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Chapter 28. Vitamin D: Production, Metabolism, Mechanism of Action, and Clinical Requirements

Daniel Bikle,[1] John Adams,[2] and Sylvia Christakos[3]

[1]Veteran Affairs Medical Center and Department of Medicine and Dermatology, University of California, San Francisco, California; [2]Orthopaedic Hospital, University of California, Los Angeles, California; [3]Department of Biochemistry and Molecular Biology, New Jersey Medical School, Newark, New Jersey

VITAMIN D_3 PRODUCTION

Vitamin D_3 is produced from 7-dehydrocholesterol (7-DHC; Fig. 1). Although irradiation of 7-DHC was known to produce pre-D_3 (which subsequently undergoes a temperature-dependent rearrangement of the triene structure to form D_3, lumisterol, and tachysterol), the physiologic regulation of this pathway was not well understood until the studies of Holick et al.[1–3] They showed that the formation of pre-D_3 under the influence of solar or UVB irradiation (maximal effective wavelength between 290 and 310 nm) is relatively rapid and reaches a maximum within hours. Both the degree of epidermal pigmentation and the intensity of exposure correlate with the time needed to achieve this maximal concentration of pre-D_3 but do not alter the maximal level achieved. Although pre-D_3 levels reach a maximum level, the biologically inactive lumisterol and tachysterol accumulate with continued UV exposure. Thus, short exposure to sunlight would be expected to lead to a prolonged production of D_3 in the exposed skin because of the relatively slow thermal conversion of pre-D_3 to D_3 and of lumisterol to pre-D_3. Prolonged exposure to sunlight would not produce toxic amounts of D_3 because of the photoconversion of pre-D_3 to lumisterol and tachysterol. Melanin in the epidermis, by absorbing UV irradiation, can also reduce the effectiveness of sunlight in producing D_3 in the skin. Sunlight exposure increases melanin production and therefore provides

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Key words: vitamin D, bone, intestine, kidney, osteomalacia, immune function, keratinocytes, vitamin D metabolism, vitamin D mechanism of action, cancer, malabsorption

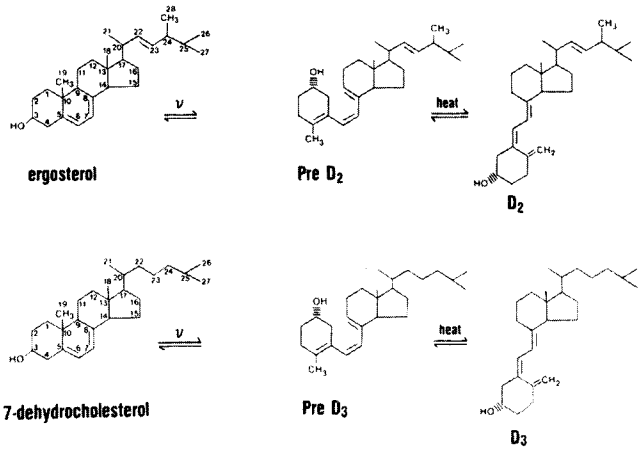

FIG. 1. The photolysis of ergosterol and 7-dehydrocholesterol to vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol), respectively. An intermediate is formed after photolysis, which undergoes a thermal-activated isomerization to the final form of vitamin D. The rotation of the A-ring puts the 3β-hydroxyl group into a different orientation with respect to the plane of the A-ring during production of vitamin D.

another mechanism by which excess D_3 production can be prevented. As just noted, the intensity of UV irradiation is also important for D_3 production and is dependent on latitude; In Edmonton, Canada (52° N), very little D_3 is produced in exposed skin from mid-October to mid-April, whereas in San Juan (18° N) the skin is able to produce D_3 all year long.[4] Clothing and sunscreen effectively prevent D_3 production in the covered areas.

VITAMIN D METABOLISM

Vitamin D, which by itself is biologically inert, must be ferried into the circulation bound to the serum vitamin D–binding protein (DBP) to be metabolically converted to the prohormone, 25-hydroxyvitamin D [25(OH)D; Fig. 2]. There are a number of cytochrome P450 enzymes capable of converting vitamin D to 25(OH)D. These enzymes are principally found in the liver, exhibit a high capacity for substrate vitamin D, and release 25(OH)D back into the circulation and not into the bile. As such, serum 25(OH)D is the most reliable indicator of whether too little or too much vitamin D is entering the host.[5]

25(OH)D is biologically inert unless present in intoxicating concentrations in the blood because of the ingestion of large amounts of vitamin D. Otherwise, it must be converted to $1,25(OH)_2D$, the specific, naturally occurring ligand for the vitamin D receptor (VDR) through CYP27B1 hydroxylase (Fig. 2). The 1-hydroxylase is a heme-containing, inner mitochondrial membrane-embedded, cytochrome P450 mixed function oxidase requiring molecular oxygen and a source of electrons for biological activity. Although the proximal renal tubular epithelial cell is the richest source of 1-hydroxylase and responsible for generating the relatively large amounts of $1,25(OH)_2D$ that are needed to achieve the endocrine functions of the hormone in mineral ion homeostasis, this enzyme is also encountered in a number of extrarenal sites, including immune cells and a variety of normal and malignant epithelia,[6] where it functions to provide $1,25(OH)_2D$ for intracrine or paracrine access to the VDR in these and neighboring cells. As discussed below, the VDR has an extraordinarily broad distribution among human tissues. There are four major recognized means of regulating the 1-hydroxylase: (1) controlling the availability of substrate 25(OH)D to the enzyme; (2) controlling the amount of CYP27B1 hydroxylase expressed; (3) altering the activity of the enzyme by co-factor availability; and (4) controlling the amount and activity of the alternatively spliced, catabolic CYP24 hydroxylase.

