Abstract

Vitamin D has classically been considered an important nutrient, but modern scientific evidence points for vitamin D a new and more critical role as ubiquos hormone at the centre of a complex endocrine, paracrine and autocrine system involved in maintaining general health. Vitamin D is found in small quantities in food; however it is also produced by the skin when exposed to certain intensities of ultraviolet light. This production of vitamin D by the skin may be limited in latitudes far from the equator where sunlight is not intense enough or due to the use of sunscreens. The active hormone, 1α,25-dihydroxycholecalciferol, may offer a defense against a growing list of health concerns, including osteoporosis and fractures, cancers, cardiovascular diseases, diabetes, hypertension and auto-immune diseases. As more people have low
levels of the precursor (25-hydroxycholecalciferol) in blood, the knowledge of vitamin D is rising increasing and the connection of vitamin D and cancer is being elucidated. The autocrine and paracrine participation of vitamin D gynaecologic carcinogenesis has been well established. Substantial epidemiological and clinical data suggest a link between low levels of vitamin D and an increased risk of a number of female specific cancers. Sustained low levels of its precursor are responsible for alterations in vitamin D tissue and cell production and metabolism. Furthermore, some recent clinical studies support the recommendation to increase vitamin D levels to a normal range in order to prevent the tissue disorders related to hypovitaminosis D which are thought to be involved in the initiation and progression of cancer.
Sunlight, the vitamin D endocrine system, and their relationships with gynaecologic cancer

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Abstract

Vitamin D has classically been considered an important nutrient, but modern scientific evidence points for vitamin D a new and more critical role as ubiquuos hormone at the centre of a complex endocrine, paracrine and autocrine system involved in maintaining general health. Vitamin D is found in small quantities in food; however, it is also produced by the skin when exposed to certain intensities of ultraviolet light. Substantial epidemiological and clinical data suggest a link between low levels of vitamin D and an increased risk of a number of female specific cancers. Different types of cancer cells present vitamin D receptors and the enzymatic system involved in both vitamin D synthesis and inhibition. Sustained low levels of its precursor are responsible for alterations in vitamin D tissue and cell production and metabolism. The active form of vitamin D, $1,25(OH)_2D_3$, can induce differentiation, inhibit proliferation, and modulate immune responsiveness of breast and a wide variety of female genital cell types. Vitamin D effects have been observed on expression of cell cycle regulators, growth factors and their receptors, apoptotic machinery, metastatic potential, and angiogenesis; all of which have some effect on hyperproliferative conditions. However, vitamin D blood levels may not be representative of the local metabolic alterations during carcinogenesis. Clinical studies support the recommendation to increase vitamin D levels to a normal range in order to prevent the tissue disorders related to hypovitaminosis D which are thought to be involved in the initiation and progression of cancer.

Key words: Vitamin D, vitamin D insufficiency, cancer, sunlight, inadequate diet, aging, sun exposure, female genital cancer, breast cancer, gynaecologic cancer.
Abbreviations:

1,25(OH)₂D₃ = 1α,25-dihydroxycholecalciferol
25(OH)D₃ = 25-hydroxycholecalciferol
CYP24A1 = 25-hydroxyvitamin D-24-hydroxylase = 25(OH)D₃ 24-hydroxylase = 24-hydroxylase
CYP27A1 = vitamin D-25-hydroxylase = 25-hydroxylase
CYP27B1 = 25(OH)D₃-1α-hydroxylase = 1α-hydroxylase,
HMEC = human mammary epithelial cells
HPV = human papilloma virus
IGF-I = insulin-like growth factor
IGFBP-3 = IGF-binding protein-3
PTH = parathyroid hormone
RXR = retinoid X receptor
UV = ultraviolet
VDR = vitamin D receptor
VDRE = vitamin D response element
VEGF = vascular endothelial growth factor

Equivalence of 25(OH)D₃: 1 ng/ml = 2.5 nmol/L
1. Introduction

The importance and therapeutic properties of the sun have been recognized since early times in human history. Egyptians worshipped the sun god Ra as the highest of gods, Greeks had Apollo and Helios and actually they made up a word, *heliotherapy*, to define the importance of the sun in health. When exposed to sunlight, bares skin produces vitamin D, and actually sunlight exposures of 10-15 minutes a day have been shown to be beneficial to the over all well being of the body. There is compelling evidence that low levels of vitamin D have led to an increased risk of diseases and abnormalities like rickets, osteomalacia, osteoporosis, different types of cancers and chronic diseases such as psoriasis, diabetes mellitus, hypertension, heart diseases, myopathy, multiple sclerosis, schizophrenia, hyperparathyroidism and others [1-6].

