stone usually branches into a staghorn formation, taking the configuration of the renal calices and pelvis. Urease-producing bacteria associated with struvite stones include *Proteus* (70%), *Klebsiella*, *Serratia*, *Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Candida*, and *Mycoplasma*. These organisms hydrolyze urea into ammonium, which complexes with magnesium and phosphate into the struvite stone. The optimal urine pH for such stone formation is 7.2. At a lower urine pH of 6.8, carbonate apatite is formed, and it is not uncommon to see the combination of magnesium ammonium phosphate and carbonate apatite in a stone. Patients with nephrolithiasis caused by metabolic disorders may also develop secondary infection. Therefore, the combined findings of infection and nephrolithiasis do not always indicate a struvite stone. Such patients should undergo appropriate metabolic workup.

Treatment of infection stones requires complete elimination of the stone, appropriate antibiotic treatment to eradicate the organism, and measures to prevent recurrence of infection and stone formation. Urinary tract obstruction must be relieved, and urinary stasis must be avoided by frequent self-catheterization if necessary. Inhibition of urease slows the growth of existing stones and reduces the risk of new stone formation. Acetohydoxaminic acid (Lithostat), a urease inhibitor, can be utilized for this purpose but is associated with significant side effects including liver and bone marrow toxicity. For

adults, the dose is 250 mg three times a day not to exceed 1.5 g/day, and in children older than 6 years of age, the recommended dose is 10 mg/kg per day divided into three or four doses. The drug is contraindicated in renal failure. Acidification of urine with high-dose vitamin C is not warranted because of its ineffectiveness and its potential to increase urine oxalate excretion.

G. Hypocitraturia (Citrate $C_6H_5O_7^{2-}$, MW 189)

Citric acid is a tricarboxylic acid with pK_a of 2.9, 4.3, and 5.6. It exists as citrate^{1-}, citrate^{2-}, and citrate^{3-}. At the physiologic pH of blood and urine, it exists predominantly as citrate^{3-}. It is filtered and taken up by proximal tubular cells both by luminal reabsorption (75%) and by peritubular uptake (10% to 30%). There is no further reabsorption beyond the proximal tubule, and there is no evidence of tubular secretion. Proximal tubular reabsorption of citrate is strongly influenced by acid–base changes, particularly cell pH. Citrate reabsorption occurs via electrogenic cotransport with sodium as 3Na-citrate^{2-}. In metabolic acidosis, citrate reabsorption is increased, resulting in decreased urine citrate excretion. The mechanisms responsible for this increased citrate reabsorption include: (1) increased acidity of the lumen, resulting in more citrate^{2-} than citrate^{3-} available for transport and (2) more intracytoplasmic citrate utilization by mitochondria resulting in diminished cellular citrate, which facilitates citrate reabsorption. Alkalosis causes citraturia by opposite mechanisms. In addition to acidosis and alkalosis, other factors may also affect urinary citrate excretion. Chronic potassium depletion is associated with hypocitraturia owing to decreased proximal sodium-citrate transport;[127] this is thought to be the consequence of low cell pH associated with potassium depletion. Women tend to have higher urine citrate excretion than men. Older adults tend to have higher urine citrate excretion than younger adults. If bacterial overgrowth is not prevented during timed urine collection, bacteria may use the citrate for metabolism, resulting in lower measured urinary citrate concentration. Factors associated with decreased urine citrate are shown in Table 7-9.

Table 7-9. Factors Associated with Low Urinary Citrate Excretion.

Age—infants
Systemic acidosis
Chronic potassium depletion
Starvation
Renal failure
Bacteriuria
Drugs—acetazolamide, ammonium chloride, ethacrynic acid?

Hypocitraturia has been documented in several groups at risk for stone formation, including patients with distal renal tubular acidosis, chronic diarrhea, and high animal protein intake. In addition, hypocitraturia can be associated with other metabolic abnormalities such as idiopathic hypercalciuria, hyperuricosuric calcium nephrolithiasis, and enteric hyperoxaluria.[128] In patients without other metabolic causes, hypocitraturia is present in 20% of patients with calcium nephrolithiasis.

Administration of oral citrate increases serum and urine citrate concentrations.[129] After ingestion of 40 mEq of citric acid, serum citrate concentration is significantly increased within 30 minutes and remains elevated for 3 hours. Urine citrate excretion peaks at 2 hours post-ingestion. With chronic citrate administration, it is very likely that the alkaline urine pH contributes to citraturia. Citrate supplementation has been shown to reduce the occurrence of calcium stones in hypocitraturic calcium stone formers in both randomized controlled[130] and uncontrolled studies.[131] In addition, in IHC patients who are poorly responsive to thiazide treatment, the addition of potassium citrate has been shown to improve outcome.[132] In this study, citrate given at a dose of 60 mEq increased urine pH, increased urine citrate excretion, and decreased stone formation. Concerns that long-term use of combined thiazide and potassium citrate therapy may result in systemic alkalosis have not been substantiated.[133] Treatment with potassium bicarbonate, but not potassium chloride, has been shown to be as effective as potassium citrate in increasing urine citrate and urine pH.[134]

Indications for citrate treatment include renal tubular acidosis, idiopathic hypocitraturic calcium oxalate nephrolithiasis, chronic diarrhea, uric acid lithiasis, thiazide-induced hypocitraturia, and enteric hyperoxaluria. These are conditions associated with low urine pH and low urine citrate excretion. As noted previously, citrate can also be used in patients with cystinuria for urinary alkalinization. Citrate is available in numerous forms. Polycitra® K contains 67 mg/ml of citric acid and 220 mg/ml of K citrate. Urocit® K contains 540 (5 mEq) or 1080 mg (10 mEq) K citrate.

