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REVIEW

Vitamin D as a regulator of steroidogenic enzymes [version 1; referees: 1 approved, 1 approved with reservations]

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Abstract

During the last decades, the outlook on vitamin D has widened, from being a vitamin solely involved in bone metabolism and calcium homeostasis, to being a multifunctional hormone known to affect a broad range of physiological processes. The aim of this review is to summarize the research on vitamin D as a regulator of steroidogenic enzymes. Steroid hormones exert a wide range of physiological responses, including functions in the immune system, protein and carbohydrate metabolism, water and salt balance, reproductive system and development of sexual characteristics. The balance of sex hormones is also of importance in the context of breast and prostate cancer. Steroid hormones are synthesized in steroidogenic tissues such as the adrenal cortex, breast, ovaries, prostate and testis, either from cholesterol or from steroidogenic precursors secreted from other steroidogenic tissues. The hormonally active form of vitamin D has been reported to act as a regulator of a number of enzymes involved in the regulation of steroid hormon production, and thereby the production of both adrenal steroid hormones and sex hormones. The research reviewed in the article has in large part been performed in cell culture based experiments and laboratory animal experiments, and the physiological role of the vitamin D mediated regulation of steroidogenic enzyme need to be further investigated.

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Vitamin D - a multifunctional hormone

Vitamin D was discovered in the early 20th century when it was described that rickets could be cured by sunlight or cod liver oil. This fat-soluble vitamin was the fourth vitamin described and therefore designated as vitamin D. Vitamin D was found to regulate intestinal absorption and renal reabsorption of calcium and bone metabolism. Later research has demonstrated that vitamin D can be obtained from the diet or be *de novo* synthesized from 7-dehydrocholesterol. Further, the active form of vitamin D, $1\alpha,25$-dihydroxyvitamin D_3, interacts with a receptor in the target cells, showing that vitamin D should be biochemically characterized as a hormone rather than as a vitamin[1].

During the last decades, the outlook on vitamin D has widened, from being a vitamin solely involved in bone metabolism and calcium homeostasis, to being a multifunctional hormone known to affect a broad range of physiological processes. This includes effects on the immune system, brain and fetal development, insulin secretion, cancer, apoptosis, cell proliferation and differentiation as well as the cardiovascular system via the vitamin D receptor (VDR)[1–4]. The vitamin D receptor is widely expressed and it has been suggested that $1\alpha,25$-dihydroxyvitamin D_3 may have other roles yet undiscovered[3,5,6]. The aim of this paper is to review the literature on effects of vitamin D and vitamin D analogs on steroidogenic enzymes.

Bioactivation and metabolism of vitamin D

There are two forms of vitamin D, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Ergocalciferol is synthesized in plants, yeast and fungi while cholecalciferol is synthesized in animals. Vitamin D_3 is synthesized in the skin from 7-dehydrocholesterol upon exposure to UV-B radiation. Vitamin D_3 is then bioactivated in two subsequent steps to gain the biologically active form of vitamin D (Figure 1). In the first step, vitamin D_3 is 25-hydroxylated to 25-hydroxyvitamin D_3 (calcidiol). 25-Hydroxylation of vitamin D is a reaction that can be catalyzed by the mitochondrial CYP27A1 and the microsomal CYP3A4, CYP2R1 and CYP2J2 in humans. 25-Hydroxylation of vitamin D is mainly performed in the liver and calcidiol is then excreted into the circulation. Calcidiol is converted to $1\alpha,25$-dihydroxyvitamin D_3 (calcitriol) by

1α-hydroxylation, mainly performed in the kidneys. The principal human 1α-hydroxylase for 25-hydroxyvitamin D_3 is CYP27B1. The $1\alpha,25$-dihydroxyvitamin D_3 produced is excreted into the circulation and acts as a hormone[1,3,4,7–9].