Over the past two decades epidemiological, experimental, and clinical evidences supporting the protective action of sunlight against cancer have been shown. It appears that vitamin D secosteroid compounds are the molecular link between sunlight and the most prevalent cancers, whose incidence rates increase with age. In the 80’s Garland et al [7-9] and Schwartz et al [10,11] proposed that hypovitaminosis D could be related with an increased risk of colon, breast, ovarian and prostate cancers. The pattern of increased cancer incidence in regions with lower solar radiation was confirmed in Europe and other parts of the world [12]. The biochemical relationship between sun exposure and cancer inhibition was first highlighted by Eisman et al [13], who reported the presence of vitamin D receptors (VDRs) in a human breast cancer cell line and their association with the incidence of bone metastases and hypercalcaemia. Furthermore, activation of the VDRs by the powerful hormone 1α,25-dihydroxyvitamin D (1,25(OH)₂D₃, dihydrocholecalciferol, or calcitriol) induces differentiation and apoptosis, inhibits proliferation, invasiveness, angiogenesis, and metastatic potential [1]. The presence of VDRs was later shown in other human breast cancer cell lines, melanoma, and in many other tumours.[14-16]. Additional evidence based on differences in incidence of cancer between Northern and Southern states of North America have found that some 17 or more different types of cancer are less common in the sunny south [1,17]. In addition, it was proposed that certain cancers could be prevented with calcium and vitamin D supplements [18,19].

Vitamin D’s role in the reproduction, osteoporosis, and musculoskeletal processes have been recently reviewed [2-6]. This is a retrospective study of published information concerning vitamin D’s influence on the origin and development of different types of female cancers retrieved from a PubMed and personal search.

2. Vitamin D acquisition and action

Most of human vitamin D₃ is synthesized by the sequential action of the solar ultraviolet (UV) radiation (wavelength 290 to 320 nm) on the skin, and cholecalciferol oxidation at the liver to
produce the principal circulating metabolite 25(OH)D3 that it is transformed into the active hormone 1,25(OH)2D3 by the kidney and to a lesser extent by other tissues [2]. Diet ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are considered to be equivalent in humans; however, although they have equivalent digestive absorption, vitamin D2 potency is less than one third that of vitamin D3 [20]. Following absorption, vitamin D is first metabolized by the liver into its principal circulating metabolite, 25(OH)D3 and by the kidneys and other tissues into its most biologically active form, 1,25(OH)2D3 (Figure 1) [2,21].

Vitamin D and its metabolites are transported in the circulation by a specific vitamin D binding protein, which is normally present in excess amounts. It acts as a hormone throughout a specific receptor, promotes the absorption of calcium and phosphorus, and helps deposit these minerals in bones and teeth. Like steroid hormones, 1,25(OH)2D3 may turn genes on and off that induce hundreds of enzymes and proteins crucial to maintain health and fight disease. The autocrine and the paracrine vitamin D systems appear to be turned on all the time since the Michaelis-Menton constant is never approached throughout the full range of physiological substrate concentrations for 1,25(OH)2D3 production in the tissues [22].

There are at least three systems that regulate the vitamin D activity within tissues: the VDR and two major vitamin D metabolising enzymes. The enzyme CYP27B1 is responsible for synthesis of 1,25(OH)2D3, and the enzyme CYP24A1 is responsible for vitamin D catabolism. All cells that express the VDR are biologically responsive to 1,25(OH)2D3 and express the CYP24A1 gene. The expression of both the CYP27B1 and CYP24A1 genes in the kidney contribute to the serum level of 1,25(OH)2D3. During growth and early adulthood, the synthesis of 1,25(OH)2D3 is a major determinant of serum levels. However, in the late adulthood and elderly, increased expression of kidney CYP24A1 is the major cause of the decreased serum 1,25(OH)2D3 levels with aging [23].

The active form of vitamin D mediates its actions through a classic steroid hormone-like transcriptional mechanism, influencing the expression of hundreds of genes. The VDR is a protein located in the cell nucleus, sensible to 1,25(OH)2D3 stimulation by altering transcription rate of target genes. VDR undergoes conformational change and forms a heterodimer with a second protein, the retinoid X receptor (RXR). This, in turn, binds to DNA elements in the promoter regions of target genes described as vitamin D response elements (VDREs). Additionally, 1,25(OH)2D3 appears to bind to one or more cell surface receptors that, through second messenger pathways, mediate certain non-genomic effects [23-25].

Besides the almost universal presence of VDRs, some cell types (e.g., keratinocytes, monocytes, bone, placenta) are capable of metabolizing 25(OH)D3 to 1,25(OH)2D3 by the enzyme 25(OH)D3-1α-hydroxylase (1α-hydroxylase, CYP27B1). 1α-hydroxylase is a cytochrome P450 enzyme expressed in kidney and other tissues that generates 1,25(OH)2D3 from the inactive vitamin D precursor 25(OH)D3 [25,26]. The combined presence of 1α-hydroxylase as well as the specific receptor in several tissues may explain the new roles non-related with calcium homeostasis for 1,25(OH)2D3. The cytochrome P450 25-hydroxyvitamin D3-24-hydroxylase (24-hydroxylase, CYP24A1) plays an important role in regulating concentrations of both the precursor
25(OH)D₃ and the hormone 1,25(OH)₂D₃ (figure 1). It converts 25OHD₃ and 1,25(OH)₂D₃ to 24,25-dihydroxyvitamin D₃ and 1,24,25-trihydroxyvitamin D₃, respectively. The 24-hydroxylase depends of VDR activity, and likely is controlled by a short-lived repressor protein. When serum calcium levels are increased, there is a reduction of the available 1,25(OH)₂D₃ [27].

