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MECHANISTIC HYPOTHESIS FOR THE INTERACTION OF DIETARY FAT, CALCIUM, AND VITAMIN D IN BREAST CANCER

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ABSTRACT. HIGH DIETARY FAT is one of several factors correlated with increased incidence of breast cancer in inter-country population studies. Laboratory animal studies suggest that high dietary fat acts as a promoter of chemical carcinogen-induced mammary cancer, and high fat diet increases epithelial cell proliferation of the mammary ducts, particularly in the small terminal ducts. The hypothesis proposes that high fat diet increases fat secretion into the small ducts leading from the terminal end buds. In non-lactating women, particularly nulliparous women, a milk-like fluid, moving very slowly in the duct can be partly metabolized, producing long chain free fatty acids (FFA's) from the secreted fat. These FFA's can be highly cytotoxic to local epithelial cells, resulting in cell damage and necrosis, followed by increased cell proliferation. The mechanism of FFA cytotoxicity has been studied in the colon, and shown to be related to the potent sequestration of calcium from the cells by the FFA's. In the colon, increased dietary calcium can readily prevent colon lumenal FFA's from these cytotoxic and hyperproliferative effects. In the breast, increase of local calcium availability from the systemic circulation depends to a greater extent on availability of calcitriol, the active metabolite of vitamin D. An inverse epidemiological correlation has been developed between sunlight availability as a source of vitamin D and the risk of breast cancer in the U.S. and Canada. A large scale prospective observation study in over 88,000 women for 16 years (the Nurses Health Study) lends support to this hypothesis. Reduction of breast cancer risk, and simultaneously osteoporosis, might be achieved by increasing dietary intake of calcium and vitamin D. This may be particularly applicable to females during the rapid growth during puberty and adolescence.

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1. INTRODUCTION

Over the last few decades several epidemiological and experimental animal studies have suggested that decreased calcium and vitamin D intake coupled with high dietary fat are associated with increased risk of mammary gland carcinogenesis (see ref. [1] for a review). The rationale for several of these studies was: *(a)* the recognition of some similarities between the epidemiological pattern of mammary cancer to that of colon cancer, and *(b)* the recognition that increased dietary calcium and vitamin D could attenuate the promotion effect of high fat diets on colon cancer. A mechanistic hypothesis for the interaction of high fat, calcium, and vitamin D in the breast was developed in the early 1980s. However, this was never previously published (in any of the colon studies) and is now presented here.

2. BACKGROUND

A summary of known or postulated associations with risk of breast cancer is shown below:

a. EPIDEMIOLOGY

- Increased incidence in postmenopausal women,
- Increased incidence with age,
- Higher incidence in nulliparous women (e.g., nuns). There is a decreased risk with increasing parity [2],
- Increased risk with later age at first birth (>30 versus <20 years of age),
- Decreased risk with breast feeding infants,
- Increased risk with early age of menarche (<12 years versus >15 years of age).

b. DIETARY

High dietary fat [3-8] leads to increased risk for development of cancer. In laboratory animal studies, high dietary fat acts a "promoter" of chemical carcinogen-induced breast cancer.

c. PATHOLOGY

In his classic textbook on pathology [2], Boyd indicates that retention of secretion in the breast is one of the significant causal factors of carcinoma of the breast: "Retention of Secretion: Breast drainage may be interfered with as the result of anomalies of the duct or a plug of desquamated cells in the duct. The breast of the typical spinster has an underdeveloped small, hard, fibrosed nipple, and we have already seen that cancer is commoner in those who have never borne children," and further states that "only 8.5% of patients with cancer of the breast give a normal nursing history." Boyd also quotes a study "in a strain of mice with a low incidence of cancer of the breast, ligation of the ducts to the nipples on one side of the body half way through pregnancy frequently produced carcinoma. Very rapid breeding without accompanying suckling also produced a high proportion of cancer. After all, the animal with the most overworked mammary gland in the world, namely, the cow, never develops mammary cancer."

Some suggestions in Boyd's textbook [2] on humans and in laboratory animal model studies indicate that breast cancer is not a single neoplastic disease, but a group of various cancers, with different tissue location and modes of origin. Several types of these cancers are hyperplastic in their early stages, suggesting the presence of a promoting hyperproliferating agent (e.g., local toxic or irritating agent). In describing the pathogenesis of intraductal carcinoma and Paget's Disease, Boyd [2] further states that "frequently the tumor appears to begin as a local lesion at or near the outlet of a lactiferous duct." In later rodent laboratory studies by our group and others, early hyperplastic and dysplasic effects were noted in the very small (lactiferous) ducts leading from the terminal end buds (small lobules) [9-11].

