ANTICANCER RESEARCH *29*: 3687-3698 (2009)

Review

Calcium, Vitamin D and Cancer

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Abstract. *A low vitamin D status and inadequate calcium intake are important risk factors for various types of cancer. Ecological studies using solar UV-B exposure as an index of vitamin D_3 photoproduction in the skin found a highly significant inverse association between UV-B and mortality in fifteen types of cancer. Of these, colon, rectal, breast, gastric, endometrial, renal and ovarian cancer exhibit a significant inverse relationship between incidence and oral intake of calcium. In addition, lung and endometrial cancer as well as multiple myeloma are considered calcium and vitamin D sensitive. Studies on tissue-specific expression of the CYP27B1-encoded 25-hdroxyvitamin D-1α-hydroxylase and of the extracellular calcium-sensing receptor (CaR) have led to an understanding how locally produced 1,25-dihydroxyvitamin D3 (1,25$(OH)_2D_3$) and extracellular Ca^{2+} act jointly as key regulators of cellular proliferation, differentiation and function. Thus, impairment of antimitogenic, proapoptotic and prodifferentiating signaling from the 1,25$(OH)_2D_3$-activated vitamin D receptor (VDR) and from the CaR in vitamin D and calcium insufficiency has been implicated in the pathogenesis of the aforementioned types of cancer. 1,25$(OH)_2D_3$ and calcium interact in modulating cell growth in different ways: (i) Signaling pathways from the VDR and the CaR converge on the same downstream elements, e.g. of the canonical Wnt pathway; (ii) high extracellular calcium modulates extrarenal vitamin D metabolism in favor of higher local steady-state concentrations of 1,25$(OH)_2D_3$; (iii) 1,25$(OH)_2D_3$ may up-regulate expression of the CaR and thus augment CaR-mediated antiproliferative responses to high extracellular Ca^{2+}. This can explain why combined supplementation is required for optimal chemoprevention of cancer by calcium and vitamin D.*

A nutritional calcium deficit and a compromised vitamin D status are risk factors for multiple chronic diseases, including various types of malignancy [for review, (1)]. A strong association between a low vitamin D status and cancer incidence or mortality has been reported for colon, rectal, breast, prostate and ovarian cancer (2). In addition, vitamin D insufficiency apparently contributes to the pathogenesis of gastric, lung, esophageal, pancreatic, renal and endometrial cancer, as well as non-Hodgkin's lymphoma (3). There is evidence that poor calcium nutrition is a significant risk factor for total cancer incidence (4), and, in particular, for colorectal (5-9), breast (10-12) and renal (13, 14) cancer. Low calcium intake may also contribute to the development of gastric (15), pancreatic (16) and ovarian cancer (17, 18), and to some extent of endometrial (19, 20) lung (21) and prostate (22) cancer, as well as multiple myeloma (23) (cf. Table 1).

Relevance of Adequate Calcium Intake for Control of Cellular Growth

Different levels of daily calcium intake according to age, sex, and hormonal status are currently recommended as a preventive measure against a negative calcium balance (24). A minimum of 1,000 mg calcium per day is required for healthy adults until age 60 years, while higher values apply for people of advanced age, or women during pregnancy and lactation as well as after menopause. Evidence is accumulating that calcium malnutrition is not only encountered in the elderly (25) but is widespread also in the younger population in Europe as well as in North America (26, 27).

A concept how signals from nutritional calcium are transduced to organs and cell systems distant from the intestinal lumen was not available until Brown and colleagues (28) cloned an extracellular calcium-sensing receptor (CaR) from the bovine parathyroid gland. Many other cells also express this receptor, among them normal and neoplastic

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Key Words: Vitamin D insufficiency, calcium intake, dairy calcium, calcium-sensing receptor, extrarenal 25-hydroxyvitamin D-1α-hydroxylase, 1,25-dihydroxyvitamin D_3, 25-hydroxyvitamin D-24-hydroxylase, review.