Extrarenal 1α-hydroxylation of 25-hydroxyvitamin D_3 has been reported for a wide range of tissues, including colon, brain, mammary tissue, breast, pancreatic islets, parathyroid glands, placenta, prostate and keratinocytes[3,7]. These findings suggest that $1\alpha,25$-dihydroxyvitamin D_3 may be produced locally and act in an intracrine or paracrine fashion. It may be speculated that the local concentration of $1\alpha,25$-dihydroxyvitamin D_3 in these tissues could be higher than the circulating levels.

The normal serum level of calcidiol is 50–100 nM and for calcitriol 50–125 pM[3]. Calcitriol is the most potent form of vitamin D even though calcidiol can exert some biological effects as well. The circulating levels of $1\alpha,25$-dihydroxyvitamin D_3 is tightly regulated via a feed-back mechanism where $1\alpha,25$-dihydroxyvitamin D_3 downregulates the expression of CYP27B1 and upregulates the expression of CYP24A1[8].

Both calcidiol and calcitriol is metabolized by CYP24A1 to the less active compounds 24,25-dihydroxyvitamin D_3 and $1\alpha,24,25$-trihydroxyvitamin D_3 respectively[10]. It has recently been reported that CYP11A1 can catalyze the production of 20-hydroxyvitamin D_3 from vitamin D_3 and the production of $1\alpha,20$-dihydroxyvitamin D_3 from 1α-hydroxyvitamin D_3[11–15]. Both these metabolites have been reported to exert biological effects on cell differentiation and gene expression in a way resembling the one of $1\alpha,25$-dihydroxyvitamin D_3[11,12]. The physiological role, if any, of this CY11A1-mediated metabolism of vitamin D remains to be clarified.

Mode of action

The bioactivated form of vitamin D alters the gene expression of a large number of genes. It is well known that $1\alpha,25$-dihydroxyvitamin D_3 can act either to increase the gene expression or decrease the gene expression, depending on the gene in question. For example, $1\alpha,25$-dihydroxyvitamin D_3 increases the gene expression of
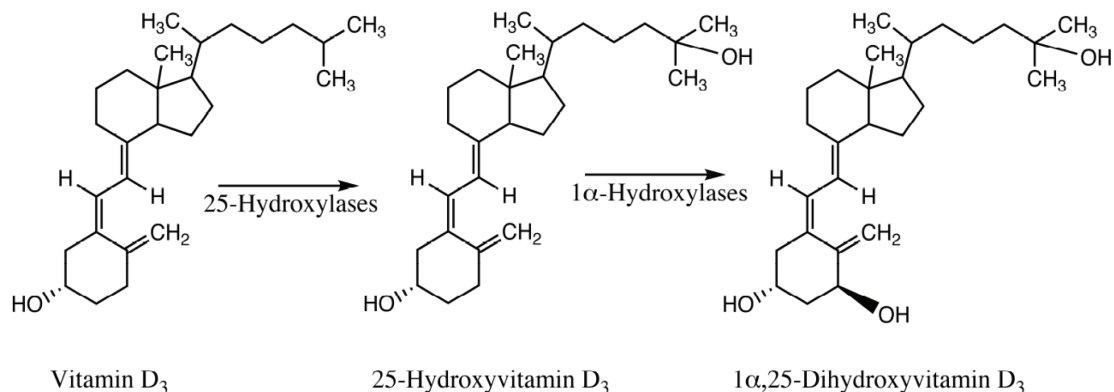

Figure 1. Bioactivation of vitamin D_3 to its hormonally active form, $1\alpha,25$-dihydroxyvitamin D_3.

CYP24A1 while it increases the gene expression of CYP27B1. Both these effects of the hormonally active form of vitamin D are mediated via a VDR dependent mechanism. The mechanism for $1\alpha,25$-dihydroxyvitamin D_3-mediated induction of gene expression is well known and based on the interaction between the $1\alpha,25$-dihydroxyvitamin D_3-activated VDR and a vitamin D responsive element (VDRE) in the gene promoter. These positive VDRE (pVDRE) consist of a hexameric direct repeat of the consensus sequence 5´-RGKTCA (R=A or G, K=G or T)[16]. The two half sites are separated by a three nucleotide spacer. The ligand-activated VDR-RXR complex interacts with the pVDRE and acts as a transcription factor to increase the transcriptional rate by recruiting coactivators[3].

