

# Vitamin D and the Renin-Angiotensin System

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## INTRODUCTION

The renin-angiotensin system (RAS) is a central regulator of renal and cardiovascular functions. Over-activation of the RAS leads to renal and cardiovascular disorders, such as hypertension and chronic kidney disease, the major risk factors for stroke, myocardial infarction, congestive heart failure, progressive atherosclerosis, and renal failure. Mounting epidemiological and clinical evidence has demonstrated an association of vitamin D deficiency or insufficiency with increased risks of renal and cardiovascular diseases, but the molecular basis remains poorly defined. The discovery of the vitamin D hormone as an endocrine repressor of the RAS provides a potential explanation for this association. 1,25-Dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) down-regulates the expression of renin, the rate-limiting enzyme of the renin-angiotensin cascade, as well as angiotensinogen, the substrate of renin. Vitamin D deficiency leads to overexpression of renin and thus activation of the RAS, causing renal and cardiovascular injuries. It is speculated that the vitamin D hormone maintains the renal and cardiovascular homeostasis via suppressing the RAS. Pharmacologically vitamin D analogs can be used to target the RAS for treatment of renal and cardiovascular diseases. This chapter will focus on vitamin D regulation of the RAS and its physiological and therapeutic implications with regard to the renal and cardiovascular systems. Other relevant chapters in this book include vitamin D effects on renal disease discussed in Chapter 70 and in cardiovascular disease and risk in Chapters 31 and 102.

## THE RENIN-ANGIOTENSIN SYSTEM

### The Renin-Angiotensin Cascade and its Biological Functions

The RAS is a systemic endocrine regulatory cascade consisting of multiple components (Fig. 40.1). The first and rate-limiting step of the RAS cascade is renin, an aspartyl protease that is primarily released from the juxtaglomerular (JG) cells in the JG apparatus of the kidney. The only known substrate for renin is angiotensinogen (AGT), produced predominantly in the liver. Renin cleaves AGT to angiotensin (Ang) I, an inactive 10-amino-acid peptide; Ang I is then converted to Ang II, an 8-amino-acid peptide, by angiotensin-converting enzyme (ACE), which primarily resides in the endothelial cells in blood vessels. Further processing of Ang II by aminopeptidase A and N produces Ang III and Ang IV [1]. ACE2, an ACE homolog, can convert Ang II to Ang (1–7) [2,3], and this enzyme is thought to play an essential role in heart functions [4].

Ang II is the central biological effector of the RAS. Systemic Ang II plays a central role in the regulation of blood pressure (Fig. 40.1). Ang II is the most potent vasoconstrictor. It acts on smooth muscle cells in the vasculature to increase vasoconstriction and thus enhances peripheral resistance. Ang II stimulates the synthesis and secretion of aldosterone from the adrenal cortex, a hormone that promotes sodium reabsorption in the renal tubular system. Ang II also stimulates the release of antidiuretic hormone (ADH, also called arginine vasopressin) from the hypothalamus/pituitary, which increases water retention from the kidney, leading

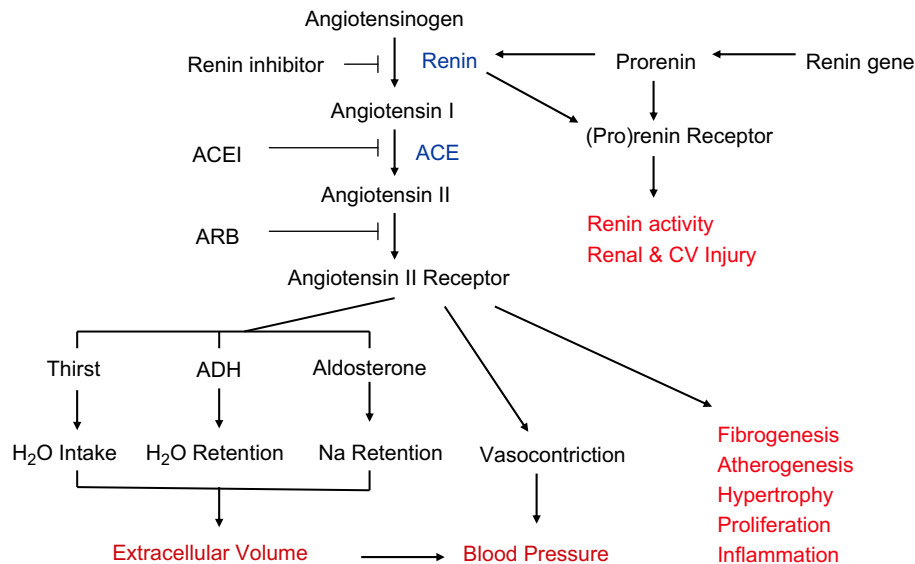


FIGURE 40.1 The renin-angiotensin system. ACE, angiotensin-converting enzyme; ADH, antidiuretic hormone; CV, cardiovascular.

to expansion of extracellular volume. Finally, Ang II stimulates thirst sensation in the central nerve system and promotes water intake. Together, activation of the renin-angiotensin cascade ultimately causes volume expansion and enhances peripheral resistance. Because blood pressure is determined by the combination of cardiac output and total vascular resistance, overactivation of the systemic RAS results in the development of hypertension [5,6].

In fact, Ang II has diverse physiological and pathological activities. In addition to blood pressure control, it has been shown to promote fibrogenesis, inflammation, and cell hypertrophy and proliferation [7–9] (Fig. 40.1). Thus activation of the RAS usually poses detrimental effects.

In addition to the systemic RAS, components of the RAS have been found inside many tissues including the brain, heart, vasculature, kidney, and reproductive system [10]. The tissue-specific RAS may function in a paracrine fashion and in some cases can cause tissue damages. The RAS within the brain is involved in the control of water drinking and blood pressure [11,12], the RAS within the heart may be involved in adaptive response to myocardial stress, and the RAS in the vasculature may be involved in vascular tone and endothelial functions [10]. The intrarenal RAS is known to play a key role in hyperglycemia-induced renal injury in diabetes mellitus [13].