In vitro, 1,25(OH)₂D₃ significantly inhibited vascular endothelial growth factor (VEGF)-induced endothelial cell sprouting and elongation in a dose-dependent manner; furthermore, there is a significant inhibitory of 1,25(OH)₂D₃ on VEGF-induced endothelial proliferation. The in vivo administration of 1,25(OH)₂D₃ induced tumors that were less vascularized than tumors in the control group without vitamin D treatment [28]. Furthermore, the induction of apoptosis of cancer cells in vivo has been postulated as the cause of tumor regression when analogues of 1,25(OH)₂D₃ are used [29]. Consequently, since angiogenesis is an essential step in initial tumor development and metastasis, the antiangiogenic properties of 1,25(OH)₂D₃ and vitamin D-related compounds may be relevant for the treatment of some tumors.

Aging is associated with a decrease in the uptake, synthesis and effect of vitamin D₃. Many changes occur during cellular aging, such as decreased membrane fluidity, increased protein oxidation, decreased DNA methylation, and defects in mitogenic signalling [30]. Aging is also associated with decreased serum vitamin D metabolites, reduced intestinal calcium absorption, and decreased bone density [31,32]. Age-related alterations of 1,25(OH)₂D₃-signal transduction have been demonstrated. For instance, the reduction of intestinal calcium absorption observed with aging has been related to an impairment in both the genomic and the non-genomic modes of action of 1,25(OH)₂D₃ [33]. Therefore, it is likely that the mechanism involved in increasing circulating 1,25(OH)₂D₃ in response to mineral deficiency changes with age, and is likely to be mediated by a decrease in metabolic clearance via the down-regulation of both renal 24-hydroxylase and VDR expression [34].

It seems quite plausible that during carcinogenesis, the local autocrine/paracrine vitamin D normal system is altered or lost. It is also likely that the chronic dysfunction of the general vitamin D endocrine system induced by inadequate vitamin D levels could alter the secosteroid metabolism, and could reduce the amount of vitamin D precursors that reach the cells.

3. Breast cancer

Breast cancer is one of the most common malignancies in women in the Western world. It is a multifactorial disease, and changes in cellular biology are affected by a large number of variables. Risk predictive models consider a combination of risk factors with good predictive but low discriminatory power which can include genetic predisposition, breast density, hormonal effects, and age. In fact, age is the most significant risk factor in breast cancer, as its incidence increases with age, doubling about every 10 years until menopause, when the rate of increase slows dramatically [35,36]. Epidemiologic and biochemical studies are searching new additional
factors to determine breast cancer risk, and new targets for preventive therapies. The incidence pattern reflects to a certain extent some basic characteristics of the society, such as life-style factors. One of the most important life-style factors is sun exposure and tanning habits as an indirect measure of vitamin D synthesis; a second factor is related with food and micronutrients.

3.1. Epidemiological and clinical evidences that link vitamin D and breast cancer

Numerous in vitro studies have demonstrated that 1,25(OH)$_2$D$_3$ can inhibit cell proliferation and promote cell differentiation in breast tumor tissue, suggesting that high levels of vitamin D metabolites may be protective against breast cancer. In addition, several ecologic studies have reported lower breast cancer incidence and mortality rates in populations with high sunlight exposure, and thus higher vitamin D levels, compared with those with lower ambient sunlight. However, it seems that there is no correlation between the vitamin D protective effect on breast cancer and circulating levels of active 1,25(OH)$_2$D$_3$, indicating a more localized activation of vitamin D. John et al [37] reported the findings of the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (NHANES I) concerning the relationships between either sunlight exposure or vitamin D supplements and breast cancer risk. Several measures of sunlight exposure and vitamin D intake were associated with reduced risk of breast cancer, with relative risks ranging from 0.67 to 0.85. Although the number of cases studied were relatively small, the data support the hypothesis that high vitamin acquisition, either by sun exposition or by dietary intake, may reduce the risk of breast cancer.

It is likely that the low concentrations of serum 1,25(OH)$_2$D$_3$ are associated with breast cancer, although the mechanism involved in these regulations is still unclear. In a small group of breast cancer patients and women submitted to stetic mammoplasties, serum levels of 25(OH)D$_3$ and 1,25(OH)$_2$D$_3$ as well as tissue expression of 1$\alpha$-hydroxylase, 24-hydroxylase, and VDRs, were determined. There were no significant differences in either serum 25(OH)D$_3$ or 1,25(OH)$_2$D$_3$, although the active hormone was slightly lower in breast cancer patients than in women without it. Furthermore, 24-hydroxylase, VDR and 1$\alpha$-hydroxylase mRNA tissue expression were similar in both groups, and no correlation between 24-hydroxylase, 1$\alpha$-hydroxylase, and VDR expression was found in patients with cancer [38]. In another study [39], the vitamin status was determined in breast cancer patients and control women by measuring serum 25(OH)D$_3$; in women with values < 50 nM the odds ratio was 3.54 for breast cancer compared to women with levels > 50 nM. In both normal and breast cancer cell lines, the 25(OH)D$_3$ activating enzyme CYP27B1 and CYP24 were detected. It seems that impaired local production of 1,25(OH)$_2$D$_3$ may contribute to the development of cancer.

