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The Emerging Role of Vitamin D in Cancer Risk Reduction

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The Emerging Role of Vitamin D in Cancer Risk Reduction

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List of abbreviations

<u>Abbreviation</u>	<u>Description</u>
25(OH)D	Calcifediol, the main form in which vitamin D is stored in the body. It is a product of enzymatic hydroxylation of vitamin D in the liver. Also known as 25-hydroxyvitamin D or calcidiol. If the source of the basic molecule is known, it may be identified with a subscript at the end (see vitamin D ₂ and vitamin D ₃ , below).
1,25(OH) ₂ D	Calcitriol, the most potent form of vitamin D in terms of calcitropic effects. This compound is also known as 1,25-vitamin D, 1alpha-25-vitamin D or 1alpha-25(OH) ₂ D. If the source of the basic molecule is known, it may be identified with a subscript.
Apc (min)	Adenomatous polyposis coli (min) mice
DAG	Diacylglycerol
EB 1089	22,24-diene-24a,26a,27a-trihomo-1,25(OH) ₂ D, a vitamin D analog
ER	Endoplasmic reticulum
IGF-I	Insulin-like growth factor I
IP3	Inositol 1,4,5-triphosphate
NHANES I	National Health and Nutrition Examination Survey Epidemiologic follow-up study, 1971-1975 to 1992.
PKC	Protein kinase C
RO 23-7553	1,25-dihydroxy-16-ene-23-yne-cholecalciferol.
RO 24-5531	1 alpha,25-Dihydroxy-16-ene-23-yne-26,27-hexafluoro-cholecalciferol
RO 25-6760	1-alpha,25-dihydroxy-16-ene-23yne-26,27-hexafluoro-19-nor-cholecalciferol
RO 27-0574	A 20-cyclopropyl analog of 1,25(OH) ₂ vitamin D ₃
TGF-a	Transforming growth factor alpha
VDR	Vitamin D receptor
Vitamin D ₂	Ergocalciferol – Vitamin D derived from plant sources (yeast).
Vitamin D ₃	Cholecalciferol – Vitamin D synthesized in the skin when ultraviolet B energy is absorbed.

Abstract

Recent advances confirming a role of vitamin D in reduction of the risk of cancer have created a new field of clinical interest. This is a review of the laboratory, clinical, and epidemiological evidence supporting the role of vitamin D in reduction of the incidence of breast, colon and prostate cancer, and the biological mechanisms of vitamin D anticarcinogenesis. It describes the prevalence of vitamin D deficiency in the United States and provides guidelines for clinical testing and interventions.

Introduction

Recent advances confirming a role of vitamin D in prevention of cancer have created a new field of clinical interest (1-15). While there is persistent interest in vitamin D for its role in reducing risk of fractures (15-20), its newly defined nonskeletal effects, including a contribution to reducing the risk of several forms of cancer, recently are becoming more widely known (21-24).

Mechanisms of Vitamin D Anticarcinogenesis

A wide variety of tissues not directly associated with calcium metabolism have vitamin D nuclear receptors (VDRs). These include tissues of the breast (25), colon (26-28), skin (melanocytes) (29), pituitary (30, 31) and CD4/CD8 lymphocytes (32, 33). The first insight into the function of $1,25(\text{OH})_2\text{D}_3$ (calcitriol) in tissues not directly related to calcium metabolism was the observation that when leukemic cells having a VDR were incubated with $1,25(\text{OH})_2\text{D}_3$, their proliferation was inhibited and they matured into granulocytes (34). It was soon learned that $1,25(\text{OH})_2\text{D}_3$ had functions in regulation of cell growth and promotion of cell maturation in experimental models of cancer (13, 35-40), and in treatment of the hyperproliferative skin disorder psoriasis (41).

Epidemiological Evidence: Latitude, Vitamin D, and Cancer

In the same time period, several epidemiologic studies demonstrated higher mortality rates of cancer of the colon (42-44), breast (43, 44), prostate (43-45), and ovary (43, 44, 46) in the northeastern sector of the United States, compared with considerably lower rates in the southern tier of states. Dietary differences could not account for this regional difference (42). While other differences may play a role (47, 48), it has been established that the northern tier of states receives less sunlight annually than the southern tier, mainly due to the longer duration and severity of winter in the north, and to far lower levels of ultraviolet B (UVB) radiation during fall through spring in the northern United States (49-52). There is a sharp threshold for biosynthesis of vitamin D in human skin (53, 54) that occurs at an intensity of UVB radiation that is reached in the southern states of the United States, but not the northern states, Canada, and Europe, during November through March (54). High age-adjusted mortality rates of female breast cancer also have been reported in areas of low winter UVB in the Northeast, where acid haze, a form of air pollution that diminishes atmospheric transmission of UVB, is a factor (87).

Similarly high rates have been reported in areas of low winter UVB in the former USSR (55). An association of low regional sunlight level with risk of colon and breast cancer also has been reported in migrant studies (56-58). The inverse association of colon, breast, prostate, and other cancers with sunlight was recently confirmed at the population level in the United States (44).

Once it was recognized that normal colon, breast, prostate and other epithelial cells have VDRs and that UVB exposure increases circulating 25(OH)D (calcifediol) levels in a dose-response manner (59), an explanation accounting for the unusual geographic distribution emerged. Increased exposure to sunlight increases the amount of circulating 25(OH)D₃ substrate, which supports increased synthesis in tissues of 1,25(OH)₂D₃ that regulates cell proliferation (35, 36, 60-62). However, increasing exposure to UVB usually does not result in marked elevation in circulating levels of 1,25(OH)₂D₃, since the synthesis of 1,25(OH)₂D₃ is tightly regulated in the kidney by serum levels of calcium, phosphorus, and parathyroid hormone (59). An important exception is individuals whose vitamin D synthesis and intake is very low, including young adults during winter months in temperate climates (63, 64). Vitamin D deficiency also is especially prevalent in older adults (65), who tend to have very low levels of 25(OH)D and 1,25(OH)₂D during the winter months in New England, the Mid-Atlantic states, and Europe (66). Sunscreen usage, which is self-reported by about half the population in the United States (67), also contributes to vitamin D deficiency; use of a sunscreen with a protection factor of 8 reduces vitamin D synthesis by 97.5% (68).

It soon was recognized that tissues other than the kidney had the enzymatic ability to synthesize 1,25(OH)₂D from the circulating substrate, 25(OH)D. It is now known that normal and malignant colon (69), prostate cells (70), keratinocytes (71, 72), lymph nodes (granulocytes) (72), pancreatic islet cells (72), cerebral cortex and cerebellar tissue cells (72), and testicular Leydig cells (72) express the mitochondrial 25(OH)D-1α-OHase enzyme that can convert 25(OH)D₃ to 1,25(OH)₂D₃ (72). This was an enormously important advance that helps to explain the influence of vitamin D from sunlight and other sources on risk of cancer. The human gene for the 1α-OHase enzyme has been cloned and localized to chromosome 12 (73). Mutations of this gene, known as the CYP 1 α gene, are the cause of vitamin D-dependent rickets, type 1 (73, 74). The 1α-OHase is not regulated by parathyroid hormone in tissues other than the kidney, and the 1α-OHase gene promoter region is regulated differently in the kidney than other tissues (75). This potent antiproliferative hormone, in turn, regulates cell growth by maintaining cells in the G₀/G₁ phase of the cell cycle, for example, in

prostate cancer cell lines (76). It also maintains normal cellular differentiation in several other tissues (77, 78), and indirectly provides a pathway for apoptosis, or programmed cellular death (78, 79).

Exposure to UVB radiation from sunlight (80, 81) or oral vitamin D intake (81, 82) increases the circulating level of 25(OH)D, which, in turn, provides a higher concentration of substrate available for the intracellular synthesis of 1,25(OH)₂D in a wide range of tissues. This is an important finding that accounts for observations that high vitamin D status and residence at sunny latitudes are associated with lower mortality rates from cancer of the colon (42, 43, 83-85), breast (5, 6, 43, 86, 87), ovary (43, 46), and prostate (43, 45, 88-90).

Mechanisms of Vitamin D and Calcium in Reduction of Risk of Colon Cancer

A key mechanism of the antineoplastic effect of vitamin D on colon cancer was elucidated by Davies and associates (91). Advances in fluorescent intracellular dye technology (92, 93) revealed that certain normally occurring pulses in the intracellular calcium concentration are needed to induce differentiation of intestinal epithelial cells, and to induce apoptosis in these cells (91). Normal human colonocytes have nuclear receptors for 1,25(OH)₂D (26) and produce calcium-binding proteins known as calbindins, which are used for temporary storage and release of calcium ions in the cytoplasm (97)(Fig. 1). Calbindin 28kD, the most common form, is a 261-amino protein coded by chromosome 8 (8q21.3-q22.1) in response to various stimuli, including vitamin D metabolites. Each calbindin molecule reversibly binds calcium ions. Calbindin molecules are storage depots for calcium in the cytoplasm that can rapidly release calcium to the cytoplasm if signaled to do so.

Colonocytes also contain calcium channels or similar structures, including a vitamin D-dependent channel-like intestinal and colonic transport protein (CaT1), which has been cloned (94). It is homologous to the kidney epithelial apical calcium channel (EcaC) (95). There is also loose binding of calcium ions to the endoplasmic reticulum (96).

Colonocytes are organized into invaginations of the mucosa known as crypts (Fig. 2) (91, 98, 99). Epithelial cells arise from the base of each crypt and migrate to the mouth of the crypt, where they undergo programmed death (91). When the colonocyte reaches the mouth of the crypt in the normal colon, the intracellular calcium pulses to a level that is lethal to the cell. The gradual increase in intracellular calcium as the colonocyte rises through and matures in the crypt is known as the intracellular calcium gradient (Fig. 3)(100). Such a gradient also has been

reported in the epidermis, where the intracellular calcium concentration is low at the dermal-epidermal junction but rises in more distal layers, reaching a peak near the proximal layer of the stratum corneum, immediately preceding apoptosis of keratinocytes into dead squames in the stratum corneum (101, 102).

When a colonocyte reaches the mouth of the crypt in the vitamin D-replete individual, calcium-mediated apoptosis occurs and the cell dies (91). Deficiency of vitamin D results in loss of the intracellular calcium gradient in the crypt architecture (Fig. 4). This loss interferes with calcium-mediated terminal differentiation of colonocytes and apoptosis of colonocytes at the mouths of the crypts (91). This loss allows aged and mutated intestinal epithelial cells to persist and accumulate for abnormally long periods of time (91, 103, 104).

Highly proliferative colonocyte populations have been documented in individuals with a family history of colon cancer (105-107). Intracellular calcium pulses of sufficient amplitude to induce immediate cell death occur only in the vitamin D-replete state (91). Pulsing of calcium is dependent on the presence of calbindin, which reversibly binds calcium and releases it in a pulsatile fashion when needed for terminal differentiation and apoptosis (91, 108). Since vitamin D is required for synthesis of calbindin, its deficiency results in destruction of the pulsatile release of intracellular ionized calcium (91), preventing apoptosis.

Lamprecht and Lipkin (109) recently critically discussed mechanisms in calcium and vitamin D inhibition of colorectal carcinogenesis involving genomic and non-genomic pathways (109). A vitamin D metabolite-activated nongenomic receptor located in the plasma membrane induces a rapid Ca^{2+} response through a G-protein-coupled Ca^{2+} membrane channel (109-111). This response sets in motion a cascade of intracellular events, including Ca^{2+} activation of phosphoinositide-specific phospholipase C, resulting in the formation of two intracellular messengers, inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 opens ER-bound channels, thus releasing stored Ca^{2+} while DAG activates protein kinase C (PKC) in the plasma membrane. Activation of specific PKC isoenzymes induces differentiation in intestinal cells (109). This cascade of biochemical events is also observed following challenge of cells of different lineages with extracellular calcium. Considerable evidence is available showing that extracellular calcium acts via a calcium-sensing receptor, a typical G-protein receptor present in the gastrointestinal tract (109).

Genomic actions of vitamin D metabolites via the nuclear VDR are well established (109). The modes of action of the secosteroids acting at various levels of cellular organization and function

in restraining growth and inducing differentiation in intestinal and colonic cells have been reviewed recently (109).

Further evidence for a predominant role of vitamin D in inhibiting development of colon cancer is that VDR expression remains sustained in low-grade adenomas, but declines to lower levels in poorly differentiated tumors exhibiting a malignant phenotype (112, 113). Consistent with these and related laboratory findings, the rate of colonic epithelial cell proliferation in humans is low when the serum 25(OH) D level is high, and high when serum 25(OH)D is low, with a dose-response gradient (98).

Vitamin D Levels and Colon Cancer: Epidemiologic Studies

It has been known for some time that colon cancer death rates tend to be low in sunny areas and high in areas of with low winter sunlight levels, particularly in the industrialized Northeast (Fig. 5). A nested case-control study designed to determine whether vitamin D might explain this geographic gradient was performed of 25,620 volunteers who provided blood samples (84). The probable association of low serum 25(OH)D with risk of colon cancer incidence was confirmed. Individuals with circulating 25(OH)D levels less than 20 ng/ml had twice the incidence rate of colon cancer during the 8-year study follow-up period as those with lower levels (84). Most of the association of serum 25(OH)D with risk of colon cancer occurred during the first decade of follow-up, suggesting that the main effect of vitamin D is during the promotional phase (114). Several epidemiological studies have reported higher risk of colon cancer in individuals who consume low amounts of vitamin D, including the Western Electric Cohort (115), the Harvard Nurses' Health Cohort (116), the Harvard Male Health Professionals Cohort (117), the Iowa Women's Health Cohort (118), and studies of residents of Stockholm (119) and Wisconsin (120). One additional study reported higher risk of colon cancer (121) and another cohort study found a higher incidence of rectal cancer in association with low intake of vitamin D, but those studies did not achieve criteria for statistical significance (122). The cohort study of rectal cancer found that individuals in the lowest third of the population for intake of vitamin D and calcium had approximately twice the incidence rate of rectal cancer as those in the highest third, indicating a synergistic effect of vitamin D and calcium intake (122). A scattering of case-control studies performed in southern Europe or other sunny climates did not find an effect of dietary vitamin D on risk, possibly because of uniformly high serum 25(OH)D levels in residents of those areas due to high solar UVB exposure (123-125).

