Reduced Renal Calcium Excretion in the Absence of Sclerostin Expression: Evidence for a Novel Calcium-Regulating Bone Kidney Axis

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ABSTRACT

The kidneys contribute to calcium homeostasis by adjusting the reabsorption and excretion of filtered calcium through processes that are regulated by parathyroid hormone (PTH) and 1α,25-dihydroxyvitamin D3 (1α,25(OH)2D3). Most of the filtered calcium is reabsorbed in the proximal tubule, primarily by paracellular mechanisms that are not sensitive to calcium-regulating hormones in physiologically relevant ways. In the distal tubule, however, calcium is reabsorbed by channels and transporters, the activity or expression of which is highly regulated and increased by PTH and 1α,25(OH)2D3. Recent research suggests that other, heretofore unrecognized factors, such as the osteocyte-specific protein sclerostin, also regulate renal calcium excretion. Clues in this regard have come from the study of humans and mice with inactivating mutations of the sclerostin gene that both have increased skeletal density, which would necessitate an increase in intestinal absorption and/or renal reabsorption of calcium. Deletion of the sclerostin gene in mice significantly diminishes urinary calcium excretion and increases fractional renal calcium reabsorption. This is associated with increased circulating 1α,25(OH)2D3 levels, whereas sclerostin directly suppresses 1α-hydroxylase in immortalized proximal tubular cells. Thus, evidence is accumulating that sclerostin directly or indirectly reduces renal calcium reabsorption, suggesting the presence of a novel calcium-excreting bone-kidney axis.


INTRODUCTION

In this review we discuss the role of an osteocyte-derived protein, sclerostin, in the regulation of calcium homeostasis and the mechanisms by which it alters renal calcium transport. The impetus for such studies has come from the study of patients with sclerosteosis and its milder variant, van Buchem disease.1–3 Affected individuals have exceptionally dense bones and skeletal overgrowth that often constrict cranial nerve foramina and the foramen magnum, resulting in premature death. Genetic studies have demonstrated that sclerosteosis is due to inactivating mutations of the sclerostin (SOST) gene, which encodes a novel cystine-knot protein that inhibits osteoblast function.4 The milder van Buchem disease is due to a deletion of a downstream enhancer element of the sclerostin gene.5 A key question is how the increased amount of calcium needed for the deposition in the skeleton is accreted. Are increases in renal calcium reabsorption and intestinal calcium transport responsible for the required augmented calcium retention? If so, what are the mechanisms by which this increase occurs? Mouse models of sclerosteosis have increases in skeletal mass similar to those found in patients with the disease.6–9 By using a Sost gene knockout model generated in our laboratory,6 we have demonstrated that sclerostin, either directly or indirectly, through an alteration in the synthesis of 1α,25-dihydroxyvitamin D (1α,25 [OH]2D), influences renal calcium reabsorption in the kidney.

Overview of Calcium Homeostasis

The pivotal role of calcium in biology is well known. Numerous biochemical and physiologic processes, including nerve conduction and function, muscle contraction, blood coagulation, enzyme activity, exocytosis, and bone mineralization, are critically dependent on normal calcium concentrations in intracellular and extracellular fluid.10–12 Not unexpectedly, finely tuned and rapidly responsive mechanisms exist to maintain intracellular and extracellular fluid calcium concentrations within a narrow

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The intestine and kidney are important in the absorption and excretion of calcium. In states of neutral calcium balance, the amount of calcium excreted by the kidney is equivalent to the net amount of dietary calcium absorbed by the intestine (net calcium absorbed = calcium absorbed – calcium secreted) (Figure 1A). The kidney, intestine, and vitamin D–parathyroid hormone (PTH)–endocrine system play important roles in the adaptation to variations in dietary calcium and phosphorus intakes. Figure 1B summarizes current information regarding changes that occur in response to decreasing dietary calcium intake. As shown, the vitamin D–PTH-endocrine system is essential for appropriate adaptations to alterations in dietary calcium intake.

