



## REVIEW ARTICLE

## VITAMIN D — NEW ACTION MECHANISMS AND EFFECTS

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The classic arena of vitamin D (cholecalciferol) action is the maintenance of calcium homeostasis. Intestinal, bone and kidney tissue interact in a tightly regulated manner to achieve this end. Since the identification of  $1,25(\text{OH})_2\text{D}_3$  (1,25-dihydroxyvitamin  $\text{D}_3$ ) as the active metabolite of vitamin D, its endocrine properties have been well documented. Calcitriol, as this metabolite is also called, acts on gene expression via a receptor in the cell nucleus. During the last decade, however, additional actions of  $1,25(\text{OH})_2\text{D}_3$  on the cell membrane that trigger rapid signal transduction mechanisms have been reported. Recently many other tissues have also joined the classic target organs of calcitriol, for example the pancreas, the immune system, the skin and the parathyroid gland, as well as an array of tumour tissues. In these instances calcitriol has intriguing non-calcaemic actions on cell differentiation and function, which opens exciting new therapeutic possibilities for the use of synthetic analogues of vitamin D.

This review gives a brief presentation of the classic hypercalcaemic and more recent non-calcaemic effects of calcitriol. It also summarises its classic slow genomic and new rapid non-genomic action mechanisms and gives a brief overview of possible clinical applications.

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It has been known for nearly a century that the cure of rickets, a disease of bone mineralisation, depends on a dietary factor that can be partly replaced by exposure to ultraviolet rays. This was reported for the first time by Mellanby in 1921.<sup>1</sup> This factor was isolated from fish liver oil in 1936<sup>2</sup> and called vitamin D. It could be formed *in vivo* by cutaneous irradiation of the steroid 7-dehydrocholesterol which is synthesised in the liver. The vitamin as such was not active in *in vitro* biological systems. Four decades went by before it could be shown that it had to be hydroxylated by the liver and kidney on positions 25 and 1, respectively, to form the active compound.<sup>3,4</sup> The latter step was

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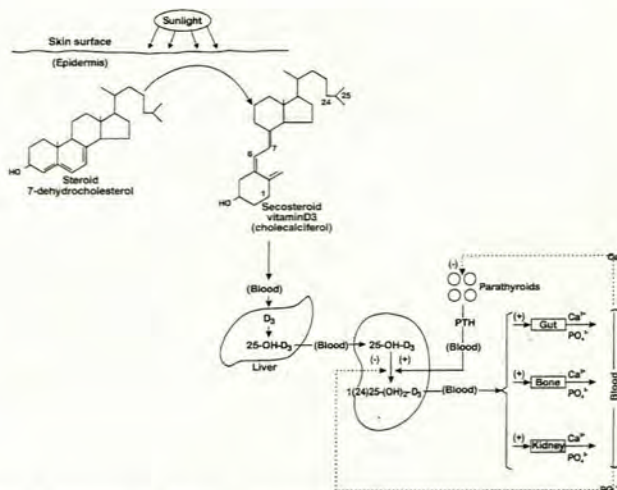


Fig. 1. Vitamin D biosynthesis and its feedback control mechanisms. PTH = parathyroid hormone.

stimulated by parathyroid hormone (PTH). Fig. 1 shows these interrelationships as well as the feedback systems regulating calcitriol biosynthesis.<sup>5</sup>

The next step in the elucidation of the action mechanism of calcitriol was the identification of a vitamin D receptor (VDR) in the cell nucleus<sup>6</sup> that could mediate messenger RNA and protein synthesis. Receptors previously found in the cytosol were shown to be artefacts in the preparation procedure. The era of the vitamin D endocrine system was born.

However, many questions have emerged. How is bone mineralisation as well as resorption influenced by calcitriol?<sup>7</sup> Does  $24,25$  dihydroxycholecalciferol ( $24,25(\text{OH})_2\text{D}_3$ ), also an important metabolite of vitamin D, have similar effects? Does calcitriol have effects on other tissues? Does it have other therapeutic uses? Do calcitriol receptors exist in the nucleus only? Can calcitriol have any more rapid, e.g. non-genomic, effects?

Many new facets of vitamin D function and clinical application have emerged during the last few years. This review gives a brief presentation of these reports.

