Vitamin D and cancer: current dilemmas and future research needs\textsuperscript{1–3}

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ABSTRACT
A diversity of scientific literature supports a role for vitamin D in decreasing colorectal cancer incidence, but the available evidence provides only limited support for an inverse association between vitamin D status and the risk of other types of cancer. We need additional studies analyzing the dose-response relation between vitamin D status and cancer risk, the optimal level of 25-hydroxyvitamin D, the length of time required to observe an effect, and the time period of life when exposure is most relevant. Studies of vitamin D receptor polymorphisms have found that not all polymorphisms have the same association with cancer, and the cancer site could further dictate which polymorphisms might be most important; this indicates a need for more research on gene-environment interactions. Several dietary components and the balance between energy intake and expenditure influence vitamin D metabolism. These studies show that scientists need to identify confounders and modifiers of the biological response to vitamin D, including dietary factors, lifestyle factors such as exercise, and race or ethnicity. Transgenic and knockout animals are powerful tools for identifying the molecular targets of bioactive food components. Scientists should therefore make increased use of these models to identify molecular targets for vitamin D. Many research gaps relate to the need to develop predictive, validated, and sensitive biomarkers, including biomarkers that researchers can use to reliably evaluate intake or exposure to vitamin D, assess one or more specific biological effects that are linked to cancer, and effectively predict individual susceptibility as a function of nutrient-nutrient interactions and genetics.

INTRODUCTION
Interest in the potential role of vitamin D in cancer prevention continues to grow among scientists, policy makers, consumers, and the media. The National Cancer Institute and the Office of Dietary Supplements of the National Institutes of Health held a conference, “Vitamin D and Cancer: Current Dilemmas/Future Needs,” on May 7–8, 2007, to assess the strengths and weaknesses of the evidence for the relation between vitamin D status and risks of developing or surviving various types of cancer, to identify gaps in knowledge, and to identify the research needed to make science-based recommendations for intake or exposure for cancer prevention (1). Conference topics included the relative importance of sunlight exposure and diet as sources of vitamin D, the epidemiologic evidence of the nature and strength of the association between vitamin D and cancer risk, how nutrigenetics has advanced our understanding of the relation between vitamin D and cancer risk, new information about the role of genes and cross-talk among genes that could influence the response to vitamin D, the interrelation between vitamin D and other dietary components that could modify the response, and information from preclinical models about the relation between vitamin D and cancer (1). This article provides a synopsis of some of the evidence presented at the conference and many of the questions that research still needs to address.

HOW STRONG IS THE EVIDENCE THAT VITAMIN D STATUS IS RELATED TO CANCER RISK?
Ecologic or geographical correlation studies were the first to offer evidence for the cancer-protective effects of vitamin D when they showed an inverse association between ultraviolet (UV) radiation exposure and cancer incidence or mortality (2). Because UV radiation can lead to vitamin D formation in the skin, this led to the hypothesis that vitamin D or one of its metabolites—25-hydroxyvitamin D [25(OH)D] or 1,25-dihydroxyvitamin D [1,25(OH)\textsubscript{2}D]—could be protective against cancer.

However, scientific acceptance of the hypothesis that vitamin D metabolites inhibit the development of cancer faced several obstacles (3). Unlike serum concentrations of 25(OH)D, which decrease with distance from the equator and are lower among persons with dark pigmentation, serum concentrations of 1,25(OH)\textsubscript{2}D are tightly regulated and do not vary with geographic latitude or race (4). The demonstration that many tissues in addition to the kidney possess the enzyme 25(OH)D-1-α-hydroxylase (CYP27B1) and, like the kidney, synthesize 1,25(OH)\textsubscript{2}D from circulating concentrations of 25(OH)D eliminated this concern (Figure 1; 3). Research has subsequently shown the autocrine synthesis of 1,25(OH)\textsubscript{2}D by many other organs, such as the prostate, colon, breast, and pancreas. This is the presumed mechanism whereby vitamin D or sunlight influences the development of cancer at these sites (5).