For the kidney, CYP27B1 hydroxylase substrate is provided by the endocytic internalization of filtered, megalin-bound DBP carrying 25(OH)D into the proximal tubular cell from the urinary side of that cell. Regulation of CYP27B1 in the proximal nephron is principally controlled at the level of transcription, with circulating PTH and fibroblast growth factor 23 (FGF-23) being the major stimulator and inhibitor of *CYP27B1* gene expression, respectively (see below). In the kidney, the CYP24 hydroxylase, also a mitochondrial P450, serves not only to limit the amount of $1,25(OH)_2D$ leaving the kidney for distant target tissues by accelerating its catabolism to $1,24,25(OH)_3D$ but also by shunting available substrate 25(OH)D away from 1-hydroxylase. In both cases, the 24-hydroxylated products are biologically inert and degraded by the same enzyme to side chain–cleaved, water-soluble catabolites. The *CYP24 hydroxylase* gene is under stringent transcriptional control by $1,25(OH)_2D$ itself, providing a robust means of proximate, negative feedback regulation of the amount of $1,25(OH)_2D$ made in and released from the kidney.[7] By comparison, the activity of some of the extrarenal, intracrine/paracrine-acting 1-hydroxylase, like that which occurs in disease-activated macrophages, seems to be primarily governed by the availability of extracellular substrate 25(OH)D to the enzyme. It is postulated that this is caused by the expression of an amino-terminally truncated splice variant of the *CYP24* gene that cannot be transported into mitochondria.[8,9] The result is generation of a noncatalytically active enzyme, albeit one that can serve as a cytoplasmic reservoir for the CYP24 substrates, $1,25(OH)_2D$ and 25(OH)D. Also contrary to renal 1-hydroxylase, the extrarenal CYP27B1 hydroxylase, at least in macrophages, (1) is immune to control by either PTH or FGF-23 (receptors for these molecules are not expressed to any degree in inflammatory cells), (2) is susceptible to induction by Toll-like receptors (TLR) ligands shed by microbial agents, and (3) can be upregulated by nontraditional electron

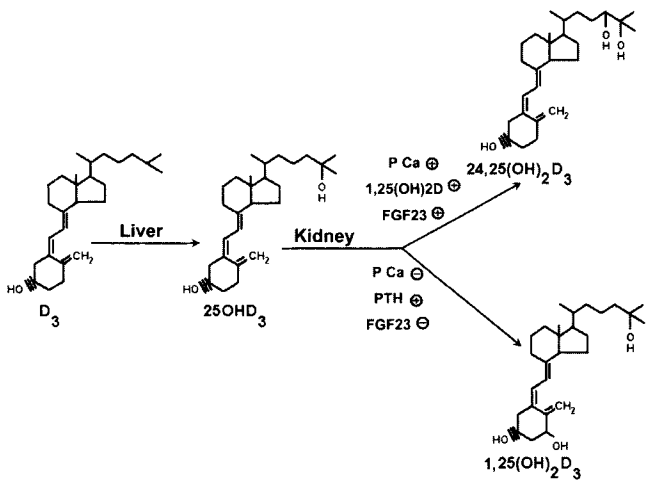

FIG. 2. The metabolism of vitamin D. The liver converts vitamin D to 25OHD. The kidney converts 25OHD to $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$. Control of metabolism is exerted primarily at the level of the kidney, where low serum phosphorus, low serum calcium, low FGF23, and high parathyroid hormone (PTH) levels favor production of $1,25(OH)_2D_3$, whereas high serum phosphorus, calcium, FGF23, and $1,25(OH)_2D_3$ and low PTH favor $24,25(OH)_2D_3$ production.

donors like NO.[6] CYP27B1 hydroxylase in keratinocytes, on the other hand, shares features of both the renal and macrophage 1-hydroxylase in that it is associated with very active CYP24 hydroxylase, which limits the levels of $1,25(OH)_2D$ in the cell, is stimulated by cytokines such as TNF-α[10] and IFN-γ[11] but not by c-AMP, and is induced by TLR2 activation.[12]