Table I. *Effect of calcium from different sources on cancer risk (with references).*

Cancer	Ca from food and/or supplements	Ca from drinking/ mineral water	Ca from dairy products	Ca + Vitamin D from food and/ or supplements
Multiple sites	Benefit (61) (n.s.) Benefit (4) (in women)			Benefit (61)
Breast, postmenopausal	No effect (10, 134) Benefit (62)		Benefit (11)	
Breast, premenopausal	Benefit (10, 62, 135) No effect (134)		Benefit (10, 135)	Benefit (62)
Colorectal	Benefit (4, 6, 7, 9, 76, 128)		Benefit (9, 128)	Benefit (6-8)
Colon	Benefit (77, 136)	Benefit (137)		Benefit (136)
Rectal	Benefit (7, 138) No effect (8, 77, 78)			
Endometrial	Possible benefit (19, 20)			
Esophageal	Possible benefit (4)			
Gastric		Benefit (15)		
Head and Neck	Possible benefit (4)			
Lung	Possible benefit (21)			
Multiple myeloma	Possible benefit (23)			
Ovarian	Possible benefit (17) Benefit (18)	No effect (139)		
Pancreatic	No effect (140)	Benefit (16)		
Prostate	Risk (105) Modest risk (133) No effect (4, 109-111) Possible benefit (22)		Risk (109) Modest risk (133) No effect (111)	
Renal	Benefit (13, 14)			

human renal (28), gastric (29), large intestinal epithelial (30), mammary gland (31), ovarian (32), prostate gland (33), and pancreatic duct cells (34). The CaR transduces minute changes in extracellular fluid Ca^{2+} concentrations to stimulatory and inhibitory G proteins in a large variety of intracellular signaling pathways. Consequently, when extracellular Ca^{2+} drops due to inadequate supply from dietary sources, not only will the parathyroid gland release more PTH, but cellular homeostasis and functions in many other tissues will also be affected. CaR-mediated changes in proliferation, differentiation, and apoptosis may thus contribute to the pathogenesis of various types of cancer (Table I).

Relevance of Adequate Plasma Vitamin D Levels for Organ-specific Control of Cell Growth

Regardless whether synthesized in the epidermis or absorbed from the diet, vitamin D_3 is converted in the liver to 25-hydroxyvitamin D_3 ($25(OH)D_3$). The serum level of 25(OH)D (the term 25(OH)D is used to denote the sum of $25(OH)D_3$ and $25(OH)D_2$, the latter from dictary sources) is considered a reliable indicator of the vitamin D status of a person. The terms vitamin D insufficiency or inadequacy are used to describe a condition in which insufficient circulating 25(OH)D is available for optimal intracellular production of $1,25(OH)_2D_3$ at extrarenal sites. As detailed in the following, this explains why serum levels of 25(OH)D are inversely associated with the incidence of many chronic diseases (1). Importantly, low serum 25(OH)D has been shown to be a reliable predictor of all-cause mortality (35, 36). Conservative calculations of the set point between vitamin D insufficiency and optimal vitamin D supply arrived at a value of 30 nM 25(OH)D (37) but there is increasing evidence that for optimal health outcomes serum 25(OH)D should be maintained at much higher levels, *i.e.* between 60 and 100 nM (38-40).

Vitamin D insufficiency is frequently observed in individuals with limited sun exposure, as in the chronically ill, in immobilized or housebound elderly people. Yet a compromised vitamin D status is also a common phenomenon in the free living normal population at any age (26, 41-43).

Conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ is catalyzed by the *CYP27B1*-encoded enzyme 25(OH)D-1α-hydroxylase and occurs primarily in the kidney. However, many extrarenal cells also biosynthesize $1,25(OH)_2D_3$. Examples are normal and neoplastic epithelial cells of the skin (44), of the gastrointestinal tract (45-48) and of female and male reproductive organs (49-51). Renal CYP27B1 activity is tightly regulated by serum Ca^{2+} and parathyroid hormone (PTH), as well as by feed-back inhibition from $1,25(OH)_2D_3$. Therefore, circulating

$1,25(OH)_2D_3$ can be maintained in the normal range, 75-200 pM, even when serum levels of 25(OH)D are relatively low (52). Extrarenal synthesis of $1,25(OH)_2D_3$ is, however, regulated differently. Expression of CYP27B1 at extrarenal sites can be modulated independently of circulating PTH, Ca^{2+} (53) or $1,25(OH)_2D_3$ (54, 55), so that 25(OH)D-1α-hydroxylase activity depends largely on ambient $25(OH)D_3$ levels. This may explain why the incidence of vitamin D insufficiency-related cancer of the colorectum (56), breast (57) and prostate gland (58) is correlated primarily with low serum 25(OH)D, and only to a lesser extent with low $1,25(OH)_2D_3$ (59). Altogether, at low serum levels of 25(OH)D, CYP27B1 activity in extrarenal cellular systems may be not high enough to achieve steady-state tissue concentrations of $1,25(OH)_2D_3$ necessary to regulate cellular growth, differentiation and apoptosis. Therefore, vitamin D insufficiency plays an important pathogenic role in many malignancies (2, 3) (see also Table I).