Measuring sunlight and UV exposure in human populations is exceedingly complex (6). Moreover, studies have not validated
measures of UV exposure or vitamin D status at an individual level (6). Thus, we need improved assessment tools to quantify UV exposure and better identification and measurement of potential confounding factors, such as the use of sunscreen, the amount of clothing worn, skin pigmentation, and medical conditions. Also, sunlight exposure might not be a viable prevention strategy because it can increase skin cancer risk, so the principal focus of this conference was on dietary vitamin D.

Evidence indicates that dietary vitamin D is protective against cancer. A diversity of scientific literature, including in vitro, animal, ecologic, and epidemiologic studies, supports a role for vitamin D in decreasing colorectal cancer incidence (7–12). In a recent meta-analysis of studies that examined serum 25(OH)D vitamin D in decreasing colorectal cancer incidence (7–12). In a animal, ecologic, and epidemiologic studies, supports a role for cancer. A diversity of scientific literature, including in vitro, focus of this conference was on dietary vitamin D.

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Some evidence indicates that vitamin D might promote cancer risk in some individuals. For example, a recent study suggested that higher baseline vitamin D status was associated with a 3-fold higher risk of pancreatic cancer incidence in Finnish smokers (highest versus lowest quintile, >65.5 versus <32.0 nmol/L; odds ratio: 2.92; 95% CI: 1.56, 5.48; P for trend = 0.001; 15). We need a better understanding of vulnerable individuals who could be placed at risk by higher vitamin D exposure.

Most scientists believe that the gold standard for research is a controlled intervention study. Recently, a double-blind, placebo-controlled trial showed that the combination of vitamin D and calcium reduced cancer incidence by >75% (16). Although these results are impressive, they are based on a secondary analysis from a study investigating indicators of bone health, and no treatment group received vitamin D only. Furthermore, the sample size was underpowered to determine whether vitamin D and calcium had differential effects on different cancer sites. Clearly, we need additional intervention studies to verify this response and to determine whether the response varies by specific tissue, age, sex, and duration of exposure.

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the start codon. Absence of the Fok I site (denoted F) results in a shorter VDR protein with higher transcriptional activity and therefore higher biological activity of the protein (17). Research has linked the Fok I f allele to decreased calcium absorption (18). Whereas dietary calcium was not important in determining colon cancer risk in individuals with the FF genotype for the VDR, low dietary calcium increased colon cancer risk with increasing copies of the f allele for the VDR (19). It is interesting that those individuals with the lowest calcium absorption because of a genetic polymorphism are the most sensitive to increased colon cancer risk with low dietary calcium.

Studies have investigated VDR polymorphisms in connection with many different types of cancer, including prostate, colorectal, bladder, breast, and melanoma (20). However, not all polymorphisms have the same association with cancer, and the cancer site could further dictate which polymorphisms might be the most important (20). The most extensively studied single nucleotide polymorphisms have been in the 3'end (BsmI, ApaI, and TaqI); these are in linkage disequilibrium (17). Unfortunately, design issues and lack of statistical power have compromised many of these studies (17).

More recent studies have investigated the relation between polymorphisms in the promoter region, such as the Cdx-2, which modulates transcription of the VDR gene expression, and GATA 3436 G→T, which influences protein-DNA complex formation (17). Studies have linked these promoter region polymorphisms to increased prostate cancer risk (21). Moreover, many different types of environmental exposures can modify the relation between VDR polymorphisms and cancer risk. For example, sunlight exposure and dietary calcium, vitamin D, energy, and fat intake could modify the association between VDR genotype and cancer risk (22). Therefore, we need a better understanding of gene-environment interactions.

WHAT OTHER GENES DETERMINE THE BIOLOGICAL RESPONSE TO VITAMIN D?