Women whose plasma 1,25(OH)₂D concentration was slightly below average also had a 60% increased risk of distal colorectal adenomas (126). The relationship was stronger for large or villous adenomas, and among women with consistent vitamin D intake over the 10 years prior to collection of the blood sample. Compared with those in the lowest quartile of plasma 25(OH)D, women in the second quartile (odds ratio [OR], 0.64; 95% confidence interval [CI], 0.41-1.00) and third quartile (OR, 0.58; 95% CI, 0.36-0.95) were at lower risk (126). There was no reduction in risk associated with the top quartile.

This inverse association of serum vitamin D metabolites with risk of colon cancer could be related to the ability of 1,25(OH)₂D₃ to block the stimulatory effect of epidermal growth factor on colon epithelial cells (127). It might also account for the approximately 40% lower risk of colon cancer in women taking estrogen, compared with placebo, that recently was observed in the Women's Health Initiative Clinical Trial (130). Exogenous estrogen increases the vitamin D receptor concentration in the bowel (128). It also upregulates serum 1,25(OH)₂D in women with abnormally low levels (129). The favorable effect of estrogen usage on colon cancer risk was even greater than the 34% reduction in incidence of hip fractures that occurred in the women taking estrogen (130). Consistent with this finding, Framingham women with high bone mass (in the top third of the cohort) had significantly reduced incidence rates of colon cancer (multiple-adjusted relative risk (RR) = 0.4) (131). Intake of milk (which contains 100 IU of vitamin D per 250-ml glass) and dairy products was associated with lower than average risk of colorectal cancer in women in the New York University Women's Health Study cohort (132), with a similar favorable association for calcium. Findings nearly identical to these were reported in two case-control studies (133, 134).

Adults who took a daily multivitamin supplement containing 400 IU of vitamin D (135) had approximately half the risk of colon cancer as those who did not, according to a large case-control study based on Surveillance, Epidemiology and End Results program cases and population controls (135). High dietary calcium intake was associated with reduced risk of adenomatous polyps (136), an effect similar to that of high serum 25(OH)D and 1,25(OH)₂D (126). Several epidemiological studies have reported lower risk of colon cancer in those with high levels of physical activity (137-140), which possibly could be explained by the increase in serum 1,25(OH)₂D concentration that results from exercise and physical activity (141, 142).

Mechanism of Vitamin D in Breast Cancer

Terminal ductal lobular units, the units of epithelial organization of the breast, have features that are analogous to intestinal crypts, such as a monolayer or bilayer of epithelial cells that originate at or near the base of the terminal duct and move distally toward the mouth of the duct (22), and the presence of vitamin D receptors (25, 143). Breast epithelial cells terminally differentiate (144, 145) in response to the high levels of circulating $1,25(\text{OH})_2\text{D}$ that are characteristic of late pregnancy (146-148) and early lactation (149).

When vitamin D intake or synthesis is high, even in the absence of pregnancy, breast epithelial cells terminally differentiate and undergo apoptosis at the mouth of the ductal units (144, 145), preventing crowding of epithelial cells at the mouths of ducts. When intake of vitamin D is low, apoptosis is considerably reduced (79, 145) and crowding occurs. The average number of epithelial cells in the terminal ducts is much greater in animals on a diet deficient in vitamin D than on a diet that is replete in vitamin D (22, 144, 145). Animals consuming low amounts of vitamin D experience reduplication of terminal ducts, with approximately twice the number of ducts as those consuming the normal intake (22, 144, 145). This mechanism, which involves the $1,25(\text{OH})_2\text{D}$ receptor, also occurs in malignant breast cancer cell lines (150).

Occurrence of mammary tumors was reduced by four fifths in animals receiving adequate vitamin D compared with those receiving the minimal dose necessary for normal growth (2, 22, 144). Vitamin D also had a larger independent effect on risk of mammary carcinogenesis than high intake of fat or deficient intake of calcium (2, 22, 144). The effect of vitamin D on mammary carcinogenesis was particularly strong early in life, suggesting that low circulating levels of $25(\text{OH})\text{D}$ in adolescence may be important contributors to later breast cancer risk (144). The roles of vitamin D, calcium, and fats may be linked (2, 22, 144). High levels of $1,25(\text{OH})_2\text{D}$ are also associated with slower progression of metastatic breast cancer (151).

Epidemiological Studies of Vitamin D and Reduction in Risk of Breast Cancer

A recent study using prediagnostic serum from cancer cases and healthy controls reported that white women in the lowest quartile of serum $1,25(\text{OH})_2\text{D}$ had five times the risk of breast cancer as those in the highest quartile (OR, 5.2, 95% CI, 2.1-12.8) (152). A prospective study of the NHANESI cohort reported lower incidence rates of breast cancer in women regularly

exposed to sunlight in the sunniest regions of the United States, and in those who consumed above-average amounts of vitamin D (6).

Role of Vitamin D in Ovarian Cancer

1,25(OH)₂D inhibits the growth of ovarian cancer cells in tissue culture (153). It counteracts the growth-stimulatory effect of dihydrotestosterone (154) and downregulates the c-myc proto-oncogene (155). These findings are supported by epidemiological data indicating a lower mortality rate of the disease in sunny areas of the United States in perimenopausal women (46). 1,25(OH)₂D inhibits growth of ovarian cancer in the OVCAR-3 cell line, where it opposes the growth-stimulatory effect of androgens (154). The effect of vitamin D metabolites occurs early in the process of ovarian carcinogenesis, so administration of 1,25(OH)₂D to women with the disease does not reduce the rate of progression (156).

Mechanisms and Clinical Effects of Vitamin D in the Prostate

Vitamin D is involved in normal differentiation of the prostate (21), vitamin D receptors are expressed in the prostatic epithelium (39, 157, 158) and malignant prostatic cells (159-161), and prostatic cells are able to convert 25(OH)₂D to 1,25(OH)₂D (70). Inhibition of proliferation of prostate cancer cells in tissue culture occurs with exposure to either 1,25(OH)₂D (70) or 25(OH)D (39, 70). 1,25 (OH)₂D also inhibits the invasiveness of human prostate cancer cells (21, 162) and cell adhesion and migration to the basement membrane matrix protein, laminin (162). Liarozole, an inhibitor of 1,25-(OH)₂D₃-24-hydroxylase makes 1,25(OH)₂D persist longer and enhances its antiproliferative effect, even in highly resistant cell lines (163). Transfection of the gene for the 1α-OHase enzyme into prostate cancer cells that have lost it reduces their growth rate (164).

In a cohort study of 19,000 men followed for 13 years, men whose 25(OH)D level was below the median had 1.7 times the incidence of prostate cancer as those above the median (89). Among younger men (under age 57 years) those with low 25(OH)D levels had 3.5 times the incidence of prostate cancer and 6.3 times the incidence of cancer that had spread beyond the prostatic capsule (89).

In a nested case-control study of stored serum based on 250,000 samples collected from members of the Kaiser Foundation Health Plan, white and black men who had lower than average levels of 1,25(OH)₂D had higher risk of prostate cancer (165). Men in the lowest

quarter of circulating $1,25(\text{OH})_2\text{D}$ had 20% higher incidence of prostate cancer than those in the highest quarter (165, 166). The influence of $1,25(\text{OH})_2\text{D}$ was greatest in men with low serum $25(\text{OH})\text{D}$ (166). A smaller nested case-control study found no association (167). Another small nested case-control study of Hawaiian Japanese men reported 20% higher risk of prostate cancer in men in the lowest quartile of serum $25(\text{OH})\text{D}$, although the trend did not achieve statistical significance, probably due to limited sample size.

Age-adjusted, race-specific prostate cancer mortality rates are generally higher in northern counties of the United States and lowest in sunnier southern counties (43, 44, 168), consistent with these laboratory and clinical findings. Men with a history of exposure to high levels of solar ultraviolet radiation are at lower than average risk of prostate cancer (169, 170). In one study (89), prostate cancer risk was highest among the group of younger men (ages 40-51 years) with low serum $25(\text{OH})\text{D}$. However low serum $25(\text{OH})\text{D}$ appeared not to be associated with an increase in risk of prostate cancer in older men, suggesting that vitamin D has a protective role against prostate cancer mainly before the andropause, when serum androgen concentrations are higher (90).

Synthetic Vitamin D Analogues

Many analogues of vitamin D have been developed as candidates for clinical use. Most of these mimic the anticarcinogenic effects of vitamin D without appreciably affecting calcium metabolism. Clinical uses of noncalcemic vitamin D analogues include pro-differentiating effects that are potentially useful against some forms of cancer, such as RO 24-5531, a fluorinated analog of $1,25(\text{OH})_2\text{D}$ (171). There was a 70% reduction in incidence of colon tumors in animals given RO 24-5531 compared with controls (171). None of the tumors in the treated group were malignant, while 40% in the control group were malignant. The malignant tumors in the control group were adenocarcinomas, while the treated group experienced only benign adenomas (171). This indicates that vitamin D analogues are able to prevent progression from adenomas to malignancies in the colon.

Another vitamin D analog, RO 25-6760 (16-ene-23yne-26, 27-hexafluoro-19-nor- $1,25(\text{OH})\text{D}_3$), has been shown to have an antimitogenic action five times greater than that of $1,25(\text{OH})_2\text{D}$ (172). The effects of $1,25$ -dihydroxyvitamin D_3 and RO 25-6760 have been evaluated in vitro and in vivo in human colorectal cancer with high (HT-29) and low (SW-620) levels of vitamin D receptor. RO 25-6760 and $1,25(\text{OH})_2\text{D}$ caused growth inhibition of both

cell lines, with a dose gradient (172). Another analog, 24R,25-dihydroxyvitamin D₃ inhibits rat colon cancer development during the post initiation period (173). Both 1,25(OH)₂D and a synthetic analog reduce intestinal tumor load in the Apc (min) mouse (28).

Treatment of breast cancer (MCF-7) cells with 1,25(OH)₂D₃ reduces estrogen receptor levels in a dose-dependent manner (174). Vitamin D analogues EB1089, KH-1060, RO 27-0574, and RO 23-7553 are more potent than 1,25(OH)₂D₃ in antiproliferative actions and estrogen receptor down-regulation (174). The vitamin D analog EB1089 (22,24-diene-24a, 26a, 27a-trihomo-1,25(OH)₂D₃ increases the rate of apoptosis sixfold in breast cancer cell cultures treated with adriamycin (11), suggesting a role in chemotherapy for vitamin D analogues. EB1089 also unexpectedly enhances the response of human breast cancer cells to therapeutic radiation (175) and produces regression of prostatic cells (176). The effect on breast cancer cells is mainly due to enhancement of induction of apoptosis of the tumor cells (177). The analog RO 25-6760 produced a dose-dependent growth inhibition of breast cancer cell lines (MCF-7 and MDAMB-468) (178) and counteracted growth stimulation of breast cancer cells by estradiol, TGF- α and IGF-I, and blocked progesterone receptor induction by estradiol (178). EB1089 also has been shown to be effective in controlling growth of breast cancer cell lines that have become resistant to tamoxifen (179). This is an active area of investigation, and new noncalcemic analogues of 1,25(OH)₂D are currently in development as potential adjunctive agents in cancer treatment. Vitamin D receptor levels usually are upregulated in human breast cancer cells (180), suggesting that breast cancer may be amenable to adjunctive approaches with vitamin D analogs or metabolites.

Prevalence of Vitamin D Deficiency

Vitamin D deficiency is common in the winter in the United States (181) and Europe (64). The vitamin is available from only a few sources (Fig. 6). Urban lifestyle has reduced average exposure of adults to midday sunlight to a low level except during summer months (64, 182, 183). Vitamin D that is photosynthesized in summer does not persist through the winter (66, 182-186). Vitamin D is metabolized within 3-5 days to 25(OH)D, the storage form (182), and the half-life of 25(OH)D is between 12 days (184) and 27 days (185).

A study of patients admitted to general hospitals found that 57% were seriously vitamin D-deficient (serum concentration of 25(OH)D $\leq$ 15 ng/ml), of whom 22% were considered severely vitamin D-deficient (serum concentration of 25(OH)D <8 ng/ml) (187); similar

deficiency states were reported in New England women (188, 189) and in black women (82, 190-193). Vitamin D deficiency is extremely prevalent in obese women, whose 25(OH)D level tends to be markedly below normal (191, 194) and whose serum 25(OH)D increases only slightly after UVB irradiation or oral intake of vitamin D (194). Serum 25(OH)D levels are, of course, also markedly low in women with hip fractures (195). Serum 25(OH)D is not well restored seasonally in black women (196); in one recent study, 42% of black women ages 15-49 years were found to have seriously deficient 25(OH)D levels (below 15 ng/ml) (193). Serum 25(OH)D levels in the range of 20-32 ng/ml are minimally required for parathyroid hormone levels to plateau at normal values (64, 197).