Combined Vitamin D and Calcium Insufficiency

In a population-based cross-sectional study on calcium and vitamin D status of healthy adults of both sexes (26, 60), daily calcium consumption was below recommended levels in 81% of the cohort. In the same study, 26% of all participants were considered vitamin D-insufficient. When calcium intake by 25(OH)D serum levels was calculated, 23% of the entire cohort exhibited combined vitamin D and calcium insufficiency (1) and, therefore, may have a particularly high risk for vitamin D and calcium insufficiency-related cancer (Table I). This notion is strongly supported by the report of Lappe *et al*. that only combined calcium and vitamin D supplements could significantly reduce the general incidence of cancer of the breast, lung, colon, and uterus as well as of the lymphoid and myeloid system (61). In particular, Cho *et al*. (6) concluded from an analysis of pooled primary data from 10 cohort studies, in which more than half a million individuals were followed up for 6-16 years, that optimal risk reduction for colorectal cancer necessitates high intake levels of both vitamin D and calcium. This notion was shown to be valid not only for Western but also for Asian populations (7, 8). Bérubé *et al*. (62) studied the relation of separate and combined intakes of vitamin D and calcium by pre-menopausal women on mammographic breast density as a surrogate marker for breast cancer risk. They found that the negative association between dietary vitamin D intake and breast density tended to be stronger when calcium intake levels were higher and *vice versa*.

Mechanisms of Calcium and Vitamin D Action in Control of Neoplastic Cell Growth

The CaR is an essential part of an intricate network of calcium signaling pathways that control normal and cancer cell growth (63-66), Depending on cell-specific coupling to appropriate G-proteins, activation of the CaR by elevated extracellular Ca^{2+} reduces the rate of cellular proliferation as in human colon carcinoma (67, 68) or ovarian surface epithelial cells (69), but may also stimulate cell growth as in malignant Leydig cells (70) and protect from apoptosis, for example, in prostate cancer cells (71).

$1,25(OH)_2D_3$ exerts antiproliferative effects on cancer cells by modulating the transcriptional activity of key genes involved in cell cycle control [for review see (72)]. $1,25(OH)_2D_3$ may also suppress tumor growth and progression indirectly by facilitating immunocytotoxic killing of tumor cells: $1,25(OH)_2D_3$ reduces levels of immunosuppressive $CD34^+$ lymphocytes, which normally limit the cytotoxic activity of infiltrating tumor-specific $CD8^+$ T lymphocytes (73). The nearly ubiquitous expression of *CYP27B1* (74) and the importance of intrinsic $1,25(OH)_2D_3$ production in controlling cell proliferation may explain why vitamin D insufficiency increases the risk of malignancies in many organs and biological systems.

Colorectal cancer. In 1980 Garland and Garland proposed that sunlight and vitamin D can protect against colon cancer (75). This hypothesis had gained strong support when in 1985 Garland *et al*. (76) published the results of a 19-year prospective trial showing that low dietary intakes of vitamin D and of calcium are associated with a significant risk of colorectal cancer. Since then many other observational studies reported a strong association between incidence or mortality for colorectal cancer and a low vitamin D status [for review, see (2)] or, respectively, low calcium intake (6-8). It should be noted that vitamin D insufficiency increases cancer risk in the colon and in the rectum, whereas calcium insufficiency does so in the colon and possibly not in the rectum (77-79).

Studies from our laboratory (80-83) have shown that $1,25(OH)_2D_3$ inhibits growth and promotes differentiation of human colon adenoma and carcinoma cells by inhibiting up-regulation of cyclin D1 expression, a key element in cell cycle control. A number of intracellular proliferative signaling pathways, *viz*. the Raf-1/MEK1/ERK and STAT-3 pathways, converge at c-Myc (84) and engage cyclin D1 as a common downstream effector. $1,25(OH)_2D_3$ therefore counteracts mitogenesis whatever the nature of cellular growth promoting factors is (85). Another antimitogenic mechanism of $1,25(OH)_2D_3$ involves direct interaction with growth factor receptor-activated pathways. For example, in human colon adenocarcinoma-derived Caco-2 cells, $1,25(OH)_2D_3$ diminishes the number of ligand-occupied epidermal growth factor receptors (EGFRs) (85).

