Abstract:

Purpose of Review: Calcium is an essential ion for the maintenance of normal bone health and physiologic functions. The extracellular and intracellular levels of calcium are maintained through hormonal regulation called homeostasis. Balance, the net intake minus excretion of calcium, is maintained by hormonal regulation of intestinal absorption and fecal/urinary excretion. In individuals without kidney disease, homeostasis and balance are connected, but in patients with CKD, this connection is lost. The purpose of this review is to understand how calcium homeostasis and balance is impaired in CKD.

Recent findings: Two formal calcium balance studies have found that an oral intake of 800 to 1000 mg of calcium in adults with CKD leads to neutral calcium balance, whereas amounts greater than that lead to positive calcium balance. In patients with CKD, the main determinant of positive calcium balance is the intake and the lack of urinary calcium excretion.

Summary: The maintenance of calcium homeostasis is impaired in CKD. Thus, the oral intake of calcium in the form of diet and binders should be less than 800 to 1000 mg per day in order to achieve neutral calcium balance in patients with CKD stages 3b/4.

Key Words: Calcium, homeostasis, balance, phosphate binders, calcification
Introduction:

Calcium homeostasis versus balance:

Calcium homeostasis refers to the regulation of blood and intracellular calcium levels. The intracellular calcium level is maintained in a very narrow range, as changes in these levels are important for cell signaling, mitochondrial function, and gene transcription. Similarly, the ratio of extracellular (intravascular) calcium levels to intracellular calcium is critical in membrane potential which is key in cardiomyocyte and skeletal muscle contractility. In contrast, calcium balance refers to the net calcium intake minus total calcium output. In an ideal situation, homeostasis and balance are linked, but in the setting of CKD, the linkage is impaired by a combination of abnormal renal handling of calcium, hormonal dysregulation, and exogenous intake of pharmacologic treatments that alter calcium homeostasis or balance. Thus, homeostasis and balance must be considered separately in order to understand the overall calcium health of CKD patients. Importantly, this disconnect in CKD also means that blood levels of calcium do not predict calcium balance, and calcium balance cannot predict blood levels of calcium.

Measurement of calcium levels in blood:

The goal of calcium homeostasis is to maintain blood levels of calcium at appropriate levels for physiologic function. The assessment of calcium measurement of blood calcium levels is limited by the fact that less than 1% is located in the extravascular space and that the total calcium measured in blood samples reflects both the physiologically active ionized calcium and that calcium bound to other anions and albumin. Unfortunately, the formulas for ‘correcting’ calcium are notoriously inaccurate in CKD[1] and in hemodialysis patients[1] in part due to differences in albumin assays[2].
The primary regulator of calcium levels is the calcium sensing receptor (CaSR) located on tissues with involvement in calcium handling including parathyroid glands, bone, kidney and many others. In health, the CaSR allows for minute-to-minute regulation of calcium levels. In the parathyroid gland normal or high calcium levels lead to the CaSR induced cell signaling that keeps the pre-packaged PTH in an ‘do not release’ setting. When the blood levels of ionized calcium drop PTH is released[3]. The CASR gene is a member of the super-family of G-protein coupled receptors. Over 400 mutations in the gene have been identified throughout the receptor (http://www.casrdb.mcgill.ca/web site). The more severe phenotypes of autosomal dominant familial hypocalciuric hypercalcemia and autosomal recessive neonatal severe hyperparathyroidism are due to inactivating mutations; activating mutations can lead to autosomal dominant hypocalcemia (reviewed in[4]). Single nucleotide polymorphisms (SNPs) in the CASR gene were responsible for 16.5% of the variance in serum ionized calcium in an Italian cohort[5]. In one genome wide association study (GWAS), the SNP rs1801725 was associated with 1.26% of the serum calcium variance in European Americans[6]. Our group also found this same SNP to be associated with serum calcium and baseline PTH in dialysis patients of European American ancestry using patients from the The Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) Trial[7]. Thus, genetics play an important role in determining an individual’s ‘normal’ calcium level.