In patients with bone metastatic breast cancer vitamin D may have a significant effect [40]. The role of vitamin D in breast cancer progression has been studied by measuring serum 25(OH)D$_3$ in women with early cancer and in those with advanced cancer. Women with early-stage breast cancer shows significantly higher serum 25(OH)D$_3$ and significantly lower PTH levels
than those with advanced breast cancer [41]. These results indicate a direct inhibition of parathyroid function by 25(OH)D₃, while there was no difference in serum calcium between the two groups due to unaltered coupling of extracellular calcium levels of PTH secretion by the calcium-sensing receptor [42].

Garland et al. [43] reviewed all observational studies that reported breast cancer risk and serum 25(OH)D₃ grouped by quintiles. The quintile values for 25(OH)D₃ were 6, 18, 29, 37, and 48, while the pooled odds ratio for breast cancer for lowest to highest quintile were 1.0, 0.90, 0.70, 0.70, and 0.50. It seems that women with 25(OH)D₃ levels of at least 52 ng/ml may halve the risk as compared to women with levels below 13 ng/ml. The authors concluded that maintaining continuous high 25(OH)D₃ levels, either by moderate sun exposure or by high vitamin D intake, may be a protective measure against breast cancer. Only about 20% of breast-cancer cases occur in women younger than 50, but the disease is often more aggressive. Though postmenopausal women can take medication to reduce their risk of developing breast cancer, nothing is available for premenopausal women. Although the evidence is not strong enough to advise all women to get more calcium and vitamin D, experts say it might help reduce the risk of breast cancer, and it is not likely to have harmful secondary effects.

The relationship between 25(OH)D₃ and 1,25(OH)₂D₃ in blood collected in 1989-1990 and breast cancer risk was studied in a case-control group nested in the Nurses’ Health Study cohort [44]. The mean serum 25(OH)D₃ level was significantly lower in women with breast cancer than in the control group, while there was no difference in mean 1,25(OH)₂D₃ between the two groups. Women in the highest quintile of 25(OH)D₃ had a relative risk of 0.73 compared with those in the lowest quintile. A United Kingdom caucasian population study concerning serum 25(OH)D₃ levels and vitamin D receptor genotype in breast cancer patients and control women, demonstrated that subjects with 25(OH)D₃ levels <50 nM and the bb BsmI VDR genotype are 6.82 times more likely to have breast cancer than subjects with levels of 25(OH)D₃>50 nM and either the BB or Bb genotype [45]. Therefore, low circulating 25(OH)D₃ levels, both alone and in combination with BsmI VDR genotype, may increase breast cancer risk.

A population-based case-control study evaluated the relationship between sources of vitamin D and breast cancer risk in women from the Ontario Cancer Registry [46]. The risk was reduced by increasing sun exposure from ages 10 to 19. The risk was also reduced with the use of cod liver and milk consumption (more than 10 glasses per week versus none). The association was weaker from ages 20 to 29, and there was no evidence for ages 45 to 54. It seems that vitamin D may have a protective effect on breast during its development. The results suggest that exposure earlier in life, particularly during breast development, may be most relevant. The study does have some limitations, notably being based on recall of dietary habits early in life as well as outdoor exposure, both of which are susceptible to recall bias from the participants. However, it supports the hypothesis that vitamin D could help to prevent breast cancer.

Lin et al. [47] have prospectively evaluated more than 30,000 women aged 45 years and older (two-thirds were postmenopausal) who were healthy at baseline in the Women's Health Study, and were followed by an average of 10 years. High intakes of calcium and vitamin D
through food sources and nutritional supplements, cut the risk of breast cancer by nearly one-third in premenopausal women. The link appeared strongest for the most aggressive tumors, and it was not seen after menopause. While most cases of breast cancer develop after menopause, premenopausal breast cancer is often more aggressive and deadly. It is noteworthy that those who had high intake of vitamin D and calcium also appeared healthier overall: they were leaner, more physically active, consumed less fat, drank less alcohol, were more likely to undergo regular mammography screening and were less likely to smoke.