3. HYPOTHESIS

a. Human milk (4-5% fat content as triglycerides) is normally produced by the small lobules of the breast in response to biochemical (e.g., hormonal) signals after parturition. Without such signaling, these small lobules probably produce a milk-like secretion at a very low rate, which moves very slowly into and down the small ducts, in a fashion almost resembling stasis.

b. High dietary fat intake could magnify the fat secretion of these small lobules, and raise the percentage of fat in the fluid secreted into the

small lactiferous ducts. It appears that circulating triglycerides (lipoprotein, chylomicrons) are a major source for milk fat in humans (Dr. Nitan Telang, Strang Cancer Research Laboratory Group, Rockefeller University, private communication), possibly augmented by diet-induced hyperlipemia.

c. Stasis of such a fat-enriched, milk-like, fluid in the small lactiferous ducts probably leads to biochemical interchange and metabolism of the fluid by the epithelial cells lining the inner wall of the ducts. A possible kinetic order of such reactions may be:

- Ions (Na, Ca, K, Mg, etc) reaching equilibrium with interstitial fluid and cells.
- Low molecular size components, e.g., lactose, which is not a normal energy source for mammary gland epithelial cells. However, in stasis it is probably partly hydrolyzed to yield glucose and galactose, which is probably slowly absorbed and metabolized.
- The epithelial cells probably have sufficient surface proteases to gradually hydrolyze proteins (e.g., casein) and recycle their amino acids.
- The lipids (fats as triglycerides) are probably almost last to be hydrolyzed by epithelial cell surface lipases, releasing the highly cytotoxic long chain free fatty acids (FFA's). Steroids (e.g., cholesterol) are far less readily metabolized, and appear in breast nipple aspirate fluid, often in oxidized form [12].

d. The focus of this hypothesis is on the FFA's, released from the high-fat containing milk-like fluid secreted by the non lactating female, especially in the small ducts, in stasis induced by "retention of secretion." The cytotoxicity of such FFA's, their mode of action by disturbing normal calcium control of cell membrane structure, viability, and normal calcium transmembrane signaling systems has been published in relation to colon cancer [13-20]. In the colon lumen concentrations and quality variations of FFA's occur as a function of diet. In similar fashion the local colon lumenal availability of calcium is largely dependent on dietary calcium intake. Vitamin D adequacy (from sunlight on skin plus dietary intake) is also needed to facilitate movement of calcium into cells, and to ensure adequate calcium systemic absorption (bioavailability), with the net effect of facilitating calcium movement from the systemic circulation to prevent or inhibit depletion in local areas. This action of vitamin D is from the active in vivo metabolite, calcitriol (1,25-dihydroxycholecalciferol). Thus, in the colon FFA cytotoxicity may be prevented by both: (i) increasing dietary calcium to elevate direct colon luminal calcium availability, and (ii) increasing vitamin D in vivo to enable rapid and higher calcitriol production (primarily in the kidney from circulating 25-hydroxycholecalciferol), which serves to facilitate calcium absorption and also movement from the systemic circulation to normal tissue cells. The circulating 25-hydroxycholecalciferol (an inactive form) is produced in the liver by a specific enzyme (25-hydroxylase found in the hepatocyte endoplasmic reticulum). It has a long half-life (3 weeks), and its circulating level is a direct function of the total vitamin D (cholecalciferol) stored in the liver. [*Note:* In studies of colon cancer in laboratory rodent models, human epidemiologic studies, and some limited intervention trials, increasing either or both dietary calcium or vitamin D (from sunlight exposure or dietary intake) have demonstrated reduction of colon cancer risk.]

e. In the tubules of the breast, however, local depletion of cell calcium from cell membrane, local surrounding fluid, etc., by strong sequestration by FFA's to form biologically unavailable calcium soaps, can only be rectified from the calcium in the central circulation, which is tightly controlled by several mechanisms to a very narrow range of concentration. Calcium movement rate into needy cells and cell areas, such as breast tubule epithelial cells, greatly depends on calcitriol regulated transfer systems, (i.e., requiring action from the active form of vitamin D).