There are multiple studies identifying associations of serum calcium levels with outcomes in CKD. Some, but not all, studies have identified both low and high calcium levels associated with mortality in patients on dialysis[8-12]. A recent study demonstrated that higher calcium in the pre-ESRD period was associated with greater mortality after starting dialysis[13]. It should be pointed out that most of these studies did not have a large population of patients receiving calcimimetics, and these drugs lower the serum calcium levels, and shift the set point
of the calcium-parathyroid relationship to the left (PTH suppression at lower levels of calcium)[14]. Therefore, the use of these agents in CKD patients may alter the outcomes or the generalizability of these association studies.

**Regulation of calcium homeostasis:**

In addition to the calcium self-regulation by the CaSR, other hormones that regulate calcium include PTH, vitamin D and FGF23. The primary function of PTH is to maintain blood ionized calcium levels by 1) increasing bone mineral dissolution, thus releasing calcium and phosphate, 2) increasing renal reabsorption of calcium and excretion of phosphate, 3) increase the activity of the renal CYP27B1 (1-alpha-hydroxylase) enzyme to convert 25(OH)D to 1,25(OH)₂D, and 4) enhancing the gastrointestinal absorption of both calcium and phosphate, indirectly through its effects to increase 1,25(OH)₂D. In healthy subjects, the increase in serum PTH concentration in response to hypocalcemia effectively restores serum calcium to normal levels. The loss of phosphate into the urine is compensated for by the enhanced bone resorption and indirect enhancement of intestinal phosphate absorption, thus maintaining normal serum phosphate levels.

Vitamin D is synthesized by skin via ultraviolet light, and is ingested in the diet in the form of vitamin D₂ (animal sources, ergocalciferol) and vitamin D₃ (plant source, cholecalciferol). Once in the blood, vitamin D₂ and D₃ bind with vitamin D binding protein (DBP) and are carried to the liver where they are hydroxylated by CYP27A1 (25-hydroxylase) in an essentially unregulated manner to yield 25(OH)D, often called calcidiol. Calcidiol is then converted in the kidney (or other cells) to 1,25(OH)₂D by the action of CYP27B1. The vitamin D is carried through the blood by D binding protein. At the cellular level, both 25(OH)D and 1,25(OH)₂D are endocytosed and bind the vitamin D response element (VDRE) of target genes and recruits transcription factors and co-repressors/activators that modulate the transcription[15, 16]. All of the key regulatory components of active calcium transport are upregulated by 1,25(OH)₂D [17]. Mice with intestinal knock down of the vitamin D receptor (VDR) are still able to maintain normal
calcium levels due to increased bone resorption. In contrast, global knock down of VDR leads to hypocalcemia but this can also be corrected with a high calcium diet[18].

FGF23 increases translocation of the calcium channel TRPV5 in the distal tubule[19], thereby leading to low urine calcium levels which consequently affects calcium balance as detailed later. In addition, FGF23 likely has indirect effects on serum calcium levels. Studies in rats with CKD have demonstrated that calcium deficiency reduces circulating FGF23[20], and that the administration of calcium in drinking water increases FGF23[21]. In CKD patients, the administration of calcitriol increases FGF23[22], whereas calcimimetics reduce FGF23[23]. FGF23 also activates 24,25-hydroxylase (CYP24A1)[24], which degrades both 25(OH)D and 1,25(OH)₂D and thus lowers vitamin D levels may impair intestinal calcium absorption.

Further complicating calcium homeostasis is the fact that calcium and phosphorus physiology are intertwined, and when calcium is released from bone, it fluxes of phosphorus. This relationship is very complex as demonstrated by recent studies that attempt to mathematically model this coupling[25, 26].