A role of the CaR in mediating the chemopreventive effects of calcium was suggested by the significant association between genetic variants of the CaR and advanced colorectal adenoma (86). Moreover, certain single nucleotide

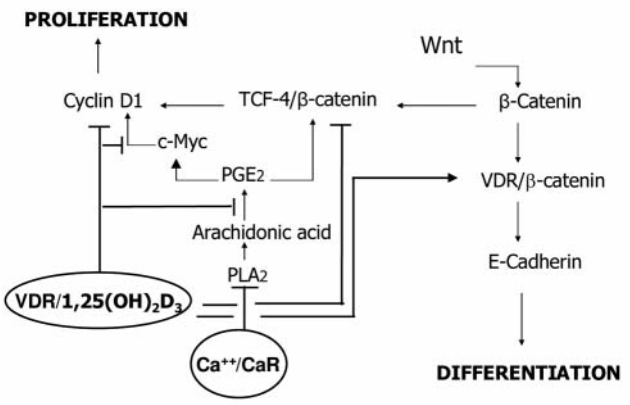

Figure 1. *Co-operative signalling from 1,25(OH)$_2$D$_3$/VDR and Ca^{2+}/CaR inhibits proliferation and promotes differentiation of human colon cancer cells.*

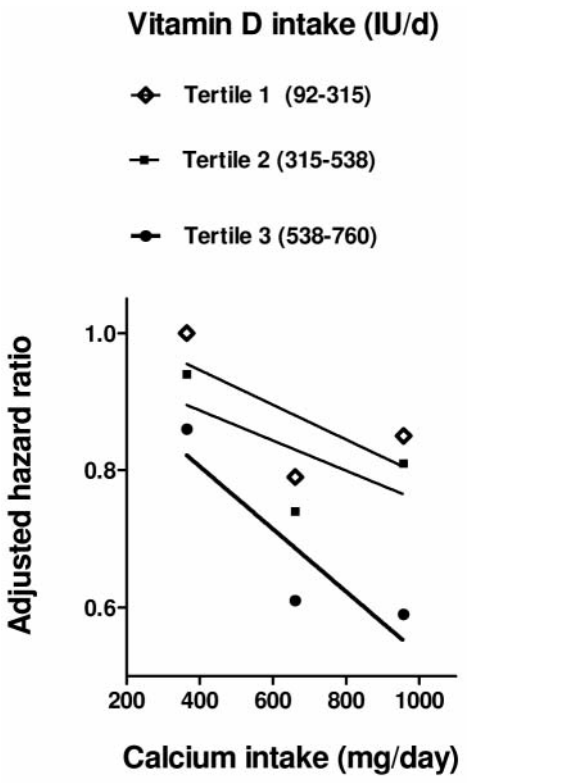

Figure 2. *Relative risk of colorectal cancer for total calcium intake by levels of total vitamin D intake. Data are from Table IV in Ishihara et al. (8).*

polymorphisms in the CaR gene were found to be associated with an increased risk of cancer in the proximal colon (87). Neoplastic human colonocytes express CaR at the mRNA and protein levels as long as they retain a certain degree of differentiation (88, 89). The sequence of events downstream of CaR activation that actually link CaR to cell cycle control starts with inhibition of phospholipase A_2 activity (67), which would reduce the amount of arachidonic acid available for synthesis of proliferation-stimulating prostaglandins. Subsequent down-regulation of *c-myc* proto-oncogene expression (30), activation of the cyclin-dependent kinase inhibitor p21 (53) and inhibition of cyclin D1 finally leads to cell cycle arrest at the G_1/S-phase transition. CaR-activated pro-differentiating signaling in colonocytes involves inhibition of the Wnt/β-catenin pathway by down-regulation of T-cell transcription factor (TCF)-4 with subsequent induction of E-cadherin expression (68, 90). Interestingly, part of the antiproliferative action of 1,25(OH)$_2$D$_3$ has been traced to a VDR-mediated negative effect on TCF-4 (68, 91) (Figure 1).

Three modes of interaction between 1,25(OH)$_2$D$_3$ and Ca^{2+} in modulating cell growth and differentiation have been identified in the colon mucosa: (i) As detailed before, activation of the VDR or the CaR is transduced to the same key elements of antiproliferative and prodifferentiating signaling, *i.e.* c-Myc and cyclin D1 as well as TCF-4 and E-cadherin (Figure 1). (ii) High luminal calcium not only inhibits cellular growth by activating the CaR, but at the same time suppresses the vitamin D catabolizing enzyme 25(OH)D-24-hydroxylase (CYP24); this very likely leads to higher steady-state local concentrations of 1,25(OH)$_2$D$_3$ (53, 92). (iii) 1,25(OH)$_2$D$_3$ may up-regulate expression of the CaR (93) and thus augment CaR-mediated antiproliferative responses to high extracellular Ca^{2+}.