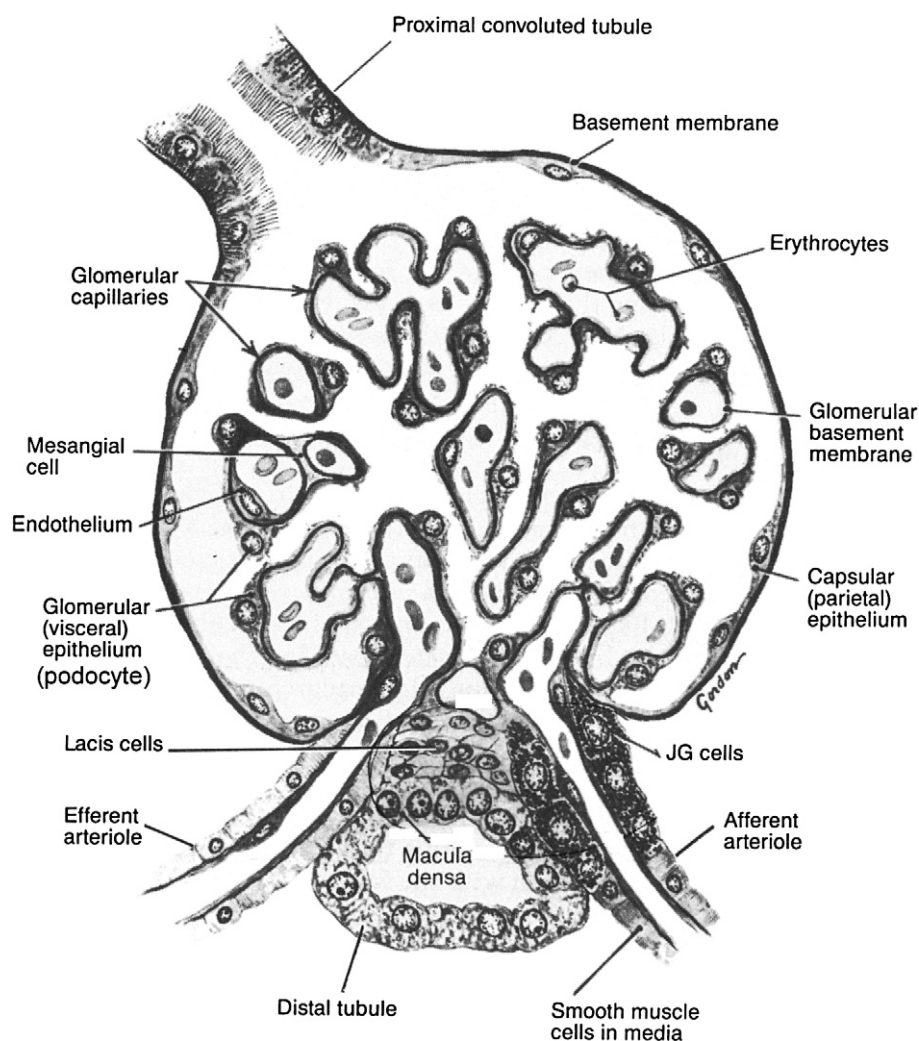
The wide range of activities of Ang II is mediated by several G-protein-coupled receptors widely distributed in tissues [14]. Among these receptors, the type 1 receptor (AT1) mediates most of the activities involved in vasoconstriction, sodium retention, and hypertrophy, whereas the type 2 receptor (AT2) is involved in

vasodilation, natriuresis, and growth inhibition. Hypertension and hypertension-related organ damage resulting from excessive activation of the RAS are mostly mediated by the AT1 receptor [14].

### Control of Renin Production and Secretion

Renin, the central regulator of the renin-angiotensin cascade, is a highly specific aspartic peptidase with AGT as the sole known substrate. Renin is also species-specific in that human renin is not able to cleave murine AGT, and vice versa [15–17]. The structure of renin is composed of two  $\beta$ -sheet domains with the enzymatic active site residing in a cleft between these two domains [18,19]. Renin and its inactive precursor, prorenin, are synthesized and secreted from the JG cells, highly granulated smooth muscle cells located in the media of the afferent arteriole at the vascular pole of the glomerulus (Fig. 40.2). Renin is synthesized as a prepolypeptide precursor during translation in the endoplasmic reticulum. The target signal sequence is cleaved during translocation in the endoplasmic reticulum, yielding the inactive prorenin. Prorenin is glycosylated and activated during intracellular transport through the Golgi apparatus, and eventually stored in secretory granules. The prosequence is removed during the activation process. Renin is secreted from these granules through exocytosis upon stimulation [20,21].

In the plasma the prorenin concentration is usually much higher (10–100 times) than the renin concentration. Prorenin is 43 amino acids longer than mature renin at the NH<sub>2</sub>-terminus, and this NH<sub>2</sub>-terminal prosegment is thought to block the enzymatic active site located in the cleft, thus preventing the interaction of



**FIGURE 40.2** Structure of the glomerulus. The tubular pole, which is where the proximal tubule leaves the corpuscle, is at the top, and the vascular pole, which is where the afferent arteriole enters and the efferent arteriole leaves the corpuscle, is at the bottom. The granulated renin-producing JG cells are located in the afferent arteriole at the vascular pole and are in close vicinity to the macula densa. (Adapted from Cormack (1984) *Introduction to Histology*, Fig. 15-5, p. 344, with permission.)

the active site with the substrate AGT. Although *in vitro* studies have shown that prorenin can be activated by endopeptidase such as trypsin and cathepsin B or by low pH, the mechanism involved in proteolytic prorenin activation *in vivo* and its physiological role remain poorly defined. High renin activity can result in inappropriate activation of RAS, leading to hypertension and end-organ damage. In fact, increased plasma renin activity is associated with hypertension [22], left ventricular hypertrophy [23], and renal dysfunction [24].

The (pro)renin receptor, a single transmembrane receptor initially identified in mesangial cells and vascular smooth muscle cells [25], binds to both renin and prorenin with high affinity. Renin bound to the receptor exhibits increased catalytic activity, and prorenin bound to this receptor exhibits full enzymatic activity comparable to that of mature renin. In addition,

binding of renin or prorenin to this receptor triggers intracellular signaling and phosphorylation of MAP kinase independent of Ang II generation. Stimulation of the (pro)renin receptor in mesangial cells with purified renin or prorenin promotes the synthesis of TGF- $\beta$  [26], a profibrotic factor involved in the development of nephropathy. Thus, increased renin/prorenin can also cause tissue injury through the (pro)renin receptor independent of Ang II (Fig. 40.1). For instance, transgenic rats overexpressing prorenin in the liver with a high level of circulating prorenin developed severe vascular damage and diabetic renal complications in the absence of high blood pressure [27]. The exact physiological roles of the (pro)renin receptor, however, remain to be fully defined.

Because of its central role in the renin-angiotensin cascade, the biosynthesis and secretion of renin is

tightly regulated. The most common physiological factors that influence renin secretion include renal perfusion pressure, renal sympathetic nerve activity, and tubular sodium chloride load [20,28]. The perfusion pressure in the renal artery is the most profound parameter to influence renin secretion: when the renal perfusion pressure falls, renin secretion rises, and vice versa. This effect is mediated by a baroreceptor or stretch receptor mechanism in the JG cells [29]. The JG apparatus has rich sympathetic nerve endings, and stimulation of renin synthesis and release by sympathetic nerve activity is mediated by  $\beta$ -adrenergic receptors and intracellular cyclic AMP [30]. This pathway may exert a tonic stimulatory influence on renin production [31]. Renin secretion is also tightly regulated by the tubular sodium chloride load [32]. There is an inverse relationship between dietary sodium chloride intake and renin secretion. Tubular control of renin release is mediated by the macula densa, which is part of the distal tubule and anatomically in close association with the JG cells (Fig. 40.2). The macula densa senses the sodium chloride load and transduces the signal, possibly via p38 MAP kinase, PGE<sub>2</sub>, NO, and ATP/adenosine, to the JG cells to influence renin production and secretion [28,33].

At the local level, renin synthesis and release are influenced by a variety of bioactive molecules. For instance, prostaglandins, nitric oxide, and adrenomedullin are known to stimulate renin secretion, whereas Ang II, endothelin, vasopressin, atrial natriuretic peptide, and adenosine are inhibitors of renin production [20,28]. Ang II is a potent negative feedback inhibitor of renin production and secretion that maintains the homeostasis of renin levels [34], and this inhibitory effect is mediated by the AT1 receptor [35,36].