The vitamin D deficiency state is defined by measurement of the serum 25(OH)D level (181, 195, 198). Relatively inexpensive, reliable clinical tests for 25(OH)D in serum are readily available through virtually all major clinical laboratories. The previously recognized normal range for prevention of osteomalacia due to vitamin D deficiency has been 15-55 ng/ml. More recent research suggests that the threshold for prevention of osteomalacia is higher, reaching to 20 ng/ml (199) or 30 ng/ml (200, 201). Serum 25(OH)D levels below 30 ng/ml also predispose to colorectal (84, 85) and breast cancer (5), and may be associated with increased incidence of prostate cancer (90). 25(OH)D levels above 150 ng/ml, by contrast, are indicative of potential toxicity (202-204).

Toxicity

Vitamin D is stored in fat and other tissues and therefore can have toxic effects when administered in large doses on a persistent basis (205). The National Academy of Sciences—Institute of Medicine, has suggested 2,000 IU per day as the safe upper limit of vitamin D intake for children over one year old and adults (206). This recommendation is considered conservative (207), but recommended daily oral intakes are usually well below this level. The recommended daily intake is 200 IU (5 µg) at ages 1-50 years, 400 IU (10 µg) at ages 51-70 years, and 600 IU (15 µg) at age 71 years and older (206, 208). Vitamin D₃ raises serum 25(OH)D more efficiently than vitamin D₂ (209). Potential toxic effects such as bone demineralization, hypercalcemia, hypercalciuria or nephrocalcinosis with renal failure are encountered rarely, and, generally, only when the daily dose exceeds 10,000 IU on a chronic basis (210). Concerns about vitamin D toxicity in the past have been due to effects of massive overdoses in the range of 50,000-150,000 IU per day on a relatively long-term basis (59, 203). No major health risks are currently known to be associated with dosages of vitamin D in the

normal range of intake (up to 2,000 IU per day) (204,206, 207, 211, 212). Serum 25(OH)D in the normal range is not associated with any adverse risk of cardiovascular disease (213, 214). On the contrary, above-average serum levels of 25(OH)D and vitamin D intake are associated with reduced blood pressure (215-217) and heart rate (217), which are both favorably associated with risk of heart disease (215-217). Above-average 25(OH)D levels are also associated with lower risk of myocardial infarction (192, 213, 214). Daily intake of 600-800 IU of vitamin D₃ will maintain serum 25(OH)D in the range for its anticarcinogenic effect, without known risk of toxicity (64).

Genetic Factors

Most actions of vitamin D metabolites are mediated by the VDR. Mice genetically lacking the classical nuclear VDR have poor skeletal mineralization and a markedly higher rate of colonic proliferation than wild-type mice (218). Gene loci related to the receptor in humans have several common polymorphisms (219). Those most closely associated with cancer risk are discussed here. The Bsm I locus is most related to risk of cancer (220-222). It has three polymorphisms: BB, Bb, and bb. The BB genotype is associated with higher circulating 1,25(OH)₂D levels (220).

A cohort study of physicians found that doctors with the BB genotype had half the risk of colon cancer (OR, 0.5, 95% CI, 0.3-0.9) (220) as those with the bb genotype. The polyA (short), and TaqI (tt) variants of the VDR gene also have been found to be associated with reduced risk of colon cancer, while the Fok I VDR variant is not associated with risk (221).

A study of women found that those with the BB genotype had less than half the risk of breast cancer (222) as those with the bb genotype. As with colon cancer, the Fok I genotype was not associated with risk of breast cancer (222, 223). Polymorphisms of the VDR ApaI VDR locus also are associated with risk of breast cancer; women with the AA genotype have three times the risk of breast cancer as those with the Aa genotype (224). Allele frequencies of the Fok I polymorphism were not significantly different in breast cancer cases than controls (223).

Physicians with the BB genotype had lower risk of prostate cancer than those with the bb genotype (220). The effect was strongest in physicians whose serum 25(OH)D level was below the median (OR, 0.43) and in older physicians (OR, 0.18). The gene frequency of the BB polymorphism is estimated to be 8-10% (224, 225). In another study, having the Bb or bb Bsm I

polymorphism was associated with having three times the risk of prostate cancer (226). A race-adjusted analysis in an earlier study found that men who were homozygous for the t allele of the Taq I VDR gene (codon 352) (for which homozygosity was previously known to associated with higher than average serum levels of 1,25(OH)₂D (227)) had one third the risk of developing prostate cancer requiring prostatectomy than men who were heterozygous or homozygous for the T allele (228). A European study found that men with no T alleles had half the risk of prostate cancer compared with those with one or more T alleles (229). Since the T polymorphism is common, approximately 50% of cases of prostate cancer in men older than 66 years in one population studied in Europe could be attributed to the effect of this polymorphism (229). In another study, the genotype TT at the Taq I locus was significantly associated (OR, 5.4) with having a high Gleason grade of tumor (grade 5) (230). While other studies have been inconclusive regarding the association of specific VDR polymorphisms with prostate cancer (231, 232), individuals with non-BB genotypes at the Bsm I site or who have one or more T alleles at the Taq I VDR site may require more vitamin D intake for reduction of risk of prostate cancer than those with other genotypes.

Recommendations for Vitamin D Intake

Vitamin D deficiency is highly prevalent in North America (181, 186, 193). This deficiency is due to inadequate dietary vitamin D intake and limited solar UVB exposure, especially during winter months (15, 182). Daily intakes of vitamin D of no less than 200-400 IU at ages 1-49 years, 400 IU at ages 50-70 years, and 600 IU at ages 71 years and older constitute the minimum dietary intakes necessary to counteract these deficiencies with regard to osteomalacia, assuming normal absorption of vitamin D. These minimal intakes would also be desirable for reducing the incidence of colon, breast, prostate, ovarian, and possibly other cancers.

Exposure to the sun during November through March in the northeastern United States does not allow any synthesis of vitamin D in the skin (53), and serum levels of vitamin D metabolites that are needed to prevent many important diseases typically cannot be maintained throughout winter. This is especially true in older persons, who cannot synthesize vitamin D as well as that of younger persons in response to ultraviolet light (17, 66) and whose vitamin D from diet is less readily absorbed in the intestine (233). It has been estimated the vitamin D

deficiency may account for more than 20,000 premature deaths from cancer annually in the U.S. (43).

When there is any doubt regarding vitamin D status, it should be tested using a simple, reliable, and widely available clinical laboratory test for serum 25(OH)D (183). The test preferably should be performed in the Fall, if possible, to identify the risk of vitamin D deficiency during the winter months. The target range of 30-50 ng/ml of serum 25(OH)D that is needed to prevent osteomalacia and fractures (200, 201) is the same as the target range for reduction of the incidence of colon, breast, prostate, and possibly other cancers throughout life.

References

1. Garland C, Garland F, Gorham E. Can colon cancer incidence and death rates be reduced with calcium and vitamin D? *Am J Clin Nutr* 1991;54 (Suppl.):193S-201S.
2. Newmark HL. Vitamin D adequacy: a possible relationship to breast cancer. *Adv Exp Med Biol* 1994;364:109-14.
3. Lipkin M. Preclinical and early human studies of calcium and colon cancer prevention. *Ann N Y Acad Sci* 1999;889:120-7.
4. Garland CF, Garland FC, Gorham ED. Calcium and vitamin D. Their potential roles in colon and breast cancer prevention. *Ann N Y Acad Sci* 1999;889:107-19.
5. Janowski EL, Weinberg GE, Millikan RC, Schildkraut JM. Garrett A, Hulka BS. Association between low levels of 1,25-dihydroxyvitamin D and breast cancer risk. *Public Health Nutrition*, 1999;2(3):283-91.
6. John EM, Schwartz GG, Dreon DM, Koo J, Vitamin D and breast cancer risk: the NHANES I Epidemiologic follow-up study, 1971-1975 to 1992. *National Health and Nutrition Examination Survey. Cancer Epidemiol Biomarkers Prev* 1999;8:399-406.
7. Campbell MJ, Gombart AF, Kwok SH, Park S, Koeffler HP. The anti-proliferative effects of 1alpha,25(OH)₂D₃ on breast and prostate cancer cells are associated with induction of BRCA1 gene expression. *Oncogene* 2000;19(44):5091-7.
8. El Abdaimi K, Dion N, Papavasiliou V, Cardinal PE, Binderup L, Goltzman D, et al. The vitamin D analogue EB 1089 prevents skeletal metastasis and prolongs survival time in nude mice transplanted with human breast cancer cells. *Cancer Res* 2000;60(16):4412-8.
9. Koli K, Keski-Oja J. 1alpha,25-dihydroxyvitamin D₃ and its analogues down-regulate cell invasion-associated proteases in cultured malignant cells. *Cell Growth Differ* 2000;11(4):221-9.
10. Mantell DJ, Owens PE, Bundred NJ, Mawer EB, Canfield AE. 1 alpha,25-dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo. *Circ Res* 2000;87(3):214-20.
11. Sundaram S, Chaudhry M, Reardon D, Gupta M, Gewirtz DA. The vitamin D₃ analog EB 1089 enhances the antiproliferative and apoptotic effects of adriamycin in MCF-7 breast tumor cells. *Breast Cancer Res Treat* 2000;63(1):1-10.

12. Wang Q, Yang W, Uytingco MS, Christakos S, Wieder R. 1,25-Dihydroxyvitamin D₃ and all-trans-retinoic acid sensitize breast cancer cells to chemotherapy-induced cell death. *Cancer Res* 2000;60(7):2040-8.
13. Chen A, Davis BH, Bissonnette M, Scaglione-Sewell B, Brasitus TA. 1,25-Dihydroxyvitamin D₃ stimulates activator protein-1-dependent caco-2 cell differentiation. *J Biol Chem* 1999;274(50):35505-13.
14. Niv Y, Sperber AD, Figer A, Igaël D, Shany S, Fraser G, et al. In colorectal carcinoma patients, serum vitamin D levels vary according to stage of the carcinoma. *Cancer* 1999;86(3):391-7.
15. Holick MF. Too little vitamin D in premenopausal women: why should we care? *Am J Clin Nutr* 2002;76(1):3-4.
16. Utiger R. The need for more vitamin D. *N Engl J Med* 1998;338(12):828-9.
17. Compston J. Vitamin D deficiency: time for action. Evidence supports routine supplementation for elderly people and others at risk. *BMJ* 1998;317(7171):1466-7.
18. Wharton B. Low plasma vitamin D in Asian toddlers in Britain. *BMJ* 1999;318(7175):2-3.
19. Garabedian, M., and Ben-Mehkbi, H. Rickets and vitamin D deficiency. In: Holick, M., (ed.), *Vitamin D: Molecular Biology, Physiology, and Clinical Applications*, pp.273-286. Totowa, NJ: Humana, 1999.
20. Holick M. Vitamin D and bone health. *J Nutr* 1996;126(4 Suppl.):1159S-64S.
21. Schwartz GG, Wang MH, Zang M, Singh RK, Siegal GP. 1 alpha,25-Dihydroxyvitamin D (calcitriol) inhibits the invasiveness of human prostate cancer cells. *Cancer Epidemiol Biomarkers Prev* 1997;6(9):727-32.
22. Lipkin M, Newmark HL. Vitamin D, calcium and prevention of breast cancer: a review. *J Am Coll Nutr* 1999;18(5 Suppl.):392S-397S.
23. Guyton KZ, Kensler TW, Posner GH. Cancer chemoprevention using natural vitamin D and synthetic analogs. *Annu Rev Pharmacol Toxicol* 2001;41:421-42.
24. Hansen CM, Binderup L, Hamberg KJ, Carlberg C. Vitamin D and cancer: effects of 1,25(OH)₂D₃ and its analogs on growth control and tumorigenesis. *Front Biosci* 2001;6:D820-48.
25. Eisman JA, Martin TJ, MacIntyre I, Moseley JM. 1,25-dihydroxyvitamin-D-receptor in breast cancer cells. *Lancet* 1979;2(8156-8157):1335-6.

26. Weeksler WR, Mason RS, Norman AW. Specific cytosol receptors for 1,25-dihydroxyvitamin D₃ in human intestine. *J Clin Endocrinol Metab* 1979;48(4):715-7.
27. Delvin EE, Lopez V, Levy E, Menard D. Developmental expression of calcitriol receptors, 9-kilodalton calcium-binding protein, and calcidiol 24-hydroxylase in human intestine. *Pediatr Res* 1996;40(5):664-70.
28. Huerta S, Irwin RW, Heber D, Go VL, Koeffler HP, Uskokovic MR, et al. 1 α ,25-(OH)₂-D₃ and its synthetic analogue decrease tumor load in the Apc (min) mouse. *Cancer Res* 2002;62(3):741-6.
29. Abdel-Malek ZA, Ross R, Trinkle L, Swope V, Pike JW, Nordlund JJ. Hormonal effects of vitamin D₃ on epidermal melanocytes. *J Cell Physiol* 1988;136(2):273-80.
30. Stumpf WE, Sar M, Clark SA, DeLuca HF. Brain target sites for 1,25-dihydroxyvitamin D₃. *Science* 1982;215(4538):1403-5.
31. Perez-Fernandez R, Alonso M, Segura C, Munoz I, Garcia-Caballero T, Diguez C. Vitamin D receptor gene expression in human pituitary gland. *Life Sci* 1997;60(1):35-42.
32. Veldman CM, Cantorna MT, DeLuca HF. Expression of 1,25-dihydroxyvitamin D₃ receptor in the immune system. *Arch Biochem Biophys* 2000;374(2):334-8.
33. Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. *FASEB J* 2001;15(14):2579-85.
34. Carling T, Rastad J, Szabo E, Westin G, Akerstrom G. Reduced parathyroid vitamin D receptor messenger ribonucleic acid levels in primary and secondary hyperparathyroidism. *J Clin Endocrinol Metab* 2000;85(5):2000-3.
35. Frampton R, Omond S, Eisman J, Martin T. Inhibition of growth in a cultured breast cancer cell line (T47D) by 1,25-dihydroxycholecalciferol. *Proc Austr Soc Med Res* 1981;14:73.
36. Eisman J, Barkla D, Tutton P. Suppression of in vivo growth of human cancer [colon cancer and melanoma] solid tumor xenografts by 1,25-dihydroxyvitamin D₃. *Cancer Res* 1987;47:21-5.
37. Chen TC, Persons K, Liu WW, Chen ML, Holick MF. The antiproliferative and differentiative activities of 1,25-dihydroxyvitamin D₃ are potentiated by epidermal growth factor and attenuated by insulin in cultured human keratinocytes. *J Invest Dermatol* 1995;104(1):113-7.