TRANSPORT OF VITAMIN D IN THE BLOOD

For the hormone $1,25(OH)_2D$ to reach any of its target tissues, with the exception of the skin where it can be both produced and act locally as just described, vitamin D must be able to escape its synthetic site in the skin or it absorption site in the gut and be transported to tissues expressing one of the vitamin D 25-hydroxylase genes. From there, 25(OH)D must travel to tissue sites expressing the *CYP27B1 hydroxylase* gene, and synthesized $1,25(OH)_2D$ must be able to gain access to target tissues containing cells expressing the VDR for the genomic actions of the sterol hormone to be realized. The serum DBP, a member of the albumin family of proteins, is the specific chaperone for vitamin D and its metabolites in the serum.[13] It has a high capacity (<5% saturated with vitamin D metabolites in humans) and is bound with high affinity (nM range) by vitamin D, particularly the 25-hydroxylated metabolites 25(OH)D, $24,25(OH)_2D$, and $1,25(OH)_2D$.[14] DBP is produced mainly in the liver and is freely filterable across the glomerulus into the urine. DBP has a serum half-life of 2.5–3.0 days, indicating that it must be largely reclaimed from the urine once filtered. Reclamation is achieved by DBP being bound by the endocytic, low-density lipoprotein (LDL)-like co-receptor molecules megalin and cubulin embedded in the plasma membrane of the proximal renal tubular epithelial cell, with eventual transcellular transport and return to the circulation through intracellular DBP (IDBP) chaperones in the heat protein-70 family.[15] No human, DBP-null homozygote has yet been described, suggesting that, unlike the DBP-null mice that are both viable and fertile,[16] such a human genotype would be embryonically lethal.

INTERNALIZATION OF VITAMIN D METABOLITES

Once bound to DBP and shuttled to sites of metabolism, action, and/or catabolism, vitamin D metabolites must gain access to the interior of their target cell and arrive safely at their intracellular destination (i.e., nucleus for transaction through the VDR, inner mitochondrial membrane for access to the CYP27B and CYP24 hydroxylases). Although possible, it seems unlikely that simple diffusion of the sterol metabolite off the serum DBP and simple diffusion through the plasma membrane to a specific intracellular destination, the so-called "free hormone" hypothesis, can account for the required specificity for targeted metabolite delivery. Current observations suggest that, similar to that which occurs in the kidney, there exists a plasma membrane–anchored receptor for DBP that is endocytically internalized with intracellular chaperones moving the metabolite(s) to specific intracellular destinations (e.g., the CYP27B1 and VDR).[17]

MECHANISM OF ACTION

The mechanism of action of the active form of vitamin D, $1,25(OH)_2D_3$, is similar to that of other steroid hormones. The intracellular mediator of $1,25(OH)_2D_3$ function is the VDR. $1,25(OH)_2D_3$ binds stereospecifically to VDR, which is a high-affinity, low-capacity intracellular receptor that has extensive homology with other members of the superfamily of nuclear receptors including receptors of steroid and thyroid hormones. VDR functions as a heterodimer with the retinoid X receptor (RXR) for activation for vitamin D target genes. Once formed, the $1,25(OH)_2D_3$-VDR-RXR heterodimeric complex interacts with specific DNA sequences (vitamin D response elements [VDRE]) within the promoter of target genes, resulting in either activation or repression of transcription.[18–21] In general, for activation of transcription, the VDRE consensus consists of two direct repeats of the hexanucleotide sequence GGGTGA separated by three nucleotide pairs. The mechanisms involved in VDR-mediated transcription after binding of the $1,25(OH)_2D_3$-VDR-RXR heterodimeric complex to DNA are now beginning to be defined. TFIIB, several TATA binding protein associated factors (TAFs), as well as the p160 co-activators known also as steroid receptor activator-1, -2, and -3 (SRC-1, SRC-2, and SRC-3), which have histone acetylase (HAT) activity, have been reported to be involved in VDR-mediated transcription. In addition to acetylation, methylation also occurs on core histones. Recent studies have indicated that cooperativity between histone methyltransferases and p160 co-activators may also play a fundamental role in VDR-mediated transcriptional activation.[22] VDR-mediated transcription is also mediated by the co-activator complex DRIP (VDR interacting protein). This complex does not have HAT activity but rather functions, at least in part, through recruitment of RNA polymerase II. It has been suggested that the SRC/CREB-binding protein (CBP) co-activator complex is recruited first for chromatin remodeling followed by the recruitment of the transcription machinery by the DRIP complex.[20,23] In addition, a number of promoter-specific transcription factors including YY1 and CCAAT enhancer binding proteins β and δ have been reported to modulate VDR-mediated transcription.[24–26] It has been suggested that cell- and promoter-specific functions of VDR may be mediated through differential recruitment of coactivators.

