Symposium: Vitamin D Insufficiency: A Significant Risk Factor in Chronic Diseases and Potential Disease-Specific Biomarkers of Vitamin D Sufficiency

Vitamin D and Calcium in the Prevention of Prostate and Colon Cancer: New Approaches for the Identification of Needs[1]

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ABSTRACT Identification of the mechanisms involved in the pathology of nutrient deficiency provides an understanding of nutrient functions, their role in metabolism, and interactions between nutrients. However, evidence has emerged in recent years that low (suboptimal) intakes of micronutrients are associated with an elevated risk of chronic diseases. The description of micronutrient associations with chronic disease as a deficiency disease does not capture the complexity of these relations. It implies a significant oversimplification of this relation and detracts from the need for development of new approaches to this area of study. Epidemiologic study designs are essential for progress in understanding the micronutrient–chronic-disease relations, and these are described. Two areas wherein epidemiological tools could be incorporated into experimental designs have been vitamin D and prostate cancer, and vitamin D and colon cancer. In each case, biomarkers of exposure, intermediary markers, and mechanisms have been identified and could be implemented in new experimental designs. Measures of exposure would be improved by incorporation of measurements of vitamin D status such as serum 25-hydroxyvitamin D measurements. Several intermediary markers are discussed and may be useful in the characterization of responses. Such developments should aid in the interpretation of studies and identify vitamin D, as well as calcium intakes, that will aid in the prevention of prostate and colon cancer. J. Nutr. 135: 326–331, 2005.

KEY WORDS: • *vitamin D* • *prostate cancer* • *colon cancer* • *biomarkers* • *epidemiology*

Our traditional understanding of nutrition has rested primarily on the concepts of energy metabolism, essential nutrients, and the prevention of deficiency diseases. Nutrients were identified by their ability to prevent deficiency diseases. These diseases generally were relatively acute pathologies (developed in <1 y), recently referred to as short-latency diseases that often occurred early in life and could be prevented or cured by restoration of missing nutrients in the diet (1). The nutrients were essential for life and for the maintenance of normal body function. The deficiency disease concept allowed for relatively straightforward experimental designs, with readily identified end points. Identification of the mechanisms involved in the pathology provided an understanding of nutrient functions, their role in metabolism, and interactions between nutrients. These discoveries of early nutritional research provided for the identification of nutrients that were required for the maintenance of normal body function. The required amounts of which may be described as fulfilling minimal essential functions.

Since these early studies, the range of nutritional research has expanded with the concept that overconsumption of otherwise nutritionally adequate diets, has long-term detrimental effects. Extensive studies suggested associations between excessive intakes of major food constituents and the risk of several cancers, diabetes, heart disease, and stroke. Most of these studies focused on fat, salt, and caloric intakes, and led to several recommendations for lower intakes. The recommendations produced a reduction in some chronic diseases, especially coronary heart disease. Thus, nutritional status was improved by these recommendations.

In another avenue of nutritional research, evidence emerged in recent years that low (suboptimal) intakes of

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micronutrients were associated with an elevated risk of chronic diseases (1). In this case, a low intake often referred to the currently recommended intakes of some nutrients. That is, the currently recommended intakes of some micronutrients appeared suboptimal for the prevention of chronic diseases. Micronutrient intakes, generally at levels above those required for the prevention of deficiency diseases, had been associated with a lower risk of certain chronic diseases. In this case, the associations were interpreted as indicators of a nutritional deficiency disease. The higher intakes of micronutrients may be essential for the maintenance of defense systems, e.g., the immune system, and provision of protective compounds that aid in the prevention of disease and degeneration. Further research is required for the identification of appropriate recommendations.