### ACTION MECHANISM

#### Classic genomic action

Calcitriol is released from its serum binding protein, diffuses through the cell membrane, and binds to a classic zinc finger-containing receptor (VDR) in the cell nucleus.<sup>7,8</sup> Interestingly, there is an interactive transcriptional control mechanism between the nuclear co-receptors of vitamins A and D<sup>8</sup> as well as the thyroxine receptor:<sup>9</sup> these receptors form heterodimers in different combinations which can bind to the genome.<sup>10</sup> Vitamin A and D receptor interplay is of special importance in the transcription of the osteocalcin gene, as illustrated in Fig. 2.<sup>8</sup> An

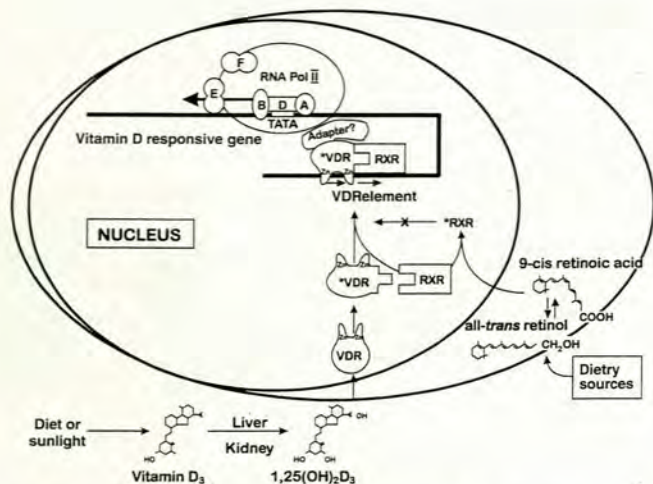


Fig. 2. Transcriptional control of the rat osteocalcin gene by vitamins A and D.<sup>10</sup> VDR = vitamin D receptor, RXR = retinoic acid receptor, VDRE = vitamin D responsive element, A, B, D, E, F, and TATA = transcription regions.

unoccupied retinoic acid receptor (RXR) is required to bind to its occupied VDR counterpart — this complex can then bind to the calcitriol-responsive element (VDRE) in nuclear DNA with the help of an adaptor protein.

The occupied VDR is subsequently phosphorylated on serine residues.<sup>11</sup> The resulting patches of negative charge interact with positive domains in transcription factors. Accordingly RNA polymerase is activated and enhanced gene expression follows: osteocalcin, osteopontin, collagen, carbonic anhydrase and alkaline phosphatase are some of the bone proteins of which the synthesis is promoted.<sup>11-13</sup>

### Rapid, non-genomic action

During the early 1990s it became clear that a number of well-known signal transduction mechanisms are activated within seconds of the addition of  $1,25(\text{OH})_2\text{D}_3$  to the cell membrane: unidirectional calcium fluxes,<sup>14,15</sup> increased phospholipase  $\text{A}_2$  activity<sup>16</sup> coupled with arachidonic acid turnover,<sup>17,18</sup> increased phosphoinositide hydrolysis<sup>15,19,20</sup> coupled with protein kinase C activation and translocation,<sup>21,22</sup> as well as the cyclic adenosine monophosphate (cAMP) pathway.<sup>23,24</sup> In 1997 the mitogen-activated protein (MAP) kinase cascade was added to this list.<sup>25,26</sup>

In September 1998 the first international conference dedicated to rapid responses of steroid hormones was held in Mannheim, Germany. It reported on additional mechanisms, namely the involvement of the tyrosine kinase cascade in activation of the  $\text{G}\alpha\text{q}$  protein as well as some protein kinase C isoforms.<sup>27-29</sup> All the above mechanisms are summarised in Fig. 3.

A specific, rapid role for  $24,25(\text{OH})_2\text{D}_3$ , opposed to that of the  $1,25$  metabolite, has also been shown by Boyan and co-

