Commentary

Women's Health

Can vitamin D reduce the risk of uterine fibroids?

"...vitamin D and/or its nonhypercalcemic potent analogs, pending appropriate clincial trials evaluation, could be viable options for medical orally adminstered treatment of symptomatic uterine fibroids."

Keywords: fibroid • leiomyoma • vitamin D

Uterine fibroid represents a localized proliferation of smooth muscle cells surrounded by a pseudocapsule of compressed muscle fibers. It is the most common benign tumor in the female genital tract. The mainstay treatment of uterine fibroid is surgery, in the form of myomectomy or hysterectomy. More than 600,000 hysterectomy procedures are performed in USA alone each year. In addition to its cost burden on the USA healthcare system, these procedures cause considerable morbidity and possible mortality. Additionally, hysterectomy will preclude future fertility. Therefore, it is crucial to search for novel nonsurgical alternatives for the management of symptomatic uterine fibroids and develop strategies to prevent its occurrencein the first place. Vitamin D is known as the main regulator of calcium homeostasis. Vitamin D3 also functions as a strong antifibrotic factor. Additionally, recent studies have demonstrated that vitamin D3 is a potent antitumor agent that effectively inhibits human uterine fibroid cells *in vitro* and shrinks fibroid lesions in preclinical animal studies; however, no human trials have been conducted in this area thus far. Here, we will discuss the different mechanisms of action of vitamin D3 in inhibiting uterine fibroid growth and proliferation.

Vitamin D was first identified as a key regulator of calcium homeostasis as its deficiency was associated with rickets and osteomalacia [1]. Humans can find vitamin D within their diet, such as oily fish, cod liver oil and dairy products [2]. However, the main source is sun exposure. UVB light transforms 7-dehydrocholesterol to vitamin D through a nonenzymatic thermal isomerization found in the skin. Vitamin D is then metabolized in the liver to 25-hydroxyvitamin D (25OHD) by 25α-hydroxylase; 25OHD is converted to the active compound 1,25-dihydroxyvitamin D (1,25[OH]2D) by 1α-hydroxylase (or CYP27B1) [3], which is predominantly expressed in the kidney [4]. A catabolic pathway involving 24α-hydroxylase (CYP24A1) is responsible for 25OHD and 1,25(OH)2D hydroxylation to inactive metabolites, named 24,25(OH)2D and 1,24,25(OH)3D, respectively (Figure 1) [5]. In the kidney, 1α-hydroxylase activity is strictly controlled by calcium homeostatic signals, especially by parathyroid hormone, whose release by parathyroid glands is elicited by hypocalcemia. 1,25(OH)2D response to low serum calcium levels by stimulation of osteoclasts to release calcium from the bone, enhances intestinal calcium absorption and reduces renal calcium excretion [6]. 1,25(OH)2D3 is a biologically active vitamin D3 and, in cell systems, it functions through interacting with the vitamin D receptor (VDR) [7]. VDR is a nuclear transcription factor that plays a major role in the modulation of gene expression. The effects of VDR on cell signaling include growth arrest, differentiation and/or induction of apoptosis, thus, demonstrating the involvement of vitamin D signaling in the inhibition of cell growth.

1,25-dihydroxyvitamin D3 (vitamin D3) is known as a strong growth inhibitor that induces apoptosis in human breast cancer cells [8]. Vitamin D3 suppresses proliferation of malignant cells, and it induces