Suboptimal micronutrient intakes as deficiency diseases

The definition of a suboptimal micronutrient intake depends on a point of reference (index disease) and clear identification of a causal relation. Identification of causal relations was relatively straightforward for traditional deficiency diseases compared with the identification of causal relations for chronic diseases. In the case of traditional deficiency diseases, a disease specific for the nutrient could be defined and relevant experimental models could be identified readily for the testing of causality. It should be recognized that the situation was quite different when the index disease was a chronic disease. Importantly, any particular micronutrient may not have had a unique biochemical function in the prevention of the chronic disease. The micronutrient may have had a generic effect, such as the suppression of cell proliferation, which was influenced by a variety of other factors. That is, other nutrients or physiological factors may be able to essentially substitute for the micronutrient and have the same effect. The implication was that demonstration of a nutrient–chronic-disease relation was highly dependent on the background diet, the background genetics, and the population characteristics. In this context, the micronutrient may be considered semiessential; it was required or effective in some but not all circumstances or persons. Another distinction was that chronic diseases were multifactorial and developed over long periods of time (i.e., long-latency diseases). Thus, epidemiologic study designs were a necessary method for the study of micronutrient-chronic disease relations. Case-control and cohort designs were frequently used for these studies. Prospective studies were generally preferred, because nutrient intakes and blood levels may be influenced by the presence of disease. Clinical and human feeding studies were generally too expensive and tested only a limited range of dietary constituents and/or did not maintain compliance for the timeframes necessary in many instances. These designs were used in the testing of very specific dietary constituents. Another reason for the use of epidemiologic study designs was that the relations being studied were very specific to human populations and did not extrapolate readily from animal models. Nonetheless, animal models were essential for the identification of fundamental mechanisms. Additional factors that may be important were as follows: *1*) the possibility of multiple mechanisms of micronutrient activity; *2*) rates of the index disease; *3*) stage of disease; and *4*) complexity of pathogenesis, adaptive responses, and complexity of the etiology. Each of these factors had to be given consideration in the design of studies of micronutrient–chronic-disease relations. Therefore the demonstration of causality often required large populations and sophisticated epidemiologic designs, as well as independent demonstrations of plausible mechanisms from animal and in vitro studies. Clinical trials generally were necessary for final evaluation of causality and nutrient amounts for the development of recommendations.

The description of micronutrient associations with chronic disease as a deficiency disease did not capture the complexity of these relations. It implied a significant oversimplification of this relation and detracted from the need for development of new approaches to this area of study. It implied that simply increasing the nutrient intake would reduce the risk of a chronic disease. The deficiency-disease label also implied that a single, and perhaps the same, mechanism as was found in the original (short-latency) deficiency disease was active in the prevention of chronic disease. A distinction from these implications should be clearly drawn, with recognition of the need for more information and integration of this information for the interpretation of experiments in this area of investigation.

The design of studies in nutritional epidemiology

Epidemiologic study designs are essential for progress in understanding the micronutrient–chronic-disease relations. However, these designs are not optimal as currently performed for the identification of micronutrient–chronic-disease studies. A major limitation is a lack of validity and reproducibility in exposure measurements. This lack of validity and reproducibility contributes to the inconsistency of results reported for many of the studies of micronutrients and chronic disease. Exposure (dietary intake) is often measured with FFQs. These instruments have an inherently low validity due to inaccurate recording of food intake. The records are influenced by poor subject recall, poor estimation of serving sizes, and incomplete reporting. Recent studies compared FFQ-based estimates of protein, potassium, and sodium with laboratory-based measurements. The studies found correlation coefficients of 0.18–0.34 between FFQ and urinary biomarker measurements of protein, potassium, and sodium in a population of men and women (2,3). The mean reproducibility correlation was 0.64 for men and 0.74 for women. Substantially higher correlations were found for 7-d diary records and urinary measures. Measurements of micronutrients by FFQ methods may be even more inaccurate than those of the major food constituents. Validity studies have found low correlation coefficients between FFQ-based estimates of micronutrient and biochemical measures of nutritional status. The correlation coefficients generally ranged from 0.1 to 0.6 for most micronutrients (4). In brief, FFQ assessments are highly variable and appear useful only in the detection of relatively large differences in dietary intakes. Interpretation of the data also suffers from the presence of numerous dietary covariates for some nutrients and confounding, which has been difficult to resolve in many instances. These findings suggest a need for the incorporation of better exposure markers, which would include the use of food diaries and biochemical markers.