Renal Calcium Excretion Is Regulated by the Calcium-Regulating Hormones, PTH and 1α,25(OH)₂D₃; Calcium-Sensing Receptor; and Klotho in Thick Ascending Limb and Distal Tubule

The kidney plays an important role in calcium homeostasis by reabsorbing filtered calcium in amounts that are subject to regulation by calcitropic hormones, PTH and 1α,25(OH)₂D₃. As a result of reabsorption processes that occur in both the proximal and the distal tubule, only 1%–2% of calcium filtered at the glomerulus appears in the urine. The amount of calcium reabsorbed in different nephron segments can be determined using micropuncture in which micropipettes are placed in accessible parts of the tubule to sample fluid, and by determining the amount of calcium present in the urine. About 60%–70% of total plasma calcium is free (not protein bound) and can be filtered at the glomerulus. A large percentage (approximately 70%) of filtered calcium (Ca²⁺) is reabsorbed in the proximal tubule mainly by paracellular processes that are linked with sodium (Na⁺) reabsorption. In this nephron segment, the reabsorption of Na⁺ and Ca²⁺ are proportional under a variety of conditions and are not dissociated following the administration of several factors that are known to alter calcium reabsorption, such as PTH, cyclic AMP, chlorothiazide, furosemide, acetazolamide, or changes in the hydrogen ion content. The precise cellular and molecular mechanisms responsible for the movement of Ca²⁺ from the lumen of the proximal tubule into the interstitial space are not clearly defined.

Figure 1. Calcium absorption in the intestine and calcium reabsorption in the kidney are influenced by calcitropic hormones. (A) Calcium balance in humans. (B) Physiologic adaptations to a low-calcium diet.
The majority of Ca\textsuperscript{2+} is believed to move in between cells (paracellular movement) with a smaller, but important, transcellular component. The components of the paracellular pathway might include claudin-2, which has a Ca\textsuperscript{2+}-binding site that competitively binds Na\textsuperscript{+}.\textsuperscript{42} The Na-K ATPase has been implicated in transcellular Ca\textsuperscript{2+} transport in the proximal tubule,\textsuperscript{43} and both the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger\textsuperscript{44} and isoforms 1 and 4 of the plasma membrane Ca\textsuperscript{2+} pump\textsuperscript{45,46} are expressed in the proximal tubule, and could be important in the movement of Ca\textsuperscript{2+} out of the proximal tubule cell. Undefined Ca\textsuperscript{2+} channels and Ca\textsuperscript{2+}-binding proteins might also influence the movement of Ca\textsuperscript{2+} into and across the cell. Suffice it to say that although the proximal tubule reabsors large amounts of Ca\textsuperscript{2+} primarily by paracellular processes, the rate of Ca\textsuperscript{2+} reabsorption is not influenced by factors or hormones that regulate calcium balance.\textsuperscript{35,36,39,40} However, in conditions such as volume depletion, where proximal tubule Na\textsuperscript{+} reabsorption is increased, one also observes enhanced Ca\textsuperscript{2+} reabsorption that can contribute to the hypercalcemia that is sometimes seen in such situations.