However, the mechanism for $1\alpha,25$-dihydroxyvitamin D_3-mediated downregulation of gene expression has in large part remained unclear[17]. Studies have only been performed for a few genes, regarding the molecular mechanism for the vitamin D-mediated downregulation of gene expression. For these genes, it has been suggested that the mechanism could include recruitment or displacement of corepressors, such as VDR interacting repressor (VDIR) and Williams syndrome transcription factor (WSTF), from the promoter sequence. Further, it has been proposed that epigenetic changes such as histone deacetylation and DNA methylation might be involved in the mechanism[18–23]. The negative vitamin D response elements (nVDRE) described show a very low level of similarity to the pVDRE described[21]. Furthermore, it has been proposed that the mechanism for vitamin D-mediated downregulation of gene expression is not based on a direct interaction between the ligand-activated VDR and the promoter sequence, but rather an in-direct interaction via comodulator VDIR[18].

Recently, it has been shown in genome-wide ChIP-seq experiments that VDR has a large number of binding sites throughout the genome[24–26]. These binding sites have been found to be located predominantly within introns and intergenic regions and often far away from the transcriptional start site[26]. The physiological role of these binding sites remains to be elucidated.

Vitamin D has also been reported to exert rapid effects only seconds or minutes after treatment. Due to the quick response, it has been suggested that these effects are non-genomic and mediated by membrane-bound receptors[27].

Steroid hormone synthesis
All steroid hormones are synthesized from the common precursor cholesterol, which can be obtained from the diet or *de novo* synthesized from acetyl CoA. The production of steroid hormones is regulated via a number of enzymes of which a majority belongs to the cytochrome P450 (CYP) superfamily.

Steroid hormones exert a wide range of physiological responses, including functions in the immune system, protein and carbohydrate metabolism, water and salt balance, reproductive system and development of sexual characteristics. Steroid hormones are synthesized in steroidogenic tissues such as the adrenal cortex, breast, ovaries, prostate and testis, either from cholesterol or from steroidogenic precursors secreted from other steroidogenic tissues.

Adrenal steroidogenesis
The adrenal cortex produces steroid hormones such as aldosterone, corticosterone, cortisol, dehydroepiandrosterone (DHEA) and androstenedione. An overview of steroids and enzyme-catalyzed reactions in the adrenal steroidogenesis is shown in Figure 2. The adrenal steroidogenesis is quantitatively regulated by the transcription of CYP11A1 (cholesterol side-chain cleavage enzyme) and the activity of steroidogenic acute regulatory protein (StAR).

There are three adrenocortical zones, each with a distinct role in the production of steroid hormones; *zona glomerulosa* produces mineralocorticoids (*e.g.* aldosterone), *zona fasciculata* produces glucocorticoids (*e.g.* cortisol) and *zona reticularis* is the point of synthesis for adrenal androgens (*e.g.* DHEA). The qualitative regulation of adrenal steroidogenesis, determining which type of steroid that will be produced, is performed by the transcription and activity of CYP17A1. CYP17A1 catalyzes two different reactions, namely the 17α-hydroxylation and the 17,20-lyase reaction. The expression and activity of CYP17A1 differs between the three adrenal zones[28–30]. CYP17A1 is not expressed in *zona glomerulosa* leading to the production of mineralocorticoids. In *zona fasciculata*, CYP17A1 is expressed and catalyzing 17α-hydroxylation, but not the 17,20-lyase activity, leading to glucocorticoid production. In *zona reticularis*, CYP17A1 catalyzes both 17α-hydroxylation and 17,20-lyase activities and adrenal androgens are therefore the main product of adrenal steroidogenesis in this adrenal zone[28]. To control the production of steroid hormones, it is essential to regulate the two activities of CYP17A1 separately. The 17α-hydroxylase activity of CYP17A1 is regulated via the gene expression of CYP17A1. On the other hand, the 17,20-lyase activity of CYP17A1 is regulated via posttranscriptional mechanisms[28–34]. The adrenal zone-specific activities of CYP17A1 are summarized in Figure 3.