### Transcriptional Regulation of Renin Gene Expression

Renin is encoded by a single gene in humans. In mice, some strains (e.g., C57BL/6) have one renin gene (Ren-1<sup>c</sup>), whereas others (e.g., DBA/2, J129) contain two renin genes (Ren-1<sup>d</sup> and Ren-2), which are closely linked and probably result from a duplication of the 21 kb Ren-1<sup>c</sup>-like ancestral gene [37]. The human renin gene and the three mouse renin genes all share the same overall genomic organization (e.g., nine exons and eight introns) and encode highly homologous proteins. For instance, Ren-1 and Ren-2 share 97% amino acid identity [38]. It is believed that the Ren-1 protein is the major source of circulating renin and thus is the major systemic regulator of the renin-angiotensin cascade. Transgenic studies demonstrate that the Ren-1<sup>d</sup> and Ren-2 genes cooperate to preserve the homeostasis of the RAS [39].

Cyclic AMP is a major mediator for renin synthesis and secretion, and several cAMP response elements (CRE) have been identified in both murine and human renin gene promoters [21,28]. But both CREB-dependent and -independent mechanisms may be involved in the cAMP-PKA pathway in human renin promoter activation [40]. Transgenic studies have demonstrated that sequences required for the tissue-specific and development stage-specific expression of the renin, as well as for the response to a variety of physiological stimuli, are located within 5 kb of the 5'-flanking region of the murine renin gene [41–43]. In the 5'-flanking region of murine Ren-1<sup>c</sup> gene, a 223-bp minimal promoter (–117 to +6) and a 242-bp enhancer (–2866 to –2625) have been found to be essential for high-level expression of the renin gene [44]. Genetic deletion of this enhancer region from the renin gene leads to reduction of renin expression in the JG cells and low blood pressure in mice [45].

Renin gene expression is regulated by a complex network of transcriptional factors [46]. In the renin gene promoter and enhancer regions, multiple transcription factor-binding sites have been identified, which are responsive to various signal transduction pathways including cAMP, retinoic acid, endothelin-1, and cytokines, to alter renin gene transcription. An array of transcriptional factors has been identified to be involved in the transcriptional regulation of renin gene expression. These factors include positive regulators such as LXR $\alpha$ , RAR/RXR, CREB/CREM, USF1/USF2, HOX genes, NFI, and SP1/SP3 [47–51], and negative regulators such as NF-Y and Ear-2 [52,53]. Thus, the production of renin is determined by a combined interplay of multiple transcriptional regulators available or activated under a specific physiological condition.

### Pharmacological Inhibition of the Renin-Angiotensin System

The RAS has been a major therapeutic target for intervention of renal and cardiovascular disorders. Small-molecule drugs that target the RAS include ACE inhibitors (ACEIs), Ang II type 1 receptor blockers (ARBs), and renin inhibitors. These drugs inhibit each major step of the renin-angiotensin cascade, respectively (Fig. 40.1). ACEIs and ARBs are probably the most successful and most widely prescribed antihypertensive drugs [54,55]. Aliskiren is the first FDA-approved renin inhibitor that specifically inhibits the enzymatic activity of renin and lowers blood pressure in hypertensive subjects [56,57].

There are a number of issues associated with the current ACEIs and ARBs that may compromise the efficacy of RAS blockade and cause undesired side effects. For example, Ang II conversion from Ang I can be

catalyzed by other enzymes such as chymase [58], thus bypassing the ACE [59], and this reduces the efficacy of ACEIs. In addition to Ang I, ACE also recognizes other substrates such as bradykinin [60]; thus inhibition of ACE may also alter bradykinin metabolism and evoke undesirable side effects. Because Ang II has multiple receptors (e.g., AT1, AT2) with different functions [14], blocking the AT1 receptor with ABRs may increase the availability of Ang II to the AT2 receptor, leading to enhancement of unwanted AT2 activity. The increase in Ang II due to AT1 receptor blockade may lead to elevation of various Ang II metabolites, such as Ang (1–7), Ang III, and Ang IV [1], which are bioactive and may cause a variety of unwanted effects.

A major problem associated with all current RAS inhibitors is the compensatory increase of renin concentration [61]. Patients receiving chronic dosing of ACEIs initially have lower plasma Ang II levels; however, Ang II, as well as aldosterone, often rises to the original baseline levels [62,63]. This phenomenon, often termed “ACE escape,” is caused by the disruption of the negative feedback loop in renin biosynthesis, leading to increased renin production. The negative feedback regulation is mediated by the AT1 receptor. The problem of plasma renin increase also exists in the case of ARBs and renin inhibitor aliskiren [64]. The huge increase in renin concentration and activity in the plasma and tissue interstitial space can stimulate the conversion of Ang I, which ultimately leads to the build-up of Ang I, Ang II, and other angiotensin metabolites in the body, through ACE-dependent and -independent pathways (see Fig. 40.5). Ang II accumulation limits the efficacy of RAS inhibition and may explain why the current RAS inhibitors are only suboptimal clinically. Therefore, it is speculated that agents that block the compensatory renin increase can enhance the efficacy of RAS inhibition.

## VITAMIN D REGULATION OF THE RENIN-ANGIOTENSIN SYSTEM

### Vitamin D Hormone as a Negative Endocrine Regulator of the Renin-Angiotensin System

More than two decades ago, two articles reported an inverse relationship between circulating  $1,25(\text{OH})_2\text{D}_3$  levels and plasma renin activity in hypertensive subjects [65,66]. The significance of these studies was hardly recognized until the discovery that  $1,25(\text{OH})_2\text{D}_3$  is a negative endocrine regulator of renin gene expression [67]. This finding has enormous physiological, pathological, and pharmacological implications.

The vitamin D receptor (VDR)-null mutant mouse, which lacks VDR-mediated vitamin D signaling, is

a complete vitamin-D-deficient model [68,69]. In 2002, we reported that VDR-null mice developed hyperreninemia due to dramatic up-regulation of renin expression in the kidney [67]. The up-regulation of renin was detected at both mRNA and protein levels, leading to marked increase in plasma renin activity and plasma Ang II levels (Fig. 40.3). The hepatic expression of AGT, the substrate of renin, was unchanged. Thus the increase in plasma Ang II production is mainly due to the increase in renin activity. As a consequence of aberrant RAS overstimulation, VDR knockout mice developed high blood pressure, cardiac hypertrophy, and an overdrinking behavior. Cardiac hypertrophy is reflected by an increase in the heart weight and in the size of left ventricular cardiomyocytes, as well as elevation of left