**Calcium balance in health and CKD:**

Calcium balance is determined by calcium intake minus calcium excretion in urine and feces. During bone growth in childhood, calcium balance is positive providing the needed calcium to enhance skeletal growth. However, once peak bone mass is achieved at age 25 to 30 years old, calcium balance becomes neutral in healthy individuals, with a slight negative balance around menopause[27]. The intricate regulators of calcium balance are similar to those regulators of calcium homeostasis, although bone released factors and gonadotropin hormones are also important.

In CKD, normal calcium balance is impaired. Studies in patients with CKD demonstrate that urinary calcium excretion levels fall as early as CKD stage 3, and remain extremely low. Although the initial thought was that this was due to PTH induced calcium reabsorption, studies
lowering PTH fail to change the urine calcium appreciably. More likely this is a result of altered regulation of the kidney TRPV5 channel in response to FGF23[19], acidosis, and calcium levels (reviewed in[28]). Theoretically, the drop in urine calcium may be an appropriate compensation in CKD to maintain overall calcium balance in the setting of decreased intestinal calcium absorption due to decreased 1,25-vitamin D.

Balance studies are very complex and difficult to conduct. All techniques (Table 1) require steady state calcium intake and are usually a crossover design in order to eliminate individual variability. Thus, one could never do a long term accurate calcium balance study in patients on dialysis due to the intermittent removal (or retention) of calcium from the dialysate.

One of the earliest balance studies in patients with CKD was published in 1978. Doses of $^{47}$Ca were ingested orally and injected intravenously. The fraction of calcium absorbed across the intestine was determined by measuring stool and urine calcium, demonstrating progressive drop in the fraction absorbed as creatinine clearance declined. Month long studies examined the whole body retention of $^{47}$Ca demonstrated net increase in calcium retention in patients with creatinine clearances less than 40-50 ml/min[29]. The conclusion was that the vitamin D deficiency of CKD impaired intestinal absorption, and that the total body retention was predominately skeletal.

More recently, Spiegel and colleagues studied calcium balance in healthy individuals and in patients with CKD stage 3b/4. Two diets were compared: 800 mg calcium and 2000 mg calcium, with diets prepared by clinical research study staff. Patients were given oral cholecalciferol for one month if baseline calcidiol levels were < 30 ng/ml. They then had two diet periods of 9 days each in which subjects were given a standardized diet containing either the 800 mg calcium or 2000 mg calcium diet (in a randomized crossover design). During the last 48 hours of each 9 day period, subjects were admitted to the clinical research center and stool and urine collected. Balance was calculated as dietary intake – output (stool plus urine). There was no difference in serum calcium or phosphorus levels, but a clear difference in
calcium balance (Figure 1). Healthy individuals on the 800 mg diet were in negative balance (net -144 ±174) whereas the CKD patients were in neutral balance (net -91 ± 113). On the 2000 mg diet, both groups were in positive calcium balance of 464 ± 225 mg in the healthy individuals, and 759 ± 120 mg in the CKD patients. Thus, in patients with CKD, a daily dietary intake of approximately 800 mg maintains neutral calcium balance.

Our group conducted a balance study using a combination of oral and intravenous calcium radionuclides in patients with CKD stage 3b/4 in order to test the hypothesis that oral calcium carbonate of 500 mg elemental calcium leads to positive calcium balance[30]. All subjects took 1000 U of cholecalciferol for 14 days, and then given three weeks of a standard diet of 1000 mg calcium with or without the 500 mg calcium binder with each of the three meals in a randomized crossover design. For the last two weeks of each three-week period, subjects stayed in the clinical research center where urine and stool were collected for 14 days. The subjects were given both oral and intravenous $^{45}$Ca, and kinetic modeling done. The results (Figure 2) demonstrated positive calcium balance when given the binder compared to placebo (average of 508 vs. 61 mg/day, p = 0.002) despite significant fecal excretion. The urine calcium excretion, even at this relatively early stage of CKD, was minimal. Similar to the study by Spiegel et al[31], there was no difference in serum calcium levels during the two arms. Calcium kinetic modeling demonstrated that there was net positive ‘bone’ balance indicating that $^{45}$Ca stay in the extracellular space in either bone or soft tissue.