The 17,20-lyase activity has been reported to be regulated via three posttranscriptional mechanism; the abundance of P450 oxidoreductase (POR)[35,36], allosteric action of cytochrome b5[37] and serine phosphorylation of CYP17A1[38–41]. In 1972, Zachman *et al.*[42] described the first case of isolated 17,20-lyase deficiency, which is a rare condition. 17,20-Lyase deficiency could be a result of a mutated cytochrome b5, according to a recent report[43]. CYP17A1 deficiency, or impaired CYP17A1 activity due to altered posttranscriptional mechanisms, may lead to hypertension, hypokalemia and impaired development of sexual characteristics due to decreased production of adrenal androgens. Patients that are genetically male often present complete male pseudohermaphroditism while female patients may be infertile[44–46].

It has been reported that CYP17A1 strongly prefers 17α-hydroxypregnenolone over 17α-hydroxyprogesterone as a substrate for 17,20-lyase activity in humans[28,47]. 17α-Hydroxyprogesterone may, however, be a substrate for CYP17A1 in other species[47].

CYP21A2 is a steroid 21-hydroxylase catalyzing the production of deoxycorticosterone and 11-deoxycortisol, which are precursors for the production of corticosterone, aldosterone and cortisol. 21-Hydroxylase deficiency may lead to severe conditions such as congenital adrenal hyperplasia and Addison´s disease[48]. CYP21A2 is exclusively expressed in the adrenal cortex[28]. Therefore, glucocorticoids
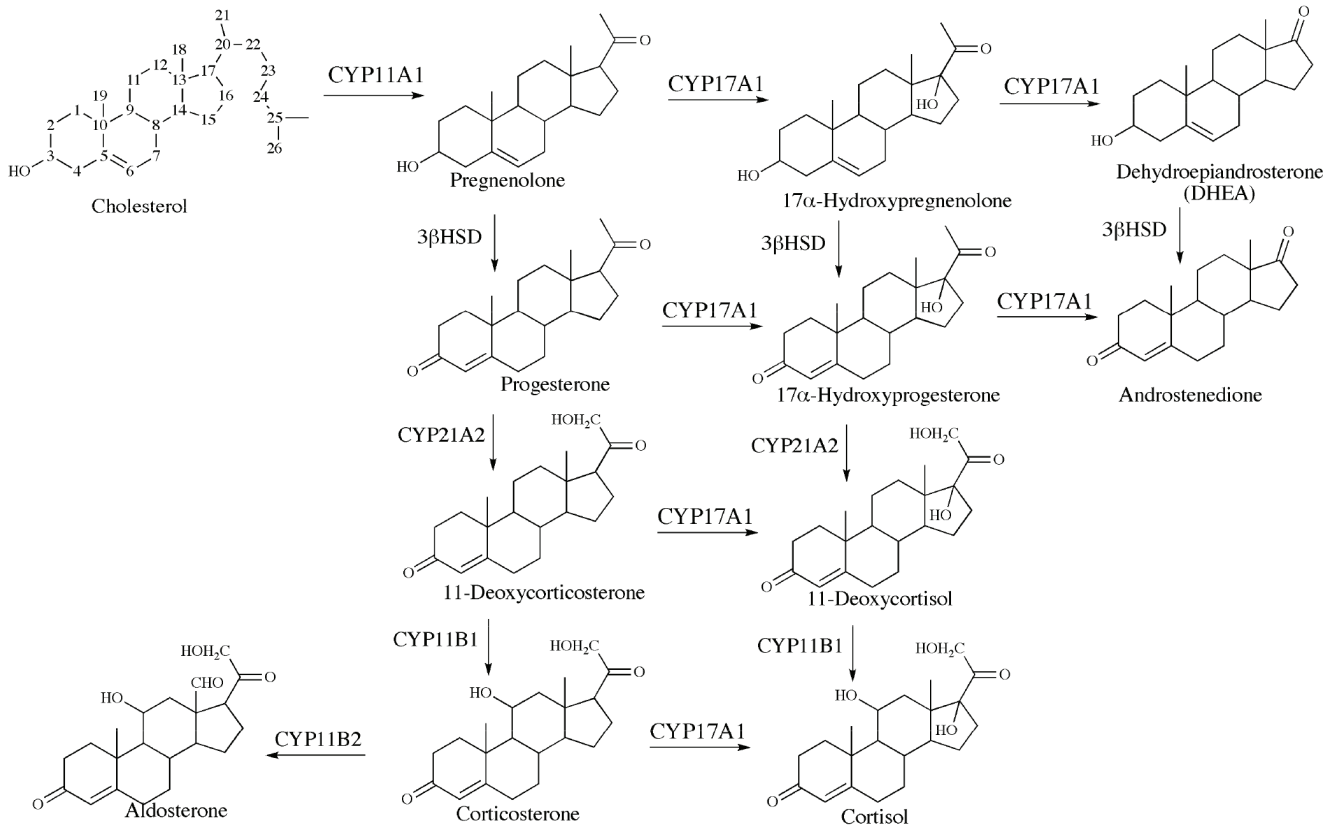
Figure 2. Overview of steroids and enzyme-catalyzed reactions in the adrenal steroidogenesis.
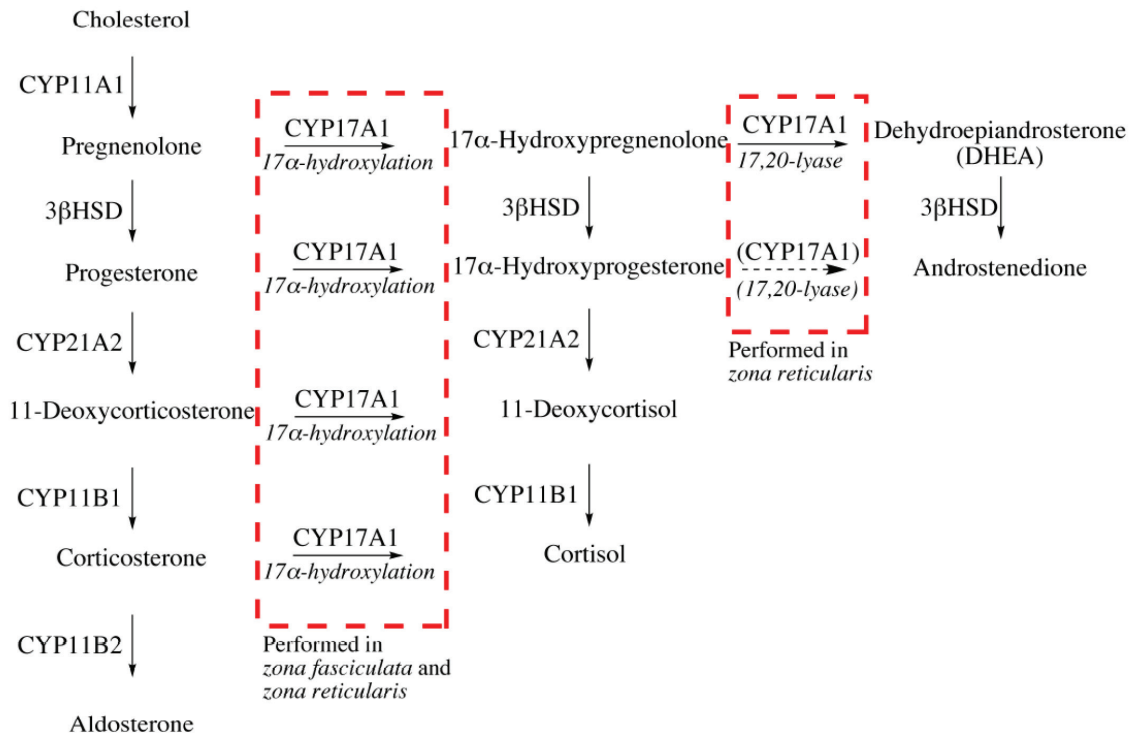

Figure 3. Adrenal zone-specific activities of CYP17A1.

and mineralocorticoids cannot be synthesized in other tissues than the adrenal cortex.

