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Bioavailable vitamin D in chronic kidney disease

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Most of the major vitamin D metabolites 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D circulates in a tightly bound state to vitamin D-binding protein (DBP), rendering this fraction unavailable for biological action. A smaller fraction, loosely bound to albumin or circulating freely, is bioavailable, and hence bioactive. This Commentary discusses the free hormone hypothesis and the role of DBP in vitamin D metabolism.

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Vitamin D metabolites are relatively hydrophobic and circulate bound to proteins, primarily to the vitamin D-binding protein (DBP), and to a lesser extent to albumin. DBP, molecular weight 58 kDa, has high affinity for the major circulating vitamin D metabolite 25-hydroxyvitamin D (25(OH)D), and a 10- to 100-fold lower affinity for the vitamin D hormone 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). Normal circulating levels of 25(OH)D are 20–80 ng/ml, whereas $1,25(OH)_2D$ levels are about 1000 times lower at 25–65 pg/ml. DBP is present at high levels in the blood (4–10 μM), more than 20 times higher than the combined concentrations of vitamin D metabolites, and has a serum half-life of 2.5–3 days. The affinities of vitamin D metabolites for albumin are lower, but because of the high concentrations of these proteins in the blood, more than 99% of the circulating vitamin D compounds are protein bound.[1]

Although approximately 85% of circulating 25(OH)D is bound by DBP, 15% is less tightly bound by albumin, and less than 0.1% circulates in the unbound state. That portion of 25(OH)D or $1,25(OH)_2D$ not bound to DBP but circulating free or bound to albumin constitutes 'bioavailable' vitamin D, which is the subject of investigation by Bhan and colleagues in this issue of *Kidney International*.[2] They report on a select subset of patients from the Accelerated Mortality on Renal Replacement (ArMORR) study, a large prospective cohort study of incident hemodialysis patients in the United States.[2,3]

When Bhan *et al.* use DBP levels to calculate bioavailable 25(OH)D and $1,25(OH)_2D$, they are able to demonstrate a relationship to serum calcium that is not present when total 25(OH)D and $1,25(OH)_2D$ levels are examined.[2] Even in healthy subjects, DBP levels vary widely (tenfold), leading to corresponding variations in total 25(OH)D and $1,25(OH)_2D$, but free levels of the hormone and its precursor remain relatively constant. This is consistent with our expansive knowledge that vitamin D and parathyroid hormone (PTH) are the major regulators of calcium homeostasis, and their roles persist into end-stage renal disease. In further support of the free hormone hypothesis, bioavailable 25(OH)D, but not total 25(OH)D, correlates with PTH. The authors found no relationship of bioavailable vitamin D levels to serum phosphorus,[2] echoing the results in the original ArMORR report that total 25(OH)D and $1,25(OH)_2D$ levels did not correlate with serum phosphorus, which is more tightly regulated by fibroblast growth factor-23.[3]

There is abundant evidence that the small fractions of unbound or free vitamin D compounds are biologically active. The free hormone hypothesis was first introduced by Mendel as a working model for the movement of lipophilic steroid hormones through cell membranes to bind to their intracellular receptors.[4] The carrier proteins are impermeable to the cell and function as a reservoir for the systemic delivery of the ligands. Entry of 25(OH)D and $1,25(OH)_2D$ into cultured keratinocytes[5] and monocytes[6] is decreased in the presence of DBP or serum. On the other hand, the 22-oxa analog of $1,25(OH)_2D$ (oxacalcitriol) has much lower DBP affinity than $1,25(OH)_2D$ and has greater access to target tissues *in vivo*, but is cleared more rapidly.[7] These and other findings demonstrate two roles of DBP in vitamin D physiology: prolonging the circulating half-lives of vitamin D metabolites and limiting their access to target tissues. DBP-ablated mice have very low levels of total 25(OH)D and $1,25(OH)_2D$, but free levels of the metabolites are normal as evidenced by the absence of a vitamin D-deficient phenotype.[8]