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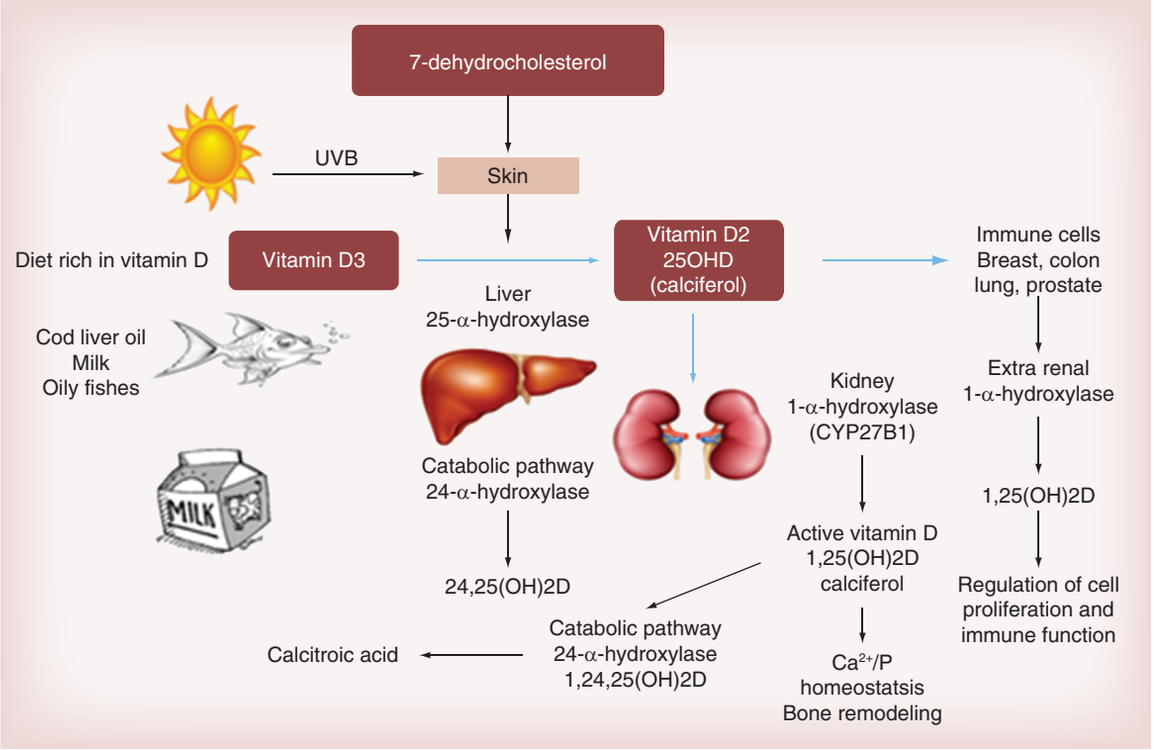

Figure 1. Vitamin D sources, metabolism and biosynthesis in humans.
1,24,25(OH)2D: 1,24,25-dihydroxyvitamin D; 1,25(OH)2D: 1,25-dihydroxyvitamin D; 24,25(OH)2D: 24,25-dihydroxyvitamin D; 25OHD: 25-hydroxyvitamin D; Ca^{2+}: Calcium; P: Phosphate.

differentiation and apoptosis [9]. Vitamin D3 analogs have also been shown to potentiate antitumor activity in a murine squamous cell carcinoma model [10].

Uterine fibroid represents a localized proliferation of smooth muscle cells surrounded by a pseudocapsule of compressed muscle fibers. They are often detected incidentally in routine health examinations, through bimanual pelvic and/or ultrasound examination or other imaging modalities; uterine fibroids are rarely associated with symptoms. Sometimes, uterine fibroids may be complicated by a variety of symptoms, including menstrual disturbance (e.g., menorrhagia, dysmenorrhea and intermenstrual bleeding), pressure symptoms, bloated sensation, increased urinary frequency, bowel disturbance or pelvic pain; therefore, definite treatment is requested [11]. The pathophysiology behind the development of uterine fibroids is still unknown, but the most accepted theory supports the fact that both estrogen and progesterone play major roles in fibroid growth [12]. Hysterectomy is by far the mainstay option presented to women who have completed child birth; however, many women may prefer to keep the uterus if the uterine fibroids-related symptoms can be appropriately controlled by some other less invasive ways. Among these conservative therapies, myomectomy is mostly used for women who would like to preserve their future fertility [13].

Recent evidence from three independent research groups in populations in North Africa, east USA and central Europe demonstrate an association between serum vitamin D deficiency and increased risk of uterine fibroids. This is not a trivial finding but a significant one that can inform clinical practice and improve management of this common disease worldwide. The Al-Hendy group was first to report on the association between lower serum vitamin D level and increased susceptibility to uterine fibroids in 2012 in a cohort of black and white women in North Africa [12]. This was followed by two other major studies including; Baird *et al.* in a cohort of women from eastern USA [13], and Paffoni *et al.* in Italian women [14]. Table 1 summarizes the main finding in these studies.

Additionally, we have reported [12] that vitamin D exhibited a significant ($r = -0.31$; $p = 0.002$) inverse dose–response relationship between its serum levels and the severity of fibroid disease in that cohort, which means the higher levels of serum vitamin D were associated with the least severe uterine fibroid burden (small fibroid size and low multiplicity) [12].

We have recently demonstrated that vitamin D3 inhibited the proliferation of human uterine fibroid cells. Blauer *et al.* confirmed the effect of vitamin D3 on the inhibition of human myometrial and fibroid cell growth [15]. Here, we will discuss the different

mechanism of action of vitamin D3 in inhibiting uterine fibroid growth and proliferation. Although studies have shown this effect of vitamin D3 on fibroid growth *in vitro* and in animal studies, no human trials have yet been conducted to translate these important preclinical observations.