f. This hypothesis then suggests that vitamin D adequacy (from sunlight production in the skin, plus dietary intake) could be a key factor in ameliorating the promotion effects of high dietary fat on breast cancer. Higher dietary calcium is probably also needed to ensure that

circulating calcium levels are maintained in the upper range of normal, but the emphasis is on maintenance of circulating 25-hydroxycholecalciferol (25-hydroxyvitamin D) at an adequate level (e.g., high end of normal range to about 100 to 150 nmol/L, or 40 to 60 ng/ml). Notably, a recent conference in October 2003 organized by the U.S. National Institutes of Health found "alarming prevalence of low circulating levels of vitamin D" [21], with an apparent resurgence of rickets in the U.S. This hypothesis suggests that such low vitamin D circulating levels could also be a significant risk factor for breast cancer.

4. EXPERIMENTAL ANIMAL STUDIES AND HUMAN STUDIES

a. PROMOTION IN CHEMICALLY-INDUCED MAMMARY CANCER

Because of the similar epidemiological correlations between colon and mammary cancer, animal studies were designed to test the influence of dietary calcium and vitamin D levels on the promotional effect of high-fat diets in chemically induced mammary cancer. In the first series [22], variation of dietary calcium and vitamin D had little effect with low-fat diets, except when the dietary calcium was very low. On high-fat diets, however, a significant increase in mammary tumorigenesis resulted from decreasing the dietary calcium and vitamin D to levels similar to those of human adults in North America.

In a second series [23,24] dietary levels of calcium, phosphate and vitamin D in high-fat diets were varied independently in studies on chemically-induced carcinogenesis in rats. The results suggested that both phosphate and vitamin D have interactive effects with dietary calcium. Dietary vitamin D had the largest individual effect, and in higher amounts it even inhibited tumorigenesis in low-calcium and high-phosphate diet, but it was most effective with a high-calcium and low-phosphate diet. These diet studies in rats are in general agreement with the mechanism postulated for the effect of high dietary fat in breast cancer promotion. According to this concept, high dietary fat produces an increased flux of fat in the breast, particularly during growth (i.e., puberty and adolescence). Movement of fats in and out of cells in the breast, particularly in and around the cancer-prone small terminal ductal epithelial cells, is largely via the hydrolysis of circulating triglycerides to free fatty acids for transport across cell membranes. This presents a risk to calcium-dependent cell structures and very sensitive cell signaling systems, due to the strong avidity of free fatty acids for calcium [13-20]. Maintenance of proper cellular calcium levels depends on adequate circulating calcium and the active hormone form of vitamin D, calcitriol (1,25-dihydroxycholecalciferol or 1,25-di-OHD_3 [18]. The blood-level of calcium is sufficiently important physiologically to be tightly controlled within a narrow range by interaction of parathyroid hormone, calcitonin, 1,25-di-OHD_3-controlled dietary absorption, losses due to gastrointestinal food component reactions, and losses in urine and sweat (25). However, blood levels of 1,25-di-OHD_3 also facilitate cellular uptake of calcium from the blood. Low total bioavailability of vitamin D (from sunlight skin exposure and dietary intake) is reflected in low serum level of circulating, but poorly active, 25-hydroxyvitamin D (25-OHD_3), with a long (2-3 week) half life. The active 1,25-di-OHD_3, enzymatically produced by the kidney in response to a small reduction of serum calcium, in contrast, has a short half-life (5-6 hrs). Low dietary vitamin D could, therefore, be expected to magnify the high-fat promoting effect on breast cancer due to limited 1,25-di-OHD_3, resulting in reduced ability of cells to replenish calcium locally lost to free fatty-acid binding. Accordingly, it was interesting to note that the greatest single effect was due to dietary vitamin D in these animal studies [23,24]. Although the data are limited, it suggests utility for this hypothesis.

b. PROLIFERATION IN MAMMARY EPITHELIAL CELLS

Zhang et al. [9] reported that high dietary fat significantly increased proliferation (~2-fold) of murine mammary epithelial cells, particularly in the epithelium of the terminal ducts.