These studies demonstrate that when CKD patients consume 800 to 1000 mg of dietary calcium, additional calcium in the form of binders leads to positive calcium balance.

Consequences of positive calcium balance:

The above studies would suggest that additional calcium in the form of binders or supplements would be retained in CKD. Ideally, the calcium influx would be deposited in bone, but that assumes normal bone remodeling which is not present in CKD. While calcium
supplements are widely prescribed for bone health, in reality there is no evidence that additional calcium alone or in addition to an adequate dietary intake reduces fractures even in healthy individuals[32]. In patients with CKD, randomized trials of calcium containing phosphate binders versus non calcium containing phosphate binders have shown increased coronary artery calcification in most studies[33-35] but not all[36]. However, in most of these studies, there was also hypercalcemia and depressed PTH in the calcium binder treated patients. This does not allow for a determination of whether it is altered calcium homeostasis (hypercalcemia and altered hormone levels) or positive calcium balance that is the culprit in arterial or cardiac calcification.

We utilized a rat model of slowly progressive CKD to distinguish the role of hypercalcemia from positive calcium intake/load (equivalent to positive calcium balance) on extraosseous calcification. In our Cy/+ rat model of progressive kidney disease, we treated rats with calcium in drinking water (to simulate increased oral intake/load), the calcimimetic R-568 (which lowers serum calcium levels), and R-568 plus calcium (low serum calcium plus calcium load) versus no treatment (normal calcium) [37]. The biochemical profile and magnitude of arterial and cardiac calcification are shown in Table 2. Treatment with calcium in the drinking water led to increased thoracic aorta, heart, and aortic valve calcification regardless of the serum level of calcium (with or without R-568). Of interest, the calcium treatment led to an even greater calcification than observed with hyperphosphatemia and normal calcium levels in the R-568 + calcium in the water treatment group[37]. These data suggest that positive calcium balance, regardless of whether hypercalcemia or hyperphosphatemia is present may induce ectopic calcification.

**Conclusion:**

Calcium balance studies, due to their small sample size and crossover design, do not meet criteria for inclusion in clinical practice guidelines, but do form the basis for human
recommended dietary intake. In the 2003 K/DOQI guidelines[38], calcium intake from binder and diet was recommended to remain below 2500 mg per day, which was the Tolerable Upper Intake Levels (ULs) for calcium established by the Food and Nutrition Board[39]. These guidelines were the first to suggesting limiting calcium intake. In the KDIGO guidelines of 2009[40], the recommendation was to “restrict the dose of calcium-based phosphate binders and/or the dose of calcitriol or vitamin D analog in the presence of persistent or recurrent hypercalcemia (1B), arterial calcification and/or if serum PTH levels are persistently low(2B)”. In the KDIGO update of 2017[41], a more general recommendation (without caveats) was provided: “in adult patients with CKD G3a-G5D receiving phosphate-lowering treatment, we suggest restricting the dose of calcium-based phosphate binders (2B)

How much is too much? In a given patient, the dose of calcium based phosphate binders that can be given without inducing positive calcium balance cannot be determined without a dietary history. A general guideline is that each serving of dairy is approximately 200 mg of calcium. Thus if a patient eats 4-5 servings per day, they are getting the recommended intake of 800 to 1000 mg. However, since most patients with CKD are told to restrict phosphate intake, and thus avoid dairy products, most patients do not consume 800 to 1000 mg. A study of 128 hemodialysis patients indicated the average calcium intake was 372 ± 364 mg/day[42] demonstrating the wide variability but generally low intake. Thus, dietary history is essential. The amount of calcium in each dose of calcium based phosphate binder also differs: Each 667 mg calcium acetate contains 169 mg per pill of elemental calcium, whereas calcium carbonate of 1200 mg contains 500 mg per pill of elemental calcium depending on the manufacturer. Thus, in a patient who consumes no calcium in the form of diet, 4 to 5 calcium acetate pills, or 2 calcium carbonate pills achieves the maximum calcium intake to maintain neutral balance. In patients on dialysis, this calculation is further complicated by differences in calcium dialysate concentration. Although the amount of calcium retention from dialysate will depend on the dialysate calcium concentration and the serum concentration, a recent randomized trial of 1.75
versus 1.5 mmol/L calcium dialysate demonstrated increased coronary calcium content in those receiving the high calcium dialysate over the one-year period[43].