Sex hormone production

Sex hormones are mainly produced in the gonads, breast and prostate. The sex hormones in these tissues are produced either by *in situ* synthesis from cholesterol or by enzyme catalyzed conversion of DHEA or androstenedione excreted to the circulation from the adrenal cortex.

The sex hormones are divided into two groups; androgens and estrogens. Androgens such as testosterone and 5α-dihydrotestosterone (DHT) are produced via reactions catalyzed by 17β-hydroxysteroid dehydrogenase (17β-HSD) and 5α-reductase. Estrogens, such as 17β-estradiol (estradiol), are produced by aromatization of androgenic precursors, a reaction catalyzed by CYP19A1 (aromatase). Hence, the tissue-selective expression and activity of 5α-reductase and aromatase regulates the production of androgens and estrogens[49,51]. For some of these steroids, it remains unclear if they act as estrogens or as androgens or if they are inactive metabolites[52]. To fully understand the estrogenic and/or androgenic signaling exerted by these steroids is of utmost importance in research on *eg.* breast and prostate carcinogenesis.

It is crucial to regulate the levels of sex hormones in order to achieve normal gonadal development. Abnormally high levels of estrogens and androgens are associated with increased risk for breast cancer and prostate cancer, respectively[50,53–55].

Vitamin D and analogs as regulators of steroidogenic enzymes

Adrenal steroidogenesis

A link between vitamin D and the adrenal steroidogenesis has been proposed in a few very early reports[56,57]. In a paper from 1959, De Toni *et al.*[56] describes several clinical cases involving children with rickets having changes in urinary 17-ketosteroid levels. It is suggested in the paper that the altered steroid production may involve some action of vitamin D. Furthermore, the authors propose that disturbances in steroid metabolism might be due to sensitivity or resistance of organisms to antirachitic vitamins[57]. More recently, a case has been described where a woman with osteomalacia was reported to have elevated serum levels of aldosterone and simultaneously low levels of 25-hydroxyvitamin D[58]. After 24 months of treatment with vitamin D, the condition was normalized.

Recently, Lundqvist *et al.*[59] investigated the effects of 1α,25-dihydroxyvitamin D$_3$ on the adrenal steroidogenesis. We studied the effects of 1α,25-dihydroxyvitamin D$_3$ on the gene expression of key steroidogenic enzymes, the enzyme activity and the hormone production. The study was performed in the human adrenocortical carcinoma cell line NCI-H295R. We found that the mRNA levels of three key enzymes in the adrenal steroidogenesis, CYP11A1, CYP17A1 and CYP21A2 were altered by 1α,25-dihydroxyvitamin D$_3$ treatment. CYP11A1 and CYP17A1 mRNA levels were upregulated after vitamin D treatment, while CYP21A2 mRNA level was suppressed by the same treatment. No significant changes were observed in the mRNA levels of CYP11B1, CYP11B2 and 3βHSD. Further, we found that 1α,25-dihydroxyvitamin D$_3$ treatment decreased the

production of corticosterone, androstenedione, dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S), while the production of aldosterone and cortisol was unaltered. Moreover, we measured the enzyme activity of CYP21A2 and CYP17A1 by adding a known amount of substrate to the cell culture and measuring the turnover of substrate to product. In resemblance with the effects on mRNA level, CYP21A2 enzyme activity was suppressed by 1α,25-dihydroxyvitamin D$_3$. CYP17A1 catalyzes two separate reactions, the 17α-hydroxylation and the 17,20-lyase reaction. We found that treatment with vitamin D resulted in increased 17α-hydroxylation activity of CYP17A1, but in decreased 17,20-lyase activity of CYP17A1.