There was a large increase in proliferation, magnified by high dietary fat, beginning at 4 weeks

of age in mice. The high proliferative rates were maintained to about 10 weeks of age during the period of normal growth and maturation of the mammary glands, before declining to lower adult levels. This period of life corresponds roughly to puberty and adolescence of the human female, a period when rapid cellular proliferation and growth of mammary epithelium are "hormonally driven" by gonad-supplied estrogens. The large increase in mammary epithelial cell proliferation caused by high dietary fat during the period of puberty and adolescence may be the key to explaining several observed phenomena:

- Epidemiological studies of dietary fat and breast cancer based on inter-country comparisons which estimate fat intake over the entire lifetime including puberty and adolescence, share a consistent association of high dietary fat with risk of breast cancer.
- Epidemiological studies based on case control of adult women, often menopausal or postmenopausal, fail to show an association between adult dietary fat intake and risk of breast cancer. Perhaps these studies should include dietary fat, calcium and vitamin D during puberty and adolescence, periods of greatest likely dietary effect on the mammary tissue based on the rodent studies of Zhang et al. [9], even though the outcome is not manifest until much later in life. Zhang et al. [9] also demonstrated that increased dietary calcium significantly reduced the increased mammary epithelial proliferation induced by high fat. The bioavailability of the dietary calcium was ensured by an optimal high level of dietary vitamin D in the AIN-76 vitamin mix [9].
- In another laboratory, a "Western Style" diet was designed with high fat, reduced calcium, reduced vitamin D and elevated phosphate as a "mimic" of the human diet based on the principle of nutrient density [26,27]. Mice on this "Western Style" diet starting at 5-6 weeks of age and fed for 20 weeks, showed mammary ductal hyperproliferation and hyperplasia, particularly in the smaller terminal ducts, in two studies [10,11].
- An observational type of epidemiology study was recently published that suggests utility for the hypothesis that increased dietary calcium and vitamin D (from sunlight exposure and/or dietary sources such as vitamin D enriched fluid milk) may reduce the risk of breast cancer. Shin et al. [28] in a 16-year prospective study of over 88,000 women in the Nurses Health Study cohort, compared dietary records starting in 1980 against incident invasive breast cancer. In premenopausal women they found that a high intake of low-fat dairy foods, especially low-fat milk, was associated with reduced risk (RR0.68, 95% CI = 0.54 to 0.86) of breast cancer. Similar inverse associations were seen with components (calcium and vitamin D) of dairy foods, but independent associations with breast cancer were too difficult to distinguish. However, in post-menopausal women, no statistically significant association of breast cancer risk with dairy foods was found.
- In a later report of a hospital based case control study of breast cancer risk in women in Korea, Shin et al. [29] found that higher dietary intake of milk and calcium have a negative association with breast cancer risk, stronger in premenopausal women. This suggests correlation with the Nurses Health Study, even though the methodologies of the two studies are different [28,29].

5. DAILY AVAILABILITY OF VITAMIN D IN THE U.S. POPULATION

The biochemistry of calcium and vitamin D are closely linked since bioavailability of calcium (e.g., absorption in the gastrointestinal tract and also into cells within the body) depends on adequate vitamin D. Dietary vitamin D in North America is low, well below the U.S. Recommended Daily Allowance. However, dietary intake of vitamin D is "supplemented" with production of vitamin D in skin exposed to clear, bright sunlight, but sunlight varies considerably with season, latitude, and degrees of haze and smog which act as ultraviolet blocking aerosols in the atmosphere. In this regard, the studies of Garland et al. [30-32] suggest a strong inverse correlation between breast cancer and

availability of solar radiation for in vivo skin production of vitamin D, especially as applied to the U.S. and Canada. There are also studies suggesting that night shift workers are at increased risk.

Lower solar radiation, particularly in urban areas where the greater part of the U.S. population lives, thus results in reduced biologically available vitamin D from this source and creates an increased dependency on dietary intake. The U.S. current Adequate Intake (A.I.) for dietary vitamin D is 5 μg (200 units) from 1-50 years of age for both males and females, except for pregnant females. However, daily dietary intakes are far lower: females' consumption averages 1.5 μg (60 units), and elderly females have a median intake of 1.35 μg (54 units) [33].

The low average intake of dietary vitamin D, coupled with the reduced capacity to convert vitamin D to 25-OHD_3 [34] and further to 1,25-di-OHD_3 in older individuals [35] may serve to explain the very poor status of active vitamin D forms in older populations [36-39]. However, this is correctable with dietary supplementation.

Studies on the etiology of osteoporosis bear a similarity to the studies of breast cancer in terms of dietary correlations, indicating inadequacy of both dietary calcium and vitamin D. The recent review of the U.S. inadequacy of vitamin D [21] thus probably applies to both risks of osteoporosis and breast cancer.