38. Kawase T, Ogata S, Orikasa M, Burns DM. 1,25-Dihydroxyvitamin D₃ promotes prostaglandin E₁-induced differentiation of HL-60 cells. *Calcif Tissue Int* 1995;57(5):359-66.
39. Barreto AM, Schwartz GG, Woodruff R, Cramer SD. 25-Hydroxyvitamin D₃, the prohormone of 1,25-dihydroxyvitamin D₃, inhibits the proliferation of primary prostatic epithelial cells. *Cancer Epidemiol Biomarkers Prev* 2000;9(3):265-70.
40. Mokady E, Schwartz B, Shany S, Lamprecht SA. A protective role of dietary vitamin D₃ in rat colon carcinogenesis. *Nutr Cancer* 2000;38(1):65-73.
41. Holick MF. 1,25-Dihydroxyvitamin D₃ and the skin: a unique application for the treatment of psoriasis. *Proc Soc Exp Biol Med* 1989;191(3):246-57.
42. Garland C, Garland F. Do sunlight and vitamin D reduce the likelihood of colon cancer? *Int J Epidemiol* 1980;9:227-31.
43. Grant WB. An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. *Cancer* 2002;94(6):1867-75.
44. Freedman D, Dosemeci M, McGlynn K. Sunlight and mortality from breast, ovarian, colon, prostate, and non-melanoma skin cancer: a composite death certificate based case-control study. *Occup Environ Med.* 2002;59:257-62.
45. Hanchette CL, Schwartz GG. Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation. *Cancer* 1992;70(12):2861-9.
46. Lefkowitz ES, Garland CF. Sunlight, vitamin D, and ovarian cancer mortality rates in US women. *Int J Epidemiol* 1994;23(6):1133-6.
47. Sturgeon SR, Schairer C, Gail M, McAdams M, Brinton LA, Hoover RN. Geographic variation in mortality from breast cancer among white women in the United States. *J Natl Cancer Inst* 1995;87(24):1846-53.
48. Prehn AW, West DW. Evaluating local differences in breast cancer incidence rates: a census-based methodology (United States). *Cancer Causes Control* 1998;9(5):511-7.
49. Frederick J, Lubin D. The budget of biologically active ultraviolet radiation in the earth-atmosphere system. *J Geophys Res* 1988;93:3825-3832.
50. Lubin D, Jensen E, Gies P. Global surface ultraviolet radiation climatology from TOMS and ERBE data. *J Geophys Res* 1998;103(D20):26,061-26,091.
51. Ainsleigh HG. Beneficial effects of sun exposure on cancer mortality. *Prev Med* 1993;22(1):132-40.

52. Garland C, Garland F, Gorham E. Epidemiology of cancer risk and Vitamin D. *In*: Holick, M. (ed.), Vitamin D: Molecular Biology, Physiology, and Clinical Applications, pp. 375-409. New Jersey: Humana, 1999.
53. Webb A, Kline L, Holick M. Influence of season and latitude on the cutaneous synthesis of vitamin D₃: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D₃ synthesis in human skin. *J Clin Endocrinol Metab* 1988;67:373-8.
54. Matsuoka LY, Wortsman J, Haddad JG, Hollis BW. In vivo threshold for cutaneous synthesis of vitamin D₃. *J Lab Clin Med* 1989;114(3):301-5.
55. Gorham ED, Garland FC, Garland CF. Sunlight and breast cancer incidence in the USSR. *Int J Epidemiol* 1990;19(4):820-4.
56. Mallin K, Anderson K. Cancer mortality in Illinois, Mexican, and Puerto Rican immigrants, 1979-1984. *Int J Cancer* 1988;41:670-6.
57. Berrino F, Gatta G. Energy-rich diet and breast cancer risk. *Int J Cancer* 1989;44:186-7.
58. Ziegler R. Epidemiologic patterns of colorectal cancer. *In*: DeVita, VT HS, and Rosenberg, S. A. (ed.), Important Advances in Oncology, pp. 209-232. Philadelphia: Lippincott, 1986.
59. Adams J, Clemens T, Parrish J, Holick M. Vitamin D synthesis and metabolism after ultraviolet irradiation of normal and vitamin D-deficient subjects. *N Engl J Med* 1982;306:722-5.
60. Abe, E.Miyaura C, Sakagami H,Takeda M, Konno K,Yamazaki T, Yoshiki S, Suda T. E, Miyaura C, Sakagimi H, et al. Differentiation of rat *myc* leukemia cells induced by 1,25-dihydroxyvitamin D. *Proceedings of the National Academy of Sciences USA* 1981;78:4990-4.
61. McCarthy D, Hibbin J, San Miguel JF, Freake H, Rodrigues B, Andrews C, et al. The effect of vitamin D₃ metabolites on normal and leukemic bone marrow cells in vitro. *Int J Cell Cloning* 1984;2(4):227-42.
62. Colston K, Berger U, Coombes R. Possible role for vitamin D in controlling breast cancer cell proliferation. *Lancet* 1989:188-91.
63. Juttman J, Visser T, Buurman C. Seasonal fluctuations in serum concentrations of vitamin D metabolites in normal subjects. *Br Med J* 1981;282:1349-52.

64. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. *Am J Med* 2002;112(8):659-62.
65. Meller Y, Kestenbaum RS, Galinsky D, Shany S. Seasonal variation in serum levels of vitamin D metabolites and parathormone in geriatric patients with fractures in Southern Israel. *Isr J Med Sci* 1986;22(1):8-11.
66. Bouillon RA, Auwerx JH, Lissens WD, Pelemans WK. Vitamin D status in the elderly: seasonal substrate deficiency causes 1,25-dihydroxycholecalciferol deficiency. *Am J Clin Nutr* 1987;45(4):755-63.
67. Johnson EY, Lookingbill DP. Sunscreen use and sun exposure. Trends in a white population. *Arch Dermatol* 1984;120(6):727-31.
68. Matsuoka L, Wortsman J, Holick M. Chronic sunscreen use decreases the concentration of 25-hydroxyvitamin D: a preliminary study. *Arch Dermatol* 1988;124:1802-4.
69. Tangpricha V, Flanagan JN, Whitlatch LW, Tseng CC, Chen TC, Holt PR, et al. 25-hydroxyvitamin D-1alpha-hydroxylase in normal and malignant colon tissue. *Lancet* 2001;357(9269):1673-4.
70. Schwartz GG, Whitlatch LW, Chen TC, Lokeshwar BL, Holick MF. Human prostate cells synthesize 1,25-dihydroxyvitamin D₃ from 25-hydroxyvitamin D₃. *Cancer Epidemiol Biomarkers Prev* 1998;7(5):391-5.
71. Lehmann B, Pietzsch J, Kampf A, Meurer M. Human keratinocyte line HaCaT metabolizes 1alpha-hydroxyvitamin D₃ and vitamin D₃ to 1alpha,25-dihydroxyvitamin D₃ (calcitriol). *J Dermatol Sci* 1998;18(2):118-27.
72. Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, et al. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. *J Clin Endocrinol Metab* 2001;86(2):888-94.
73. Fu GK, Lin D, Zhang MY, Bikle DD, Shackleton CH, Miller WL, et al. Cloning of human 25-hydroxyvitamin D-1 alpha-hydroxylase and mutations causing vitamin D-dependent rickets type 1. *Mol Endocrinol* 1997;11(13):1961-70.
74. Hewison M, Zehnder D, Bland R, Stewart PM. 1alpha-Hydroxylase and the action of vitamin D. *J Mol Endocrinol* 2000;25(2):141-8.
75. Flanagan JN, Whitlatch LW, Chen TC, Zhu XH, Holick MT, Kong XF, et al. Enhancing 1 alpha-hydroxylase activity with the 25-hydroxyvitamin D-1 alpha-hydroxylase gene in cultured human keratinocytes and mouse skin. *J Invest Dermatol* 2001;116(6):910-4.

76. Blutt SE, McDonnell TJ, Polek TC, Weigel NL. Calcitriol-induced apoptosis in LNCaP cells is blocked by overexpression of Bcl-2 [see comments]. *Endocrinology* 2000;141(1):10-7.
77. James SY, Williams MA, Newland AC, Colston KW. Leukemia cell differentiation: cellular and molecular interactions of retinoids and vitamin D. *Gen Pharmacol* 1999;32(1):143-54.
78. Johansen C, Iversen L, Ryborg A, Kragballe K. 1alpha,25-dihydroxyvitamin D3 induced differentiation of cultured human keratinocytes is accompanied by a PKC-independent regulation of AP-1 DNA binding activity. *J Invest Dermatol* 2000;114(6):1174-9.
79. Welsh J, VanWeelden K, Flanagan L, Byrne I, Nolan E, Narvaez CJ. The role of vitamin D3 and antiestrogens in modulating apoptosis of breast cancer cells and tumors. *Subcell Biochem* 1998;30:245-70.
80. Holick M. Photosynthesis of vitamin D in the skin: effect of environment and life-style variables. *Fed Proc* 1987;46:1876-82.
81. Webb A, Pilbeam C, Hanofin N, Holick M. An evaluation of the relative contributions of exposure to sunlight and of diet to the circulating concentrations of 25-hydroxyvitamin D in an elderly nursing home population in Boston. *Am J Clin Nutr* 1990;51:1075-81.
82. Kyriakidou-Himonas M, Aloia JF, Yeh JK. Vitamin D supplementation in postmenopausal black women. *J Clin Endocrinol Metab* 1999;84(11):3988-90.
83. Garland C, Shekelle R, Barrett-Connor E, Criqui MH, Rossof AH, Paul O. . Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* 1985;1:307-9.
84. Garland C, Comstock G, Garland F, Helsing K, Shaw E, Gorham E. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet* 1989; 2:1176-8.
85. Tangrea J, Helzlsouer K, Pietinen P, Taylor P, Hollis B, Virtamo J, et al. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. *Cancer Causes Control* 1997;8(4):615-25.
86. Garland F, Garland C, Gorham E, Young J, Jr. Geographic variation in breast cancer mortality in the United States: a hypothesis involving exposure to solar radiation. *Prev Med* 1990;19:614-22.

87. Gorham E, Garland C, Garland F. Acid haze air pollution and breast and colon cancer in 20 Canadian cities. *Can J Public Health* 1989;80:96-100.
88. Schwartz G, Hulka B. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). *Anticancer Res* 1990; 10(5A):1307-11.
89. Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control* 2000;11(9):847-52.
90. Tuohimaa P, Lyakhovich A, Aksenov N, Pennanen P, Syvala H, Lou YR, et al. Vitamin D and prostate cancer. *J Steroid Biochem Mol Biol* 2001;76(1-5):125-34.
91. Brenner B, Russell N, Albrecht S, Davies R. The effect of dietary vitamin D₃ on the intracellular calcium gradient in mammalian colonic crypts. *Cancer Letters* 1998;12(7):43-53.
92. Tsien R, Pozzan T. Measurement of cytosolic free Ca²⁺ with quin2. *Methods Enzymol* 1989;172:230-62.
93. Bischof G, Brenman J, Bredt DS, Machen TE. Possible regulation of capacitative Ca²⁺ entry into colonic epithelial cells by NO and cGMP. *Cell Calcium* 1995;17(4):250-62.
94. Peng JB, Chen XZ, Berger UV, Vassilev PM, Tsukaguchi H, Brown EM, et al. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. *J Biol Chem* 1999;274(32):22739-46.
95. Muller D, Hoenderop JG, Meij IC, van den Heuvel LP, Knoers NV, den Hollander AI, et al. Molecular cloning, tissue distribution, and chromosomal mapping of the human epithelial Ca²⁺ channel (ECAC1). *Genomics* 2000;67(1):48-53.
96. Sitrin MD, Bissonnette M, Bolt MJ, Wali R, Khare S, Scaglione-Sewell B, et al. Rapid effects of 1,25(OH)₂ vitamin D₃ on signal transduction systems in colonic cells. *Steroids* 1999;64(1-2):137-42.
97. Parmentier M, De Vijlder JJ, Muir, E, Szpirer C, Islam MQ, Geurts van Kessel A. The human calbindin 27-kDa gene: structural organization of the 5' and 3' regions, chromosomal assignment, and restriction fragment length polymorphism. *Genomics* 1989;4:309-19.
98. Holt P, Arber N, Halmos B, Forde K, Kissileff H, Moss S, et al. Colonic epithelial cell proliferation decreases with increasing levels of serum 25-hydroxy vitamin D. *Cancer Epidemiology Biomarkers and Prevention* 2002;11:113-119.