Postmenopausal women aged 55 years and older who lived in rural Nebraska (latitude 41 degrees North) who were free of cancer for 10 years before entering the study, were randomly assigned to one of three groups and followed for four years. One group took 1,400 to 1,500 mg a day of supplementary calcium, another group took that same amount of calcium plus 1,100 IU of vitamin D$_3$ daily, while the third took placebo pills every day [48]. The primary objective was to determine the fracture incidence, and the most important secondary objective to determine the incidence of cancer in those populations. The unadjusted relative risks of incident cancer in the Ca + D and Ca-only groups were 0.402 and 0.532, respectively. After four years, those in the combination vitamin D and calcium group had a 60% lower risk of developing cancer, compared to the placebo group, and the calcium-only group had a 47% reduced risk. When data from the first year of the study were eliminated, figuring some women may have entered the study with cancer that had not yet been diagnosed, the results become clearer for the combination calcium and vitamin D with a relative risk = 0.232, while there was not a significant effect of calcium alone when compared to placebo. Although the results are quite interesting, the study has some limitations: it was designed to study the influence of calcium and vitamin D$_3$ on bone health, and the number of patients with cancer was relatively low. Therefore, the results should be confirmed by a more appropriate study, with other populations, including women of all ages, and different ethnic groups. However, the study allows to recommend food that contain large quantities of vitamin D (salmon, tuna, sardines, milk, mushrooms, etc), and vitamin D supplements to overcome its scanty skin formation from sun exposure, specially at present time when it is widely recommended to avoid natural and artificial UV rays. The vitamin D treatment used in the study by Lappe et al [48] is nearly three times the US government's recommended daily amount for middle-age adults [49].

3.2. X-ray breast density and vitamin D

Controversial results have been reported concerning the acquisition of vitamin D and calcium versus x-ray breast density. Mammographic breast density, which reflects breast epithelial and/or stromal proliferation, is one of the strongest breast cancer risk indicators [36,47,50], and seems to decrease as vitamin D and calcium acquisition increased [47,51-53]. This association between cancer risk and breast density seems to be limited to premenopausal women. Indeed, in premenopausal women, total daily intakes of vitamin D and calcium -determined by a food
frequency questionnaire- were inversely related to breast density as assessed using a computer-assisted method, while there was not any relation in postmenopausal women between food supplements and breast density [53].

Knight et al [54] studied the possible association between serum 25(OH)D3 and mammographic density, adjusting results according to dietary calcium intake, body mass index, menopause, season and other covariates. Although there was no evidence for an association between 25(OH)D3 and either percent density or total dense area, both percentage of density and area of high density were lowest among those in the highest vitamin D quartile with calcium intake above the median. Although there was no evidence for any association between 25(OH)D3 and mammographic findings, the percentage of density and dense area of high density were lowest among the women within the highest 25(OH)D3 quartile. This study does not preclude the possible effect of vitamin D at breast cell level. Furthermore, the effects of vitamin D on breast density may be related with other biochemical factors like insulin-like growth factor (IGF)-I and IGF-binding protein-3 (IGFBP-3) that act on human breast cancer cells. Diorio et al [55] studied the links between breast density, vitamin D and calcium intakes, and levels of plasma IGF-I and IGFBP-3 in premenopausal women. The negative association of vitamin D or calcium intakes with breast density was stronger among women with IGF-I levels above the median compared with those with IGF-I below or equal to the median. Similar results were observed within levels of IGFBP-3. Therefore, there are complex and sustained relations between breast metabolism, vitamin D, and the radiologic expression of breast density.

The circannual rhythm of 25(OH)D3 has been related with the effect of UV light on the skin [3]. In a cross-sectional study of Canadian premenopausal women recruited at screening mammography, the highest mean 25(OH)D3 levels were measured at the end of July, and the lowest in mid-April. Breast density also showed a minor and significant seasonal variation, with the lowest mean breast density in early December [56]. It seems that breast density and 25(OH)D3 has a negative correlation with a lag time of around 4 months; however, it remains to be determined whether vitamin D acquisition may prevent the increase in breast density.

It seems that the relation between breast density, vitamin D metabolites, and other biochemical markers is quite complex and need further studies.

### 3.3. Metabolism of vitamin D in normal and malignant breast tissue

The active hormone 1,25(OH)2D3 acts as an autocrine regulator of cell turnover in nontumorigenic human mammary epithelial cells (HMEC). In vitro studies demonstrate that HMEC cultures are dose dependently growth inhibited by different concentrations of 25(OH)D3. Similar results were observed in two independently derived immortalized HMEC lines as well as in primary cultures derived from human breast epithelium [57]. Normal breast cells may synthesize 1,25(OH)2D3 using the circulating precursor and its own enzymatic system of CYP27B1 (figure 1), and the active hormone inhibits cell growth. Local synthesis of 1,25(OH)2D3 may contribute to
maintain normal mammary cell function and could be impaired with low 25(OH)D$_3$ circulating levels. In fact, normal human mammary epithelial cells are dose dependently growth inhibited by physiological concentrations of 25(OH)D$_3$; and this effect is preceeded by the conversion to 1,25(OH)$_2$D$_3$, the ligand for the VDR. In basal conditions CYP27B1 activity predominates over CYP24 activity, resulting in net conversion of 25(OH)D$_3$ into 1,25(OH)$_2$D$_3$. The local transformation has also been reported in homogenates of human breast tumors and adjacent normal breast tissue [57,58]. Furthermore, in immortalized normal breast epithelial cells both 25(OH)D$_3$ and 1,25(OH)$_2$D$_3$ activate VDR, as measured by induction of a vitamin D responsive gene and upregulation of CYP24A1 [58].