The thin descending and thin ascending limbs of the loop of Henle do not transport substantial amounts of Ca\textsuperscript{2+}.\textsuperscript{47,48} Another 20%–25% of filtered Ca\textsuperscript{2+} is reabsorbed in the thick ascending loop of Henle, primarily by the paracellular route involving claudins 16 and 19.\textsuperscript{32,47–58} Thick ascending limb cells express the furosemide-sensitive Na-K-Cl cotransporter, NKCC2,\textsuperscript{59–62} which contributes to the driving force for paracellular Ca\textsuperscript{2+} transport. Accordingly, mutations of NKCC2 are associated with the common form of Bartter syndrome, which, like the other forms of the syndrome, can be associated with calciumuria.\textsuperscript{63} The calcium-sensing receptor (CaSR) is expressed in the basolateral membrane of the thick ascending limb of the loop of Henle, where its activation inhibits the reabsorption of Ca\textsuperscript{2+}.\textsuperscript{64,65} In accordance, patients heterozygous (familial benign hypocalciuric hypercalciuria) or homozygous (neonatal severe hyperparathyroidism) for inactivating mutations of the CaSR have hypocalcuiuria.\textsuperscript{66} Because these patients have intact parathyroid glands and either detectable (in familial benign hypocalciuric hypercalciuria) or high (in neonatal severe hyperparathyroidism) PTH concentrations,\textsuperscript{67} PTH concentrations, an additional effect of PTH on renal Ca\textsuperscript{2+} absorption in such patients cannot be ruled out. Mice in which the Casr and Pth genes have been deleted in the germ line, do not dispose of a Ca\textsuperscript{2+} load as efficiently as mice lacking just the Pth gene demonstrating the importance of the CaSR in facilitating renal calcium excretion.\textsuperscript{67} Recently, Toka et al. deleted the CaSR just in the kidney and confirmed the expected reduced capacity to excrete Ca\textsuperscript{2+} in these mice.\textsuperscript{68} There is considerable species heterogeneity with respect to responses to calcium-regulating hormones by the thick ascending limb; in the mouse, PTH and calcitonin stimulate Ca\textsuperscript{2+} transport in the cortical thick ascending limb,\textsuperscript{51,53,69,70} whereas in the rabbit calcitonin stimulates calcium reabsorption in the medullary thick ascending limb but not in the cortical thick ascending limb.\textsuperscript{56}

In the distal convoluted tubule (primarily DCT2) and connecting tubule (together abbreviated as DT), 5%–10% of filtered Ca\textsuperscript{2+} is reabsorbed\textsuperscript{71–73} by active transport processes against both an electrical and concentration gradient. Ca\textsuperscript{2+} reabsorption in this segment of the nephron is regulated by PTH,\textsuperscript{21–23} calcitonin,\textsuperscript{69,70} and 1α,25(OH)\textsubscript{2}D\textsubscript{3}\textsuperscript{24–28}—these hormones increase the efficiency of Ca\textsuperscript{2+} reabsorption in this nephron segment. Ca\textsuperscript{2+} reabsorption in the DT occurs via a transcellular pathway. Mediators of Ca\textsuperscript{2+} transport in the renal DT include apically situated, transient receptor potential cation channels, subfamily V, type 5 and 6 channels (TRPV5, TRPV6), which mediate the increase in Ca\textsuperscript{2+} uptake from the lumen into the cell\textsuperscript{19,74–79}; micropuncture studies in knockout mice indicated that TRPV5 is the gatekeeper of Ca\textsuperscript{2+} reabsorption in the accessible DT in mice (Figures 2C and 3, A and B).\textsuperscript{76} Intracellular Ca\textsuperscript{2+} binding proteins, such as calbindin D\textsubscript{9k} and D\textsubscript{28k}, facilitate the movement of Ca\textsuperscript{2+} across the cell\textsuperscript{29,80–81}, and the basolateral plasma membrane calcium (PMCA) pump,\textsuperscript{16,17,29} Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger,\textsuperscript{82–85} and the Na\textsuperscript{+}-Ca\textsuperscript{2+}-K\textsuperscript{+} exchanger\textsuperscript{86} increase the rate of extrusion of Ca\textsuperscript{2+} across the basolateral membrane (Figure 3, A and B). The Na\textsuperscript{+} gradient for the activity of the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger and the Na\textsuperscript{+}-Ca\textsuperscript{2+}-K\textsuperscript{+} exchanger is provided by the Na-K ATPase situated at the basolateral cell membrane (not shown).

**Figure 2.** Regulated calcium reabsorption occurs in the distal convoluted tubule. (A) Segment-specific calcium reabsorption in the kidney. (B) Tubular fluid can be sampled by micropuncture in the late proximal tubule and along the distal tubule, allowing a determination of reabsorption of analytes in the proximal tubule, in the loop of Henle, and along the DT (early to late). The difference between values obtained at late DT and in the urine (there is even evidence for Ca\textsuperscript{2+} leaking back into the lumen, possibly by paracellular routes); TRPV6 may partially compensate in the collecting duct. Data in error bars are presented as mean±SEM. Adapted from reference 76.
The calcium-regulating hormones alter the expression of calcium channels, calcium-binding proteins, calcium pumps, and exchangers by varied mechanisms. PTH increases the activity of TRPV5 channels in the kidney by activating cAMP-protein kinase A signaling, and phosphorylating a threonine residue within the channel, resulting in an increase in the open probability of the channel. PTH also activates the protein kinase C pathway and increases the numbers of TRPV5 channels on the cell surface.