99. Xue L, Lipkin M, Newmark H, Wang J. Influence of dietary calcium and vitamin D on diet-induced epithelial cell hyperproliferation in mice. *J Natl Cancer Inst* 1999;91(2):176-81.
100. Lipkin M, Newmark H. Effect of added dietary calcium on colonic epithelial cell proliferation in subjects at high risk for familial colon cancer. *N Engl J Med* 1985;313:1381-4.
101. Kruszewski FH, Hennings H, Yuspa SH, Tucker RW. Regulation of intracellular free calcium in normal murine keratinocytes. *Am J Physiol* 1991;261(5 Pt 1):C767-73.
102. Kruszewski FH, Hennings H, Tucker RW, Yuspa SH. Differences in the regulation of intracellular calcium in normal and neoplastic keratinocytes are not caused by ras gene mutations. *Cancer Res* 1991;51(16):4206-12.
103. Shih IM, Wang TL, Traverso G, Romans K, Hamilton SR, Ben-Sasson S, et al. Top-down morphogenesis of colorectal tumors. *Proc Natl Acad Sci U S A* 2001;98(5):2640-5.
104. Lamprecht SA, Lipkin M. Migrating colonic crypt epithelial cells: primary targets for transformation. *Carcinogenesis* 2002;23(11):1777-80.
105. Buset M, Lipkin M, Winawer S, Swaroop, S, Friedman, E. Inhibition of human colonic epithelial cell proliferation in vivo and in vitro by calcium. *Cancer Res* 1986;46:5426-30.
106. Lipkin M. Biomarkers of increased susceptibility to gastrointestinal cancer: new application to studies of cancer prevention in human subjects. *Cancer Res* 1988;48:235.
107. Lipkin M, Friedman E, Winawer S, Newmark H. Colonic epithelial cell proliferation in responders and nonresponders to supplemental dietary calcium. *Cancer Res* 1989;49:248-54.
108. Welsh J. Induction of apoptosis in breast cancer cells in response to vitamin D and antiestrogens. *Biochem Cell Bio* 1994;72:537-45.
109. Lamprecht SA, Lipkin M. Cellular mechanisms of calcium and vitamin D in the inhibition of colorectal carcinogenesis. *Ann N Y Acad Sci* 2001;952:73-87.
110. Kim K, Brasitus T. The role of vitamin D in normal and pathological processes in the colon. *Curr Opin Gastroenterol* 2001;17:72-77.

111. Revelli A, Massobrio M, Tesarik J. Non-genomic effects of 1alpha-25-hydroxyvitamin D. *Trends Endocrinol Metabolism* 1998;9:419-27.
112. Sheinin Y, Kaserer K, Wrba F, Wenzl E, Kriwanek S, Peterlik M, et al. *In situ* mRNA hybridization analysis and immunolocalization of the vitamin D receptor in normal and carcinomatous human colonic mucosa: relation to epidermal growth factor receptor expression. *Virchows Arch* 2000;437(5):501-7.
113. Bareis P, Kallay E, Bischof MG, Bises G, Hofer H, Potzi C, et al. Clonal differences in expression of 25-hydroxyvitamin D(3)-1alpha- hydroxylase, of 25-hydroxyvitamin D(3)-24-hydroxylase, and of the vitamin D receptor in human colon carcinoma cells: effects of epidermal growth factor and 1alpha,25-dihydroxyvitamin D(3). *Exp Cell Res* 2002;276(2):320-7.
114. Braun M, Helzlsouer K, Hollis B, Comstock G. Colon cancer and serum vitamin D metabolite levels 10-17 years prior to diagnosis. *Am J Epidemiol* 1995;142:608-11.
115. Garland C, Shekelle RB, Barrett-Connor E, Criqui MH, Rossof AH, Paul O. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* 1985;1(8424):307-9.
116. Martinez ME, Giovannucci EL, Colditz GA, Stampfer MJ, Hunter DJ, Speizer FE, et al. Calcium, vitamin D, and the occurrence of colorectal cancer among women. *J Natl Cancer Inst* 1996;88(19):1375-82.
117. Kearney J, Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, et al. Calcium, vitamin D, and dairy foods and the occurrence of colon cancer in men. *Am J Epidemiol* 1996;143(9):907-17.
118. Bostick RM, Potter JD, Sellers TA, McKenzie DR, Kushi LH, Folsom AR. Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. *Am J Epidemiol* 1993;137(12):1302-17.
119. Pritchard RS, Baron JA, Gerhardsson de Verdier M. Dietary calcium, vitamin D, and the risk of colorectal cancer in Stockholm, Sweden. *Cancer Epidemiol Biomarkers Prev* 1996;5(11):897-900.
120. Marcus PM, Newcomb PA. The association of calcium and vitamin D, and colon and rectal cancer in Wisconsin women. *Int J Epidemiol* 1998;27(5):788-93.

121. Kampman E, Slattery ML, Caan B, Potter JD. Calcium, vitamin D, sunshine exposure, dairy products and colon cancer risk (United States). *Cancer Causes Control* 2000;11(5):459-66.
122. Zheng W, Anderson KE, Kushi LH, Sellers TA, Greenstein J, Hong CP, et al. A prospective cohort study of intake of calcium, vitamin D, and other micronutrients in relation to incidence of rectal cancer among postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 1998;7(3):221-5.
123. Benito E, Obrador A, Stiggelbout A, et al. A population-based case-control study of colorectal cancer in Majorca. I. Dietary factors. *Int J Cancer* 1990;45:69-76.
124. Peters RK, Pike MC, Garabrant D, Mack TM. Diet and colon cancer in Los Angeles County, California. *Cancer Causes Control* 1992;3(5):457-73.
125. Ferraroni M, La Vecchia C, D'Avanzo B, Negri E, Franceschi S, Decarli A. Selected micronutrient intake and the risk of colorectal cancer. *Br J Cancer* 1994;70(6):1150-5.
126. Platz EA, Hankinson SE, Hollis BW, Colditz GA, Hunter DJ, Speizer FE, et al. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and adenomatous polyps of the distal colorectum. *Cancer Epidemiol Biomarkers Prev* 2000;9(10):1059-65.
127. Tong W, Kallay E, Hofer H, Hulla W, Manhardt T, Peterlik M, et al. Growth regulation of human colon cancer cells by epidermal growth factor and 1,25-dihydroxyvitamin D3 is mediated by mutual modulation of receptor expression. *Eur J Cancer* 1998;34:2119-25.
128. Chen C, Noland KA, Kalu DN. Modulation of intestinal vitamin D receptor by ovariectomy, estrogen and growth hormone. *Mech Aging Dev* 1997;99(2):109-22.
129. Aloia JF, Vaswani A, Yeh JK, Russo L. Differential effects of dietary calcium augmentation and hormone replacement therapy on bone turnover and serum levels of calcitrophic hormones. *Osteoporos Int* 1996;6(1):55-62.
130. Women's Health Initiative Writing Group. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA* 2002;288(3):321-33.
131. Zhang Y, Felson DT, Ellison RC, Kreger BE, Schatzkin A, Dorgan JF, et al. Bone mass and the risk of colon cancer among postmenopausal women: the Framingham study. *Am J Epidemiol* 2001;153(1):31-7.

132. Kato I, Akhmedkhanov A, Koenig K, Toniolo P, Shore R, Riboli E. Prospective study of diet and female colorectal cancer: the New York University Women's Health Study. *Nutr Cancer* 1997;28:276-81.
133. De Stefani E, Mendilaharsu M, Deneo-Pellegrini H, Ronco A. Influence of dietary levels of fat, cholesterol, and calcium on colorectal cancer. *Nutr Cancer* 1997;29(1):83-9.
134. Zaridze D, Filipchenko V, Kustov V, Serdyuk V, Duffy S. Diet and colorectal cancer: results of two case-control studies in Russia. *Eur J Cancer* 1992;1:112-5.
135. White E, Shannon JS, Patterson RE. Relationship between vitamin and calcium supplement use and colon cancer. *Cancer Epidemiol Biomarkers Prev* 1997;6(10):769-74.
136. Martinez ME, McPherson RS, Annegers JF, Levin B. Association of diet and colorectal adenomatous polyps: dietary fiber, calcium, and total fat. *Epidemiology* 1996;7(3):264-8.
137. White E, Jacobs EJ, Daling JR. Physical activity in relation to colon cancer in middle-aged men and women. *Am J Epidemiol* 1996;144(1):42-50.
138. Fredriksson M, Bengtsson NO, Hardell L, Axelson O. Colon cancer, physical activity, and occupational exposures. A case-control study. *Cancer* 1989;63(9):1838-42.
139. Slattery ML, Schumacher MC, Smith KR, West DW, Abd-Elghany N. Physical activity, diet, and risk of colon cancer in Utah. *Am J Epidemiol* 1988;128(5):989-99.
140. Hardman AE. Physical activity and cancer risk. *Proc Nutr Soc* 2001;60(1):107-13.
141. Garland C, Gorham E, Garland F. Physical activity, diet, and risk of colon cancer in Utah. *Am J Epidemiol* 1990;131:567-9.
142. Yeh JK, Aloia JF. Effect of physical activity on calciotropic hormones and calcium balance in rats. *Am J Physiol* 1990;258(2 Pt 1):E263-8.
143. Buras RR, Schumaker LM, Davoodi F, Brenner RV, Shabahang M, Nauta RJ, et al. Vitamin D receptors in breast cancer cells. *Breast Cancer Res Treat* 1994;31(2-3):191-202.
144. Xue L, Newmark H, Yang K, Lipkin M. Model of mouse mammary gland hyperproliferation and hyperplasia induced by a Western-style diet. *Nutr Cancer* 1996;26(3):281-7.

145. Carroll K, Jacobson L, Eckel L, Newmark H. Dietary calcium, phosphate and vitamin D in relation to mammary carcinogenesis. *In*: Lipkin M, Newmark H, Kelloff G, editors. Calcium, Vitamin D, and Prevention of Colon Cancer. Boca Raton: CRC Press; 1991.
146. Wilson SG, Retallack RW, Kent JC, Worth GK, Gutteridge DH. Serum free 1,25-dihydroxyvitamin D and the free 1,25-dihydroxyvitamin D index during a longitudinal study of human pregnancy and lactation. *Clin Endocrinol (Oxf)* 1990;32(5):613-22.
147. Ardawi MS, Nasrat HA, HS BAA. Calcium-regulating hormones and parathyroid hormone-related peptide in normal human pregnancy and postpartum: a longitudinal study. *Eur J Endocrinol* 1997;137(4):402-9.
148. Seki K, Makimura N, Mitsui C, Hirata J, Nagata I. Calcium-regulating hormones and osteocalcin levels during pregnancy: a longitudinal study. *Am J Obstet Gynecol* 1991;164(5 Pt 1):1248-52.
149. Fry JM, Curnow DH, Gutteridge DH, Retallack RW. Vitamin D in lactation. I. The localization, specific binding and biological effect of 1,25-dihydroxyvitamin D₃ in mammary tissue of lactating rats. *Life Sci* 1980;27(14):1255-63.
150. Wang Q, Lee D, Sysounthone V, Chandraratna RAS, Christakos S, Korah R, et al. 1,25-dihydroxyvitamin D₃ and retinoic acid analogues induce differentiation in breast cancer cells with function- and cell-specific additive effects. *Breast Cancer Res Treat* 2001;67(2):157-68.
151. Mawer EB, Walls J, Howell A, Davies M, Ratcliffe WA, Bundred NJ. Serum 1,25-dihydroxyvitamin D may be related inversely to disease activity in breast cancer patients with bone metastases. *J Clin Endocrinol Metab* 1997;82(1):118-22.
152. Janowsky EC, Lester GE, Weinberg CR, Millikan RC, Schildkraut JM, Garrett PA, et al. Association between low levels of 1,25-dihydroxyvitamin D and breast cancer risk. *Public Health Nutr* 1999;2(3):283-91.
153. Saunders DE, Christensen C, Williams JR, Wappler NL, Lawrence WD, Malone JM, et al. Inhibition of breast and ovarian carcinoma cell growth by 1,25-dihydroxyvitamin D₃ combined with retinoic acid or dexamethasone. *Anticancer Drugs* 1995;6(4):562-9.