A second area of possible improvement is a need for the incorporation of intermediary (subclinical) markers of disease into nutritional epidemiologic studies. These markers include measures such as coronary artery calcification as an indicator of an elevated risk of coronary heart disease, prostate-specific antigen as an indicator of prostate cancer risk, and aberrant crypt foci as indicators of an elevated risk of colon cancer. Several of these intermediary markers are currently in validation studies. The markers are essential for the demonstration that nutrient effects are specific and have a functional effect on the pathogenesis of the disease of interest. Prevention of early pathogenic lesions is of most concern in disease preven-

tion, and intermediary markers generally involved these early lesions. In some instances, measurements of the intermediary markers may confirm hypothesized mechanisms of micronutrient action. It should be recognized that usually only a small proportion of subjects who were positive for intermediary markers ultimately develop the disease, but they have enhanced levels of risk. Intermediary markers preceded the development of clinical disease and develop over a relatively short timeframe by comparison to clinical disease. This aspect has a major impact on study design, with the requirement of lower subject numbers and shorter timeframes. Further intermediary markers are needed and require validation for nutritional studies. The performance of validation studies has been the major limiting step in the development of most intermediary markers. Another area of investigation related to the nutritional epidemiologic studies was the demonstration of mechanisms of micronutrient action. These frequently are defined by in vitro and animal studies. When possible, these mechanisms should be demonstrated in tightly controlled clinical studies with human subjects. These studies will identify relevant mechanisms, nutrient effects, and associations with intermediary markers of disease.

Taken together, the suggested changes in experimental design should aid in a clearer identification of causal micronutrients in the prevention of chronic disease. Two areas wherein the suggested changes could be incorporated into experimental designs have been vitamin D and prostate cancer, and vitamin D and colon cancer. In each case, biomarkers of exposure, intermediary markers, and mechanisms have been identified and could be implemented in new experimental designs. These areas are given as examples, wherein a new approach and an experimental design could be implemented for clarification of the micronutrient–chronic-disease relation.

Vitamin D and chronic disease

Vitamin D is an example of a micronutrient that is essential for the prevention of deficiency (short-latency) disease and that also may act in the prevention of chronic diseases (1). Vitamin D regulates calcium absorption and is essential for the maintenance of systemic calcium homeostasis. Its effects are very evident during early growth when deficiencies have short-term effects, most notably rickets. Vitamin D intakes and synthesis in the skin are generally adequate for the prevention of rickets and provide for normal bone development. However, vitamin D levels may be marginal in some populations (elderly and women) for the prevention of some long-latency (chronic) diseases, most notably osteoporosis and related bone diseases. Some studies have shown a relation between vitamin D intakes or blood levels and the risk of osteoporosis (5). Numerous approaches have been used in addressing this disease, of which the pathogenesis involved perturbations of known mechanisms of calcium homeostasis.

In recent years, it has been suggested that vitamin D has an even wider range of biological activities, which may influence the development of several chronic diseases, including several cancers, diabetes, and cardiovascular disease (1). A possible association between vitamin D and 2 cancers, prostate cancer and colon cancer, has been of particular interest. These cancer studies illustrate the difficulties of micronutrient–cancer-research and some of the possibilities for inroads to understanding the relations between vitamin D and cancer. Most of the information in this area has been based on observational, generally, case-control or cohort, studies. The first of which was a finding of an association between geographic location and an increased risk of dying from cancer. Those subjects living in the northern regions of the United States had an approximately 2-fold higher risk of dying of cancer than those living in southern regions (6). Recently, several more studies found an association between latitude and the risk of prostate, breast, and colon cancer in the United States and Europe (7–9). In these studies, living at higher latitudes was accompanied by a higher risk of dying of cancer. Presumably, the subjects living further north synthesized less vitamin D, which promoted tumor development. In another study, men with elevated levels of sunlight exposure had a later onset of prostate cancer than those with low levels of sunlight exposure (10). Solar UV-B exposure was inversely related to the risk of dying of cancer in men and women in the United States (11). This effect affected the risk of numerous cancers, such as prostrate, breast, colon, ovarian, non-Hogkins lymphoma, esophageal, stomach, pancreatic, rectal, kidney, corpus uteri, lung, and bladder. These studies provide indirect evidence of a possible causal relation between low vitamin D status and the risk of cancer.