Elucidation of the molecular and cellular mechanisms of action of calcium and vitamin D on growth rate and differentiation of human colon carcinoma cells helped to understand why the efficiency of vitamin D in reducing the risk of colorectal cancer depends very much on the calcium status of an individual and *vice versa*, so that optimal prevention of the disease necessitates high intake levels of both vitamin D and calcium. Cho *et al.* (6) analyzed pooled primary data from 10 large cohort studies and found, as illustrated in Figure 2, that a significant effect of calcium intake on colorectal cancer risk can be observed only at the highest level of vitamin D intake. Additional strong support for a joint action of calcium and vitamin D in the prevention of colorectal carcinogenesis is provided by two recent large cohort studies from Japan (7, 8).

Breast cancer. The long-standing assumption that low vitamin D intake is associated with increased breast cancer risk (94-96) has been supported by a recent study of Shin *et al.* (10), who showed in an analysis of data from the Nurses' Health Study that premenopausal women with a daily vitamin D intake of >500 IU had a significantly lower risk (RR=0.72) of breast cancer than those ingesting only 150 IU and less. The importance of adequate vitamin D supply for the prevention of breast cancer had been particularly

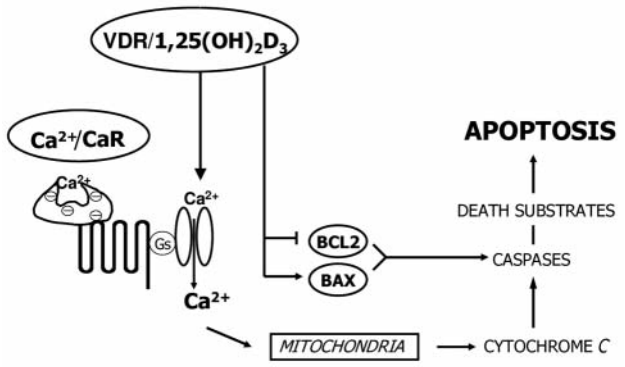

Figure 3. *Proapoptotic signalling from VDR/1,25(OH)$_2$D$_3$ and Ca^{2+}/CaR in MCF-7 breast cancer cells.*

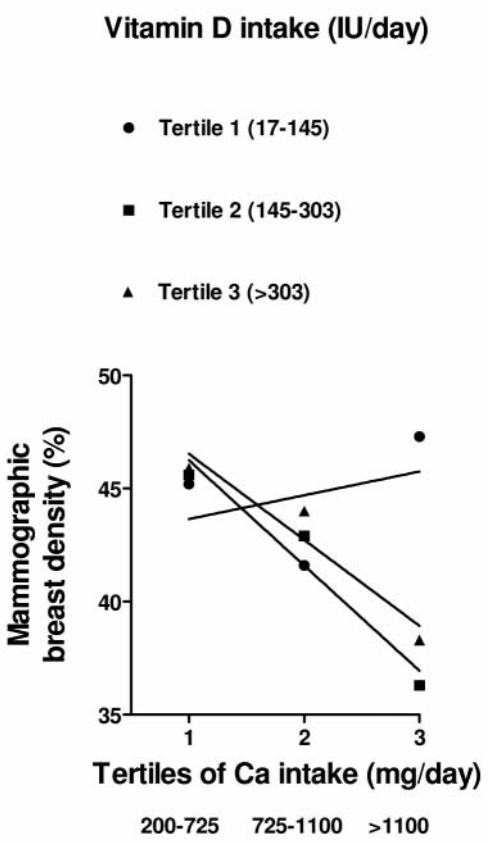

Figure 4. *Effect of Ca intake on breast density in premenopausal women by levels of vitamin D intake. Adapted from Table III in Bérubé et al. (62).*

emphasized by Grant (97-99) and Garland *et al*. (2), who estimated that in the U.S., more than 10% of premature mortality from breast cancer could be attributed to insufficient UV-B radiation.

Lin *et al*. (12) studied the effects of vitamin D and calcium intake from nutrient sources and supplements on breast cancer risk in a large cohort of premenopausal women. They found that higher intakes of total calcium and vitamin D were associated with a lower risk of premenopausal breast cancer (RR=0.61; 95% CI: 0.40-0.92, for calcium, and RR=0.65; 95% CI: 0.42-1.00, for vitamin D intake). McCullough *et al*. (11) analyzed data from nearly 70,000 postmenopausal women participating in the Cancer Prevention Study II Nutrition Cohort and found a moderately lower risk of breast cancer (RR=0.80) with intake of dietary calcium >1,250 mg/day compared to <500 mg/day. This association was even stronger (RR=0.67) in women with estrogen receptor (ER)-positive tumors.