154. Ahonen MH, Zhuang YH, Aine R, Ylikomi T, Tuohimaa P. Androgen receptor and vitamin D receptor in human ovarian cancer: growth stimulation and inhibition by ligands. *Int J Cancer* 2000;86(1):40-6.
155. Saunders DE, Christensen C, Wappler NL, Schultz JF, Lawrence WD, Malviya VK, et al. Inhibition of c-myc in breast and ovarian carcinoma cells by 1,25-dihydroxyvitamin D₃, retinoic acid and dexamethasone. *Anticancer Drugs* 1993;4(2):201-8.
156. Rustin GJ, Quinnell TG, Johnson J, Clarke H, Nelstrop AE, Bollag W. Trial of isotretinoin and calcitriol monitored by CA 125 in patients with ovarian cancer. *Br J Cancer* 1996;74(9):1479-81.
157. Hedlund TE, Moffatt KA, Miller GJ. Vitamin D receptor expression is required for growth modulation by 1 alpha,25-dihydroxyvitamin D₃ in the human prostatic carcinoma cell line ALVA-31. *J Steroid Biochem Mol Biol* 1996;58(3):277-88.
158. Hedlund TE, Moffatt KA, Miller GJ. Stable expression of the nuclear vitamin D receptor in the human prostatic carcinoma cell line JCA-1: evidence that the antiproliferative effects of 1 alpha, 25-dihydroxyvitamin D₃ are mediated exclusively through the genomic signaling pathway. *Endocrinology* 1996;137(5):1554-61.
159. Zhao XY, Ly LH, Peehl DM, Feldman D. 1alpha,25-dihydroxyvitamin D₃ actions in LNCaP human prostate cancer cells are androgen-dependent. *Endocrinology* 1997;138(8):3290-8.
160. Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. Antiproliferative effects of 1,25-dihydroxyvitamin D₃ on primary cultures of human prostatic cells. *Cancer Res* 1994;54(3):805-10.
161. Feldman D. Androgen and vitamin D receptor gene polymorphisms: the long and short of prostate cancer risk. *J Natl Cancer Inst* 1997;89(2):109-11.
162. Sung V, Feldman D. 1,25-Dihydroxyvitamin D₃ decreases human prostate cancer cell adhesion and migration. *Mol Cell Endocrinol* 2000;164(1-2):133-43.
163. Ly LH, Zhao XY, Holloway L, Feldman D. Liarozole acts synergistically with 1alpha,25-dihydroxyvitamin D₃ to inhibit growth of DU 145 human prostate cancer cells by blocking 24-hydroxylase activity. *Endocrinology* 1999;140(5):2071-6.
164. Whitlatch LW, Young MV, Schwartz GG, Flanagan JN, Burnstein KL, Lokeshwar BL, et al. 25-Hydroxyvitamin D-1alpha-hydroxylase activity is diminished in human

- prostate cancer cells and is enhanced by gene transfer. *J Steroid Biochem Mol Biol* 2002;81(2):135-40.
165. Corder E, Guess H, Hulka B, Friedman G, Sadler M, Vollmer R, et al. Vitamin D and prostate cancer: a prediagnostic study with stored sera. *Cancer Epidemiol Biomarkers Prev* 1993;2:467-72.
 166. Corder EH, Friedman GD, Vogelmann JH, Orentreich N. Seasonal variation in vitamin D, vitamin D-binding protein, and dehydroepiandrosterone: risk of prostate cancer in black and white men. *Cancer Epidemiol Biomarkers Prev* 1995;4(6):655-9.
 167. Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. Prostate cancer and prediagnostic levels of serum vitamin D metabolites (Maryland, United States). *Cancer Causes Control* 1995;6(3):235-9.
 168. Schwartz GG, Hulka BS. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). *Anticancer Res* 1990;10(5A):1307-11.
 169. Luscombe CJ, Fryer AA, French ME, Liu S, Saxby MF, Jones PW, et al. Exposure to ultraviolet radiation: association with susceptibility and age at presentation with prostate cancer. *Lancet* 2001;358(9282):641-2.
 170. Luscombe CJ, French ME, Liu S, Saxby MF, Jones PW, Fryer AA, et al. Prostate cancer risk: associations with ultraviolet radiation, tyrosinase and melanocortin-1 receptor genotypes. *Br J Cancer* 2001;85(10):1504-9.
 171. Wali RK, Bissonnette M, Khare S, Hart J, Sitrin MD, Brasitus TA. 1 alpha,25-Dihydroxy-16-ene-23-yne-26,27-hexafluorocholecalciferol, a noncalcemic analogue of 1 alpha,25-dihydroxyvitamin D₃, inhibits azoxymethane-induced colonic tumorigenesis. *Cancer Res* 1995;55(14):3050-4.
 172. Evans SR, Schwartz AM, Shchepotin EI, Uskokovic M, Shchepotin IB. Growth inhibitory effects of 1,25-dihydroxyvitamin D₃ and its synthetic analogue, 1alpha,25-dihydroxy-16-ene-23yne-26,27-hexafluoro-19-nor-cholecalciferol (Ro 25-6760), on a human colon cancer xenograft. *Clin Cancer Res* 1998;4(11):2869-76.
 173. Taniyama T, Wanibuchi H, Salim EI, Yano Y, Otani S, Nishizawa Y, et al. Chemopreventive effect of 24R,25-dihydroxyvitamin D(3) in N, N'-dimethylhydrazine-induced rat colon carcinogenesis. *Carcinogenesis* 2000;21(2):173-8.

174. Swami S, Krishnan AV, Feldman D. 1 α ,25-Dihydroxyvitamin D₃ down-regulates estrogen receptor abundance and suppresses estrogen actions in MCF-7 human breast cancer cells. *Clin Cancer Res* 2000;6(8):3371-9.
175. Sundaram S, Gewirtz DA. The vitamin D₃ analog EB 1089 enhances the response of human breast tumor cells to radiation. *Radiat Res* 1999;152(5):479-86.
176. Nickerson T, Huynh H. Vitamin D analogue EB1089-induced prostate regression is associated with increased gene expression of insulin-like growth factor binding proteins. *J Endocrinol* 1999;160:223-9.
177. James SY, Mercer E, Brady M, Binderup L, Colston KW. EB1089, a synthetic analogue of vitamin D, induces apoptosis in breast cancer cells in vivo and in vitro. *Br J Pharmacol* 1998;125(5):953-62.
178. Fioravanti L, Miodini P, Cappelletti V, DiFronzo G. Synthetic analogs of vitamin D₃ have inhibitory effects on breast cancer cell lines. *Anticancer Res* 1998;18(3A):1703-8.
179. Larsen SS, Heiberg I, Lykkesfeldt AE. Anti-oestrogen resistant human breast cancer cell lines are more sensitive towards treatment with the vitamin D analogue EB1089 than parent MCF-7 cells. *Br J Cancer* 2001;84(5):686-90.
180. Friedrich M, Axt-Flidner R, Villena-Heinsen C, Tilgen W, Schmidt W, Reichrath J. Analysis of vitamin D-receptor (VDR) and retinoid X-receptor alpha in breast cancer. *Histochem J* 2002;34(1-2):35-40.
181. Holick MF. Calcium and vitamin D. Diagnostics and therapeutics. *Clin Lab Med* 2000;20(3):569-90.
182. Adams JS, Clemens TL, Parrish JA, Holick MF. Vitamin-D synthesis and metabolism after ultraviolet irradiation of normal and vitamin-D-deficient subjects. *N Engl J Med* 1982;306(12):722-5.
183. Vieth R, Cole DE, Hawker GA, Trang HM, Rubin LA. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. *Eur J Clin Nutr* 2001;55(12):1091-7.
184. Haddad JG, Jr., Rojanasathit S. Acute administration of 25-hydroxycholecalciferol in man. *J Clin Endocrinol Metab* 1976;42(2):284-90.

185. Batchelor AJ, Compston JE. Reduced plasma half-life of radio-labelled 25-hydroxyvitamin D3 in subjects receiving a high-fibre diet. *Br J Nutr* 1983;49(2):213-6.
186. Tangpricha V, Pearce E, Chen T, Holick M. Vitamin D insufficiency among free-living healthy young adults. *Am J Med* 2002;112((8) Jun 1):659-62.
187. Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med* 1998;338(12):777-83.
188. Rosen CJ, Morrison A, Zhou H, Storm D, Hunter SJ, Musgrave K, et al. Elderly women in northern New England exhibit seasonal changes in bone mineral density and calciotropic hormones. *Bone Miner* 1994;25(2):83-92.
189. Storm D, Eslin R, Porter ES, Musgrave K, Vereault D, Patton C, et al. Calcium supplementation prevents seasonal bone loss and changes in biochemical markers of bone turnover in elderly New England women: a randomized placebo-controlled trial. *J Clin Endocrinol Metab* 1998;83(11):3817-25.
190. Aloia JF, Mikhail M, Pagan CD, Arunachalam A, Yeh JK, Flaster E. Biochemical and hormonal variables in black and white women matched for age and weight. *J Lab Clin Med* 1998;132(5):383-9.
191. Awamey E, Hollis B, Bell N. Low serum 25-hydroxyvitamin D in blacks results from decreased production rate and not increased metabolic clearance rate (Abstract). *J Bone Mineral Res* 1996;11:S165.
192. Mitra D, Bell N. Racial, geographic, genetic and body habitus effects on vitamin D metabolism. In: Feldman D GF, Pike J, editor. *Vitamin D*. San Diego: Academic Press; 1997.
193. Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr* 2002;76(1):187-92.
194. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000;72(3):690-3.
195. LeBoff MS, Kohlmeier L, Hurwitz S, Franklin J, Wright J, Glowacki J. Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. *JAMA* 1999;281(16):1505-11.

196. Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. *Am J Clin Nutr* 1998;67(6):1232-6.
197. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly. *Endocrinol Rev Monogr* 2000;22:477-501.
198. Lips P, Chapuy MC, Dawson-Hughes B, Pols HA, Holick MF. An international comparison of serum 25-hydroxyvitamin D measurements. *Osteoporos Int* 1999;9(5):394-7.
199. Lips P, Duong T, Oleksik A, Black D, Cummings S, Cox D, et al. A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. *J Clin Endocrinol Metab* 2001;86(3):1212-21.
200. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. *Lancet* 1998;351(9105):805-6.
201. Harris SS, Soteriades E, Coolidge JA, Mudgal S, Dawson-Hughes B. Vitamin D insufficiency and hyperparathyroidism in a low income, multiracial, elderly population. *J Clin Endocrinol Metab* 2000;85(11):4125-30.
202. Holick MF, Shao Q, Liu WW, Chen TC. The vitamin D content of fortified milk and infant formula. *N Engl J Med* 1992;326(18):1178-81.
203. Jacobus CH, Holick MF, Shao Q, Chen TC, Holm IA, Kolodny JM, et al. Hypervitaminosis D associated with drinking milk. *N Engl J Med* 1992;326(18):1173-7.
204. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* 1999;69(5):842-56.
205. Parfitt A, Gallagher J, Heaney R, Johnson C, Neer R, Whedon G. Vitamin D and bone health in the elderly. *Am J Clin Nutr* 1982;36:1014-31.
206. National Academy of Sciences–Institute of Medicine Food and Nutrition Board. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press; 1997.
207. Vieth R, Chan PC, MacFarlane GD. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. *Am J Clin Nutr* 2001;73(2):288-94.

208. Holick MF. Vitamin D requirements for humans of all ages: new increased requirements for women and men 50 years and older. *Osteoporos Int* 1998;8(8 Suppl.):S24-S29.
209. Trang HM, Cole DE, Rubin LA, Pierratos A, Siu S, Vieth R. Evidence that vitamin D₃ increases serum 25-hydroxyvitamin D more efficiently than does vitamin D₂. *Am J Clin Nutr* 1998;68(4):854-8.
210. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* 1999;69:842-56.
211. Davies M, Adams PH. The continuing risk of vitamin-D intoxication. *Lancet* 1978;2(8090):621-3.
212. Curhan GC, Willett WC, Rimm EB, Stampfer MJ. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med* 1993;328(12):833-8.
213. Scragg R, Jackson R, Holdaway IM, Lim T, Beaglehole R. Myocardial infarction is inversely associated with plasma 25- hydroxyvitamin D₃ levels: a community-based study. *Int J Epidemiol* 1990;19(3):559-63.
214. Holick MF. Sunlight and vitamin D: both good for cardiovascular health. *J Gen Intern Med* 2002;17(9):733-5.
215. Krause R, Buhning M, Hopfenmuller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. *Lancet* 1998;352(9129):709-10.
216. Thierry-Palmer M, Carlyle KS, Williams MD, Tewolde T, Caines-McKenzie S, Bayorh MA, et al. Plasma 25-hydroxyvitamin D concentrations are inversely associated with blood pressure of Dahl salt-sensitive rats. *J Steroid Biochem Mol Biol* 1998;66(4):255-61.
217. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab* 2001;86(4):1633-7.
218. Kallay E, Pietschmann P, Toyokuni S, Bajna E, Hahn P, Mazzucco K, et al. Characterization of a vitamin D receptor knockout mouse as a model of colorectal hyperproliferation and DNA damage. *Carcinogenesis* 2001;22(9):1429-35.
219. Zmuda JM, Cauley JA, Ferrell RE. Molecular epidemiology of vitamin D receptor gene variants. *Epidemiol Rev* 2000;22(2):203-17.

220. Ma J, Stampfer MJ, Gann PH, Hough HL, Giovannucci E, Kelsey KT, et al. Vitamin D receptor polymorphisms, circulating vitamin D metabolites, and risk of prostate cancer in United States physicians. *Cancer Epidemiol Biomarkers Prev* 1998;7(5):385-90.
221. Slatter ML, Yakumo K, Hoffman M, Neuhausen S. Variants of the VDR gene and risk of colon cancer (United States). *Cancer Causes Control* 2001;12(4):359-64.
222. Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW. Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population. *Br J Cancer* 2001;85(2):171-5.
223. Curran JE, Vaughan T, Lea RA, Weinstein SR, Morrison NA, Griffiths LR. Association of A vitamin D receptor polymorphism with sporadic breast cancer development. *Int J Cancer* 1999;83(6):723-6.
224. Hou MF, Tien YC, Lin GT, Chen CJ, Liu CS, Lin SY, et al. Association of vitamin D receptor gene polymorphism with sporadic breast cancer in Taiwanese patients. *Breast Cancer Res Treat* 2002;74(1):1-7.
225. Zhang H, Tao G, Wu Q, Liu J, Gao Y, Chen R, et al. Vitamin D receptor gene polymorphism in postmenopausal women of the Han and Uygur nationalities in China. *Chin Med J (Engl)* 2000;113(9):787-9.
226. Habuchi T, Suzuki T, Sasaki R, Wang L, Sato K, Satoh S, et al. Association of vitamin D receptor gene polymorphism with prostate cancer and benign prostatic hyperplasia in a Japanese population. *Cancer Res* 2000;60(2):305-8.
227. Morrison N, Qi J, Tokita A, Kelly P, Crofts L, Nguyen T, et al. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994;367:284-7.
228. Taylor JA, Hirvonen A, Watson M, Pittman G, Mohler JL, Bell DA. Association of prostate cancer with vitamin D receptor gene polymorphism. *Cancer Res* 1996;56(18):4108-10.
229. Medeiros R, Morais A, Vasconcelos A, Costa S, Pinto D, Oliveira J, et al. The role of vitamin D receptor gene polymorphisms in the susceptibility to prostate cancer of a southern European population. *J Hum Genet* 2002;47(8):413-8.
230. Hamasaki T, Inatomi H, Katoh T, Ikuyama T, Matsumoto T. Clinical and pathological significance of vitamin D receptor gene polymorphism for prostate cancer which is associated with a higher mortality in Japanese. *Endocr J* 2001;48(5):543-9.