OVERVIEW OF VITAMIN D REGULATION OF CALCIUM AND PHOSPHATE METABOLISM

The classic actions of $1,25(OH)_2D_3$ involve its regulation of calcium and phosphate flux across three target tissues: bone, gut, and kidney. The mechanisms by which $1,25(OH)_2D_3$ operates in these tissues will be described in more detail below. However, the receptor for $1,25(OH)_2D_3$ (VDR) is widespread and not limited to these classic target tissues. Indeed, the list of tissues not containing the VDR is probably shorter than the list of tissues that contain the VDR. Furthermore, as discussed previously, a number of these tissues express CYP27B1 and therefore can make their own $1,25(OH)_2D_3$. The biological significance of these observations is found in the large number of nonclassical actions of vitamin D including effects on cellular proliferation and differentiation, regulation of hormone secretion, and immune modulation. Examples of these actions will be discussed below. In at least the classic actions of vitamin D, $1,25(OH)_2D_3$ acts in concert with two peptide hormones, PTH and FGF-23 (Fig. 3). In each case, feedforward and feedback loops are operative. PTH is the major stimulator of $1,25(OH)_2D_3$ production in the kidney. $1,25(OH)_2D_3$ in turn suppresses PTH production directly by a transcriptional mechanism and indirectly by increasing serum calcium levels. Calcium acts through the calcium receptor (CaR) in the parathyroid gland to suppress PTH release. $1,25(OH)_2D_3$ increases the levels of CaR in the parathyroid gland just as calcium increases the $1,25(OH)_2D_3$ receptor (VDR) in the parathyroid gland, further enhancing the negative influence of calcium and $1,25(OH)_2D_3$ on PTH secretion. FGF-23, on the other hand, inhibits $1,25(OH)_2D_3$ production by the kidney while increasing the expression of CYP24, whereas $1,25(OH)_2D_3$ stimulates

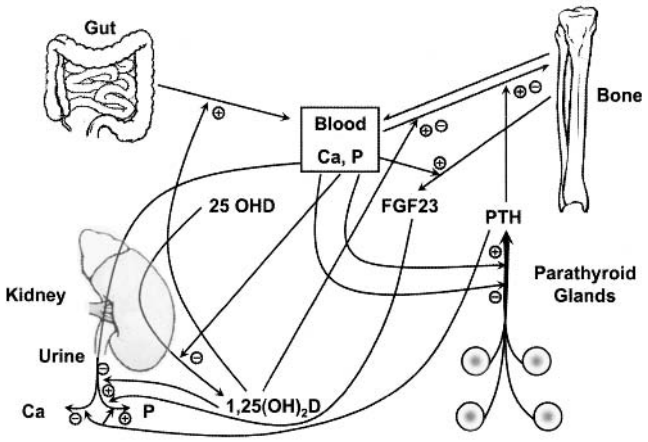

FIG. 3. $1,25(OH)_2D_3$ interacts with other hormones, in particular FGF23 and PTH, to regulate calcium and phosphate homeostasis. As noted in the legend to figure 2, FGF23 inhibits whereas PTH stimulates $1,25(OH)_2D_3$ production by the kidney. In turn $1,25(OH)_2D_3$ inhibits PTH production but stimulates that of FGF23. Calcium and phosphate in turn regulate FGF23, PTH, and so $1,25(OH)_2D_3$ indirectly.

FGF-23 production. Dietary phosphate also regulates FGF-23 levels [high phosphate stimulates, an effect independent of $1,25(OH)_2D_3$ as shown by phosphate regulation of FGF-23 in the VDR-null mice]. Whether phosphate has its own receptor like calcium is unclear. FGF-23 expression is found in a number of tissues including the parathyroid gland, but its greatest expression is in osteocytes, bone-lining cells, and active osteoblasts. Thus, in considering the mechanisms of action of vitamin D and its active metabolite $1,25(OH)_2D_3$ in vivo, the roles of PTH and FGF-23 must also be considered.

CLASSIC TARGET TISSUES

Bone

Whether $1,25(OH)_2D_3$ acts directly on bone or whether the anti-rachitic effects of $1,25(OH)_2D_3$ are indirect and are caused by $1,25(OH)_2D_3$ stimulation of intestinal calcium and phosphorus absorption resulting in increased incorporation of calcium and phosphorus into bone has been a matter of debate. VDR ablated mice (VDR knockout mice) develop secondary hyperparathyroidism, hypocalcemia, and rickets after weaning.[27,28] However, when VDR KO mice are fed a rescue diet containing high levels of calcium, phosphorus, and lactose, serum ionized calcium and PTH levels are normalized, and rickets and osteomalacia are prevented, suggesting that a major effect of $1,25(OH)_2D_3$ is the provision of calcium and phosphate to bone from the intestine rather than a direct action on bone.[29] In vitro studies, however, support a direct effect of $1,25(OH)_2D_3$ on bone.[30] $1,25(OH)_2D_3$ can stimulate the formation of bone-resorbing osteoclasts.[30] However, VDR is not present in osteoclasts but rather in osteoprogenitor cells, osteoblast precursors, and mature osteoblasts. Stimulation of osteoclast formation by $1,25(OH)_2D_3$ involves upregulation by $1,25(OH)_2D_3$ in osteoblastic cells of RANKL (osteoclast differentiating factor) and requires cell to cell contact between osteoblastic cells and osteoclast precursors.[31] Osteoclastogenesis inhibitory factor/osteoprotegerin, which is a decoy receptor for RANKL and antagonizes RANKL function thus blocking osteoclastogenesis, is downregulated by $1,25(OH)_2D_3$.[31] $1,25(OH)_2D_3$ has also been reported to stimulate the production in osteoblasts of the calcium-binding proteins osteocalcin and osteopontin.[32,33] Runx2, a transcriptional regulator of osteoblast differentiation, is also regulated by $1,25(OH)_2D_3$.[34] Transgenic mice overexpressing VDR in osteoblastic cells have increased bone formation, further indicating direct effects of $1,25(OH)_2D_3$ on bone.[35] Thus, effects of $1,25(OH)_2D_3$ on bone are diverse and can affect formation or resorption.