In a recent study, we have evaluated the efficacy and safety of vitamin D3 for the potential treatment of fibroid in the immune-competent authentic Eker rat model. The mutation of tuberous sclerosis suppressor gene makes the Eker rat more prone to fibroid development; its detailed phenotype has been described recently in a publication from our laboratory group [16]. We randomly assigned rats harboring visible fibroid tumors into two groups, with six in each group: the control group was administered with ethylene glycol (vehicle); and the treatment group was given vitamin D3 delivered by micro-osmotic pumps (Alzet Inc., CA, USA) implanted into the dorsal subcutaneous space at a rate of 0.5 µg/kg/day for 3 weeks. Methodological details of this experiment have been recently described by our group [16].

The physiological level of vitamin D is 30–80 ng/ml for 25-hydroxyvitamin D3 and can usually be accomplished by intake of 2000 IU/day. Chronic or acute administration of higher doses of vitamin D3 can lead to hypervitaminosis D, inducing hypercalcemia and functional hypoparathyroidism or adynamic bone disease in which the serum parathyroid hormone levels are suppressed below 100 pg/ml (100 ng/l), as well as the frequent fractures and bone pain usually seen in this condition. Hypercalcemia has been reported with daily doses of greater than 50,000 IU of vitamin D3. Studies have demonstrated that vitamin D toxicity is very unlikely in healthy people at intake levels lower than 10,000 IU/day [17]. In our study, we have used 0.5 µg/kg/day dose of vitamin D3, which is equivalent to 1400 IU for an adult individual with average bodyweight of 70 kg. We have calculated the dose of vitamin D3 using the conversion equation 1 µg of vitamin D3 is equivalent to 40 IU [18].

> "...the expression of the proapoptotic Bad protein to be increased in fibroid tumors in Eker rats treated with vitamin D3 when compared with vehicle-treated controls."

To understand the different antiproliferative mechanism of action of 1,25 dihydroxyvitamin D3, we are going to discuss each mechanism separately in the following discussion.

We detected that vitamin D3 reduced the expression levels of the cell proliferation marker PCNA on vitamin D3-treated Eker rats when compared with vehicle-treated controls. We used the western blot analyses using uterine fibroid tumor lysates obtained from both groups to determine the effects of vitamin D3 on protein expression associated with fibroid growth. Vitamin D3 also significantly reduced the expression levels of cell cycle regulatory proteins, such as CDK1, CDK2 and CDK4, in fibroid tumors [19].

In addition, we have found the expression of the proapoptotic Bad protein to be increased in fibroid tumors in Eker rats treated with vitamin D3 when compared with vehicle-treated controls. The effects of vitamin D3 treatment on cellular apoptosis-related protein expression were verified using western blot analyses using the same fibroid tumor lysates. Vitamin D3 treatment significantly reduced the antiapoptotic BCL2 and BCL2L1 protein expressions in fibroid tumors. The

Table 1. A recently published study demonstrated the relationship between vitamin D and uterine fibroids.

	Sabry *et al.* (2013)	Baird *et al.* (2013)	Paffoni *et al.* (2013)
Total number of participants	Total of 154 patients were included; 50 control group, 104 with fibroid. A total of 87 were black and 67 were white	1036 (620 blacks and 416 whites)	128 with fibroid; 256 control
OR/CI	Not reported	Adjusted protective OR: 0.68; 95% CI: 0.48–0.96 for 25OHD >20 ng/ml	2.5 (95% CI: 1.2–4.9; p = 0.016)
Ethnicity	Different ethnicity (women; black and white)	African and Caucasian American women	Italian women
Serum level 25OHD assay	UF: 19.7 ± 11.8 ng/ml Control: 22.3 ± 6.5 ng/ml Radio-immunoassay	Only 10% of blacks and 50% of whites had levels of 25OHD regarded as sufficient (>20 ng/ml) Radio-immunoassay	UF: 18.0 ± 7.7 ng/ml Control: 20.8 ± 11.1 ng/ml Chemiluminescence

25OHD: 25-hydroxyvitamin D; OR: Odds ratio; UF: Uterine fibroid.

cell proliferation marker cyclin D1 and the proto-oncogene MYC has been reported to be overexpressed in uterine fibroid compared with normal myometrium [20]. We have shown vitamin D3 treatment significantly reduced both cyclin D1 and MYC protein expression in Eker rat fibroid tumors by using western blot analyses. Additionally, vitamin D3 treatment induced the caspase-3-cleaved product in fibroid tumors in the treatment group, suggesting the activation of the caspase signaling cascade. These last two mechanisms of actions demonstrate the strong antiproliferation function of 1,25-dihydroxyvitamin D3 in controlling fibroid growth [19].

As mentioned above, both estrogen and progesterone play major roles in fibroid growth and function through their nuclear receptors [11]. We used western blot analysis to verify the regulatory effect of vitamin D3 treatment on uterine fibroid steroid receptor expression. These results demonstrated that vitamin D3 reduced fibroid tumor size by decreasing the protein expressions of estrogen receptor ESR1, as well as progesterone receptor PGR-A and PGR-B and increasing the VDR expression in Eker rats [19].