The regulation of the two activities of CYP17A1 uses two principally different mechanisms. The 17α-hydroxylase activity is regulated by alterations of the gene expression of CYP17A1. The 17,20-lyase activity, on the other hand, is regulated via posttranscriptional mechanisms[28,31–34]. It has been reported that the 17,20-lyase activity is regulated via three mechanisms; the abundance of P450 oxidoreductase (POR)[35,36], allosteric action of cytochrome b5[37] and serine phosphorylation of CYP17A1[38–41]. The discrepancy between the 1α,25-dihydroxyvitamin D$_3$-mediated increase in expression of CYP17A1 mRNA and the suppression of 17,20-lyase activity could be a result of posttranscriptional mechanisms affected by 1α,25-dihydroxyvitamin D$_3$.

In a subsequent report[22], we investigated the molecular mechanism for the effect of 1α,25-dihydroxyvitamin D$_3$ on CYP21A2 gene expression. We found that 1α,25-Dihydroxyvitamin D$_3$ altered the promoter activity of CYP21A2 via a mechanism involving VDR and a vitamin D response element in the CYP21A2 promoter. Further, we found that the mechanism included interaction of the comodulators VDR interacting repressor (VDIR) and Williams syndrome transcription factor (WSTF) to the gene promoter.

Chatterjee and collaborators[60,61] have reported that ligand-activated VDR upregulates the expression of sulfotransferase 2A1 (SULT2A1), an enzyme that catalyzes the conversion of dehydroepiandrosterone (DHEA) to dehydroepiandrosterone-sulfate (DHEA-S).

Sex hormone production

The production of sex hormones is regulated by multiple enzymes. Vitamin D has been reported to affect the expression and activity of several of these enzymes.

17β-hydroxysteroiddehydrogenases. Wang and Tuohimaa[62] have reported that 1α,25-dihydroxyvitamin D$_3$ upregulates the mRNA level for 17β-hydroxysteroid dehydrogenases (17β-HSD) type 2, 4 and 5 in cell lines derived from human prostate. In keratinocytes, Hughes *et al.*[63] have reported that 1α,25-dihydroxyvitamin D$_3$ stimulates the expression of 17β-HSD type 1 and 2.

Aromatase. It has been shown that 1α,25-dihydroxyvitamin D$_3$ alters the aromatase activity in placental cells[64,65], prostate cells[66] and osteoblasts[67,68]. Kinuta *et al.*[69] have reported that vitamin D receptor null mutant mice have a decreased aromatase activity in the ovary, testis and epididymis. Recently, it was reported that 1α,25-dihydroxyvitamin D$_3$ alters the gene expression of aromatase in a tissue-selective manner[70]. In breast cancer cell lines, vitamin D

treatment resulted in decreased aromatase gene expression, while the same treatment increased the aromatase gene expression in osteosarcoma cell lines. 1α,25-Dihydroxyvitamin D_3 has therefore been proposed to be a tissue-selective aromatase modulator[70].

We have investigated the effects of 1α,25-dihydroxyvitamin D_3 on the aromatase gene expression and estradiol production in human breast carcinoma MCF-7 cells, human adrenocortical carcinoma NCI-H295R cells and human prostate cancer LNCaP cells[71]. We found that the hormonally active form of vitamin D altered the estrogen and androgen metabolism in a cell line specific manner. Aromatase gene expression and estradiol production was found to be decreased in breast cancer cells, while the androgen production was markedly increased in the same cell line. 1α,25-dihydroxyvitamin D_3 was found to increase the aromatase gene expression and decrease dihydrotestosterone production in adrenocortical cells. In prostate cancer cells, aromatase gene expression was found to be increased after 1α,25-dihydroxyvitamin D_3 treatment. Furthermore, we studied the effects of 1α,25-dihydroxyvitamin D_3 on three different aromatase promoters, and found that the transcriptional rate of these promoters were affected by 1α,25-dihydroxyvitamin D_3 in a cell line-specific manner.

In a subsequent study, we have shown that the substance EB1089 (a vitamin D analog with decreased hypercalcemic effect) is able to inhibit the aromatase gene expression by dissociation of comodulator WSTF from the CYP19A1 promoter in human breast cancer MCF-7 cells[23]. Furthermore, 1α,25-dihydroxyvitamin D_3 and analogs have been reported to alter sex hormone signaling by suppressing the expression of estrogen receptor α[72–77]. It has also been reported that vitamin D deficiency alters reproductive functions in both male and female rats, indicating that vitamin D may affect sex hormone signaling[78,79].