In women with 25(OH)D$_3$ of less than 50 nM the odds ratio for breast cancer compared with those with values higher than 50 nM was 3.54. Therefore, impaired local generation of 1,25(OH)$_2$D$_3$ may contribute to the development of breast cancer [59]. 1,25(OH)$_2$D$_3$ interacts with the VDR to regulate proliferation, differentiation, and survival of breast cancer cells. In addition, epidemiologic, clinical, and animal studies suggested that vitamin D status is important for protection against the development of breast cancer. Both VDR and vitamin D 1$\alpha$-hydroxylase are expressed and dynamically regulated in the normal mammary gland. In animal models, vitamin D exerts a negative control of the normal mammary gland, and the disruption of VDR signaling is associated with abnormal ductal morphologic features, increased incidence of preneoplastic lesions, and accelerated mammary tumor development. It seems quite plausive that suboptimal generation of 1,25(OH)$_2$D$_3$ in the mammary gland might sufficiently deregulate VDR-mediated gene expression to sensitize mammary cells to transformation [60]. In breast cancer cells, the VDR signaling may be attenuated due to alterations in the receptor expression mechanisms [61]. Therefore, 1,25(OH)$_2$D$_3$ resistance seems to be a new molecular alteration in breast cancer cells. Furthermore, some changes mediated by VDR may be relevant to metastatic progression of breast cancer [61].

4. Endometrial cancer

Endometrial cancer is the most common type of gynecologic cancer in the Western hemisphere. In the United States, approximately 37,000 new cases are diagnosed and about 6000 women die from the disease each year. Incidence of uterine cancer increases after menopause and approximately 75% of cases are diagnosed in postmenopausal patients. Adenocarcinoma accounts for approximately 90% to 95% of cases of endometrial cancer. It is more common during perimenopause and is usually associated with an early onset of symptoms [62]. Human cycling endometrium may be included among the extrarenal sites that synthesize vitamin D. The induction of 1 $\alpha$-hydroxylase gene and the hormonal modulation of osteopontin support a role for the hormone in the immunological mechanisms underlying uterine function. The
capacity for $\alpha$-hydroxylation and the presence of VDR in endometrial cells have also been demonstrated. The active form of the $\alpha$-hydroxylase gene was expressed in human endometrial stromal cells independent of the cycle phase but with a significant increase in early pregnant decidua. Both cycling and early pregnant endometrial cells also expressed the VDR [63].

Mohr et al [64] reported the relationship between low levels of UV B exposure and incidence of endometrial cancer incidence by country, age, and controlling by known confounders (overweight, skin pigmentation, cigarette consumption, health expenditure, and fertility) in 107 countries. The authors found an association between low UV B dose and high caloric intake from animal source per capita health expenditure, overweight, and incidence rate. Several dietary factors have been also related with endometrial cancer risk. In a case-control study, it was found that calcium and vitamin D were inversely associated with endometrial cancer [65]. In a previous dietary analysis, the only relation detected in food intake was for processed meat and fish, with a 50% excess risk of endometrial cancer [66]. The human endometrium express of $\alpha$-hydroxylase in normal and malignant tissue, and may contribute to the antiproliferative effect of $1,25(\text{OH})_2\text{D}_3$ [67]. This finding opens a new application for vitamin D related therapies in endometrial cancer.

5. Ovarian cancer

Ovarian cancer is the leading cause of death among gynecologic malignancies, which results in more deaths than all other gynecologic cancers combined. In contrast to declining death rates for cervical and uterus cancers, the annual report of ovarian cancer mortality has risen by 250% since 1930 [68]. Ovarian cancer is a disease that principally affects middle and upper-class women in industrialized nations. It is uncommon in developing countries, perhaps because of different dietary factors in these regions.

Evans et al [69] have demonstrated that the $\alpha$-hidroxilase expression is increased in ovarian dysgerminoma, as compared to the normal ovary tissue. This enzymatic activity is five-fold higher in ovarian dysgerminoma than in normal ovary tissue, and the increased $\alpha$-hydroxilase expression is present in both the cells from the dysgerminoma and in the macrophages. These findings are different to the changes induced by tumours that produce the PTH-related peptide. The hypercalcemia associated with cancerous disease is a disorder that courses with increased PTH-related peptide. In the ovarian dysgerminoma the situation is different, and there is hypercalcemia associated to high $1,25(\text{OH})_2\text{D}_3$ blood levels.