The Kidney Is the Major Site for Metabolic Transformation of 25(OH)D

See references 106–110. The 25(OH)D-1α-hydroxylase (Cyp27b1) mRNA and protein expression were likewise increased in Sost−/− mice, strongly suggesting that the increase in serum 1α,25(OH)2D concentrations was due to increased 1α,25(OH)2D synthesis. When recombinant sclerostin was added to cultures of proximal tubular cells, the expression of the messenger RNA for Cyp27b1, the 1α-hydroxylase cytochrome P450, was diminished. Serum 24, 25(OH)2D concentrations were diminished in Sost−/− mice, and PTH concentrations were similar in knockout and wild-type mice. The lack of change in PTH is consistent with previous studies in humans. The data suggest that in addition to the hormones traditionally thought to alter calcium reabsorption in the kidney (PTH and 1α,25(OH)2D), sclerostin plays a significant role in altering renal calcium excretion. While PTH and 1α,25(OH)2D decrease fractional excretion of calcium by increasing the efficiency of calcium reabsorption in the DT, sclerostin increases fractional excretion of calcium (the absence of sclerostin expression being

Evidence for Regulation of Renal Calcium Excretion by the Bone-Derived Protein Sclerostin

To address how calcium is retained in increased amounts in sclerosteosis, and to determine whether increased renal calcium reabsorption can play a role in this regard, we generated a mouse model of sclerosteosis in which the sclerostin (Sost) gene was deleted (Sost−/− mice).

As expected, serum sclerostin concentrations were readily detectable in wild-type mice but were not measurable in Sost−/− mice. We found that absolute urinary calcium excretion and renal fractional excretion of calcium were decreased in Sost−/− mice. Serum 1α,25(OH)2D concentrations were increased without attendant hypercalcemia; renal 25(OH)D-1α-hydroxylase (Cyp27b1) mRNA and protein expression were likewise increased in Sost−/− mice, strongly suggesting that the increase in serum 1α,25(OH)2D concentrations was due to increased 1α,25(OH)2D synthesis.

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associated with a reduced fractional excretion of calcium). Thus, the scheme of adaptations to reductions in calcium intake and resultant downstream alterations in hormones (see Figure 1B for current understanding) may need to be amended to include changes in sclerostin expression (Figure 4). In the modified scheme, reduced sclerostin expression, which result from increases in PTH,126–129 would enhance renal Ca²⁺ reabsorption directly or through changes in 1α,25(OH)₂D synthesis (Figure 4). The change in 1α,25(OH)₂D synthesis might be direct or mediated through changes in FGF-23 concentrations.

Unanswered Questions regarding the Mechanism of Action of Sclerostin in the Kidney

Several unanswered questions remain regarding the manner in which sclerostin affects kidney functions. For example, do patients with inactivating mutations of the SOST gene also have increased serum 1α,25(OH)₂D concentrations? In what nephron segment does sclerostin alter Ca²⁺ transport? What Ca²⁺ channels and Ca²⁺ transporters/exchangers mediate the effect of sclerostin on renal Ca²⁺ reabsorption? Is the inhibition of 1α,25(OH)₂D synthesis essential for sclerostin’s action on renal tubular Ca²⁺ transport? Does FGF-23 mediate the inhibitory effect of sclerostin on 1α,25(OH)₂D synthesis? Are other signaling pathways, such as the Wnt/β-catenin pathway (which mediates some of the effects of sclerostin in bone), responsible for the action of sclerostin in the renal tubules? Are serum sclerostin concentrations altered by changes in dietary calcium, and do such changes in serum sclerostin help regulate calcium balance?