From a mechanistic standpoint, investigators are increasingly realizing that many tissues in addition to the kidney contain enzymes that metabolize 25(OH)D (Figure 1). However, extra-renal 1,25(OH)2D synthesis influences local cell proliferation and differentiation and depends on serum 25(OH)D concentrations (23). Moreover, parathyroid hormone does not regulate the renal 1,25(OH)2D synthesis from 25(OH)D; however, we do not know whether this reflects a direct mechanism for this effect, hypotheses include sequestration in obesity is associated with lower circulating concentrations of 25(OH)D (27). Although researchers have not identified the mechanism for this effect, hypotheses include sequestration in fat, negative feedback from higher circulating 1,25(OH)2D concentrations in obesity, and lower sun exposure due to avoidance of outdoor activity by the obese (27). Higher physical activity levels are linked to higher circulating concentrations of 25(OH)D; however, we do not know whether this reflects a direct relation between physical activity and vitamin D metabolism or is a result of confounding by body fat or sun exposure. Furthermore, the relations between both vitamin D status and obesity and vitamin D status and physical activity were stronger in whites than in African Americans (27). These types of relations highlight the importance of identifying confounders and modifiers of the biological response to vitamin D, including dietary factors, lifestyle factors such as exercise, and race/ethnicity.

WHAT ARE THE IMPORTANT DIETARY COMPONENTS THAT MODIFY THE EFFECT OF VITAMIN D?

Several dietary components and the balance between energy intake and expenditure influence vitamin D metabolism. Although research has shown that low serum calcium concentrations stimulate renal 1,25(OH)2D synthesis from 25(OH)D, evidence presented at the conference indicated that dietary genistein, an important bioactive component of soy, can also influence the metabolism of 25(OH)D and its biological effects. Genistein inhibits CYP24 activity, which, in turn, increases the production and serum half-life of 1,25(OH)2D and the expression of the VDR (26). Dietary folate can also inhibit CYP24 activity by increasing the methylation status of the promoter region and by down-regulating expression of the gene (25). These results show that establishing a definitive concentration of 25(OH)D will be very difficult because multiple dietary components can influence the metabolism of 25(OH)D. Researchers must also therefore consider nutrient-nutrient interactions.

Vitamin D status is also related to body fat and physical activity. Several large observational studies, including the third National Health and Nutrition Examination Survey, showed that obesity is associated with lower circulating concentrations of 25(OH)D (27). Although researchers have not identified the mechanism for this effect, hypotheses include sequestration in fat, negative feedback from higher circulating 1,25(OH)2D concentrations in obesity, and lower sun exposure due to avoidance of outdoor activity by the obese (27). Higher physical activity levels are linked to higher circulating concentrations of 25(OH)D; however, we do not know whether this reflects a direct relation between physical activity and vitamin D metabolism or is a result of confounding by body fat or sun exposure. Furthermore, the relations between both vitamin D status and obesity and vitamin D status and physical activity were stronger in whites than in African Americans (27). These types of relations highlight the importance of identifying confounders and modifiers of the biological response to vitamin D, including dietary factors, lifestyle factors such as exercise, and race/ethnicity.
WHAT INFORMATION HAVE PRECLINICAL MODELS PROVIDED ABOUT THE RELATION BETWEEN VITAMIN D, CALCIUM, AND CANCER?

Transgenic and knockout animals are powerful tools for identifying the molecular targets of bioactive food components. For example, VDR knockout animals provide a tool for evaluating the potential biological effects of vitamin D that are not mediated through the VDR. Studies using these animals have clearly demonstrated the importance of the VDR in influencing cancer risk. VDR knockout animals have increased numbers of chemically induced tumors in many (mammary, prostate, skin, and colon) but not all (ovary, liver, lung, and uterus) organs (28). Interestingly, supplemental vitamin D had no effect on mammary tumor development in VDR knockout animals with the neu protooncogene, which suggests that the VDR must be present for vitamin D to exert its cancer-protective effects (28). These types of studies demonstrate the need for increased use of transgenic and knockout animal models to identify molecular targets for vitamin D.

Typically, rodents do not develop colon cancer. However, when mice eat a diet similar to that of humans, a so-called Western diet that is high in fat and phosphorous and low in vitamin D, calcium, fiber, choline, methionine, and folate, approximately 25% spontaneously develop colon cancer in the absence of carcinogen treatment (29). If researchers supplement the Western diet with calcium and vitamin D, both colon tumor incidence and frequency decrease significantly. This suggests that vitamin D is protective against cancer and that this is a new mouse model of sporadic colon cancer (29).