More recently, DBP was found to maintain vitamin D levels through a reabsorption mechanism in the proximal tubules.[9] Filtered DBP binds to megalin (LRP2) and is taken up by proximal tubular cells by endocytosis; the DBP is degraded, and its vitamin D cargo is either metabolized or released into the circulation (Figure 1). Megalin-ablated mice become vitamin D deficient and rachitic because of loss of DBP and its bound vitamin D metabolites in the urine. While there is evidence that conversion of 25(OH)D to $1,25(OH)_2D$ is impaired in megalin-deficient mice, DBP-ablated mice produce sufficient $1,25(OH)_2D$ to maintain serum calcium and phosphate, indicating that uptake of free 25(OH)D at the basolateral surface of proximal tubular cells also occurs. Thus, the primary role of DBP in the endocytic pathway appears to be in the reabsorption of vitamin D metabolites. Not surprisingly, then, proteinuria results in losses of DBP and may contribute to vitamin D deficiency.

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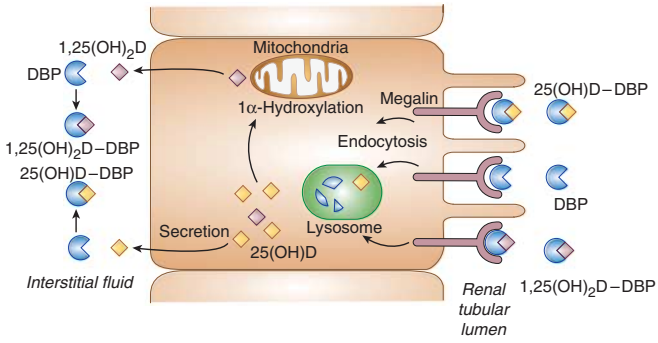

Figure 1 | Schematic of a proximal tubular cell demonstrating the role of megalin located in the brush border membrane in reclaiming DBP and any 25(OH)D or 1,25(OH)$_2$D bound to DBP. Heavy proteinuria can lead to substantial DBP losses and potentially contribute to vitamin D deficiency. DBP, vitamin D-binding protein; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

Circulating DBP is produced by the liver and cleared by the kidney. Its levels are increased by estrogens and pregnancy and decreased in patients with cirrhosis and nephrotic syndrome. On the basis of the equations used by Bhan *et al.* to calculate bioavailable D, if DBP is twofold lower, free 25(OH)D will be about twofold higher. In the study by Bhan *et al.*, the mean DBP level in black dialysis patients was less than half that observed in white patients.[2] Five black patients had DBP levels below the limit of detection for the assay. In two prior studies in healthy young adults and pregnant women, Bhan's group also found black ethnicity associated with significantly lower DBP levels,[10] whereas at least one other group has reported no relationship of black ethnicity to lower DBP levels in healthy young women.

If DBP levels are routinely lower among blacks, then we are probably overestimating the degree and severity of vitamin D deficiency in the black population. In 2007, the first ArMORR study in 825 incident hemodialysis patients noted that 25(OH)D levels were less than 30 ng/ml in 78% of patients and less than 10 ng/ml in 18%.[3] Women, blacks, and diabetics were particularly likely to be severely vitamin D deficient. If DBP levels in the overall ArMORR cohort parallel the results from the Bhan report,[2,3] then bioavailable 25(OH)D levels among black patients are likely not different from those among white patients.

The 2007 ArMORR study also noted 1,25(OH)$_2$D levels were also low (~80% of patients had levels less than 20 pg/ml), and these levels were not influenced by sex, ethnicity, or diabetic status.[3] Low levels of both 25(OH)D and 1,25(OH)$_2$D correlated with increased 90-day mortality. However, patients treated with active vitamin D (calcitriol), as is commonly done for secondary hyperparathyroidism or hypocalcemia, did not have increased mortality despite low incident vitamin D levels.[3] Treatment with calcitriol will of course normalize 1,25(OH)$_2$D levels and may account for this observation. The finding of a significant relationship between endogenous vitamin D levels, subsequent active vitamin D therapy, and survival strongly suggests but does not prove a causal role for vitamin D deficiency in the high mortality observed in incident dialysis patients.[3] If bioavailable 25(OH)D and 1,25(OH)$_2$D have even stronger relationships to survival among dialysis patients, then the causal role for vitamin D in survival is strengthened even more. Use of active vitamin D may have salutary effects on bone by stimulating bone formation, and inhibiting osteoclast production. Conversely, there are legitimate concerns that administration of active vitamin D could lead to adynamic bone disease via oversuppression of PTH, induce hypercalcemia, or promote vascular calcification. Clearly, randomized clinical trials are needed in this area.