H. Drug-Induced Nephrolithiasis

Drugs may cause nephrolithiasis by two mechanisms. First, the drug may induce metabolic alterations in the blood or urine that predispose to stone formation. Second, the high urine concentration of the drug itself or its metabolic end-product(s) can be responsible for stone formation. Furosemide and acetazolamide are examples of the former. Furosemide by its calciuric effect causes calcium oxalate stone in low-birth-weight infant receiving this drug.[135,136] It is unclear why this particular population is susceptible because this finding is rare in adults receiving furosemide treatment. Carbonic anhydrase inhibitors cause

systemic acidosis and increase urine pH. As noted previously, systemic acidosis is associated with decreased urine citrate excretion. This combination of low citrate and high urine pH is responsible for calcium phosphate stones associated with the use of carbonic anhydrase inhibitors. The increased stone risk was first reported with acetazolamide used for long-term treatment of glaucoma.[137,138] Recently two other carbonic anhydrase inhibitors, topiramate and zonisamide, have also been shown to cause calcium phosphate nephrolithiasis.[139,140] These two drugs have been primarily utilized as antiseizure medications. The use of the antacids aluminum hydroxide and magnesium hydroxide has been reported to increase stone risk. The mechanism is believed to be an increase in urine calcium excretion during phosphate depletion.

Drugs that directly induce nephrolithiasis include ceftriaxone, ciprofloxacin, ephedrine, indinavir, magnesium trisilicate, sulfadiazine, sulfamethoxazole, and triamterene. Ceftriaxone is widely used for treatment of bacteremia and serious bacterial infection in children. Avci et al.[141] have recently described the evolution of nephrolithiasis in 4 of 51 children treated with intravenous or intramuscular ceftriaxone for 1 week. In each case, identified stones were small (2 mm). In three of four cases, nephrolithiasis resolved spontaneously within 4 weeks. The stones were not analyzed, and no clinical sequelae of stone formation were noted. Ciprofloxacin stones have been reported by Chopra et al.[142] Ephedrine stones have been described in patients taking the herbal supplement ma-huang, which contains ephedrine, and in patients taking cough medications.[143,144] Indinavir is used to treat patients with human immunodeficiency virus (HIV). It can cause indinavir crystalluria, acute renal failure, and indinavir stones. Silicate stones have been reported in patients taking magnesium trisilicate for gastroesophageal reflux disease.[145] Sulfadiazine is well known to cause crystalluria and acute renal failure. Sulfadiazine nephrolithiasis is rare but has been reported both in adults and children.[146,147] Sulfamethoxazole has been reported to cause urolithiasis and ureteral obstruction.[148] Triamterene, a potassium sparing diuretic, is well known to cause triamterene stones.[149,150] Of drug-induced stones, indinavir and triamterene are the most common. Kidney stones caused by the aforementioned are radiolucent and are usually unsuspected until stone analysis is performed. Management includes either stopping the offending drug or increasing urine output to more than 2 L per day, especially in the case of indinavir.

I. Prognosis

The prognosis for nephrolithiasis depends on whether an underlying metabolic disorder is present. Specific etiologies of nephrolithiasis with treatment and outcome have been described in the preceding text. The prognosis for

patients with idiopathic calcium oxalate or calcium phosphate stones is generally good. During acute episodes of stone passage, the patient is likely to cope with acute pain, hematuria, and possibly urinary tract obstruction. However, once the stone has passed spontaneously, which is usually the case for stones measuring less than 5 mm, or it has been removed through urologic surgery, the patient is likely to be symptom-free until the next recurrence. Occasional patients may develop urinary tract infection, which may be recurrent especially in patients with structure abnormalities of the urinary tract. Loss of kidney function occasionally occurs in patients with chronic nephrolithiasis. The three major causes of kidney loss include stone burden, recurrent infection, and recurrent obstruction.[151] Fortunately, progressive loss of renal function due to chronic nephrolithiasis is not common. Jungers has reported an incidence of nephrolithiasis-related ESRD of 3.2% from a total of 1391 consecutive adult patients beginning maintenance dialysis therapy.[152] Conditions that are more likely to be associated with progressive renal failure include hereditary stone diseases such as primary hyperoxaluria, cystinuria, and Dent's disease; primary struvite stones; recurrent urinary tract infection associated with structural abnormalities and spinal cord injury; and nephrocalcinosis.[151–153] In patients with a single remaining kidney as a consequence of kidney loss associated with nephrolithiasis, the rate of loss of kidney function based on age was not different from that of women with two kidneys or men with a single kidney who are older than 45 years of age. The rate of loss was higher among younger men with a single kidney.[151] The recent report of papillary calcification, fibrotic changes, and cortical tubular atrophy in renal biopsy specimens obtained during nephrostomy in patients with calcium phosphate (brushite) stones suggests that with time and recurrent stones, impaired kidney function can be expected[10] (see section on pathogenesis). Finally, the role of repeated extracorporeal shock wave lithotripsy (ESWL) on long-term renal function remains unclear. Short-term studies in pigs and humans have shown a transient decrease in glomerular filtration rate and renal blood flow accompanied by tissue damage.[154,155] Permanent damage may also occur.[156]

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