Investigators are using transcriptomic or microarray approaches to understand the molecular targets for 1,25(OH)2D in both cell culture and animal models (29, 30). Researchers have generated gene expression profiles for 1,25(OH)2D treatment in both classic (ie, bone, kidney, intestine, Caco-2, and ROS/17/2.8) and nonclassic (ie, HL-60, squamous cell carcinoma, B, and prostate epithelial) cells (30). These studies have identified a large number of genes that are differentially expressed. However, for meaningful interpretation of this plethora of information, researchers will need to compare studies to identify particular genes targeted by vitamin D by using functional annotation to define the processes involved. They also need to assign these processes to known metabolic and signaling pathways when possible (30).

Researchers successfully applied a comparative microarray approach in 3 different mouse models of colon cancer (APC1638+/−, Muc2−/−, and wild-type C57Bl6 mice) fed a Western diet (29). In all 3 models, increasing dietary calcium and vitamin D was effective in inhibiting tumor formation. Moreover, gene expression profiling of the colonic mucosa in each model identified genes and pathways that track dietary calcium and vitamin D and, hence, also track the probability of tumor formation (29). Results of studies with these models reveal genes and pathways that commonly respond to calcium and vitamin D in all 3 models and other genes and pathways specific to 1 of the 3 models.

FUTURE RESEARCH DIRECTIONS

Many of the research gaps that participants identified at the May 2007 conference related to the need to develop predictive, valid, and sensitive biomarkers. These include biomarkers that researchers can use to reliably evaluate intake or exposure to vitamin D, assess one or more specific biological effects that are linked to cancer, and effectively predict individual susceptibility as a function of nutrient-nutrient interactions and genetics (Figure 2). This information is fundamental to evaluating who will benefit most from, and who might be placed at risk by, increased vitamin D intake or exposure.

First, we need a better understanding of biomarkers of exposure, including improved databases for measuring dietary and supplemental vitamin D intake. Although researchers usually use 25(OH)D as a biomarker of vitamin D status, laboratory analysis results vary widely depending on the assay used (31). Furthermore, no standards are currently available and a one-time analysis probably does not reflect long-term exposure because of wide seasonal variability. Many other dietary factors, such as calcium, folate, and soy, can modify the metabolism of 25(OH)D. Thus, we do not understand the relation between serum 25(OH)D and tissue exposure to 1,25(OH)2D. We also need more research on the optimal concentration of 25(OH)D for cancer prevention, whether this varies among various racial and ethnic groups, whether it varies by life cycle stage, and whether it changes during the transition from a healthy to a diseased state.

Second, we need a better understanding of susceptibility biomarkers. Although research has linked many different polymorphisms in the VDR to cancer risk, we need additional information about gene-environment interactions and organ specificity. We need more research on the relation between polymorphisms in other genes in the vitamin D metabolic pathway (ie, CYP24 and CYP27B1), vitamin D status, and cancer risk. In addition, because obese individuals and African Americans have lower 25(OH)D concentrations than do other populations, studies need to determine whether this contributes to their increased cancer susceptibility. Additional studies should address whether persons with cancer respond to vitamin D in the same way as persons without cancer and, if so, whether their response depends on their cancer type.

Finally, we still need a better understanding of the molecular targets for vitamin D. Researchers should use various “omics”
technologies, such as transcriptomics, proteomics, and metabolomics, to elucidate the mechanism of action of vitamin D in both animal models and normal versus malignant tissues of human subjects. We need additional information to determine whether the biological response differs among different tissues and between normal and neoplastic tissue.

Although a wealth of information is available from preclinical and epidemiologic studies, we need more controlled intervention studies. We also need to evaluate potential adverse effects of long-term, high-dose vitamin D exposure, including the dose of vitamin D associated with toxicity. For example, research should show whether this varies by age, race, sex, body size, and health status. Finally, we need to understand better potential adverse outcomes and how to best monitor them; animal models could be useful for this purpose. Thus, although the current body of evidence is intriguing, many unanswered questions remain.

The author had no conflicts of interest.

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