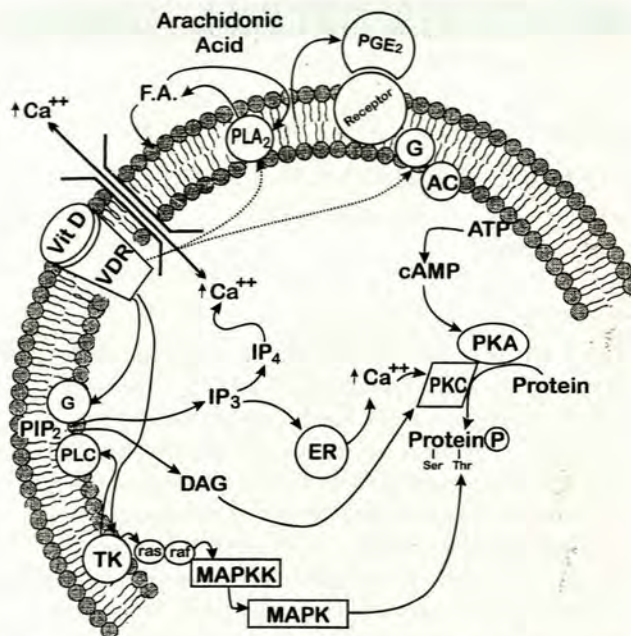


Fig. 3. Rapid signalling mechanisms of vitamin D. VDR = vitamin D receptor,  $\text{IP}_3$  and  $\text{IP}_4$  = inositoltris- and tetrakisphosphates,  $\text{PIP}_2$  = phosphatidylinositol bisphosphate, PLC = phospholipase C, G = G protein, TK = tyrosine kinase, MAPK = mitogen-activated protein kinase, MAPKK = mitogen-activated protein kinase kinase, PKA = protein kinase A, PKC = protein kinase C, AC = adenylate cyclase, DAG = diacyl glycerol,  $\text{PGE}_2$  = prostaglandin  $\text{E}_2$ , FA = fatty acid,  $\text{PLA}_2$  = phospholipase  $\text{A}_2$ , ER = endoplasmic reticulum.

workers.<sup>14,30,31</sup> The  $1,25$  metabolite affected primarily growth zone and the  $24,25$  moiety mainly resting zone cells in costochondral chondrocyte cultures. Phospholipase  $\text{A}_2$  and protein kinase C activities, arachidonic acid as well as prostaglandin  $\text{E}_2$  ( $\text{PGE}_2$ ) levels are decreased by the  $24,25$  and increased by the  $1,25$  compound.

All the above mechanisms result in modulation of protein phosphorylation. Some documented examples include the following: the protein kinase C (PKC) pathway phosphorylates membrane proteins of 42 and 48 kDa in rat colonocytes,<sup>32</sup> whereas protein kinase A<sup>33</sup> phosphorylates heart membrane proteins of 45 and 70 kDa. The nuclear VDR in rat osteoblasts is also phosphorylated after treatment with calcitriol.<sup>34</sup> Whether a calcitriol receptor situated in the cell membrane really exists has been a subject of contention for a long time.<sup>35</sup> A first definite report on identification of a membrane receptor that mediates rapid activation of PKC was published by Nemere *et al.*<sup>36</sup> in 1998.

The configuration of the calcitriol molecule is of paramount importance in its action on the genomic as well as the non-genomic receptor;<sup>37</sup> the natural secosteroid rotates easily around its 6,7 carbon bond. Calcitriol analogues locked in the 6-s-cis formation are potent agonists for rapid membrane responses, whereas the 6-trans analogues tested performed poorly in both membrane and nuclear assays.<sup>38</sup>



To see the rapid, non-genomic effects of calcitriol in perspective it must be mentioned that they are not specific to calcitriol — estradiol<sup>39-41</sup> and aldosterone<sup>42</sup> can generate similar rapid effects.

## EFFECTS

Both the genomic and non-genomic calcitriol action mechanisms described above are involved in a myriad of both calcaemic and non-calcaemic effects.

### Hypercalcaemic effects

These effects are achieved by 1,25(OH)<sub>2</sub>D<sub>3</sub> action on the intestine, bone and kidney.

#### Intestinal calcium uptake

Vitamin D administration increases the three basic steps of this process.<sup>43</sup> Calcium (Ca) inflow through Ca channels in the apical membrane, Ca transport through the enterocyte (bound to calbindin), and extrusion against a considerable concentration gradient through the basolateral membrane by a Ca-adenosine triphosphatase (ATPase) pump as well as a sodium (Na)/Ca exchanger.<sup>44</sup> Genomic mechanisms promote the synthesis of both calbindin and the Ca-ATPase within 4 - 8 hours.