231. Kibel AS, Isaacs SD, Isaacs WB, Bova GS. Vitamin D receptor polymorphisms and lethal prostate cancer. *J Urol* 1998;160(4):1405-9.
232. Blazer DG, 3rd, Umbach DM, Bostick RM, Taylor JA. Vitamin D receptor polymorphisms and prostate cancer. *Mol Carcinog* 2000;27(1):18-23.
233. Ebeling P, Sandgren M, Lane A, DeLuca H, Riggs B. Evidence of an age-related decrease in intestinal responsiveness to vitamin D: relationship between serum 1,25-dihydroxyvitamin D₃ and intestinal vitamin D receptor concentrations in normal women. *J Clin Endocrinol Metab* 1992;75:176-82.
234. Gagnon AM, Simboli-Campbell M, Welsh JE. Induction of calbindin D-28K in Madin-Darby bovine kidney cells by 1,25(OH)₂D₃. *Kidney Int* 1994;45(1):95-102.
235. Lipkin M, Yang K, Edelmann W, Xue L, Fan K, Risio M, W, et al. Preclinical mouse models for cancer chemoprevention studies. *Ann N Y Acad Sci* 1999;889:14-19.
236. Goss KH, Groden J. Biology of the adenomatous polyposis coli tumor suppressor. *J Clin Oncol* 2000;18(9):1967-79.
237. Scaglione-Sewell BA, Bissonnette M, Skarosi S, Abraham C, Brasitus TA. A vitamin D₃ analog induces a G₁-phase arrest in CaCo-2 cells by inhibiting cdk2 and cdk6: roles of cyclin E, p21Waf1, and p27Kip1. *Endocrinology* 2000;141(11):3931-9.
238. Jensen SS, Madsen MW, Lukas J, Binderup L, Bartek J. Inhibitory effects of 1 α ,25-dihydroxyvitamin D₃ on the G₁-S phase-controlling machinery. *Mol Endocrinol* 2001;15(8):1370-80.

Figure Legends

Figure 1. Data are accumulating that support a mechanism for vitamin D in reducing the risk of malignancy. The effect is based on the known role that intracellular Ca^{2+} plays in differentiation and apoptosis, and is based on research by Davies and colleagues (92). It is built on the existence of an intracellular Ca^{2+} gradient from the base to the top of the colonic crypts that was first described by Lipkin and colleagues (3, 99). This figure shows Ca^{2+} channels in the colonocyte membrane that function as receptors for Ca^{2+} , allowing entry of vitamin D metabolites that are thought to exist in the cell membrane, and calbindin molecules that are synthesized in the cell in response to stimulation of the nuclear vitamin D receptor by $1,25(\text{OH})_2\text{D}$ (234). Each calbindin molecule reversibly binds four Ca^{2+} ions, making them available for pulsatile release when needed for intracellular physiological roles, including differentiation and apoptosis. If intracellular vitamin D metabolites are deficient, calbindin is virtually absent from the cytoplasm. As a result, rapid pulsatile release of Ca^{2+} is impossible at the level needed to sustain differentiation and apoptosis. This may be because there is an inadequate concentration of the intracellular calbindin molecules that normally serve as Ca^{2+} “capacitors” and rapidly release Ca^{2+} ions for these roles.

Figure 2. Normal structure and pattern of differentiation and apoptosis in colonocytes, based on the concept developed by Lipkin and colleagues (3, 99). As the colonocytes migrate from the base of the crypt to the top, they differentiate and ultimately undergo apoptosis. These functions are mediated by the intracellular Ca^{2+} concentration, which increases under normal conditions as the cells migrate upward. There is also a gradient in the luminal Ca^{2+} concentration that is thought to contribute to differentiation and apoptosis at the top of the crypt if the luminal contents are replete with Ca^{2+} .

Figure 3. Colonic crypt in the vitamin D-replete individual. In the cell of a vitamin D-replete individual, there is a strong gradient in the intracellular Ca^{2+} from a low concentration at the base to a high concentration at the top (91). Calbindin provides a means of storing and releasing Ca^{2+} , and it is thought to mediate the intracellular Ca^{2+} gradient from the low concentration at the base of the crypt to the usually high concentration at the top. When $1,25(\text{OH})_2\text{D}$ is abundant in the colonocyte, a high concentration of calbindin is produced (97). The intracellular free Ca^{2+}

ions are needed for terminal differentiation and, ultimately, apoptosis in the vitamin D-replete cell (91).

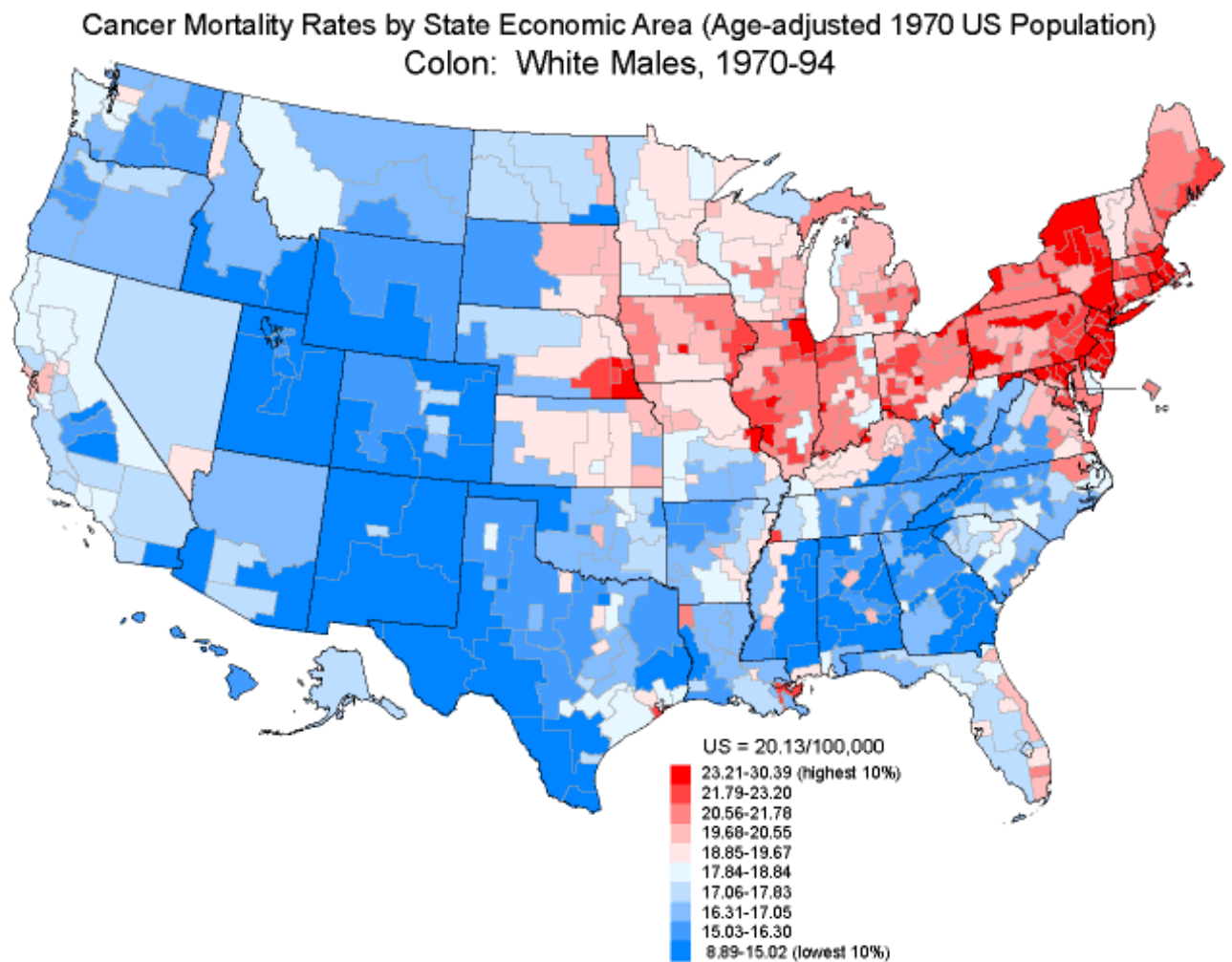
Figure 4. Colonic crypt in the vitamin D-deficient individual (91). When vitamin D metabolites are deficient in the cell, calbindin is present in low concentration or nonexistent. There are no means of reversibly storing and rapidly releasing the Ca^{2+} that mediates the intracellular Ca^{2+} gradient from the base of the crypt to the mouth. When this gradient is absent, the colonocytes do not terminally differentiate as they migrate up the crypt, and the normal occurrence of apoptosis at the mouth of the crypt is inhibited or prevented (3, 99). An adenomatous polyp results from buildup of senescent colonocytes that have not undergone apoptosis, and ultimately a cancer may evolve in the polyp or nearby if other conditions are appropriate. The effect of loss of the intracellular Ca^{2+} gradient is thought to be exaggerated when the luminal Ca^{2+} concentration is low. Other genomic and nongenomic mechanisms for the anticarcinogenic role of vitamin D in colonocytes have also been described (109-111, 235-238).

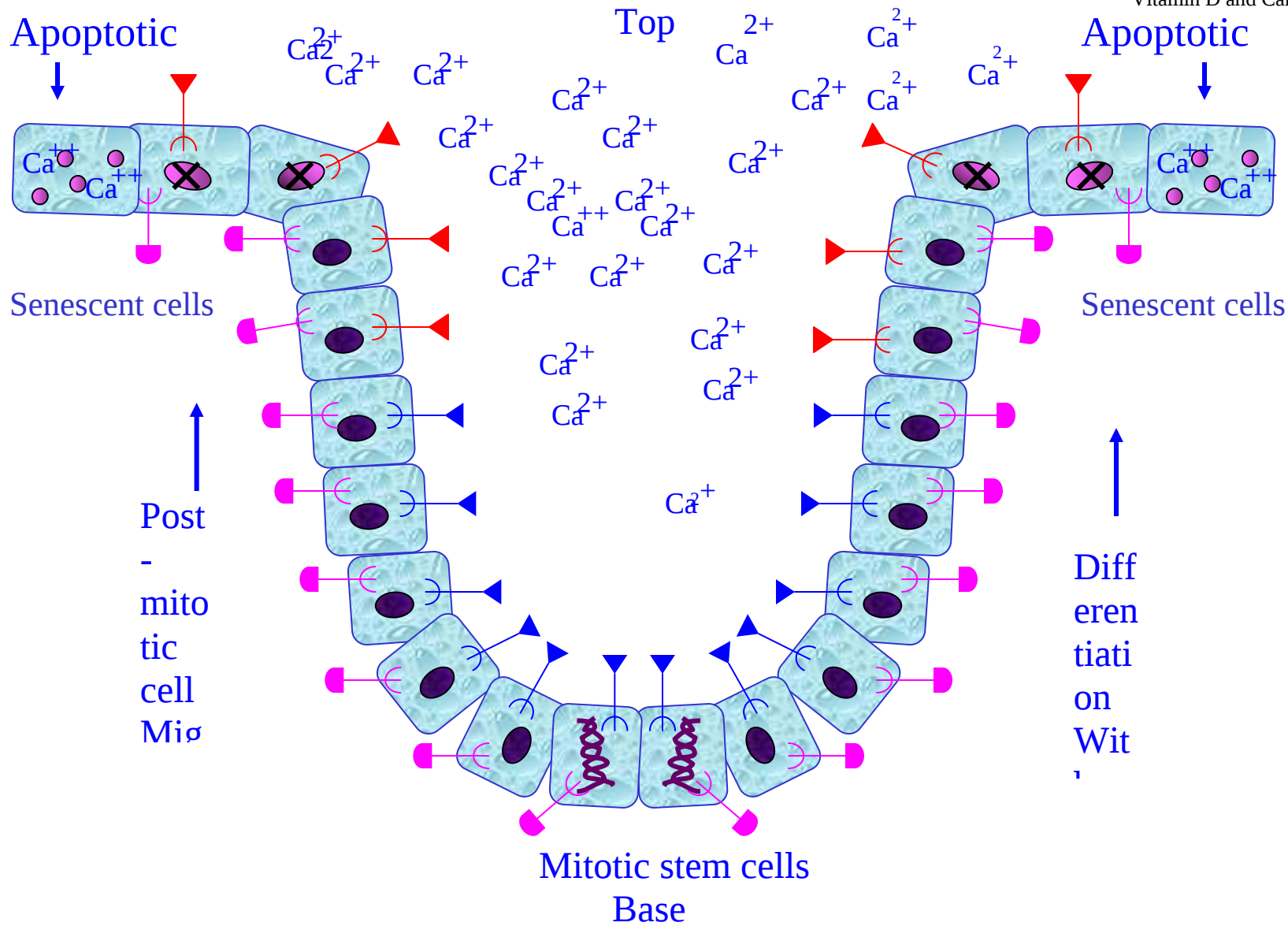
Figure 5. Colon cancer mortality rates are uniformly low (blue tints) south of about 37 degrees latitude in the United States, especially in the sunniest areas (New Mexico, Western Texas, Utah, and Arizona). By contrast, rates are highest (pink and red tints) in areas that receive the least solar ultraviolet B (UVB), including New York, New Jersey, Pennsylvania, and parts of New England. Winter UVB radiation levels in the Northeastern industrial sector are so low that no vitamin D can be photosynthesized during November through February (53). The low UVB levels are due to climate (cold and cloudy arctic and Canadian air masses transported to the region during the late fall and winter), relatively high latitude, removal of UVB from sunlight by a type of air pollution (acid haze) that predominates in the region (86, 87), and a thick stratospheric ozone layer that exists over the Northeastern sector of the United States (86). Death rates in the areas with the highest rates are approximately twice those in areas with the lowest rates. (Source: National Cancer Institute, 2002. Rates shown are age-adjusted mortality rates for white men during 1970-1994. The pattern of rates was virtually identical for white women.)