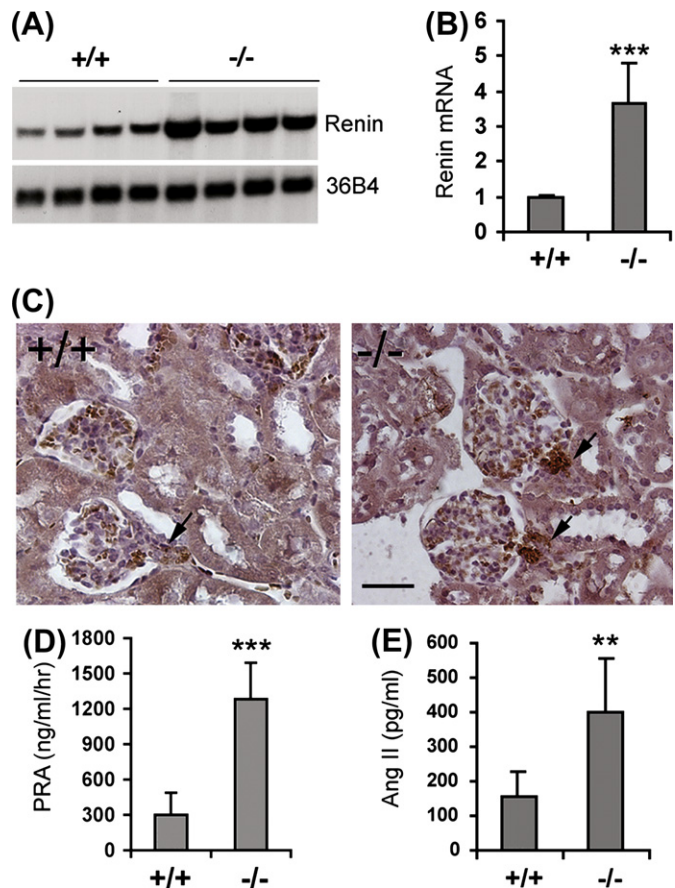


FIGURE 40.3 VDR-null mice develop hyperreninemia. (A) Northern blot showing marked up-regulation of renin mRNA expression in the kidney of VDR-null mice. (B) Quantitative results of the Northern blot data; \*\*\*,  $P < 0.001$  vs. +/+ mice. (C) Immunostaining of kidney cortex sections with renin antiserum. Arrows indicate the afferent glomerular arterioles in the juxtaglomerular region. Note the marked increase in renin staining in VDR-null kidney. (D) Plasma renin activity in wild-type and VDR-null mice. (E) Plasma Ang II concentrations in wild-type and VDR-null mice. \*\*  $P < 0.01$ ; \*\*\*,  $P < 0.001$  vs. +/+ mice. +/+, wild-type; -/-, VDR-null; PRA, plasma renin activity; Ang II, angiotensin II. (From Li et al. (2002), with permission.) Please refer to color plate section.

ventricular ANP and plasma ANP levels, the surrogate marker of cardiac hypertrophy [70]. Urinary volume and urinary salt excretion are also increased, whereas plasma sodium and potassium concentrations remain normal in the mutant mice. All these abnormalities can be corrected by treatment with an ACEI or an ARB, confirming that RAS activation is responsible for these phenotypes [67,70]. As expected, plasma and urinary aldosterone levels are also markedly elevated in VDR knockout mice [71]. Moreover, renin expression in the brain is also up-regulated in VDR-null mice, leading to activation of the local RAS in the brain, which is mostly responsible for the overdrinking and polyuric phenotypes seen in the mutant mice [72].

Inactivation of VDR leads to development of hypocalcemia, secondary hyperparathyroidism, and alopecia [68], which may potentially influence renin production and secretion. Several lines of evidence demonstrate that vitamin D regulation of renin is independent of calcium, parathyroid hormone, or alopecia. First, normocalcemic neonatal VDR knockout mice have elevated renin expression, indicating that hyperreninemia develops prior to hypocalcemia; second, renin expression remains elevated in adult VDR-null mice whose serum calcium levels are maintained normal with a high-calcium diet; third, renin expression is normal in *Gcm2*-null mice that are as hypocalcemic as VDR-null mice [73,74]; and fourth, renin expression remains up-regulated in VDR knockout mice whose alopecia is rescued by targeted expression of human VDR in the skin [75]. Moreover, despite the high basal renin synthesis, the basic regulatory mechanisms that control renin production, including the Ang II negative feedback and salt- and volume-sensing mechanisms, remain intact in VDR-null mice [67,71], indicating that the vitamin D repression is independent of these mechanisms.

The inhibitory role of vitamin D in renin biosynthesis has been confirmed by a transgenic approach. We recently produced transgenic mice that overexpress the human VDR in the JG cells. In these transgenic mice, renal renin mRNA levels and plasma renin activity were significantly suppressed while serum calcium and parathyroid hormone levels were normal. When the human VDR transgene was bred into VDR knockout mice to generate knockout mice that express VDR only in the JG cells, renin up-regulation was markedly reduced in these mice compared to VDR-null mice [76]. These data further prove that  $1,25(\text{OH})_2\text{D}_3$  suppresses renin expression *in vivo* independent of parathyroid hormone and calcium. This conclusion is consistent with previous observations in humans that the inverse relationship between serum  $1,25(\text{OH})_2\text{D}_3$  levels and plasma renin activity or blood pressure is independent of serum calcium levels [65,77].

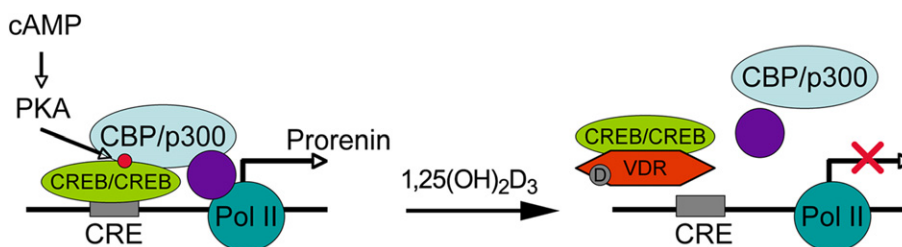
The critical role of vitamin D in the regulation of the RAS *in vivo* has also been confirmed in another genetic mutant mouse model of vitamin D deficiency. These mutant mice lack *Cyp27b1*, the rate-limiting  $1\alpha$ -hydroxylase required for the biosynthesis of  $1,25(\text{OH})_2\text{D}_3$  in the kidney. As seen in VDR knockout mice, *Cyp27b1* knockout mice also developed hyperreninemia, hypertension, and cardiac hypertrophy as a result of renin up-regulation. Importantly, in this model these abnormalities were corrected not only by ACEI or ARB, but also by treating the mice with exogenous  $1,25(\text{OH})_2\text{D}_3$  [78]. Consistently, in wild-type mice rendered vitamin-D-deficient by dietary strontium treatment, which inhibits  $1,25(\text{OH})_2\text{D}_3$  biosynthesis [79], renin expression in the kidney was markedly up-regulated. On the other hand, treatment of wild-type mice with  $1,25(\text{OH})_2\text{D}_3$  significantly reduced renin expression [67]. Together these data firmly establish vitamin D hormone as a crucial negative endocrine regulator of the RAS.

### Molecular Mechanism for Vitamin D Repression of Renin Expression

As a ligand-activated transcription factor, VDR is involved in both positive and negative transcriptional regulations (see Chapters 7 and 8). While most positive regulations are mediated by vitamin D response elements (VDRE) in vitamin D target gene promoters, the mechanisms for negative regulation are diverse. Liganded VDR has been shown to physically interact with a variety of regulatory proteins including Smad3,  $\beta$ -catenin, and p65 NF- $\kappa$ B to down-regulate gene expression [80–83]. In the case of renin repression,  $1,25(\text{OH})_2\text{D}_3$  targets the cyclic AMP signaling pathway [84], a central stimulatory pathway involved in renin biosynthesis [28].

Cyclic AMP signals through cAMP response element (CRE), which interacts with members of the ATF/CREB/CREM bZIP transcription factor family in homodimeric or heterodimeric forms. Intracellular cAMP is converted from ATP by adenylate cyclase. Cyclic AMP binds to the regulatory subunit of protein kinase A to free the catalytic subunit; the latter enters the nucleus and phosphorylates CREB at serine-133 or CREM at serine-117, resulting in the recruitment of ubiquitous co-activators CBP/p300 to promote gene transcription [85–87].