Intestine

When the demand for calcium increases from a diet deficient in calcium, from growth, or from pregnancy or lactation, the synthesis of $1,25(OH)_2D_3$ is increased, stimulating the rate of calcium absorption. In VDR KO mice, a major defect is in intestinal calcium absorption, suggesting that a principal action of $1,25(OH)_2D_3$ to maintain calcium homeostasis is increased intestinal calcium absorption.[36–38] It is thought that intestinal calcium absorption is comprised of two different modes of calcium transport: the saturable process that is mainly transcellular and a diffusional mode that is nonsaturating, requires a lumenal free calcium concentration >2–6 mM and is paracellular (the movement of calcium is across tight junctions and intracellular spaces and is directly related to the concentration of calcium in the intestinal lumen). The saturable component of intestinal calcium absorption is observed predominantly in the duodenum. The diffusional, nonsaturable process is observed all along the intestine (duodenum, jejunum, and ileum). $1,25(OH)_2D_3$ has been reported to affect both the transcellular and the paracellular path.[37,38] The transcellular process is comprised of three $1,25(OH)_2D_3$-regulated steps: the entry of calcium across the brush border membrane, intracellular diffusion, and the energy requiring extrusion of calcium across the basolateral membrane.[37,38] It is thought that the calcium-binding protein, calbindin, which is induced by $1,25(OH)_2D_3$ in the intestine, acts to facilitate the diffusion of calcium through the cell interior toward the basolateral membrane. Recent studies in which calbindin-D_{9k}–null mutant mice showed no change in $1,25(OH)_2D_3$-mediated intestinal calcium absorption and in serum calcium levels compared with wildtype mice[36,39] provide evidence that calbindin alone is not responsible for $1,25(OH)_2D_3$-mediated intestinal calcium absorption.

$1,25(OH)_2D_3$ also affects calcium extrusion from the enterocyte. The plasma membrane calcium pump (PMCA) has been reported to be stimulated by $1,25(OH)_2D_3$, suggesting that intestinal calcium absorption may involve a direct effect of $1,25(OH)_2D_3$ on calcium pump expression.[38]

The rate of calcium entry into the enterocyte is also increased by $1,25(OH)_2D_3$. Recently a calcium selective channel, TRPV6, which is co-localized with calbindin and is induced by $1,25(OH)_2D_3$, was cloned from rat duodenum.[40,41] It has been suggested that TRPV6 plays a key role in vitamin D–dependent calcium entry into the enterocyte.

In addition to intestinal calcium absorption, $1,25(OH)_2D_3$ also results in enhanced intestinal phosphorus absorption. Although the mechanisms involved have been a matter of debate, it has been suggested that $1,25(OH)_2D_3$ stimulates the active transport of phosphorus.[42]

Kidney

A third target tissue involved in $1,25(OH)_2D_3$-mediated mineral homeostasis is the kidney. $1,25(OH)_2D_3$ has been reported to enhance the actions of PTH on calcium transport in the distal tubule, at least in part, by increasing PTH receptor mRNA and binding activity in distal tubule cells.[43] $1,25(OH)_2D_3$ also induces the synthesis of the calbindins in the distal tubules.[37] It has been suggested that calbindin-D_{28k}

stimulates the high-affinity system in the distal luminal membrane, and calbindin-D_{9k} enhances the ATP-dependent calcium transport of the basolateral membrane.[37] Similar to studies in the intestine, an apical calcium channel, TRPV5, which is co-localized with the calbindins and induced by $1,25(OH)_2D_3$, has been identified in the distal convoluted tubule and distal connecting tubules.[40] Calbindin-D_{28k} was reported to associate directly with TRPV5 and to control TRPV5-mediated calcium influx.[44] Thus, $1,25(OH)_2D_3$ affects calcium transport in the distal tubule by enhancing the action of PTH and by inducing TRPV5 and the calbindins. Another important effect of $1,25(OH)_2D_3$ in the kidney is the inhibition of the $25(OH)D_3$ 1 α-hydroxylase enzyme (CYP27B1) and the induction of the 24-hydroxylase enzyme (CYP24).[45] Besides calcium transport in the distal nephron and modulation of the $25(OH)D_3$ hydroxylases, effects of $1,25(OH)_2D_3$ on phosphate reabsorption in the proximal tubule have been suggested. Vitamin D has been reported to increase or decrease renal phosphate reabsorption depending on the parathyroid status and on experimental conditions.

NONCLASSICAL TARGET TISSUES

Parathyroid Glands

The parathyroid glands are an important target of $1,25(OH)_2D_3$. As discussed previously, $1,25(OH)_2D_3$ inhibits the synthesis and secretion of PTH and prevents the proliferation of the parathyroid gland to maintain normal parathyroid status.[46,47] It has also been shown that $1,25(OH)_2D_3$ upregulates calcium sensing receptor (CaSR) transcription,[48] suggesting that $1,25(OH)_2D_3$ sensitizes the parathyroid gland to calcium inhibition.