Several limitations can be found in the observational studies of vitamin D and the risk of cancer. First, there was an elevated risk of several cancers with differences in latitude or sunlight exposure. Thus, the relation was not specific for particular cancers, for instance, only those in tissues containing the vitamin D receptor, and raises the possibility that unmeasured lifestyle, dietary, or cultural differences were responsible for differences in risk. This observation illustrates the difficulty of interpretation of these observational studies and the need for extensive collection of lifestyle data. The exposure measures (latitude) are not specific and may be confounded by several factors. Dietary intakes of vitamin D and exposure practices, e.g., the use of sunscreen, may confound exposure estimates (12). This concern can be addressed partially with measurement of blood 25-hydroxyvitamin D [25(OH)D][3] concentration, because this is a good indicator of vitamin D status. It provides an integrated measure of vitamin D from both the diet and in vivo (skin) synthesis. Incorporation of this measure into epidemiologic studies provides a specific estimate of vitamin D status. Vitamin D was measured in a few studies, and an apparent elevation in circulating vitamin D was associated with a lower risk of cancer (6,8).

Many of the studies use cancer mortality as an end point; cancer incidence would be a better indicator. Mortality is influenced by available treatment options and reporting practices. The incorporation of incidence measures and unmeasured confounders may influence the relation between latitude and cancer mortality. The effect of latitude or a low 25(OH)D concentration on the risk of dying of cancer is low (1.5–2.0) in most studies (8). Very few studies have used both measures of 25(OH)D and cancer incidence; additional studies with improved designs are necessary.

In the case of prostate cancer, mixed results have been found in several nested case-control studies. Each of the studies measured 25(OH)D and the active form of vitamin D, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. The results included no association (13), a U-shaped association (14), and an inverse association (15). These findings suggest a complex relation between 25(OH)D and prostate cancer, with the possibility of unmeasured confounders. It has been hypothesized that elevated 25(OH)D concentrations have an autocrine effect on 1,25$(OH)_2$D formation, which has several anticancer activities, including the inhibition cell prolifera-

[3] Abbreviations used: 1,25(OH_2)D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; PSA, prostate-specific antigen.

tion, induction of apoptosis, and induction of cell differentiation. Several studies have demonstrated these activities in prostate cancer cell lines (16–18). Thus, an important question is the following: do elevated 25(OH)D concentrations promote the inhibition of cell proliferation, the induction of apoptosis, and/or the induction of cell differentiation in vivo? Whether in vivo 25(OH)D induces these activities is unknown and is difficult to address experimentally. One approach that may shed light on the in vivo effect of 25(OH)D is an evaluation of the relation between 25(OH)D concentrations and blood prostate-specific antigen (PSA) concentrations. A main advantage of PSA is its very high specificity for prostate cancer. It is a serine protease that is involved in the activation of several cellular polypeptides. Levels of PSA vary between individuals and increase with prostate tumor stage and tumor size. One clinical study found reductions in PSA concentrations after vitamin D treatment in patients with androgen-independent prostate cancer (19). The simultaneous measurement of PSA may provide an in vivo measure of the effect of differences in 25(OH)D concentrations in human populations. This possibility should be explored, because it may provide a better understanding of the factors influencing relations between vitamin D and prostate cancer.

Vitamin D, calcium, and colon cancer

The relations between vitamin D, calcium, and colon cancer have been studied extensively. Colon cancer develops as a result of tissue injury, which is followed by the hyperproliferation of cells, and, in some cases, inflammation. Genetic mutations may be a major consequence of tissue injury and were associated with the initiation of transformed cells (20). These genetic and cellular changes are the first events in steps toward the development of polyps (adenomas). A polyp is a precancerous lesion, which is characterized by high rates of cell proliferation and accumulation. There are several histological types and forms of polyps, with adenomatous polyps being the primary form believed to develop into colon cancer. The polyps develop malignant cells and additional genetic mutation on the pathway to colon cancer (21). A central feature at each of these steps of colon-cancer pathogenesis is an elevation in cell proliferation and loss of growth control. Thus, the inhibition of cell proliferation is a primary target in the prevention colon cancer.