Through systematic deletion analyses of the mouse renin gene promoter, we demonstrated that the CRE at  $-2688$  is necessary to mediate the suppression of renin gene transcription by  $1,25(\text{OH})_2\text{D}_3$ . This CRE is critical for the basal expression of renin [49]. Experimental data obtained from EMSA, ChIP assays, GST pull-down assays, and cell transfection experiments



**FIGURE 40.4** Schematic model of  $1,25(\text{OH})_2\text{D}_3$ -induced transrepression of renin gene transcription. PKA, protein kinase A; D,  $1,25(\text{OH})_2\text{D}_3$ ; Pol II, RNA polymerase II.

demonstrated that  $1,25(\text{OH})_2\text{D}_3$  disrupts the formation of the DNA–protein complex on the CRE site. The complex contains CREB/CREM and CBP/p300.  $1,25(\text{OH})_2\text{D}_3$ -activated VDR physically interacts with CREB, thus blocking CREB binding to this CRE. Consequently the DNA–protein complex cannot be assembled on this CRE [84]. These data establish that  $1,25(\text{OH})_2\text{D}_3$  suppresses renin gene transcription, at least in part, by direct inhibition of CRE-mediated transcriptional activity. The proposed model is that in the basal state CREB, CREM, and CBP/p300 are recruited to the CRE to drive renin gene transcription; in the presence of  $1,25(\text{OH})_2\text{D}_3$ , liganded VDR binds to CREB, blocking CREB binding to the CRE and disrupting the formation of CRE-CREB-CBP/p300 complex. As a result, renin gene expression is inhibited (Fig. 40.4). Besides renin, it is possible that  $1,25(\text{OH})_2\text{D}_3$  may also target the cAMP-CRE-CREB pathway in the regulation of other genes.

This renin repression model has important physiological implications, because cAMP is the central intracellular signal that stimulates renin production and release in the JG cells. For instance, intracellular cAMP is critically involved in renin synthesis and release in response to sympathetic nerve stimulation (mediated by  $\beta$ -adrenergic receptor), as well as to stimulation by prostaglandins, dopamine, adrenomedullin, calcitonin gene-related peptide, and pituitary adenylyl cyclase activating polypeptide [28]. It is speculated that, by targeting the cAMP signaling pathway,  $1,25(\text{OH})_2\text{D}_3$  may function as a general gatekeeper to counterbalance other renin-stimulating factors and prevent detrimental overproduction of renin.

### Vitamin D Analogs as Renin Inhibitors

A large number of vitamin D analogs have been synthesized with a wide range of pharmacological potency and calcemic index (see Section IX of this book). A few vitamin D analogs have been approved for clinical use [88,89]. Therefore, the concept of vitamin D analogs as therapeutic drugs is already sound. The notion that  $1,25(\text{OH})_2\text{D}_3$  suppresses renin biosynthesis

provides a molecular basis to explore vitamin D analogs as renin synthesis inhibitors for therapeutic purposes. Low calcemic vitamin D analogs that have potent renin-inhibiting activity are particularly valuable. The vitamin D analog-based renin inhibitors, which inhibit renin gene expression, are different from another class of aliskiren-like renin inhibitors, which inhibit renin enzymatic activity. There are advantages to having two classes of renin inhibitors. For example, combination of these two classes of drugs simultaneously will inhibit renin at both the biosynthetic and enzymatic levels and thus may increase therapeutic efficacy.

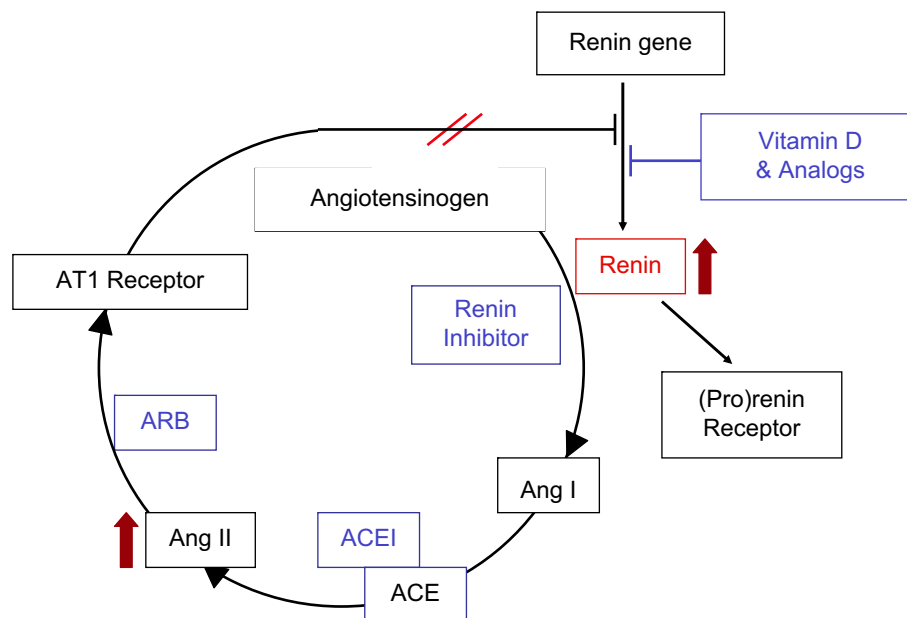
The renin-inhibiting activity of vitamin D analogs has been reported in a number of *in vitro* and *in vivo* models. Through cell culture screening, we have identified a group of vitamin D analog compounds that inhibit renin expression *in vitro* and *in vivo* without invoking severe hypercalcemia in mice, and some analogs are much more potent than  $1,25(\text{OH})_2\text{D}_3$  in terms of renin repression [90]. Paricalcitol (19-nor- $1,25$ -dihydroxyvitamin  $\text{D}_2$ ), an activated vitamin D analog, has been shown to suppress renin expression in mice with the same potency as  $1,25(\text{OH})_2\text{D}_3$  but without induction of hypercalcemia [91]. Paricalcitol can also suppress renin expression in kidney mesangial cells [92]. In the rat model of chronic renal failure (5/6 nephrectomy) paricalcitol treatment was shown to significantly lower blood pressure and suppress the RAS in the remnant kidney [93]. Recently we showed that paricalcitol or doxercalciferol ( $1\alpha$ -hydroxyvitamin  $\text{D}_2$ ) is able to suppress the RAS and effectively block cardiac hypertrophy in spontaneously hypertensive rats [94]. We also reported that patients with chronic kidney disease receiving vitamin D analog therapy have significantly lowered plasma renin activity, suggesting that vitamin D analogs can also suppress the RAS in humans [94]. Therefore down-regulation of the RAS appears to be a major therapeutic mechanism underlying the beneficial effects of vitamin D analogs in renal and cardiovascular diseases.

As discussed above, the dramatic compensatory increase in plasma renin concentration that is associated with the current RAS inhibitors may compromise the

efficacy of these drugs. One important application of vitamin D analogs is to block the compensatory renin increase at the transcriptional level when used together with the classic RAS inhibitors. The combination is expected to enhance the efficacy of RAS inhibition and achieve better therapeutic outcomes [95] (Fig. 40.5). We have tested the combination strategy (an ARB plus a vitamin D analog) in models of diabetic nephropathy and cardiac hypertrophy. We showed that combination of losartan and paricalcitol or doxercalciferol produced synergistic effects in prevention of albuminuria and glomerulosclerosis, the main pathogenic hallmarks of diabetic renal injury. The molecular basis underlying the therapeutic synergism is the blockade of the compensatory renin increase and intrarenal Ang II accumulation [96–98]. Similarly, in spontaneously hypertensive rats, the combination therapy markedly shrunk cardiac hypertrophy in a synergistic fashion, also as a result of inhibition of the compensatory rise of renin in the kidney and heart [94]. Clinical trials of vitamin D analogs in patients with chronic kidney disease who are already on ARB or ACEI therapy suggest that the concept of combination synergy also works in human patients [99]. Given the wide use of the RAS inhibitors, the combination strategy warrants more and larger clinical trials to test its therapeutic efficacy in humans.