1,25(OH)$_2$D$_3$ exerts antiproliferative effects on breast cancer cells by changing the expression of oncogenes and tumor suppressor genes, such as retinoblastoma tumor suppressor protein, cyclins A1, D1, D3 and E1, as well as cyclin-dependent kinase inhibitors *p21$^{WAF-1/CIP-1}$* and *p27^{kip1}* (72, 100). In addition, 1,25(OH)$_2$D$_3$ induces apoptosis in breast cancer cells by stimulating Ca^{2+} release from intracellular stores. The resulting rise in cytosolic Ca^{2+} triggers calpain-mediated caspase-independent programmed cell death (101).

A role for a functional CaR in breast cancer can be inferred from the fact that in premenopausal women the serum calcium level varies inversely with breast cancer risk in a concentration-dependent manner (102). Both normal and malignant mammary gland epithelial cells are endowed with the CaR (31). However, little is known how the CaR mediates changes in ambient Ca^{2+} to regulate cellular growth. In MCF-7 breast cancer cells, activation of the CaR is transduced into enhanced Ca^{2+} influx across the plasma membrane through non-selective cation channels (103). The resulting increase in intracellular Ca^{2+} may conceivably activate proapoptotic intracellular signaling (63), similar to that caused by 1,25(OH)$_2$D$_3$ (101) (Figure 3).

The effect of the apparent cross-talk between Ca^{2+}/CaR and 1,25(OH)$_2$D$_3$/VDR signaling on cytosolic Ca^{2+} may explain, at least in part, how vitamin D and calcium together efficiently inhibit mammary gland cell growth *in vivo*. Bérubé *et al*. (62) found that combined intake of vitamin D and calcium by pre-menopausal women was superior to separate intakes in reducing mammographic breast density (Figure 4). Synergistic actions of calcium and vitamin D are likely to be the reason why high intake of low-fat dairy products is associated with a reduced risk of breast cancer in premenopausal women (10) (cf. Figure 5).

Prostate cancer. Although there is firm evidence that low 25(OH)D serum levels are associated with increased risk of and mortality from prostate cancer (58, 104), rather conflicting data have been reported on the effect of calcium intake on the incidence and prognosis of prostate cancer.

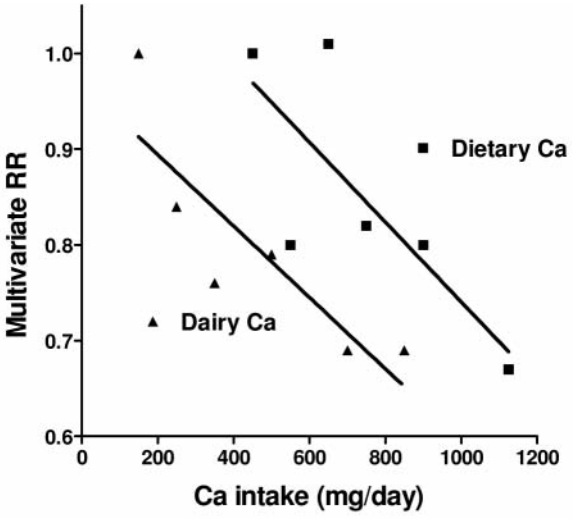

Figure 5. *Effect of Ca from different sources on breast cancer risk in premenopausal women. Data are from Table III in Shin et al. (10).*

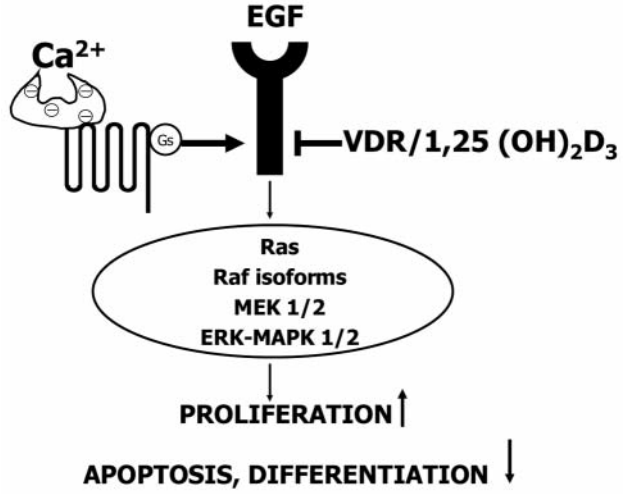

Figure 6. *Antagonistic effects of Ca^{2+} and vitamin D on cellular homeostasis in prostate cancer.*