> "Vitamin D3 also functions as a strong antifibrotic factor by inducing a remarkable reduction in the expression of collagen and other TGF-β3-dependent key profibrotic factors in human fibroid cells in a dose-dependent fashion."

We have found that the expression of cell proliferating markers PCNA and MKI67 was significantly lower ($p < 0.05$) in fibroid tumors obtained from vitamin D3-treated Eker rats when compared with vehicle-treated controls. In addition, the hematoxylin and eosin staining revealed a reduced number of tumor cells in vitamin D3-treated Eker rats when compared with vehicle-treated controls. Caspase-3 immunoreactive cells denote the high apoptotic activity in a given tissue; we have found a significantly higher levels of caspase-3 immunoreactive cells in fibroid tumors derived from vitamin D3-treated Eker rats when compared with vehicle-treated controls. Taken all together, we can suggest that vitamin D3 shrinks uterine fibroid tumor size in the Eker rat preclinical model by reducing cell proliferation and by activating the intrinsic apoptosis pathway [19].

In addition, there were a remarkable reduction of the mRNA levels of MMP-1, MMP-3, MMP-13 and MMP-14 in human fibroid cells after treatment with vitamin D. The reduction of MMP-2 and MMP-9 protein were in a vitamin D concentration-dependent manner in uterine fibroid cells ($p > 0.05$ to $p > 0.001$). Uterine fibroids grow slowly by the deposition of a wide array of extracellular matrix (ECM) components. This ECM is under continuous physiological degradation process important for development, tissue repair and remodeling. Although the major proteinases enzymes called matrix metalloproteinases (MMPs) are involved in ECM degradation [21], the MMP family of proteases can digest fibrillar collagen and collagen type I via collagenases such as MMP-1, MMP-8, MMP-13, MMP-14, gelatinase A (MMP-2) and gelatinase B (MMP-9). In addition, the dysfunction and dysorganization of ECM homeostasis causes a paradoxical increase in MMPs in uterine fibroids. Vitamin D treatment led to a decrease in the expression of these MMPs, down to the level noticed in the normal myometrium [19].

TGF-βs, which are multifunctional peptides, are the key regulators of cell growth, differentiation, inflammation, apoptosis and tissue remodeling, and these processes are important contributors to tissue fibrosis [22]. The mRNAs and proteins for TGF-β1, TGF-β2 and TGF-β3 and their receptors have been detected in both human myometrium and fibroids [23]. TGF-βs also upregulate the synthesis of many of the ECM proteins that are involved in fibrosis [24]. TGF-β3 is elevated three- to five-fold in fibroid compared with adjacent myometrium tissues [25]. TGF-β3 plays an essential role in ECM overproduction in uterine fibroids by inducing the expression of collagen type 1, fibronectin, laminin and proteoglycans [26]. These ECM-related genes such as collagen type 1 and fibronectin are overexpressed in uterine fibroids. Vitamin D3 also functions as a strong antifibrotic factor by inducing a remarkable reduction in the expression of collagen and other TGF-β3-dependent key profibrotic factors in human fibroid cells in a dose-dependent fashion [27].

Conclusion

Vitamin D is known as the main regulator of calcium homeostasis. Recent studies have demonstrated that vitamin D3 is a potent antitumor agent that shrinks uterine fibroids *in vitro* and in appropriate preclinical animal studies; however, human trials are yet to be conducted in this important area of women's health, which should be considered a high clinical research priority to verify these important preclincial observations. 1,25(OH)2D3 is the biologically active form of vitamin D3 and, in cell systems, functions through interacting with the VDR. VDR is a cell membrane as well as a nuclear transcription factor that includes growth arrest, differentiation and/or induction of apoptosis demonstrating the involvement of vitamin D signaling in the inhibition of cell growth. Vitamin D3

reduces the expression levels of cell proliferation marker PCNA; MKI67 protein expressions; antiapoptotic BCL2, BCL2L1, ESR1, PGR-A, PGR-B protein expressions; and the expression levels of cell cycle regulatory proteins (CDK1, CDK2 and CDK4) in fibroid tumors, but increases the expression of the proapoptotic Bad protein and the VDR itself. Vitamin D3 also functions as a strong antifibrotic factor as it dramatically inhibit the expression of collagen and other TGF-β3-dependent key profibrotic factors in human fibroid cells in a dose-dependent fashion. Finally, there is a paradoxical increase in MMPs in uterine fibroids. The MMP system is responsible for ECM homeostasis and a decrease in the expression of MMPs caused by 1,25 dihydroxyvitamin D3 is associate with fibroid shrinkage. We believe vitamin D and/or its nonhypercalcemic potent analogs, pending appropriate clincial trials evaluation, could be viable options for medical orally adminstered treatment of symptomatic uterine fibroids.

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