## Pathophysiological Implications

The finding that  $1,25(\text{OH})_2\text{D}_3$  regulates the RAS is consistent with the view that the vitamin D endocrine system plays multiple physiological roles.  $1,25(\text{OH})_2\text{D}_3$  is a principal regulator to maintain calcium homeostasis. The notion of  $1,25(\text{OH})_2\text{D}_3$  as a negative regulator of renin production implies that vitamin D deficiency or insufficiency can cause activation of the RAS. Adding to the complexity and controversy in defining vitamin D deficiency, the threshold of vitamin D status to induce hyperreninemia is unknown and might be different from that for hypocalcemia. Nevertheless, as described in the following sections, vitamin D deficiency is highly prevalent in patients with renal and cardiovascular problems. Given the broad pathological roles of the RAS in the development of renal and cardiovascular disorders [100,101], the notion of vitamin D affecting the RAS status suggests that vitamin D deficiency is not only associated with renal and cardiovascular diseases but may also promote them. This view has already gained support from clinical data [102]. Therefore, at least in part because of the RAS, long-term vitamin D deficiency may increase the risk of renal and cardiovascular diseases, whereas vitamin D supplementation and therapies with vitamin D and its analogs should be beneficial. In the following sections we will further discuss



**FIGURE 40.5** Compensatory increase of renin and its inhibition by vitamin D. Ang II feedback inhibition of renin expression is mediated by angiotensin type I (AT1) receptor. This negative feedback mechanism is required to maintain the homeostasis of the RAS. When the RAS is inhibited with renin enzymatic inhibitor, ACE inhibitor (ACEI) or AT1 receptor blocker (ARB), the feedback loop is disrupted, leading to overexpression of renin. Renin overproduction can increase Ang I conversion, which ultimately leads to Ang II increase, thus reducing the efficacy of RAS inhibition. Renin accumulation can also activate the (pro)renin receptor independent of Ang II to cause organ damage. When a vitamin D analog is used together with one of the three classes of RAS inhibitors, vitamin D analog will block the compensatory renin expression, thus enhancing the efficacy of RAS inhibition. That is the molecular basis for the synergism between vitamin D analogs and classic RAS inhibitors in combination therapy.



the relationship between vitamin D status and renal and cardiovascular diseases in the human population and the potential of vitamin D and its analogs in the prevention and intervention of these diseases.

## VITAMIN D, BLOOD PRESSURE AND HEART DISEASE

### Sunlight and Blood Pressure

Hypertension, or high blood pressure, is one of the most prevalent health problems in the world. Hypertension increases the risk of heart attack, heart failure, stroke, atherosclerosis, and kidney disease. Data from the National Health and Nutrition Examination Surveys (NHANES) show that in the United States 29% adults had hypertension in 2005–2006. Despite intense prevention and intervention efforts, there was still no change in the prevalence of hypertension during 1999–2006 [103].

An increasing body of evidence has suggested a link between sunlight exposure, vitamin D, and blood pressure. As ultraviolet (UV) irradiation is essential for the cutaneous synthesis of vitamin D, circulating 25-hydroxyvitamin D (25(OH)D) levels are influenced by geographic locations, seasonal changes, and skin pigmentations (see Chapter 2). UV irradiation decreases with the increase in latitude, and the data from the INTERSALT study show that the increase in latitude is correlated with the rise of blood pressure and the prevalence of hypertension in the general population around the globe [104]. An increasing gradient of hypertension prevalence and stroke incidents from south to north has also been reported in China [105]. Seasonal variations in blood pressure are seen in temperate climates, with blood pressure higher in the winter (low UV irradiation) than in the summer (high UV irradiation) [106,107]. Winter season is also associated with high incidence of myocardial infarction [108]. Dark skin pigmentation in the black population, which inhibits an efficient UV light penetration, is associated with higher blood pressure [109,110]. A small clinical study has shown that direct UVB irradiation is able to lower blood pressure in patients with mild essential hypertension [111].

### Vitamin D Deficiency and High Blood Pressure

Epidemiological studies have established a correlation between vitamin D deficiency and high blood pressure and cardiovascular risk factors. The NHANES III, a cross-sectional survey representative of the US civilian population from 1988 to 1994, shows an inverse relationship between serum 25(OH)D and blood pressure [112]. In this nonhypertensive population ( $n > 2500$ ), systolic

and diastolic blood pressures are 3 and 1.6 mmHg higher, respectively, in the lowest quintile of serum 25(OH)D compared to the highest quintile. This difference, although modest, has public health significance, as a 2–3 mmHg decrease in blood pressure could account for 10–15% decline in cardiovascular mortality on a population basis [113]. Analysis of the NHANES III database ( $n > 15\,000$  adults) revealed that serum 25(OH)D levels are also inversely associated with increased prevalence of cardiovascular risk factors including hypertension, diabetes, obesity, and hyperlipidemia [114].

Other epidemiological studies confirm the association between vitamin D deficiency and hypertension. Prospective cohorts from the Health Professionals' Follow-Up Study (HPFS) ( $n = 613$ ) and the Nurses' Health Study (NHS) ( $n = 1198$ ) showed that serum 25(OH)D levels are inversely associated with the risk of incident hypertension during 4 years of follow-up [115]. A nested case–control prospective study using the HPFS database ( $n > 18\,000$ ) also demonstrated an association of low serum 25(OH)D levels with higher risk of myocardial infarction, even after adjusting for factors known to be associated with coronary artery disease [116]. An examination of the Framingham Offspring Study participants without prior cardiovascular disease ( $n = 1739$ ) concluded that during the mean follow-up of 5.4 years low serum 25(OH)D ( $< 10\text{--}15$  ng/ml) is associated with incident cardiovascular disease after adjustment for C-reactive protein, physical activity, or vitamin use [117].

A similar inverse association is also found between serum levels of  $1,25(\text{OH})_2\text{D}_3$  and blood pressure. A cross-sectional multivariate study with normotensive male industrial employees ( $n = 100$ ) showed an inverse and statistically significant association between serum  $1,25(\text{OH})_2\text{D}_3$  levels and systolic blood pressure independent of serum parathyroid hormone and calcium levels [77]. In another population-based study with 34 middle-aged men serum levels of  $1,25(\text{OH})_2\text{D}_3$  were also found to be inversely correlated to blood pressure [118].

### Interventional Effects of Vitamin D on Blood Pressure

Many clinical studies have reported cardiovascular benefits of vitamin D supplementation or therapy. In a double-blinded, placebo-controlled clinical trial with 39 hypertensive patients, blood pressure was significantly reduced after 4 months of  $1\alpha$ -hydroxyvitamin  $\text{D}_3$  treatment [119]. In a clinical trial involving 148 elderly women, 8-week supplement of vitamin  $\text{D}_3$  (800 IU) plus calcium (1200 mg) significantly reduced systolic blood pressure in these subjects [120]. A large prospective study with 28 886 middle-aged women in

the US found that dietary intake of dairy products, calcium, and vitamin D are each inversely associated with risk of hypertension during 10 years of follow-up [121].