Black dialysis patients have significantly higher PTH levels than white patients, and therefore blacks are more likely to receive active vitamin D and more likely to receive larger doses.[11] Among patients treated with active vitamin D, lower DBP levels could result in higher bioavailability and greater PTH-suppressing effect, but also enhanced active vitamin D clearance. Therefore, it is not clear whether lower DBP levels would enhance or impair the PTH-suppressing effects of administered active vitamin D. Further study is warranted to examine the relationship of DBP levels to control of secondary hyperparathyroidism by active vitamin D.

The potential effects of variable serum DBP on total versus free vitamin D metabolite levels are an important consideration in examining associations between these metabolites and various biological end points. As Bhan and colleagues demonstrate, the associations of bioavailable metabolites with calcium are significantly stronger than those of total metabolites.[2] While further observational studies of the relationship of vitamin D levels and active vitamin D to clinical end points will undoubtedly continue to appear, clinical trials are needed to test the hypotheses that vitamin D use improves clinical outcomes.

DISCLOSURE
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REFERENCES

1. Bouillon R. The vitamin D binding protein DBP. In: Feldman D, Pike JW, Adams JS (eds). *Vitamin D*, 3rd edn Academic Press: San Diego, 2011, pp 57–72.
2. Bhan I, Powe CE, Berg AH *et al.* Bioavailable vitamin D is more tightly linked to mineral metabolism than total vitamin D in incident hemodialysis patients. *Kidney Int* 2012; **82**: 84–89.
3. Wolf M, Shah A, Gutierrez O *et al.* Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney International* 2007; **72**: 1004–1013.
4. Mendel CM. The free hormone hypothesis: a physiologically based mathematical model. *Endocr Rev* 1989; **10**: 232–274.
5. Bikle DD, Gee E. Free, and not total, 1,25-dihydroxyvitamin D regulates 25-hydroxyvitamin D metabolism by keratinocytes. *Endocrinology* 1989; **124**: 649–654.
6. Chun RF, Lauridsen AL, Suon L *et al.* Vitamin D-binding protein directs monocyte responses to 25-hydroxy- and 1,25-dihydroxyvitamin D. *J Clin Endocrinol Metab* 2010; **95**: 3368–3376.
7. Brown AJ, Finch J, Grieff M *et al.* The mechanism for the disparate actions of calcitriol and 22-oxacalcitriol in the intestine. *Endocrinology* 1993; **133**: 1158–1164.
8. Safadi FF, Thornton P, Magiera H *et al.* Osteopathy and resistance to vitamin D toxicity in mice null for vitamin D binding protein. *J Clin Invest* 1999; **103**: 239–251.

9. Christensen EI, Willnow TE. Essential role of megalin in renal proximal tubule for vitamin homeostasis. *J Am Soc Nephrol* 1999; **10**: 2224–2236.
10. Powe CE, Ricciardi C, Berg AH *et al.* Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship. *J Bone Miner Res* 2011; **26**: 1609–1616.
11. Wolf M, Betancourt J, Chang Y *et al.* Impact of activated vitamin D and race on survival among hemodialysis patients. *J Am Soc Nephrol* 2008; **19**: 1379–1388.

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Corin: a key protein of an adaptive renal mechanism to respond to salt variation?