Giovannucci *et al.* (105) found a positive correlation between calcium intake from food sources and supplements and risk of prostate cancer. Skinner and Schwartz analyzing data from the National Health and Nutrition Examination Surveys I and III found that high serum calcium is associated with an increased risk of fatal prostate cancer though not with incident prostate cancer (106, 107). Any notion that high serum calcium has a direct cancerogenic effect is not supported by the report of Leifsson and Ahren (108) that in men under 50 years of age the risk of obtaining a diagnosis of malignant disease in the future was not found to increase with rising serum Ca^{2+} levels. No effect of calcium intake on prostate cancer risk was seen in two large observational studies (109, 110). In a meta-analysis of 45 observational studies, Huncharek *et al.* (111) found that calcium data from cohort studies were heterogenous. Case control analyses, however, demonstrated no association between calcium and increased risk of prostate cancer. Notably, data from a randomized prospective clinical trial (22) indicated that calcium supplements did not increase but even appeared to lower the incidence of prostate cancer.

These discordant findings on the influence of calcium on prostate cancer risk may be better understood if one considers the possibility of a dual effect of CaR activation on prostate epithelial cell growth. It is not clear whether activation of the CaR on prostate epithelial cells by high calcium will only inhibit cell growth. Due to transactivation by the CaR of the EGFR (112), high calcium concentrations could also induce proliferation and prevent apoptosis (71). In the presence of EGFR agonists, activation of the CaR could then effectively counteract any VDR-mediated growth inhibitory effect of $1,25(OH)_2D_3$ and *vice versa* (Figure 6). We hypothesize that depending on the outcome of CaR-mediated growth modulation, calcium intake could be associated with an increased risk (105), with no risk (109, 110), or even with a reduced risk (22) of prostate cancer.

Other types of cancer. Vitamin D sensitivity has been reported in many malignancies, including endometrial, gastric, ovarian, pancreatic and renal cancer (3, 113-115). Since all of these cancer cells express a functional CaR (29, 32-34), an inverse relation between calcium intake and disease incidence is not unexpected. In a prospective study on a large cohort of post-menopausal women, Prineas *et al.* (13) found that total dietary calcium was an independent predictor of renal cell carcinoma incidence. Women taking >1,280 mg calcium per day had a 35% lower risk of the disease compared to those on less than 800 mg/day. The beneficial effect of calcium supplements on renal cell carcinoma risk particularly in women was confirmed by a case-control study by Hu *et al.* (14). Isolated reports on a risk-reducing effect of nutrient calcium on head and neck, esophageal (4), gastric (15), pancreatic (16), ovarian (17), endometrial (19, 20) cancer certainly need to be confirmed by further studies.

Calcium, Vitamin D and Cancer Prevention

Because Ca^{2+}/CaR and VDR/$1,25(OH)_2D_3$ signaling interact positively in growth control of cancer cells (Figures 1 and 3), it can be expected that an adequate vitamin D status is required to achieve the benefits of high calcium intake and *vice versa*. In fact, there is evidence from epidemiological as well as interventional studies that optimal reduction of

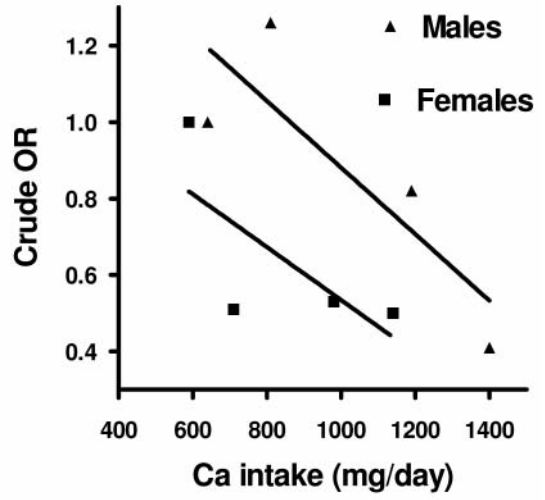

Figure 7. *Differential effect of Ca intake on risk of colon cancer in women and men.*

cancer risk can be achieved only by a high intake of both calcium and vitamin D. For example, in a study on the effect of vitamin D and calcium supplementation on recurrence of colorectal adenomas, Grau *et al*. (116) found that calcium supplementation was only effective in patients if their serum 25(OH)D values were normal. Conversely, high 25(OH)D levels were associated with a reduced risk of adenoma recurrence only among those on calcium supplements. Holt *et al*. (117) gave adenomatous polyp patients high doses of supplemental calcium in combination with vitamin D. After six months of treatment they observed a significant reduction in the rate of polyp formation that was accompanied by an increase in expression of apoptotic markers. Similar results were reported recently by Fedirko *et al*. (118). Cho *et al*. (6) concluded from an analysis of pooled primary data from 10 cohort studies with a follow-up of more than half a million individuals for 6-16 years, that optimal risk reduction for colorectal cancer necessitates high intake levels of both vitamin D and calcium (Figure 2).