However, not all reported studies support a role of vitamin D in blood pressure control. In a clinical trial involving 189 elderly subjects, a single oral dose of 2.5 mg cholecalciferol (100 000 IU) in winter failed to change blood pressure [122]. A possible explanation is that a single dose of vitamin D supplement in the winter is not sufficient to raise the circulating vitamin D level to the blood-pressure-affecting threshold. Results from three large prospective cohort studies including NHS I and II and HPFS failed to find an association between high intake of vitamin D and low risk of incident hypertension [123]. These conflicting reports call for more rigorous and well-controlled investigations into the effects of vitamin D on blood pressure.

### Vitamin D Status and the Renin-Angiotensin System

Many studies have investigated the correlation between vitamin D status and the RAS in humans. Resnick et al. first demonstrated an inverse correlation between serum levels of  $1,25(\text{OH})_2\text{D}_3$  and plasma renin activity in a study with 51 patients with essential hypertension [65] (Fig. 40.6). Subsequently, another smaller study confirmed the inverse correlation between the change in circulating  $1,25(\text{OH})_2\text{D}_3$  and the change in plasma renin activity in subjects with high renin hypertension [66]. In the Ludwigshafen Risk and Cardiovascular Health (LURIC) study that includes more than 3000 patients referred for coronary angiography, both

serum  $25(\text{OH})\text{D}$  and  $1,25(\text{OH})_2\text{D}_3$  levels were found to be independently and inversely associated with plasma renin concentration and Ang II levels [124]. Another study reported an inverse association between serum  $25(\text{OH})\text{D}$  levels and Ang II levels in normotensive individuals ( $n = 184$ ); it also showed an association of lower serum  $25(\text{OH})\text{D}$  levels with increased renal plasma flow in response to Ang II infusion [125], suggesting increased intrarenal RAS activity in individuals with lower serum  $25(\text{OH})\text{D}$  levels. Consistent with these observations, data from clinical studies also support a connection between vitamin D and renin. In a double-blinded, placebo-controlled clinical trial (32 subjects), 16 weeks of daily oral calcium supplementation, which suppresses plasma  $1,25(\text{OH})_2\text{D}_3$  levels, resulted in a significant elevation of plasma renin activity, suggesting a suppressive role of vitamin D in renin regulation (although an effect of calcium independent of vitamin D is possible) [126]. In a clinical study involving 25 hypertensive patients with end-stage renal disease, 15 weeks of  $1,25(\text{OH})_2\text{D}_3$  treatment reduced myocardial hypertrophy, with a concomitant reduction in plasma renin activity, Ang II and ANP levels [127].  $1,25(\text{OH})_2\text{D}_3$  treatment was also reported to reduce blood pressure and plasma renin activity in a patient with pseudohyperparathyroidism and high plasma renin activity [128]. Therefore, the beneficial effects of vitamin D on the cardiovascular system reported in the literature, including reduction of high blood pressure, likely include the down-regulation of the RAS [129,130]. Given the critical role of the RAS in the cardiovascular system, the relationship between vitamin D and plasma renin activity is likely part of the mechanism underlying the relationship between vitamin D and blood pressure. This notion is worth more translational and clinical investigations.

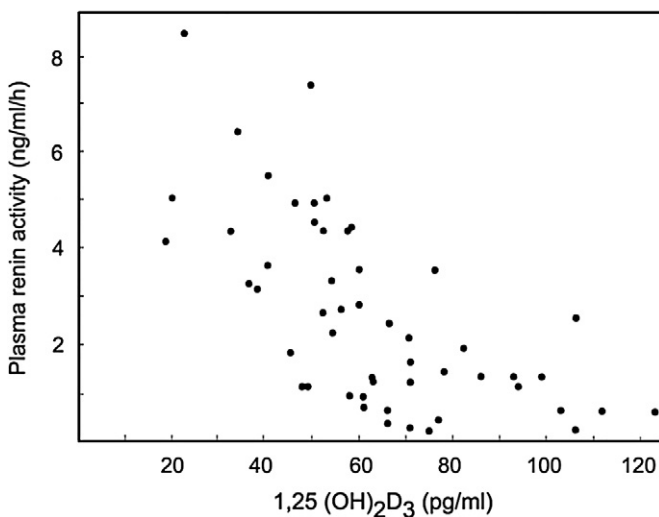


FIGURE 40.6 Inverse correlation between circulating  $1,25(\text{OH})_2\text{D}_3$  levels and plasma renin activity in patients with essential hypertension. (Adapted from Resnick et al. (1986) Fig. 2, p. 652, with permission.)

## VITAMIN D AND CHRONIC KIDNEY DISEASE

### Increasing Prevalence of Chronic Kidney Disease

Chronic kidney disease (CKD) affects more than 50 million people worldwide. The prevalence of CKD and kidney failure is continuously rising with the growing global epidemic of metabolic syndrome and diabetes. In the United States, the incidence and prevalence of end-stage renal disease have doubled in the past 10 years. The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative estimates that CKD affects 11% of the US population. Diabetic nephropathy is the most common renal complication of diabetes. Diabetes is by far the leading cause of

CKD, accounting for 44% of new cases of end-stage renal disease in 2005. Current clinical care and management of kidney disease are costly with poor outcomes. Thus, new therapeutic strategies and methods for CKD treatment are urgently needed.

### Vitamin D Deficiency in Chronic Kidney Disease

Vitamin D deficiency has been increasingly recognized as a prominent feature of CKD. This is in part because the kidney is a key organ involved in vitamin D metabolism. The kidney not only provides the enzymatic system for the synthesis of  $1,25(\text{OH})_2\text{D}_3$ , but also is involved in the uptake of filtrated  $25(\text{OH})\text{D}$  from the urine, in the form of vitamin-D-binding protein (DBP)– $25(\text{OH})\text{D}$  complex, through megalin-mediated endocytosis [131]. Renal  $1\alpha$ -hydroxylase activity starts to decline even in the early stages of CKD. Continued progression of CKD leads to accumulation of phosphate in the serum, which suppresses  $1\alpha$ -hydroxylase activities. Proteinuria, a hallmark of renal disease, results in loss of DBP– $25(\text{OH})\text{D}$  from the urine. Therefore vitamin D deficiency, particularly  $1,25(\text{OH})_2\text{D}_3$  deficiency, is very common in patients with CKD even at the early stages [132] (Fig. 40.7). Accumulating evidence has demonstrated a correlation between vitamin D deficiency and progression of CKD, and plasma vitamin D status is an independent inverse predictor of disease progression and death in patients with CKD [102]. Thus vitamin D deficiency may in fact accelerate the progression of kidney disease. In fact, a large number of retrospective observational studies have demonstrated multiple beneficial effects of calcitriol or vitamin

D analog therapy in both hemodialysis and nondialysis CKD patients, leading to significant survival advantage for the patients receiving the therapy [133–137]. Because the risk of death is significantly lower in the treated patients with all levels of serum calcium, phosphorus, and PTH, the underlying protective mechanism of vitamin D likely extends beyond the impact on PTH and mineral metabolism. See Chapters 70 and 81 for further discussion of vitamin D therapy in renal disease patients.