Figure 6. Vitamin D deficiency is common in the winter in North America (180, 181) because the vitamin is available from only a few sources. Urban lifestyle has reduced average exposure of

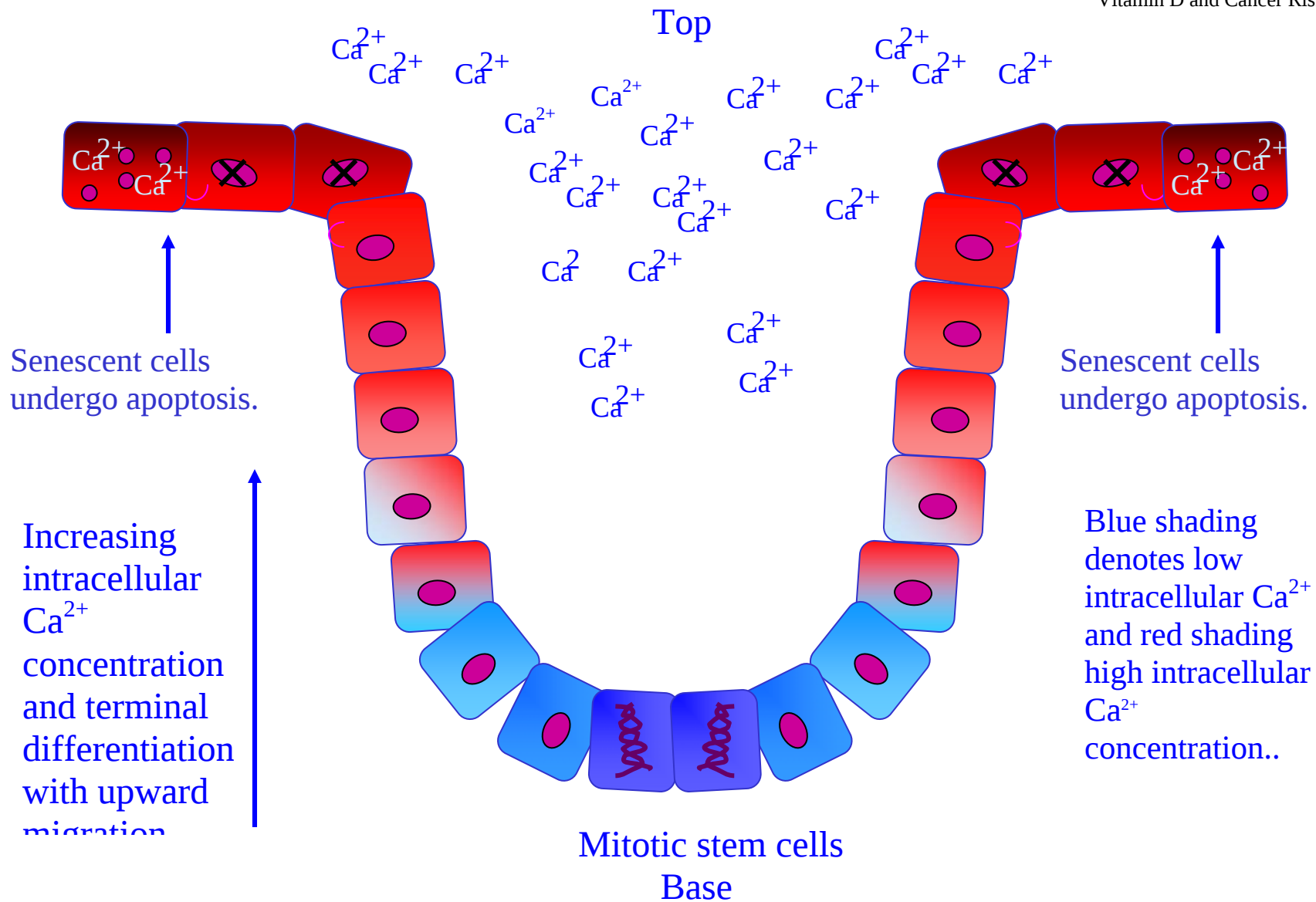
adults to midday sunlight to a low level except during summer months. Vitamin D that is photosynthesized in the summer does not persist through the winter (66, 182, 184-186). Vitamin D is metabolized within 3-5 days to 25(OH)D, the storage form (182). The half-life of 25(OH)D is between 12 days (184) and 27 days (185).

Figure 5. (Figures 1-4 and 6 are in a PowerPoint file (attached) named VitDMech_Figs1-4_&_6_TRv3_REVISED.ppt

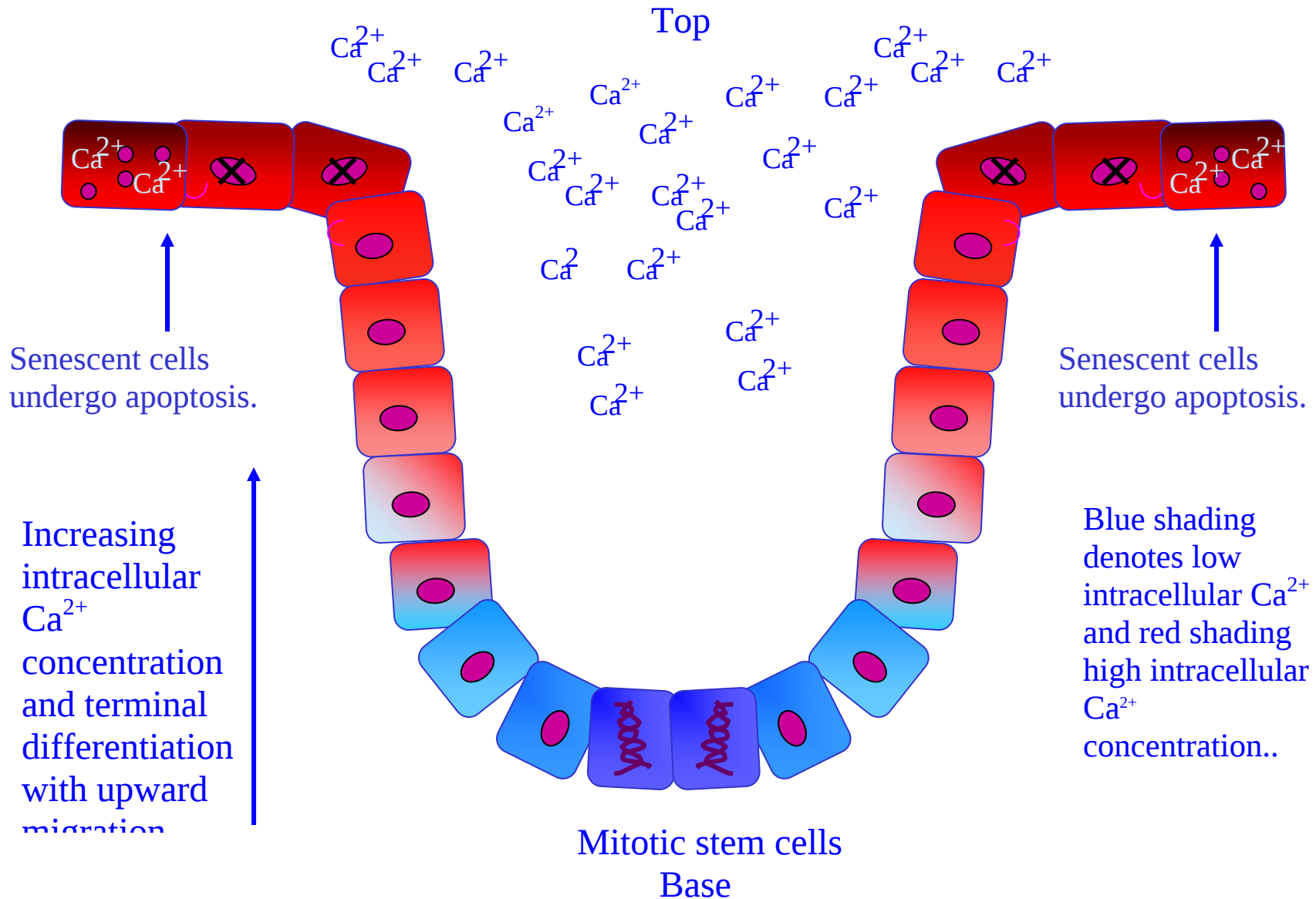




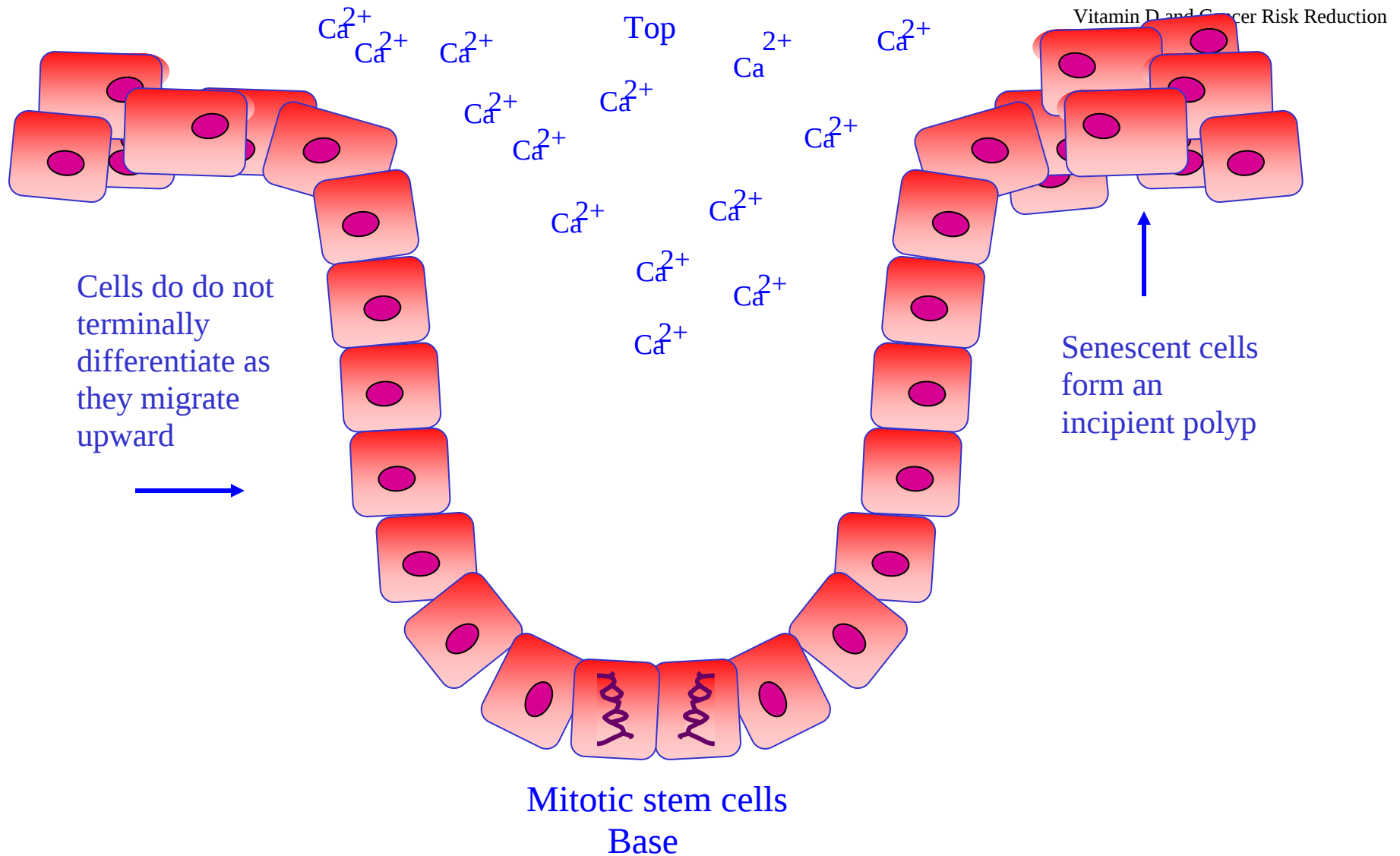
Normal colonic crypt differentiation and



Vitamin D replete crypt: intracellular Ca^{2+} concentration gradient is



Vitamin D replete crypt: intracellular Ca^{2+} concentration gradient is



Vitamin D deficient crypt: intracellular Ca^{2+} concentration gradient is abolished