Pancreas

The pancreas was one of the first nonclassical target tissues in which receptors for $1,25(OH)_2D_3$ were identified.[49] Although $1,25(OH)_2D_3$ has been reported to play a role in insulin secretion, the exact mechanisms remain unclear. Autoradiographic data and immunocytochemical studies have localized VDR and calbindin-D_{28k},respectively, in pancreatic β cells.[50,51] Studies using calbindin-D_{28k}–null mutant mice have indicated that calbindin-D_{28k}, by regulating intracellular calcium, can modulate depolarization-stimulated insulin release.[52] In addition to modulating insulin release, calbindin-D_{28k}, by buffering calcium, can protect against cytokine-mediated destruction of β cells.[53] These findings have important therapeutic implications for type 1 diabetes and the prevention of cytokine destruction of pancreatic β cells, as well as type 2 diabetes and the potentiation of insulin secretion.

IMMUNOBIOLOGY OF VITAMIN D

Nonclassical regulation of immune responses by $1,25(OH)_2D$ was first reported 25 yr ago with the discovery of the presence of the VDR in activated human inflammatory cells[54] and the ability of disease-activated macrophages to make $1,25(OH)_2D$.[55] Recent studies have shown that $1,25(OH)_2D$ regulates both innate and adaptive immunity, but in opposite directions, namely promoting the former while repressing the latter.

Vitamin D and Innate Immunity

Innate immunity encompasses the ability of the host immune system to recognize and respond to an offending antigen. In 1986, Rook et al.[56] described studies using cultured human macrophages in which they showed that $1,25(OH)_2D$ can inhibit the growth of *Mycobacterium tuberculosis*. Although this seminal report was widely cited, it is only in the last 3 yr that more comprehensive appraisals of the antimicrobial effects of vitamin D metabolites have been published. In silico screening of the human genome showed the presence of a VDRE in the promoter of the human gene for cathelicidin, whose product LL37 is an antimicrobial peptide capable of killing bacteria.[57] Subsequent studies confirmed the ability of $1,25(OH)_2D$[58] and its precursor $25(OH)D$[59] to induce expression of cathelicidin in cells of the monocyte/macrophage and epidermal lineage, respectively, highlighting the potential for intracrine/autocrine induction of antimicrobial responses in cells that also express the 25(OH)D-activating enzyme, CYP27B1. Although detectable in many cell types, functionally significant expression and activity of CYP27B1 seem to be dependent on cell-specific stimulation of a broad spectrum of immune surveillance proteins, the TLRs. The TLRs are an extended family of host, noncatalytic transmembrane pattern-recognition receptors (PRRs) that interact with specific pathogen-associated membrane patterns (PAMPs) shed by infectious agents and trigger the innate immune response in the host.[60]

In this regard, Liu et al.[61] recently used DNA array to characterize changes in gene expression after activation of the human macrophage TLR2/1 dimer by one of the PAMPs for *M. tuberculosis*. Macrophages, but not dendritic cells, thus treated showed increased expression of both *CYP27B1* and *VDR* genes and gene products and showed intracrine induction of the antimicrobial cathelicidin gene with subsequent mycobacterial killing in response to 25(OH)D and $1,25(OH)_2D$. In fact, microbial killing was more efficiently achieved with the prohormone 25(OH)D than with $1,25(OH)_2D$ at similar extracellular concentrations, indicating that the robustness of the human innate response to microbial challenge is dependent on the serum 25(OH)D status of the host. This concept was confirmed in these studies by the ability of 25(OH)D-sufficient serum to rescue a deficient, cathelicidin-driven antimicrobial response in human macrophages conditioned in vitamin D–deficient serum. A similar vitamin D–directed antimicrobial-generating capacity has been recently observed in wounded skin,[12] suggesting that TLR-driven expression of cathecidin, requiring the intracellular synthesis and genomic action of $1,25(OH)_2D$, is a common response feature to infectious agent invasion. Although not yet proven in a clinical setting, it is possible that increasing the serum 25(OH)D level of the host to the normal range (>30 ng/ml) will augment the effectiveness of the innate immune response to such commonly encountered microbial agents as *M. tuberculosis* and HIV known to trip macrophage TLR signaling pathways. Reinforcing these events is the ability of locally generated $1,25(OH)_2D$ to escape the confines of the cell in which it is made to act on neighboring VDR-expressing monocytes to promote their maturation to mature macrophages,[62] thus acting as a feedforward signal to further enhance the innate immune response.

Vitamin D and the Adaptive Immune Response

The adaptive immune response is generally defined by T and B lymphocytes and their ability to produce cytokines and immunoglobulins, respectively, to specifically combat the source of the antigen presented to them by cells (i.e., macrophages, dendritic cells) of the innate immune response. As previously noted,[54] the presence of VDR in activated, but not resting, human T and B lymphocytes was the first observation implicating these cells as targets for the noncalciotropic responses to $1,25(OH)_2D$. Contrary to the role of locally produced $1,25(OH)_2D$ to promote the innate immune response,

the hormone exerts a generalized dampening effect on lymphocyte function. With respect to B cells, $1,25(OH)_2D$ suppresses proliferation and immunoglobulin production and retards the differentiation of B-lymphocyte precursors to mature plasma cells. With regard to T cells, $1,25(OH)_2D$, acting through the VDR, inhibits the proliferation of uncommitted T_H (helper) cells. This results in diminished numbers of T_H capable of maturing to IFNγ-elaborating, macrophage-stimulating T_{H1} cells and, to a lesser extent, to interleukin (IL)-4–, IL-5–, and IL-13–producing, B cell–activating T_{H2} cells. On the other hand, the hormone promotes the proliferation of immunosuppressive regulatory T cells, so called T_{REG}s,[63] and promotes their accumulation at sites of inflammation by stimulating expression of the T-cell homing molecule, CCL22, by dendritic cells[64] in the local inflammatory microenvironment. In fact, it is this generalized ability of $1,25(OH)_2D$ to quell the adaptive immune response, which has prompted the use of the hormone and its analogs in the adjuvant treatment of inflammatory autoimmune and neoplastic disorders.