There are many factors involved in ovarian carcinogenesis, including genes, diet, hormones, lifestyle habits, reproductive choices, etc. The study by Garland et al [70] shows a link between vitamin D deficiency and increased incidence of ovarian cancer, suggesting that adequate levels of vitamin D may reduce the incidence of this aggressive cancer. The paper was based on worldwide data developed by the World Health Organization’s International Agency for
Research on Cancer that includes cancer incidence, mortality and prevalence for 175 countries; the authors correlated the results with information on latitude and atmospheric levels of ozone. The findings show only an association between latitude and ovarian cancer, but not a protective role for vitamin D against this disease. The association of milk nutrients and ovarian cancer risk has been also studied in a population of almost 32,000 women adjusted by age, parity and other factors [71]. High intake of total dairy food was associated with a significant decrease in ovarian cancer risk. A non significant inverse association between high dietary calcium intake and ovarian cancer was demonstrated, while there was no association for consumption of specific dairy foods, lactose, or vitamin D and ovarian cancer. Tworeger et al. [72] used data from three prospective cohorts to study plasma concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ the Nurses’ Health Study (NHS), NHSII, and the Women's Health Study. They did not found significant associations between 25(OH)D₃ or 1,25(OH)₂D₃ distributed by quartiles and ovarian cancer risk, although there was a significant inverse association among overweight and obese women for 25(OH)D₃ levels. In addition, those with adequate (≥32 ng/mL) versus inadequate 25(OH)D₃ levels had a modest decrease in the risk for serous ovarian cancer.

The ovary, from birds and mammals, is a target organ for 1,25(OH)₂D₃ throughout a specific VDR, and 1,25(OH)₂D₃ inhibits cell growth in a dose-dependent manner [73]. Ovarian cancer in nude mice responds in vivo to treatment with a 1,25(OH)₂D₃ compound; the tumor suppression is associated with an increase in apoptotic rate and a reduction in cell proliferation [74]. VDR has been also demonstrated in human ovarian cancers, and Scatchard plots showed that labelled 1,25(OH)₂D₃ was bound to a single class of high affinity sites characteristic of authentic VDR. Immunohistochemical studies have analyzed the expression of VDR in normal and carcinomatous ovarian tissues. Using a semiquantitative evaluation, 83% of the normal surface ovarian epithelium had weak to moderate VDR immunoreactivity, and cancers had moderate to strong nuclear immunoreactivity for almost all cases. The intensity and number of VDR-positive cells were increased in carcinomas as compared to normal ovarian tissue, and the expression of VDR in ovarian carcinomas is independently influenced from the expression of estrogen receptors and progesterone receptors [75]. Androgens and 1,25(OH)₂D₃ control the growth and differentiation in reproductive tissues. The presence of VDRs in ovarian cancer cases has been reported in 43% of patients, whilst androgen receptors are present in 64% of ovarian cancers [76].

All these investigations support the search of new vitamin D analogues for use as therapy for human ovarian cancer.

6. Cervix and vulva cancer

Cancer of the cervix is the second most common cancer in women worldwide and is a leading cause of cancer-related deaths in women in developing countries. Worldwide,
approximately 500,000 cases are diagnosed each year. According to well-established knowledge, its development is related with high-risk human papilloma virus (HPV) infection. Invasive cervical cancer is more common among middle-aged women and older and among women of lower socioeconomic status, who are less likely to receive regular screening and early treatment. HPV is found in nearly 80% of cases and it may reduce the immune system’s ability to fight infection, increasing the likelihood of precancerous cells to progress into cancerous cells. VDR immunoreactivity is increased in cervix carcinoma as compared to healthy cervical tissue, indicating that it may be considered as a potential target for prevention or therapy with new vitamin D analogs that exert little or no calcemic effects or by pharmacological modulation of 1,25(OH)2D3 synthesis and metabolism in cervical tumor cells [77,78]. Systemic treatment of mice with 1,25(OH)2D3 significantly decreased tumor cell-induced angiogenesis; while in vitro 1,25(OH)2D3 inhibits angiogenesis induced by cell lines harboring DNA of HPV types 16 or 18 [79].

Vitamin D, acquired primarily through skin exposure to the sun, seems to inhibit tumor development and growth at the vulva [80].

7. Sunlight and vitamin D supplements in cancer prevention

There is a strong relationship between moderate exposure to sunlight and reduction of many prevalent female malignant tumors. On the other hand, sun exposure is a significant risk for skin aging, cancer and melanoma. Therefore, it is difficult to combine the sunlight benefits from mild exposure to prevent tumour development, and the alarming data about the cumulative risk of UV radiation. Sun exposure is the natural way to obtain the benefits from a dose of vitamin D able to maintain the endocrine, paracrine and autocrine equilibrium; 10 to 15 minutes of daily sun exposure during spring and summer may be enough to maintain a normal metabolism of the vitamin D secosteroids [2]. Obviously, there is not any reason to sunbathe or spend a lot of time on the beach and other outdoor activities without appropriate sunscreen protection. Adequate information should find an intermediate point between careless/dangerous behaviour and the utopia of absolute protection from natural sunlight. Extreme positions have always been bad recommendations. Along the evolution, the human beings have been always exposed to sunlight, although the present atmospheric pollution and ozone conditions as well as the longevity have created a new scenario: the number of women with age-related diseases and cancer are continuously growing.