6. HUMAN INTERVENTION STUDIES

No intervention human studies of dietary vitamin D and/or calcium and human breast cancer have been reported, to the best of the author's knowledge. A main difficulty is the lack of suitable, established, acceptable biomarkers for the early preclinical manifestations of breast cancer. Another difficulty is the lack of sufficiently nontoxic vitamin D analogs that could be tested as drugs in prevention studies. Consideration has been given in the past to potential responses to vitamin D dietary supplementation in nipple aspirates based on the studies reported by Petrakis [12] and its derivative, nipple lavage, and also fine needle aspiration cytology developed by Fabian [40]. However, for a variety of reasons, no intervention studies have yet been instituted.

Epidemiological studies, based on potential correlations (preferably prospective) of circulatory serum levels of 25-hydroxyvitamin D, with consideration for seasonal variation, to incidence of breast cancer, have also been proposed, but not yet implemented.

Long term intervention of the effect of dietary vitamin D supplementation on potential reduction of incidence of cysts, "lumps" or other early events related to breast cancer, as well as fully diagnosed breast cancer, have also been proposed, but not implemented.

7. HYPOTHESIS SUMMARY

a. High dietary fat is one of several factors correlated with increased incidence of breast cancer in inter-country population studies. Laboratory animal studies suggest that high dietary fat acts as a promoter of chemical carcinogen-induced mammary cancer, and that high fat diet is associated with increased epithelial cell proliferation of the mammary ducts, particularly in the small terminal ducts.

b. The hypothesis proposes that high fat diet increases fat secretion into the small ducts leading from the terminal (lactiferous) end buds. In non-lactating women, particularly nulliparous women, a milk-like fluid, moving very slowly in the duct (stasis-like) can be partly metabolized, producing long-chain free fatty acids (FFA's) from the secreted fat. These FFA's can be highly cytotoxic to local epithelial cells, resulting in cell damage and necrosis, followed by increased cell proliferation (probably from basal cells) [9-11,22-24].

c. The mechanism of FFA cytotoxicity has been studied in the colon, and shown to be related to the potent sequestration of calcium from the cells by the FFA's. In the colon, increased dietary calcium can readily prevent colon lumenal FFA's from these cytotoxic and hyperproliferative effects [13-20].

d. In the breast, increase of local calcium availability from the systemic circulation (where its concentration is tightly controlled) depends to a greater extent on availability of calcitriol, the

active metabolite of vitamin D. This, in turn, depends on total vitamin D stored in the liver, which depends on dietary vitamin D (currently quite low) or sunlight exposure on the human skin (seasonable and geographically very variable).

e. Laboratory animal studies suggest usefulness for this hypothesis.

f. An inverse epidemiological correlation has been developed between sunlight availability as a source of vitamin D and the risk of breast cancer in the U.S. and Canada.

g. Current vitamin D and calcium dietary intake in the U.S. is far below the recommended adequate intake (A.I.) levels in all female age groups, particularly for the elderly.

h. Reduction of breast cancer risk, and simultaneously osteoporosis, might be achieved by increasing dietary intake of calcium and vitamin D, possibly by increased and broader food enrichment with calcium and vitamin D. This may be particularly applicable to females during puberty and adolescence.

i. A large-scale prospective observation study in >88,000 women for 16 years (the Nurses Health Study) lends support to this hypothesis, although only in premenopausal but not postmenopausal women [28]. A hospital based case-control study of breast cancer in Korean women also adds support for this hypothesis [29].

j. *Note:* This hypothesis was designed to relate increased intake of calcium and vitamin D to reduction of breast cancer to only one of the factors (i.e., high dietary fat intake) related to risk of breast cancer. As indicated in the section on Background in the early part of this paper, there are several other factors involved. Reduction of breast cancer risk by increasing dietary intake of calcium and vitamin D can only be expected to produce a modest, but probably significant effect in a low-cost, safe way, probably by adding these components to foods, such as cereal grain products, similar to the five items currently added. Such additions are being now considered to reduce osteoporosis and colon cancer risk. Estimates suggest that a 20-30% reduction of colon cancer risk may result. Perhaps breast cancer risk may be reduced similarly, but more research is needed to establish a reasonable estimate.

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ABBREVIATIONS USED:

FFA's, free fatty acids; 25-OHD_3, 25-hydroxy-vitamin D or 25-hydroxycholecalciferol; 1,25-di-OHD_3, calcitriol, 1,25-dihydroxycholecalciferol.

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