Concluding remarks

In conclusion, 1α,25-dihydroxyvitamin D_3 has been shown to regulate the gene expression and enzyme activity of a number of steroidogenic enzymes, and the corresponding hormone production. The physiological role of these vitamin D effects remains to be elucidated in many cases, especially for the adrenal steroidogenic enzymes. More research has been conducted on the physiological role and potential medical use of the effects of vitamin D on estrogen production and action in breast cancer. Cell culture based experiments and laboratory animal experiments has clearly shown that 1α,25-dihydroxyvitamin D_3 is able to decrase the production and action of estrogens in breast cancer models, and also the growth of breast cancer xenotumors[70,72–75,80]. Based on these findings, vitamin D or analogs has been proposed to be of potential use as an anti-cancer agent[76,81–84] and a large number of clinical trials (*e.g.* ClinicalTrials.gov identifiers NCT00656019, NCT01472445, NCT01965522, NCT01948128, NCT01787409, and NCT01097278) are currently being conducted using vitamin D or analogs in different settings to investigate the potential use in the prevention or treatment of breast cancer.

Competing interests

No competing interests were disclosed.

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Current Referee Status: ✔ ?

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Referee Report 18 May 2015

? **Takashi Yazawa**

Department of Biochemistry, Asahikawa Medical University, Asahikawa, Japan

Lundqvist has overviewed the effects of Vitamin D on steroidogenesis. Overall, the manuscript is disorganized. In addition, there are many serious issues:

1. The references are improper. There are too few for a review article. Additionally, the author references some articles which are already retracted.

2. There are some unclear sentences. For example:

   Page 2, last line: "*For example, 1α,25-dihydroxyvitamin D3 increases the gene expression of CYP24A1 while it increases the gene expression of CYP27B1.*" Why are both genes are upregulated by vitamin D?

   Page 3, Adrenal steroidogenesis: "*[...] and zona reticularis is the point of synthesis for adrenal androgens (e.g. DHEA)*" What is the point?

3. The description of steroidogenesis is not sufficient.

4. Abstract: "*Steroid hormones are synthesized in steroidogenic tissues such as [...]*" Why are breast and prostate tissues steroidogenic? They hardly perform de novo synthesis of steroid hormones, at least under normal conditions.

5. Page 3, Adrenal steroidogenesis: "*The adrenal steroidogenesis is quantitatively regulated by the transcription of CYP11A1 (cholesterol side-chain cleavage enzyme) and the activity of steroidogenic acute regulatory protein (StAR).*" The transcriptional regulation of StAR gene is also a rate-limiting step for steroidogenesis.

6. P3, Adrenal steroidogenesis: "*The qualitative regulation of adrenal steroidogenesis, determining which type of steroid that will be produced, is performed by the transcription and activity of CYP17A1.*" This sentence is an overstatement. The expression of 3β-HSD is also important for zone-specific adrenal steroidogenesis.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Daniel Bikle

Department of Medicine and Dermatology, University of California San Francisco, San Francisco, CA, USA

This review is comprised of three parts: first a review of the mechanisms of action of vitamin D, second a review of steroid hormone metabolism, and third a review of primarily the authors research into the role of 1,25(OH)2D in regulating steroid hormone metabolism. The first sections provide a brief but effective introduction to the authors main focus on his own work regarding the regulation by vitamin D of steroidogenesis. The major criticism I have is that this last section reveals how preliminary the evidence is that vitamin D has a physiologically significant role in steroidogenesis. The work has been done primarily in cancer cells *in vitro*. No compelling *in vivo* data are provided to show its physiologic importance, although animal models with deletions in VDR and CYP27B1 have been available for years. Nevertheless, this review opens up an interesting area for future research, and as such serves as a useful stimulus for that research to occur.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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