### Therapeutic Mechanisms of Vitamin D: Targeting the Local Renin-Angiotensin System

It is well established that activation of the local, intrarenal RAS plays a key role in kidney damage [13]. Kidney cells have the capacity to synthesize all components of the RAS. Many pathological factors such as hyperglycemia, renal insufficiency, and vitamin D deficiency can activate the intrarenal RAS, leading to increased local production of Ang II that acts in a paracrine manner within the kidney. For example, in diabetes the intrarenal interstitial Ang II levels can reach as much as 1000 times higher than in the plasma. Ang II has a range of pathological activities that promote the progression of renal injury and renal failure [100,101]. Thus inhibition of the RAS has been the first line of treatment for kidney disease. ACEIs, ARBs, and the renin inhibitor aliskiren have been shown to reduce the progression of diabetic nephropathy in a number of large clinical trials [138–142]; however, the growing number of patients with CKD attests that these therapies are insufficient and ineffective to halt the epidemic of kidney disease.

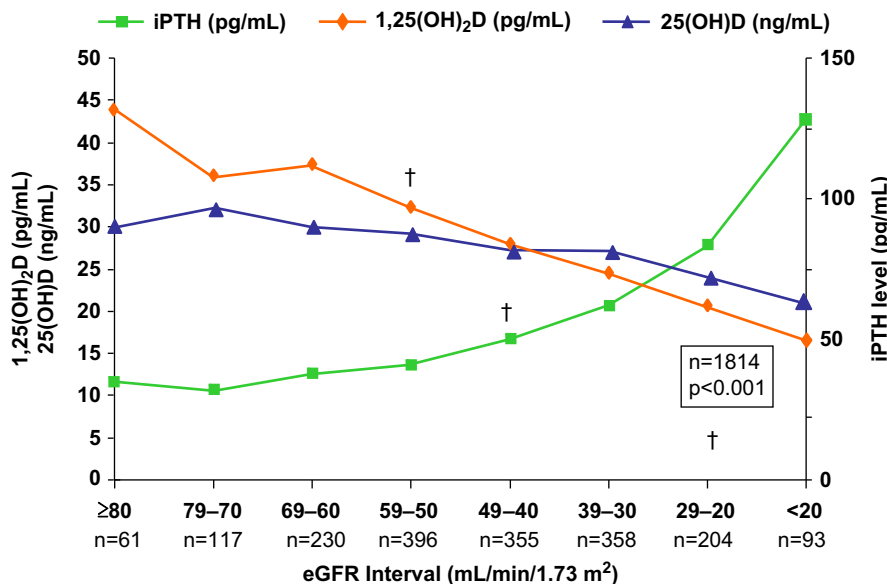


FIGURE 40.7 Vitamin D status decreases along with progression of chronic kidney disease. Serum 25-hydroxyvitamin D ( $25(\text{OH})\text{D}$ ) and 1,25-dihydroxyvitamin D ( $1,25(\text{OH})_2\text{D}$ ) levels begin to decline in CKD patients in early Stage 2. iPTH, intact parathyroid hormone. Adapted from Levin A et al. *Kidney International* (2007) 71:31–38, Figure 5.

Proteinuria, glomerulosclerosis, and interstitial fibrosis are some of the key features of kidney disease. That vitamin D protects the kidney by suppressing the local RAS has been demonstrated in a number of kidney disease models. We have shown that in diabetic state or under unilateral ureteral obstruction (UUO) VDR-null mutant mice (with normal serum calcium) developed more severe renal injury compared with wild-type mice. Diabetic VDR-null mice showed earlier and more robust albuminuria and glomerulosclerosis, and VDR-null mice with UUO showed increased interstitial fibrosis in the obstructed kidney. In both models, the more severe kidney injury is largely due to the increased activation of the local RAS [92,143].

The subtotaly nephrectomized rat model recapitulates renal insufficiency seen in advanced-stage CKD. The pathophysiology of this model is characterized by progressive glomerular and tubulointerstitial damage, glomerular hypertension and proteinuria, in which the activation of the RAS plays a pivotal role. Treatment with  $1,25(\text{OH})_2\text{D}_3$  can reduce glomerulosclerosis and albuminuria and prevent podocyte injury in 5/6 nephrectomized rats [144,145]. Freundlich et al. reported that paricalcitol suppressed the activation of the local RAS in the kidney remnant, attenuated glomerular and tubulointerstitial damage and reduced high blood pressure and proteinuria in rats with 5/6 nephrectomy, confirming the importance of RAS blockade in prevention of renal function deterioration [93].

The mainstay treatment of diabetic nephropathy is to inhibit the RAS, but RAS inhibitors induce a compensatory rise of renin activity (see Fig. 40.5). We have demonstrated that blocking the compensatory increase of renin with vitamin D analogs markedly enhances the reno-protective efficacy in experimental models of both type 1 and type 2 diabetes mellitus [96–98]. In these studies, diabetic mice were treated with a combination of an ARB (losartan) and a vitamin D analog (paricalcitol or doxercalciferol) or with monotherapy. The combination strategy produced additive or synergistic therapeutic effects much better than the monotherapies, including inhibition of albuminuria and glomerulosclerosis and amelioration of glomerular filtration barrier damage. This is largely a result of blockade of renin induction and Ang II accumulation within the kidney. Similar combination therapies have also been used in the models of subtotal nephrectomy and UUO with significant better therapeutic outcomes compared to the monotherapy [146,147]. These preclinical investigations have important implications for the therapeutic treatment of kidney disease in humans.

## Renoprotection of Vitamin D Therapy in Chronic Kidney Disease

Albuminuria is a major risk factor for progressive renal function decline and is believed to be the initial step in an inevitable progression to proteinuria and renal failure in humans. Thus reduction of albuminuria is a major target for renoprotective therapy in CKD. A number of epidemiological and clinical studies have demonstrated potent antiproteinuric activity of vitamin D and vitamin D analogs. In a large cohort cross-sectional analysis of data from the NHANES III, vitamin D insufficiency was found to be associated with increased prevalence of albuminuria [148], suggesting that vitamin D has an intrinsic antiproteinuric property. The therapeutic antiproteinuric activity of vitamin D analogs was first reported in a retrospective analysis of patients with CKD [149]. In that study the antiproteinuric effect of paricalcitol was seen even in subjects already treated with ACEI or ARB, indicating that the effects of vitamin D analogs are on top of those of ACEI and ARB. A recent randomized double-blinded pilot trial in patients with stage 2–3 CKD ( $n = 24$ ) showed that paricalcitol treatment for 1 month significantly reduced albuminuria and inflammation status in the drug-treated subjects, and these effects were independent of its effects on hemodynamics and PTH suppression [99]. Again, as the CKD patients in this study were already on ACEI or ARB treatment, the beneficial effects of vitamin D analogs are additive or synergistic to those of RAS inhibitors. These clinical data warrant larger and long-term randomized, controlled trials to confirm the therapeutic benefits of vitamin D and its analogs.

## CONCLUSION

Recent genetic, physiological, biochemical, and molecular studies have established  $1,25(\text{OH})_2\text{D}_3$  as a negative endocrine regulator of the RAS. This discovery reveals an important physiological function of the vitamin D endocrine system. As such, long-term vitamin D deficiency can lead to overactivation of the RAS. Because of the broad involvement of the RAS in the development of renal and cardiovascular diseases, this finding has invaluable pathophysiological and therapeutic implications. It provides a mechanistic insight into the ever-increasing epidemiological and clinical evidence linking vitamin D deficiency to renal and cardiovascular problems in the general population. It also provides a molecular basis to explore the therapeutic potentials of vitamin D and its analogs in the prevention and intervention of these diseases. In

this regard, the rising prevalence of hypertension and chronic kidney disease around the world attests the urgent need for new and more effective therapeutic methods. Given the promising data obtained from recent translational and clinical studies, the future for vitamin D analogs to become renal and cardiovascular drugs appears to be bright.

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