In summary, the collective, concerted action of $1,25(OH)_2D$ is to promote the host's response to an invading pathogen while simultaneously acting to limit what might be an overzealous immune response to that pathogen, representative of the process of tolerance. Once again, a good example is that of infection with the intracellular pathogen *M. tuberculosis*. In this case, the pathogen evokes an exceptionally robust innate immune response that is fueled by the endogenous production of $1,25(OH)_2D$ by macrophages and dendritic cells, which also express the CYP27B1, at the site of host invasion. If substantial amounts of $1,25(OH)_2D$ escape the confines of the macrophage or the dendritic cell, the immunostimulation of VDR-expressing, activated lymphocytes in that environment is quelled by $1,25(OH)_2D$. If the innate immune response is extreme and enough $1,25(OH)_2D$ finds its way into the general circulation, as may occur in human granuloma-forming disorders such as sarcoidosis and tuberculosis, an endocrine effect of the hormone, most notably hypercalciuria and hypercalcemia, can be observed.

KERATINOCYTE FUNCTION IN EPIDERMIS AND HAIR FOLLICLES

$1,25(OH)_2D$-Regulated Epidermal Differentiation

$1,25(OH)_2D_3$ is likely to be an autocrine or paracrine factor for epidermal differentiation because it is produced in the keratinocyte by the same enzyme, CYP27B1, as found in the kidney, but under normal circumstances, keratinocyte production of $1,25(OH)_2D_3$ does not seem to contribute to circulating levels.[65] The receptors for and the production of $1,25(OH)_2D_3$ decrease with differentiation. Stimulation of differentiation is accompanied by the rise in mRNA and protein levels of involucrin and transglutaminase,[66] as well as the late differentiation markers filaggrin and loricrin.[67] The mechanisms by which $1,25(OH)_2D_3$ alter keratinocyte differentiation are multiple and include induction of the calcium receptor enhancing the effects of calcium on differentiation and induction of the phospholipase C family, which provide second messengers such as diacyl glycerol and inositol trisphosphate to the differentiation process. Although the most striking feature of the VDR-null mouse is the development of alopecia (also found in many but not all patients with mutations in the VDR), these mice also exhibit a defect in epidermal differentiation as shown by reduced levels of involucrin and loricrin, loss of keratohyalin granules, loss of the calcium gradient, and disruption of lamellar body production and secretion resulting in defective barrier function. Furthermore, both VDR and $1,25(OH)_2D_3$ production are required for normal antimicrobial peptide expression in response to epidermal injury.[12]

VDR Regulation of Hair Follicle Cycling

As noted above, alopecia is a well-known part of the phenotype of many patients with mutations in their VDR.[68] Vitamin D deficiency or lack of CYP27B1 per se is not associated with alopecia, and the alopecia can be rescued with mutants of VDR that fail to bind $1,25(OH)_2D_3$ or its co-activators.[69] Recent attention has been paid to both hairless (Hr), a putative transcription factor capable of binding the VDR and suppressing at least its ligand-dependent transcriptional activity, and β-catenin, which like Hr binds to VDR and may regulate its transcriptional activity (or vice versa). Hr mutations in both mice and humans and transcriptionally inactivating β-catenin mutations in mice result in phenocopies of the VDR-null animal with regard to the morphologic changes observed in hair cycling. In these models, the abnormality leading to alopecia develops during catagen at the end of the developmental cycle, precluding the re-initiation of anagen. Both VDR-null mice and those with disrupted Wnt signaling lose stem cells from the bulge, perhaps as a result of the loss of the interaction of the bulge with the dermal papilla.[70,71] Thus, although the mechanism by which VDR controls hair follicle cycling is not established, hair follicle cycling represents the best example by which VDR regulates a physiologic process that is independent of its ligand, $1,25(OH)_2D_3$, and therefore points to a novel mechanism of action for this transcriptional regulator.