It is well known that women are protected particularly from more aggressive colorectal cancer [cf. (119)]. It has been argued that this may be a result of long-time exposure to estrogens before menopause or of hormone replacement therapy thereafter (120, 121). Antiproliferative effects of 17β-estradiol are mediated through the ER-β, which is the predominant ER subtype in the human colon mucosa (122). In addition, there is evidence to suggest that the chemopreventive effect of estrogen against colorectal cancer is mediated in part through VDR-activated antiproliferative intracellular signaling from $1,25(OH)_2D_3$: Preliminary data from our laboratory indicate that 17β-estradiol up-regulates *CYP27B1* expression in human rectal epithelium *in vivo* (unpublished observation). 17β-Estradiol and ER-β-activating phytoestrogens such as genistein have been shown to increase *VDR* and *CYP27B1* expression and activity in human colonocytes (123). Similar effects were seen in MCF-7 breast cancer cells (123) and DU-145 prostate cancer cells (124).

Estrogens stimulate intestinal calcium absorption by a vitamin D-independent mechanism (125) and have thus a positive effect on calcium metabolism in women. This may be the reason that dietary calcium is approximately twice as effective in reducing colon cancer risk in women compared to men (Figure 7). Taken together, by appropriate modulation of vitamin D metabolism and by improving the calcium status, estrogenic compounds have the potential to intensify the antiproliferative actions of vitamin D and calcium. Based on these findings, Cross *et al*. (126, 127) developed the concept of chemoprevention of colorectal, breast and prostate cancer by phytoestrogens, vitamin D and calcium.

Berubé *et al*. (62) concluded from the results of their study on the effects of calcium and/or vitamin D on breast density that increasing the intake of both vitamin D and calcium "may represent a safe and inexpensive strategy for breast cancer prevention". A way to raise calcium and vitamin D intake is by increased consumption of milk and dairy products. There is firm evidence that higher consumption of milk and dairy products reduces the risk of colorectal cancer (128). Studies by Kesse *et al*. (9), Shin *et al*. (10) and McCullough *et al*. (11) strongly suggest that the protective effect of dairy products on colon and breast cancer is due to dietary calcium in combination with some other components in dairy products, one of which could be vitamin D. Figure 5 indicates that calcium is more effective in reducing breast cancer risk when derived from dairy than from other sources. However, it must be noted that milk and dairy products contain not only vitamin D_3 and its biologically more active metabolites but may also contain carcinogenic substances such as fat and fatty acids, insulin-like growth factor and bovine growth hormone (129). Therefore, dairy product consumption, while not a risk factor for breast cancer (129), may be a risk factor for pancreatic cancer (130, 131) and possibly for prostate cancer. Using mortality and ecological data from 41 countries, Grant (132) identified the non-fat portion of milk as the dairy component with the highest association with prostate cancer. This may explain why dairy calcium seems to be associated with a modest risk of non-aggressive prostate cancer (133).

Lappe *et al*. (61) reported evidence from a four-year, population-based, double-blind, randomized placebo-controlled trial that in post-menopausal women combined high-dose calcium (1,500 mg/day) and vitamin D_3 (1,100 IU/day) supplementation reduced the cumulative risk of cancer of the breast, lung, colon, uterus, lymphoid and myeloid system to 0.232 after four years of trial. Survival at the end of the study was significantly higher in the calcium/vitamin D treatment

group compared to the placebo group. This study provides an impressive example of the efficacy of combined calcium and vitamin D supplementation in cancer prevention in general.

We want to emphasize that high intake of vitamin D together with calcium is relevant not only for cancer prevention and, as is well known, for osteoporosis therapy, but has benefits for many other calcium and vitamin D insufficiency-related pathologies (for review see (1)), including infectious, chronic inflammatory and autoimmune diseases as well as incipient and end-stage cardiovascular disorders.

Disclosure

WBG receives funding from the UV Foundation (McLean, VA, USA), the Vitamin D Society (Canada), and the European Sunlight Association (Brussels).

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Received April 15, 2009
Revised June 8, 2009
Accepted June 18, 2009