Additionally, a very rapid (within minutes) stimulation of intestinal Ca transport was reported by the groups of Lieberherr *et al.*,<sup>19</sup> de Boland and Nemere<sup>45</sup> and Zhou *et al.*,<sup>23</sup> and called transcaltachia. The non-genomic mechanisms discussed above are involved in this mechanism: calcitriol stimulates phosphorylation events via both the protein kinase A (PKA) and PKC pathways. This could control the opening of basolateral Ca channels as well as the activity of the Ca pump mechanism. However, the physiological importance of these rapid mechanisms remains to be seen.

#### Calcium reabsorption by the kidney

Similar to the process in the gut, calcium and phosphate reabsorption are also promoted by calcitriol-induced calbindin synthesis. Both the 28 and 10 kDa forms are active in the distal kidney tubule.<sup>46,47</sup>

#### Calcium liberation from bone

Blood Ca levels can be increased when bone salts are dissolved by hydrogen ions secreted by osteoclasts into the lacunae formed by their ruffled borders. Hydrogen ions are produced by carbonic acid anhydrase, an enzyme that is induced by calcitriol, directly or indirectly, in osteoclast precursors.<sup>12</sup>

However, the VDR has only been found in osteoblasts, which have an indirect influence on osteoclast differentiation: certain cytokines, especially interleukin-1, are secreted by calcitriol-stimulated osteoblasts. These cytokines, in turn, promote

differentiation of monocyte precursors into osteoclasts<sup>48,49</sup> capable of carbonic anhydrase secretion. It has also been shown<sup>12</sup> that calcitriol can induce carbonic anhydrase in bone marrow macrophages directly.

## Non-calcaemic effects

### Bone

Given the complexity of bone cells (osteoblasts, osteoclasts, osteocytes), of bone metabolism, and of bone remodelling (mineral accretion versus bone resorption), it has been a major challenge to identify the cellular sites and molecular contributions of each of the different calcitriol metabolites in the overall process of bone biology.<sup>50</sup>

It is clear that osteoblasts have a VDR and that their differentiation is promoted by calcitriol.<sup>51</sup> Osteoblasts synthesise a variety of bone proteins: the production of collagen and alkaline phosphatase is, however, dependent on the stage of osteoblast differentiation.<sup>52,53</sup> In undifferentiated cells calcitriol can promote their synthesis, driving the cells to higher bone-forming capabilities, whereas it depresses these functions in the mature cell; at these concentrations calcitriol can stimulate bone resorption by osteoclasts.<sup>54</sup> The latter, however, is not a direct effect on osteoclasts since they do not possess a VDR. Mature osteoblasts secrete cytokines<sup>55</sup> that promote the differentiation of monocytic osteoclast precursors, which in their mature state are capable of bone resorption.

The role of 24,25(OH)<sub>2</sub>D<sub>3</sub> in bone biology has been studied intensively, but still remains elusive.<sup>50</sup> It has been known for 20 years that administration of 1,25(OH)<sub>2</sub>D<sub>3</sub> does not have the same beneficial effect as its parent compound, vitamin D, on calcification processes,<sup>56</sup> indicating that another metabolite, for example 24,25(OH)<sub>2</sub>D<sub>3</sub>, is also involved. Some important findings include the following: rats with high levels of 24,25(OH)<sub>2</sub>D<sub>3</sub> have a reduced rate of bone turnover;<sup>57</sup> 24,25(OH)<sub>2</sub>D<sub>3</sub> inhibits the bone resorptive function of calcitriol or PTH-stimulated osteoclasts;<sup>58</sup> it promotes the healing of chick tibia fractures;<sup>59</sup> and its receptor is found in epiphyseal cartilage<sup>60</sup> where [<sup>3</sup>H]24,25(OH)<sub>2</sub>D<sub>3</sub> is also found to accumulate.<sup>61</sup>

Some rapid, non-genomic effects of 24,25(OH)<sub>2</sub>D<sub>3</sub> in chondrocytes that are mediated by PKC have been mentioned above.<sup>14,30,31</sup> It is known that effects mediated by protein PKC are often linked to cell differentiation. This metabolite has additional effects: it has rapid biphasic effects on L-type calcium channels in UMR 106 cells<sup>62</sup> (mediated by PKC and PKA), and acts synergistically with transforming growth factor (TGF)-beta in resting zone chondrocytes<sup>63</sup> to activate PKC. Finally, chondrocyte metalloproteins can degrade extracellular matrix proteins, a process necessary in the remodelling of the matrix during endochondral development. 24,25(OH)<sub>2</sub>D<sub>3</sub> results in PKC-mediated phosphorylation and thus inhibition of stromelysin-1, an important metalloproteinase.<sup>64</sup>