In summary, calcium is an essential ion and necessary to maintain good bone health and normal cellular physiology. During periods of bone growth in childhood, nature has devised an intricate homeostatic system that helps ensure positive calcium balance. Once in adulthood, those patients without kidney disease tend towards neutral balance. In the presence CKD, most patients will be in positive calcium balance when prescribed calcium based binders with serum levels of calcium that may be low, normal or high- the result of abnormal calcium homeostasis in kidney disease.
Key Points:

- In CKD, calcium homeostasis is perturbed leading to abnormal calcium balance.
- Tightly controlled balance studies demonstrate positive calcium balance in patients with CKD stage 3b/4 with oral intake of calcium from diet or binders of more than 800 to 1000 mg/day.
- In patients with CKD, dietary calcium intake should be estimated by history in order to safely determine dosage of calcium based binders that will not lead to positive calcium balance.
References and recommended reading:

*of special interest

** of outstanding interest


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Figure Legends:

**Figure 1**: Estimated calcium balance in control and chronic kidney disease (CKD) subjects on 800- and 2000-mg calcium diets. Filled circles, CKD subjects; filled triangles, controls. Ca, calcium; Cont., control. Reprinted with permission from[31].

**Figure 2**: Calcium balance in stage 3/4 CKD patients with and without calcium carbonate. Calcium balance was greater with calcium carbonate compared with placebo. Ca intake was experimentally controlled and statistical analysis does not apply. White bars=placebo; black bars=calcium carbonate. Ca, calcium; NS, not significant ($P>0.05$). Data are presented as least squares mean±pooled s.e.m. Reprinted with permission from[30].
Figure

Calcium balance (mg/day)

Control low Ca
Control high Ca
CKD low Ca
CKD high Ca

$P < 0.05$
vs. low Ca

$P < 0.05$
vs. cont and CKD low Ca and cont high Ca
Table 1: Methods for measuring calcium balance

1. Intestinal absorption
   - Plasma appearance after oral radiocalcium
   - Metabolic balance studies (collection of feces only)

2. Net balance
   - Fecal and urinary recovery of radiocalcium
   - Whole body retention after oral radiocalcium

3. Kinetics (distribution)
   - Double isotope method (radiocalcium given orally and intravenously) with fecal and urinary recovery of radiocalcium
Table 2: Is it hypercalcemia or increased calcium intake that matters?

<table>
<thead>
<tr>
<th>Treatment</th>
<th>“clinical” Scenario</th>
<th>Thoracic calcification</th>
<th>Heart calcification</th>
<th>Valve calcification</th>
</tr>
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<tbody>
<tr>
<td>None (control CKD animals)</td>
<td>SHPT High phosph, nl Ca</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>R-568</td>
<td>Controlled PTH High phosph Low calcium</td>
<td>↔</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>R-568 + calcium</td>
<td>Controlled PTH Normal phosph Normal calcium *calcium load</td>
<td>↔↑</td>
<td>↑↑</td>
<td>↔↑</td>
</tr>
<tr>
<td>Calcium alone</td>
<td>Controlled PTH Normal phosph Normal calcium *hypercalcemia and calcium load</td>
<td>↔↑</td>
<td>↑↑</td>
<td>↔↑</td>
</tr>
</tbody>
</table>

Studies utilizing advanced stage CKD rats treated as in the first column. SHPT = secondary hyperparathyroidism; PTH = parathyroid, phosph = phosphorus. Arrows represent increase or decrease compared to control CKD animals. Adapted from [37] and reprinted with permission from Elsevier.