Epidemiological Studies of Vitamin D and Breast Cancer

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Abundant experimental evidence provides a rationale for examining the role of vitamin D in relation to breast cancer risk. Specifically, the main actions of the biologically active form of vitamin D, $1,25(OH)_2D$, are mediated via the vitamin D receptor.[1,2] The vitamin D receptor (VDR) is present in normal breast tissue. $1,25(OH)_2D$ has antiproliferative effects on and promotes the differentiation of breast cancer cells. In MCF-7 cells, vitamin D and vitamin D analogs have been shown to induce cell cycle arrest and apoptosis, to downregulate estrogen receptor expression, to limit responsiveness to the mitogenic effects of 17beta-estradiol, and to limit induction of the progesterone receptor.

Epidemiologic evidence bearing on the relationship between vitamin D and breast cancer risk has come from several sources: ecologic studies, studies of vitamin D in relation to breast density, studies of VDR polymorphisms and breast cancer risk, studies of circulating vitamin D levels and risk, and studies of dietary and supplemental vitamin D intake and risk.

There have been several ecologic studies of the association between sunlight or solar radiation exposure and breast cancer incidence or mortality.[2] These are of interest because synthesis in the skin resulting from exposure to sunlight (in particular, exposure to UV-B radiation) is the major source of endogenous vitamin D production in humans,[3] given that few food sources contain vitamin D. For example, one study showed a strong inverse association between per capita average annual total solar energy exposure (calories/cm^2/d) and breast cancer incidence rates in republics of the former Soviet Union.[4] However, as is well known, ecologic studies suffer from a number of limitations, including the fact that because measurements are averaged over individuals, associations observed at the population level may not reflect associations at the individual level and generally do not account for potential confounding. Therefore, this type of information is suggestive (hypothesis-generating) at best.

Mammographic (breast) density reflects the epithelial and stromal components of the breast; fat appears dark and epithelium and stroma appear light or white.[5] Women with very dense breast tissue, as seen on mammography, are at increased risk of subsequent breast cancer. Mammographic density can be modified, and such changes represent a potential biological marker for assessing the effects of dietary/supplemental factors on breast cancer risk. Four studies have examined the association between vitamin D and breast density.[6-9] Of these, two showed an inverse association between vitamin D intake and breast density in premenopausal women only (one focused on vitamin D from foods only[7] and the other on total vitamin D intake from foods and supplements),[9] one study showed an inverse association in both premenopausal and postmenopausal women (dietary vitamin D only[8]), and one study showed no association.[6] However, all of these studies were cross-sectional, which limits the inferences that can be drawn. Interestingly, in one study,[9] the association was independent of sunlight exposure but weakened after adjustment for calcium intake, highlighting the need to include assessment of calcium intake in studies of vitamin D. A recent cross-sectional study showed no association between serum 25-hydroxyvitamin D levels and mammographic density.[10]

The vitamin D receptor is a nuclear transcription regulating factor[11] that is the crucial mediator of the cellular growth and differentiation effects of vitamin D.[12] It is expressed in normal and malignant breast cells.[13] Genetic polymorphisms in the VDR might influence breast cancer risk due to their effects on VDR gene expression and protein function.[2] Of the polymorphisms that have been identified in the VDR gene (largely through their restriction endonuclease cleavage sites), Fok1, Bsm1, Apa1, Taq1, and Poly(A) have been studied most frequently.[2] Whereas the Fok1 polymorphism (a T-to-C transition in exon 2) has functional consequences, the functional significance of the Bsm1, Apa1, Taq1, and Poly(A) polymorphisms, located at the 3′ end of the

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doi: 10.1301/nr.2007.aug.S80–S83

VDR gene and in strong linkage disequilibrium, is not well understood. However, the length polymorphism Poly(A) may influence the transcriptional efficiency and stability of VDR mRNA.[2] To date, there have been 14 studies that have examined VDR polymorphisms in relation to breast cancer risk.[2,14] Three of the studies were nested within cohorts, while the remainder were either hospital- or population-based case-control studies. In brief, the results of studies to date for all of the aforementioned polymorphisms have been inconsistent, perhaps reflecting study design issues (e.g., small sample size for many of the studies, potential selection bias in case-control studies, and failure to control for potential confounding factors in some studies) and intrinsic population differences (i.e., between-population differences in the extent of linkage disequilibrium).[2]

Circulating levels of vitamin D are directly related to dietary vitamin D intake and cutaneous synthesis of vitamin D.[15] The active form of vitamin D, 1,25-dihydroxyvitamin D, abbreviated $1,25(OH)_2D$, is produced by hydroxylation of the major circulating form of vitamin D, 25-hydroxy vitamin D, abbreviated 25(OH)D, a reaction catalyzed by the enzyme 25-hydroxy vitamin D-1α-hydroxylase.[15] $1,25(OH)_2D$ is produced in the breast (amongst other anatomic sites, including the kidney, colon, and prostate), and the extent of its production there is probably dependent upon the availability of 25(OH)D for 1α-hydroxylation. Therefore, it has been hypothesized that low circulating levels of 25(OH)D might impair local production of $1,25(OH)_2D$ in breast tissue and thereby increase risk of breast cancer.[15] To date, there have been four studies of circulating levels of vitamin D in relation to breast cancer risk. Of the two studies that reported results for 25(OH)D, the hospital-based case-control study of Lowe et al.[15] showed a strong inverse association, whereas the nested case-control study of Bertone-Johnson et al.[16] showed only a weak, statistically non-significant inverse association with risk. The latter study also showed a weak, statistically non-significant inverse association with $1,25(OH)_2D$. Similar discrepancies were observed with respect to the results of the remaining two studies, which focused on $1,25(OH)_2D$: the hospital-based case-control study of Janowsky et al.[17] showed a strong, inverse association, whereas the nested case-control study of Hiatt et al.[18] showed no association. The discrepancies between the results of the two types of study designs raises the possibility that the results of the retrospective case-control studies were influenced by effects of breast cancer on circulating vitamin D levels. Furthermore, an individual's serum level of 25(OH)D represents short-term vitamin D exposure during the preceding few months at most[19]; a single measure may not reflect habitual long-term exposure.[20]