It will be important to ascertain where sclerostin functions along the nephron. The effects of sclerostin probably occur in the DT because this is the nephron segment in which hormone-regulated Ca²⁺ transport occurs. It is plausible, although unproven, that the effects of sclerostin on the tubule are direct and not through any intermediary molecules. Alternatively, or in addition, the effects of sclerostin on renal Ca²⁺ transport may be indirect and mediated through changes in the synthesis of 1α,25(OH)₂D. Because 1α,25(OH)₂D increases the re-absorption of Ca²⁺ in the kidney, it is conceivable that a calciuric effect of sclerostin is mediated through its inhibitory effects on 1α,25(OH)₂D synthesis. It is likely that sclerostin-induced changes in renal Ca²⁺ handling result from changes in the expression of various transporters responsible for the re-absorption of calcium in the DT. Preliminary (unpublished) micro-array data suggest that Sost⁻/⁻ mice have increased renal expression of mRNAs for TrpV5, the 1α,25(OH)₂D-regulated gate keeper of DT calcium transport (Figure 2C), and the basolateral Na⁺–Ca²⁺–K⁺ exchanger, member 3 (NCKX3, Slc24a3) (R. Kumar, unpublished data).

How does sclerostin alter 1α,25(OH)₂D concentrations? Because the addition of sclerostin to immortalized proximal tubular cells decreases Cyp27b1 expression,6 there is a known direct effect of sclerostin on the expression of renal Cyp27b1, and thus renal 1α,25(OH)₂D synthesis. Sclerostin may have an additional indirect inhibitory

![Figure 4](https://www.jasn.org)
effect on Cyp27b1 expression since FGF-23 concentrations are diminished in Sost−/− mice along with an increase in serum P1 concentrations.8 The bone-derived FGF-23 is a known suppressor of 25(OH)D-1α-hydroxylase,116 suggesting that the increase in 1α,25(OH)2D concentrations in the absence of sclerostin may have resulted from lower FGF-23 levels. In other words, sclerostin may stimulate FGF-23 release from bone, which suppresses renal 1α,25(OH)2D synthesis. Whether plasma levels of sclerostin are regulated (e.g., stimulated by dietary calcium) and can directly affect and regulate kidney functions, however, remains to be determined. Additional information is needed regarding the regulation of sclerostin in humans by dietary calcium, phosphorus, and vitamin D. As noted earlier, PTH appears to inhibit sclerostin concentrations.126–139 Care, however, needs to be exercised in interpreting sclerostin concentrations in humans because available assays give different values when serum or plasma is used to measure the protein and reproducibility in the lower range of the assay is poor.130

The molecular signaling mechanisms by which sclerostin affects renal tubule function are unknown, but an alteration of Wnt signaling seems attractive. A function are unknown, but an alteration by which sclerostin affects renal tubule Wnt signaling begins with Wnts binding to receptor complexes consisting of Lrp5/6 and Frizzled proteins.131 The canonical pathway involves β-catenin as a key intermediate signaling molecule. Several laboratories have demonstrated that canonical Wnt signaling and β-catenin expression are blocked by sclerostin in bone and osteoblasts through the sequestration of the LRP 5/6 coreceptor by sclerostin.9,132–136 Increased β-catenin in bone of Sost knockout mice is associated with enhanced fracture repair.137 “Noncanonical” Wnt pathways may also play a role in sclerostin signaling in bone. Lrp5/6 also stimulates signaling via Rac1, mTorc2/Akt, and other molecules142 suggesting that sclerostin may function by pathways other than the classic β-catenin pathway. Sclerostin also binds to BMP6, BMP2, noggin, and several other proteins133–135,139–145 in bone, and it is plausible but undetermined that BMP signaling might help regulate sclerostin-mediated changes in renal Ca2+ transport.

The studies outlined introduce sclerostin as part of a novel “bone-kidney” axis that suppresses renal 25(OH)D-1α-hydroxylase and 1α,25(OH)2D3 synthesis and enhances renal calcium excretion. Further studies are required to delineate whether and how sclerostin is regulated in bone and the circulation, how this protein affects the kidney (including the involved molecular mechanisms), and whether sclerostin might be involved in the pathogenesis and/or has therapeutic potential in disorders associated with renal calcium wasting and associated bone loss.146

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DISCLOSURES

None.

REFERENCES

BRIEF REVIEW


69. De Rouf