NUTRITIONAL CONSIDERATIONS

Defining Vitamin D Sufficiency

Serum 25(OH)D levels provide a useful surrogate for assessing vitamin D status, because the conversion of vitamin D to 25(OH)D is less well controlled (i.e., primarily substrate dependent) than the subsequent conversion of 25(OH)D to $1,25(OH)_2D$. $1,25(OH)_2D$ levels, unlike 25(OH)D levels, are well maintained until the extremes of vitamin D deficiency because of secondary hyperparathyroidism and therefore do not provide a useful index for assessing vitamin D deficiency, at least in the initial stages. Historically, vitamin D sufficiency was defined as the level of 25(OH)D sufficient to prevent rickets in children and osteomalacia in adults. Levels of 25(OH)D <5 ng/ml (or 12 nM) are associated with a high prevalence of rickets or osteomalacia, and current "normal" levels of 25(OH)D are often stated to include levels as low as 15 ng/ml. However, there is a growing consensus that these lower limits of normal are too low. Although there is currently no consensus on the optimal levels, most experts define vitamin D deficiency as levels of 25(OH)D <30 ng/ml.[5] With this definition of vitamin D deficiency, a very large proportion of the population in both the developed and developing world are vitamin D deficient.

Impact on the Musculoskeletal System

This rethinking of the definition of vitamin D sufficiency comes from the appreciation that vitamin D affects a large number of physiologic functions in addition to bone mineralization. 25(OH)D levels are inversely proportional to PTH levels such that PTH levels increase at levels of 25(OH)D <30–40 ng/ml. Intestinal calcium transport increases 45–65% when the 25(OH)D levels are increased from 20 to 32 ng/ml. Large epidemiologic surveys showed a positive correlation between 25(OH)D levels and BMD, with no evidence for a plateau <30 ng/ml, and vitamin D and calcium supplementation

showed improvement in BMD in older individuals. Similarly a positive association between 25(OH)D levels and muscle function (e.g., walking speed, sit-to-stand) has been shown, even over the interval of 20–38 ng/ml, although the correlation is strongest at lower levels. Vitamin D supplementation with at least 800 IU improved lower extremity function, decreased body sway, and reduced falls. Most importantly, adequate levels of vitamin D and calcium supplementation prevent fractures.[72,73]

Impact Beyond the Musculoskeletal System

The impact of vitamin D extends beyond the musculoskeletal system and the regulation of calcium homeostasis. Vitamin D deficiency is a well-known accompaniment of various infectious diseases such as tuberculosis, and $1,25(OH)_2D_3$ has long been recognized to potentiate the killing of mycobacteria by monocytes. The nutritional aspect of these observations has recently been illuminated by the observation that the monocyte, when activated by mycobacterial lipopeptides, expresses CYP27B1, producing $1,25(OH)_2D_3$ from circulating 25(OH)D, and in turn inducing cathelicidin, an antimicrobial peptide that enhances killing of the *Mycobacterium*. Inadequate 25(OH)D levels abort this process.[61] Vitamin D deficiency and/or living at higher latitudes (with less sunlight) is associated with a number of autoimmune diseases including type 1 diabetes mellitus, multiple sclerosis, and Crohn's disease.[74] 25(OH)D levels are also inversely associated with type 2 diabetes mellitus and metabolic syndrome, and some studies have shown that vitamin D and calcium supplementation may prevent the progression to diabetes mellitus in individuals with glucose intolerance. Improvements in both insulin secretion and action have been observed. The potential to prevent certain cancers may be the most compelling reason for adequate vitamin D nutrition. A large body of epidemiologic data exists documenting the inverse correlation of 25(OH)D levels, latitude, and/or vitamin D intake with cancer incidence.[5] Although numerous types of cancers show reduction,[75] most attention has been paid to breast, colon, and prostate. A prospective 4-yr trial with 1100 IU vitamin D and 1400–1500 mg calcium showed a 77% reduction in cancers after the first year of study,[76] including a reduction in both breast and colon cancers. In this study, vitamin D supplementation raised the 25(OH)D levels from a mean of 28.8 to 38.4 ng/ml, with no changes in the placebo or calcium-only arms of the study.

Vitamin D Treatment Strategies

Adequate sunlight exposure is the most cost-effective means of obtaining vitamin D. Whole body exposure to sunlight has been calculated to provide the equivalent of 10,000 IU vitamin D_3.[77] A 0.5 minimal erythema dose of sunlight (i.e., one half the dose required to produce a slight reddening of the skin) or UVB radiation to the arms and legs, which can be achieved in 5–10 min on a bright summer day, has been calculated to be the equivalent of 3000 IU vitamin D_3.[5] However, concerns regarding the association between sunlight and skin cancer and/or aging have limited this approach, perhaps to the extreme, although it remains a viable option for those unable or unwilling to benefit from oral supplementation. Current recommendations for daily vitamin D supplementation (200 IU for children and young adults, 400 IU for adults 51–70 yr of age, and 600 IU for adults >71 yr of age) are too low and do not maintain 25(OH)D at the desired level for many individuals. Studies have shown that for every 100 IU vitamin D_3 supplementation administered, the 25(OH)D levels rise by 0.5–1 ng/ml.[77,78] Seven hundred to 800 IU seems to be the lower limit of vitamin D supplementation required to prevent fractures and falls. Unfortified food contains little vitamin D, with the exception of wild salmon and other fish products such as cod liver oil. Milk and other fortified beverages typically contain 100 IU/8-oz serving. Vitamin D_2 is substantially less potent than vitamin D_3, in part because it is more rapidly cleared. Therefore, if vitamin D_2 is used, it needs to be given at least weekly. Toxicity caused by vitamin D supplementation has not been observed at doses <10,000 IU/d.[79]

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