Dairy products have constituents that been postulated to increase breast cancer risk and others that have been postulated to decrease risk; the former include relatively high total and saturated fat intake and the presence in milk of contaminants (e.g., pesticides) and of growth factors such as insulin-like growth factor (IGF-I); the latter include conjugated linoleic acid, calcium, and vitamin D. There have been many (about 50) epidemiologic studies of the association between intake of dairy products and breast cancer risk, and, overall, the results of these studies have been inconsistent [21]

Fewer studies have focused on the role of vitamin D intake in the etiology of breast cancer.[2] To date, there have been 10 studies of the relationship between vitamin D intake and breast cancer risk, five case-control studies[22-26] and five cohort studies.[20,26-29] Of these, two cohort studies addressed diet in adolescence in relation to subsequent breast cancer risk.[28,29] Neither showed an association with vitamin D intake, but both involved recall of diet in the distant past, raising concerns about the validity of the exposure measurements. The case-control study of Knight et al.[26] examined sunlight exposure and intake of vitamin D-rich foods as well as vitamin D supplements at various ages (10–19, 20–29, 45–54 years). Sun exposure and use of vitamin D supplements or multivitamins between ages 10 to 19 and 20 to 29 were associated with reduced risk of breast cancer.

The remaining studies focused on diet in adulthood. Of these, the hospital-based case-control study of Nunez et al.[23] and the cohort studies of John et al.,[20] Shin et al.,[27] and McCullough et al.[30] provided some evidence for inverse associations, while the results of most of the remaining studies were essentially null. The study of John et al.,[20] conducted in NHANES I, did not show an association for vitamin D overall, but did suggest that the combination of high solar radiation exposure and relatively high dietary vitamin D intake (3200 IU/d) was associated with a small reduction in risk (RR = 0.71, 95% CI = 0.44–1.14), but this was not statistically significant. However, the number of cases of breast cancer was relatively small, the estimate of vitamin D intake was based on a 24-hour recall (which probably does not represent an individual's usual intake), and uncontrolled confounding may also have been a problem. In the study by Shin et al.,[27] total vitamin D intake was associated with a reduction in risk in premenopausal women (RR = 0.72, 95%CI = 0.55–0.94, for the highest vs. the lowest quintile level), and dietary vitamin D intake was associated with a similar reduction in risk of borderline statistical significance. The estimates were adjusted for history of outdoor sun exposure and participant's residential area, but the measure of sun exposure was rather crude. Furthermore, supplemental vitamin D intake was associated with a weak, statistically non-

significant reduction in risk in premenopausal women, particularly in the low dietary vitamin D intake group. This study involved use of a comprehensive, validated food frequency questionnaire that was completed by the participants several times over the course of the study, and estimates of cumulative average intake were related to risk. In the study of McCullough et al.,[30] there was no association between dietary or total vitamin D intake and risk in postmenopausal women; however, there was a small reduction in risk in association with dietary vitamin D intake in women from states with lower UV exposure (RR = 0.81, 95% CI = 0.67–0.97). A limitation of this study is that sun exposure was not assessed.

So, what does the currently available evidence show?

- Ecologic studies have suggested that an inverse association exists between sunlight or solar radiation exposure and breast cancer incidence rates.
- Cross-sectional studies have shown an inverse association between vitamin D intake and mammographic density, predominantly in premenopausal women.
- Studies of VDR polymorphisms and breast cancer risk have been inconclusive.
- Studies of circulating levels of vitamin D and risk have mostly been null.
- Studies of vitamin D intake and risk have been inconclusive.

Overall, despite abundant experimental evidence in support of an inverse association between vitamin D and breast cancer risk, the available epidemiologic evidence provides, at best, limited support for such an association. Establishing an independent association between vitamin D intake and breast cancer risk from observational epidemiologic studies will be challenging for a number of reasons, relating mostly to assessment of vitamin D status. Given that only a few foods contain vitamin D, exposure to UV-B radiation is the major source of vitamin D in humans.[3] Therefore, there is a need for studies using validated methods for collecting quantitative information on the intensity and duration of sunlight exposure, as well as on other factors that influence vitamin D synthesis, such as skin pigmentation, use of sunscreen and protective clothing, medical conditions, and medications.[20] Furthermore, a comprehensive assessment of vitamin D status will also require consideration of the determinants of circulating 25(OH)D, the principal form of circulating vitamin D, and $25(OH)_2D$, the form of vitamin D that is most active biologically. Among these are: glycemic index, which may affect cellular uptake of calcium,[27] the major known, modifiable determinant of $1,25(OH)_2D$ levels[31]; retinol, which can antagonize the actions of vitamin D[31]; and body mass index, which is inversely associated with serum levels of 25(OH)D, probably as a result of decreased bioavailability of 25(OH)D due to its deposition in body fat compartments.[32] Finally, the association between vitamin D status and risk might vary according to VDR genotype. These are issues that have not been addressed comprehensively in most studies to date.

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