Vitamin D and calcium may have roles in the control of cell proliferation beyond those associated with the maintenance of blood-calcium concentrations. Cell culture studies consistently have shown decreases in cell proliferation in the presence of elevated vitamin D and calcium concentrations (22–28). There are several possible mechanisms of action. $1,25(OH)_2D$ induces cell differentiation and inhibits cell proliferation. It induces changes in gene expression involved in the control of cell proliferation. These include cyclin D1, Kip1, and WAF1, as well as c-Fos, c-Myc, cyclin C, c-JUN, and members of the TBF-beta family (29). Cell differentiation and/or apoptosis are promoted by $1,25(OH)_2D$ through changes in gene expression (30–32). Together, these mechanisms are potent regulators of cell proliferation and may have a strong antitumor activity.

It may be argued that $1,25(OH)_2D$ is tightly controlled, and blood concentrations only respond to changes in calcium status. However, recently, receptors for $1,25(OH)_2D$ have been found in numerous tissues that are not involved in the maintenance of blood-calcium concentrations (33,34). These tissues also contained 1-alpha hydroxylase, which converts 25(OH)D to $1,25(OH)_2D$ (35,36). This production of $1,25(OH)_2D$ was formed in an autocrine or, possibly, a paracrine manner and may not be regulated by the typical feedback control of 1-alpha hydroxylase in the kidney. Thus, local concentrations of $1,25(OH)_2D$ may become elevated with increases in circulating 25(OH)D.

Calcium may act in the regulation of cell proliferation by an indirect route involving the precipitation of fatty acid and bile acids. Calcium was absorbed poorly, with only an uptake of about 30% of dietary calcium by the digestive tract. The excess calcium complexes with free fatty acids and bile acids that otherwise will promote carcinogenesis. Fecal water from calcium-supplemented subjects compared with unsupplemented subjects has a lower cytotoxicity (37) and may aid in preventing tumor promotion. Additional mechanisms may be active, because some studies suggest an interaction between calcium and vitamin D in the inhibition of cell proliferation. This interaction may be the result of common effects on the expression of several genes involved in the regulation of cell proliferation. Extracellular calcium is sensed by a calcium receptor, which transduces a signal along intracellular pathways, e.g., MAPK. These pathways act in the induction of cell proliferation. This activity can be modulated by protein kinase C, which phosphorylates the calcium receptor and blocks the induction of cell proliferation by calcium. The protein kinase C pathway also downregulates cyclin D1 and upregulates WAF1/CIP1 (p21) and KIP1 (p27) (29). These genes were cyclin-dependent kinase inhibitors and inducers of cell differentiation.

Several animal studies have shown a reduction in colonic cell proliferation with the administration of vitamin D (38–41). Colonic cell proliferation was enhanced with the intake of a stress diet (low calcium and high phosphorus-to-calcium ratio). This effect was abrogated by the administration of supplemental dietary vitamin D. Moreover, the vitamin D supplement reduced formation of 1,2-dimethylhydrazine-induced tumors (39). One study suggested an interaction between dietary calcium and vitamin D was necessary for a reduction in cell proliferation and colonic tumors (38).

Epidemiologic studies

The results of epidemiologic studies are mixed, with several finding a significant inverse relation between vitamin D/calcium and several colon cancer-related end points (42) and others not finding any association (43). A nested case-control study found a higher risk of colorectal adenomas among subjects with the lowest plasma concentrations of $1,25(OH)_2D$ and 25(OH)D (44). The concentration–risk relations were not entirely consistent but generally showed lower risk with higher vitamin D concentrations. A Swedish study (569 cases) found significant inverse associations between dietary vitamin D and the risk of colorectal cancer (45). The odds ratios were 0.5 for rectal cancer and 0.6 for colon cancer. No effect of calcium was found on the risk of rectal or colon cancer. Two large U.S. cohorts did not find an association between dietary calcium or vitamin D and the risk of colorectal adenomas (46). Also, a cohort of Iowa women did not find significant associations between dietary calcium or vitamin D and the risk of colon cancer (47). Calcium supplementation decreased rectal epithelial cell proliferation by similar amounts, whether given as calcium tablets or as low-fat dairy foods (48). Subjects received 900 mg/d of calcium in two 4-wk treatment periods. The labeling index of cell proliferation decreased by about 25% with each treatment. An 8-week calcium supplementation study did not find significant changes in colorectal epithelial cell proliferation (43). Subjects were given 1200 mg/d