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The protease corin generates atrial natriuretic peptide and affects blood pressure and salt–water homeostasis. Under dietary salt challenge, corin knockout mice show blood pressure exacerbation and significant weight gain due to water and salt retention. This phenotype involves the epithelial sodium channel but is independent of the renin–angiotensin–aldosterone system. This suggests that corin has an important role in a new adaptive mechanism of the response to variations of salt in the diet.

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Wang *et al.*[1] (this issue) show with simple experiments the role of corin in blood pressure adaptation to salt diet challenge and highlight the importance of this enzyme in the homeostatic regulation mechanism of blood pressure.

Corin is a transmembrane serine protease of the trypsin superfamily. This protease is abundant in atrial and ventricular cardiac myocytes. Corin was also detected in the kidney and uterus. Interestingly, soluble fragments of corin have been detected in human blood. The plasma corin concentration is reduced in the case of heart failure, but increased in hypertension of pregnancy.[2] The soluble corin proteolytic activity *in vivo* is still elusive, but *in vitro* assays suggest that both active and inactive fragments of corin were generated either by the action of the metalloprotease or by corin autocleavage.[3] More work is needed to answer this question, but these observations support the idea that soluble corin concentration in the bloodstream might be a promising biomarker in diseases such as heart failure, and during salt-related hypertension or pregnancy. Corin mediates the cleavage of pro-atrial natriuretic peptide (Pro-ANP), an inactive hormone, to an active hormone, atrial natriuretic peptide (ANP). ANP reduces sodium and water reabsorption and plays a role in blood pressure homeostasis.

It is known that plasma ANP levels are elevated in mice on a high-salt diet and that the absence of ANP or its receptor leads to hypertension. It was not surprising, therefore, to find that deletion of the corin gene in the knockout mouse ($Cor^{-/-}$) causes hypertension and cardiac hypertrophy. In African Americans, polymorphisms in the corin gene are associated with high blood pressure and cardiac hypertrophy. Corin is not the first protease identified as playing a major role in blood pressure homeostasis. Thus, renin and angiotensin-converting enzyme have also been shown to be involved in the pathophysiology of hypertension (Figure 1).

Renin and angiotensin-converting enzyme produce the active fragment angiotensin II, which increases sodium reabsorption by the Na^+/H^+ exchanger in proximal tubules, where most of the filtered sodium is reabsorbed. A second hormone, aldosterone, plays a role in the fine tuning of sodium reabsorption. Aldosterone increases the reabsorption of sodium in the collecting ducts by its effect on the epithelial sodium channel (ENaC). In contrast to angiotensin II and aldosterone, ANP inhibits the reabsorption of sodium by ENaC in the collecting ducts. Furthermore, the localization of corin in the kidney and its proximity to the site of action of ANP support the idea that corin might play an important physiological role in this organ (Figure 1).

Wang *et al.*[1] (this issue) show that a 13-fold increase in sodium concentration did not affect blood pressure in normal mice. However, the same high-salt diet significantly affects the already high blood pressure observed in mice that do not express corin ($Cor^{-/-}$ mice). Interestingly, previous studies using 26 times more sodium reported an increased blood pressure in both wild-type and $Cor^{-/-}$ mice.[4] This observation suggests first that corin expression and its production of ANP can help to handle variations of salt without affecting any major regulatory pathways. Second, this mechanism can be overloaded in the presence of a significant amount of sodium. The effect of a high-salt diet on the high blood pressure of $Cor^{-/-}$ mice was observed 2 weeks after they were returned to a normal diet. Interestingly, during a high-salt diet, both plasma renin and aldosterone concentrations are reduced. This effect is observed in both wild-type and $Cor^{-/-}$ mice and may represent an attempt to reduce sodium reabsorption. A similar reduction of renin and aldosterone is observed with a physiological dose of ANP.[5] In addition, the knockout mice that did not express ANP receptor type 1 showed an increase in serum aldosterone.[6] Thus, the reduced level of aldosterone in $Cor^{-/-}$ mice is unexpected. Is it possible that ANP is produced in the adrenal gland? Yet, the mechanism by which aldosterone but not renin is reduced in $Cor^{-/-}$

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