### Other tissues

During the last decade the pluripotent actions of calcitriol on a wide variety of unexpected tissues that all contain VDRs has been reported.<sup>52</sup> A brief summary follows:

**Pancreas.** Vitamin D deficiency is associated with the diabetic state. Optimal vitamin D levels are therefore needed for an adequate insulin secretion response to glucose or arginine.<sup>65,66</sup>

**Skin.** Vitamin D is a powerful modulator of keratinocyte proliferation and differentiation.<sup>67</sup> It also blocks cytokine production by infiltrating lymphocytes,<sup>68</sup> which is an important factor in the inflammatory aspect of psoriasis.

**Immune system.** The production of a variety of cytokines, for example interleukins 1 and 2 and gamma interferon by lymphocytes and monocytes,<sup>69</sup> is modulated by calcitriol.

**Parathyroid gland.** Calcitriol decreases PTH secretion by inhibiting its synthesis at the level of gene transcription.<sup>70</sup> A VDRE has been identified in the PTH gene.<sup>71</sup>

**Cancer.** Vitamin D inhibits proliferation and promotes differentiation of various cancer cell lines and can diminish tumour size in certain cases.<sup>52,72-74</sup>

### POTENTIAL CLINICAL APPLICATIONS

Many analogues of vitamin D have been synthesised<sup>50</sup> in the hope of producing non-calcaemic, non-toxic and specific therapeutic agents. Their use has been complicated by the finding of polymorphism in the vitamin D receptor gene in different population groups.<sup>75,76</sup> This gives rise to different forms of the VDR that have differing affinities and activities and therefore variations in their biological outcome. This phenomenon influences most clinical applications of calcitriol (CT) and its analogues, and is discussed in the following section.

In the field of bone pathology certain new analogues of CT, notably 20-epimers,<sup>50</sup> can be used to promote calcaemic responses, for example, increased duodenal calcium absorption and osteoclast activity. Other analogues promote osteoblast differentiation and can therefore be used in an anti-osteoporosis strategy.<sup>50</sup> Calcitriol treatment is also of use in renal osteodystrophy<sup>50</sup> and X-linked hypophosphataemic rickets.<sup>77</sup> As mentioned above, VDR gene polymorphism predisposes to low duodenal Ca absorption in postmenopausal women<sup>78</sup> and low bone density in children.<sup>79</sup> However, conflicting results were reported by the study of Hansen *et al.*<sup>80</sup> on Danish perimenopausal women. Furthermore, suppression of PTH secretion by CT is an option in clinical management of different types of hyperparathyroidism.<sup>50</sup> The influence of polymorphic VDR gene alleles in treatment of both primary<sup>81</sup> and secondary<sup>82</sup> hyperparathyroidism has also been reported.

The pro-differentiation effects of the CT analogue calcipotriol

can be put to excellent use in the treatment of psoriasis<sup>83</sup> and possibly radiation-induced alopecia.<sup>84</sup> As in the above instances, allelic variations in the VDR gene can predispose to psoriasis.<sup>85</sup> Treatment of cancers with CT analogues is still in an experimental stage.<sup>50</sup> VDR polymorphism can play a role in predisposition to cancer of the breast,<sup>86</sup> but its effect in prostate cancer is questioned.<sup>87</sup>

The use of 22-oxa-calcitriol, a non-calcaemic analogue of CT, has been described in treatment of immune disorders.<sup>88</sup> A plethora of infective diseases can be treated with CT.<sup>52</sup> In addition, a hyperfunctioning immune system (as in the chronic inflammatory state of psoriasis, as well as in arthritis<sup>89</sup>) as well as some auto-immune diseases<sup>90</sup> have responded well to CT treatment. Recent studies have reported a role for VDR gene allelism in the occurrence of chronic hepatitis B and tuberculosis<sup>91</sup> as well as rheumatoid arthritis<sup>92</sup> in certain population groups.

Lastly, vitamin D has a potential role to play in the treatment of diabetes mellitus and hypertension.<sup>52</sup> All the clinical possibilities described above lead us into a new era of understanding and appreciation of its potential use.

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