Health consequences associated with low vitamin D levels should warn an adequate intake year round. People at risk include those with darker skin, older people who do not spend enough time outdoors and wear clothing covering most of the skin, individuals living in a geographical area with fewer hours of sunlight, pregnant women, breast feeding and newborns
who are fed only on mother’s milk, among others. Vitamin D refers to two biologically inactive precursors - D$_3$, also known as cholecalciferol, and D$_2$, also known as ergocalciferol. The former, produced in the skin on exposure to UV B radiation, is said to be more bioactive [21]. The latter is derived from plants and some animal products and adequate intake is assured thorough a diet rich in foods such as mushrooms, oily fish, egg yolk and liver. Both D$_3$ and D$_2$ precursors are hydroxylated in the liver and kidneys to form the biologically active form that is tightly controlled by the body. A selection of food may contribute to receive vitamin D with diet, although the amount needed may alter its equilibrium and other essential contents.

Vitamin D supplements may be a solution to maintain adequate levels. The US Institute of Medicine, which issues recommendations on vitamin and mineral requirements, considers 200 IU of vitamin D as adequate for children and adults up to age 50; 400 IU for adults 51 to 70, and 600 IU for those 71 and older [49]. The levels are not Recommended Dietary Allowances, because the Institute does not consider there is enough evidence to establish an RDA for vitamin D. According the scientific evidence published during the last 10 years these amounts should be considered insufficient. Furthermore, fifteen experts from universities, research institutes, and university hospitals around the world recently called for international agencies to "reassess as a matter of high priority" dietary recommendations for vitamin D because current advice is outdated and puts the public at risk of deficiency [4-6,81].

Very recently, the Canadian Cancer Society released its recommendation to consider taking a vitamin D supplement of 1,000 IU daily during fall and winter [82]. Adults at risk of having lower vitamin D levels should consider maintaining the recommended intake level year round. This includes people who are older, have darker skin, do not spend time outdoors often and wear clothing covering most of their skin. The daily supplements should not be more than 2,000 IU to prevent side effects and because there is not evidence that a higher dosis will produce a better protection. For the first time, there will be a confirmation –or not- of the evidence discussed in this article that vitamin D used in young women may reduce the most prevalent cancers. The Canadian recommendation allows to get adequate vitamin D levels without risking UV radiation due to sunlight exposure. The results of the initiated widespread use of vitamin D will provide a lot of information concerning the value of maintaining adequate vitamin D levels and the prevalence of cancer.

From a broader health perspective, a more proactive attitude to identify, prevent and treat vitamin D deficiency should be part of the standard medical care. For several types of cancer (e.g., breast and vulva), the relative risk of mortality is higher if there is hypovitaminosis D, suggesting that maintenance of adequate vitamin D levels is more important in limiting tumour progression than in preventing tumour initiation [80]. Studies have now suggested that sunlight and adequate vitamin D levels may be beneficial in some cancers. However, further research is needed to elucidate the future precise place of vitamin D and related compounds in both cancer prevention and treatment.
References

[18] Grant WB, Garland CF, Gorham ED. An estimate of cancer mortality rate reductions in Europe and the US with 1,000 IU of oral vitamin D per day. Recent Results Cancer Res 2007;174:225-34.


Figure 1. The synthesis and action of vitamin D. Vitamin D$_2$ is derived from fungal and plant sources, and is not produced by the human body. Vitamin D$_3$ is derived from animal sources and is made in the skin when 7-dehydrocholesterol reacts with UV B light at wavelengths between 270–290 nm. The majority of human vitamin D requirements are met through the UV dependent conversion of 7-dehydrocholesterol to vitamin D$_3$ in the skin, and its transfer to the blood where it is attached to a vitamin D binding protein. In the liver, it is then converted to 25(OH)D$_3$, which is the major circulating form of vitamin D, and is the best indicator of its sufficiency. The major active form of vitamin D is formed in the kidney where it is converted to 1,25(OH)$_2$D$_3$. Due to its effects on calcium homeostasis, the formation of 1,25(OH)$_2$D$_3$ is tightly controlled by the body. Liganded vitamin D receptor (VDR) undergoes conformational change and forms a heterodimer with a second protein, the retinoid X receptor (RXR). This, in turn, binds to DNA elements in the promoter regions of target genes described as vitamin D response elements (VDRE). The synthesis of 1,25(OH)$_2$D$_3$ from the circulating precursor 25(OH)D$_3$, and the regulation of VDRs are also expressed as paracrine and autocrine regulatory systems at different tissues, with their own set-points and metabolism.
Diet

Vitamin D<sub>2</sub>
Vitamin D<sub>3</sub>

Vitamin D<sub>3</sub>

7-dehydrocholesterol

CYP27A1

25(OH)D<sub>3</sub>

CYP24A1

24,25(OH)<sub>2</sub>D<sub>3</sub>

CYP27B1

Vitamin D-Binding Protein

1,25(OH)<sub>2</sub>D<sub>3</sub>

CYP24A1

1,24,25(OH)<sub>3</sub>D<sub>3</sub>

VDRE

R<sub>XR</sub> V<sub>D</sub>R

Vitamin D responsive genes