of supplemental calcium, and biopsy specimens were taken at baseline and after treatment. The thymidine labeling index did not differ between the placebo and the calcium groups, and did not change significantly between baseline and end of the treatment period. A large case-control study (1953 cases) found an inverse association between dietary intakes of calcium and vitamin D with the risk of colorectal cancer (49). The odds ratios were 0.85 and 0.93 for calcium and vitamin D, respectively. Another large case-control study found that a significant decrease in the risk of colon cancer was associated with calcium intake (12). The odds ratio was 0.6 between the highest and the lowest quintile for men and women. A similar relation was found for low-fat dairy product intakes and the use of calcium supplements. No significant relations were found between indicators of vitamin D exposure and the risk of colon cancer. A recent calcium supplementation study found significant interactions between calcium and vitamin D in the prevention of colorectal adenoma recurrence. The supplemented group was given 1200 mg of calcium per day for 4 years (42). A significant reduction in adenoma occurrence was found in the supplemented group. Serum 25(OH)D concentrations were inversely associated with a protective effect but only in the treatment group. No association was found between serum 25(OH)D concentrations and colorectal adenoma recurrence in the placebo group. The findings suggested that the vitamin D effect only occurred in the presence of high dietary-calcium intakes and that vitamin D did not have an independent effect. Taken together, these results suggest the possible presence of unmeasured confounders and the possible dependence of a response on background diet, genetics, and other population characteristics.

New approaches for vitamin D, calcium, and colon cancer

Studies in this area may benefit from better measures of exposure, a better understanding of interactions between mechanisms of vitamin D and calcium action, and characterization of in vivo cellular responses, intermediary markers. The measures of exposure would be improved by incorporation of 25(OH)D measurements. This measure was incorporated into several studies and may be expanded for the inclusion of replicate measurements to reduce misclassification of exposure and to provide an indication of the timeframe for changes in exposure. Additional mechanistic studies may define the circumstances required for interactions between vitamin D and calcium in the regulation of cell proliferation. These experiments could be performed with cell culture and animal models.

Further characterization of in vivo cellular responses may provide insights regarding the relation between calcium, vitamin D, and the risk of colon cancer. The mainstay of this area has been polyp formation and recurrence. The main limitation of this indicator is the slow rate of recurrence and formation. Vitamin D and calcium also may alter cell proliferation, apoptosis, and cell differentiation. The in vivo effects of vitamin D and calcium remain largely unknown for these colon cancer-related parameters.

Several intermediary markers have been proposed and may be useful in the characterization of responses. These include aberrant crypt foci (presence of abnormal morphology), Ki67 (indicator of cell proliferation), markers of apoptosis, and indicators of cellular differentiation (lectin-binding proteins, mucin distribution) (50). Many of these markers still require substantial validation, but the application of these techniques is becoming more feasible for use in human studies. Measurement of these intermediary markers may provide a basis for understanding the inconsistent relations between vitamin D, calcium, and colon cancer in human populations.

Summary

In recent years, low intakes of micronutrients, e.g., vitamin D, have been associated with an elevated risk of certain chronic diseases, including prostate and colon cancer. Many believe that intakes of vitamin D may be suboptimal for the prevention of these diseases. Substantial cell culture and animal data support an anticancer effect for vitamin D and calcium in both prostate and colon cancer. Whether these activities occur in humans is uncertain, because epidemiologic studies have yielded mixed results. The results suggest a complex relation between vitamin D/calcium and the risk of prostate and colon cancer, wherein the effect may be dependent on background diet, lifestyle, genetics, and other population characteristics. A better understanding of the relation between vitamin D/calcium and prostate and colon cancer may be gained with changes in methodology and experimental design of nutritional epidemiologic studies. These changes would include further studies of mechanisms, better indicators of exposure, and the development of intermediary markers. Taken together, developments in these areas should aid in the interpretation of studies and would identify vitamin D and calcium intakes that will aid in the prevention of prostate and colon cancer.

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