

Dairy Foods and Prevention of Colon Cancer: Human Studies

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Colon cancer is the commonest gastrointestinal cancer and the second leading cause of cancer deaths in the United States. Recent approaches to lowering the incidence of colon cancer have included attempts at dietary prevention and chemoprevention. International and national incidence rates for colon cancer suggest an inverse relationship with dietary calcium and/or vitamin D intake (or sun exposure). Several human intervention studies have suggested that supplemental calcium administration will change proliferative indices of risk for colon cancer from high to lower risk patterns. The principal current hypothesis for the action of calcium implies that calcium may precipitate or bring out of solution fatty acids and bile acids that are potentially toxic to the colorectal epithelium. Both calcium administration and dairy food administration are associated with lowering aqueous fecal concentrations of bile acids and fatty acids accompanied by a highly significant lowering of cytotoxicity in studies *in vitro*. There is biochemical and biological evidence in cell culture systems that exposure to calcium and/or vitamin D reduces the oncogenic properties of colon cancer cells. A recent blinded study of the administration of low-fat dairy foods demonstrated a significant improvement in several parameters of proliferation as well as in two differentiation markers from a high to a lower risk pattern. Furthermore, administration of calcium also has been shown to reduce the incidence of recurrent adenomatous polyps in individuals at increased risk for colon polyp formation because of the presence of prior colon adenomata. These combined data suggest that administration of supplemental calcium or low-fat dairy foods may have a significant effect upon colonic polyp and perhaps colon cancer incidence.

Key teaching points:

- Epidemiologic variation in the incidence of colon cancer principally is related to environmental (i.e. dietary) differences.
- Epidemiologic data indicate that the prevalence of colon cancer generally is inversely related to the intake of calcium and/or vitamin D or exposure to sunlight.
- Intermediate biomarkers of risk for colon cancer, including proliferation and differentiation markers, are used to evaluate risk and effect of intervention.
- Calcium or dairy foods lower the aqueous concentration of bile acids and fatty acids in fecal contents.
- Dairy products are very effective at altering biomarkers of risk for colon cancer from a high risk to lower risk levels.

INTRODUCTION

Colon cancer is one of the commonest cancers in the United States and the Western world. The incidence also recently has increased rapidly in less-developed parts of the world that have adopted Western customs and diet. Colon cancer is the end result of a combination of genetic and environmental changes.

The degree to which these two etiologic factors play a role in individual patients with colon cancer varies widely. For example, familial adenomatous polyposis, an autosomal dominant hereditary disease with 90% to 100% penetrance with essentially all affected individuals developing colon adenoma, and later colon cancer [1], is thought to have almost a 100% genetic basis. On the other hand, epidemiologic studies, such as those

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in Japanese migrants to Hawaii and the mainland United States, have indicated that changing to a Western lifestyle and diet will rapidly increase the incidence of colon cancer, particularly of the sigmoid colon [2]. This change in incidence is seen within one generation, from a low incidence in homeland Japan, to a high incidence in Japanese migrants in Hawaii and the U.S. mainland. These studies, as well as several others, have focused upon the importance of the environment and, in particular the diet, in colon cancer causation.

The present approach to reducing colon cancer mortality and morbidity is directed to early detection of malignant tumors by routine tests for blood in the stools on an annual basis in the average risk population over age 50 and the detection of pre-malignant colorectal adenomas by sigmoidoscopy or colonoscopy and polypectomy [3]. Populations at increased risk are studied by colonoscopy at an earlier age and more frequently. These approaches have had a modest impact upon colon cancer deaths [4–6], and there is some evidence for a reduction in colon cancer incidence in the United States [7,8]. However, the major recent investigational approach to altering the incidence of colon cancer in the United States is directed to finding new approaches to colon cancer prevention [9,10].

EPIDEMIOLOGY

Several separate observations contributed to the concept that calcium supplementation might be a preventive approach to reduce the high prevalence of colon cancer in the developed countries of the world. First, human calcium intake in food presently is far lower than the amounts that were estimated to be consumed in prehistoric times [11], serial epidemiologic data have indicated that dietary levels of calcium are inversely related to the incidence of colon cancer; finally, the experimental hypothesis of Newmark, Wargovich and Bruce suggests that dietary calcium might precipitate bile salts and fatty acids in the colonic lumen [12].

The epidemiologic evidence for an inverse relationship between the dietary intake of calcium and the incidence of colon cancer is quite strong [13]. The classical long-term prospective study of Garland and coworkers in Chicago examined the association of dietary calcium and vitamin D and the risk of colon cancer. They found a strong inverse correlation with these dietary components [14]. A protective effect of calcium also was described from case control studies in the Southwest United States [15] and in Australia [16]. In 1988, Sorensen *et al.* summarized all of these studies and emphasized the inverse relationship with calcium consumption in age-adjusted colon cancer incidence rates [17]. The first human study of the effect of calcium administration upon the colon was reported by Lipkin and Newmark in 1985 [18]. In that uncontrolled study, administration of 1.25 gm of elemental calcium to subjects with

a familial history of sporadic adenomas resulted in pronounced changes in proliferation in the direction of normality (a 40% reduction in whole-crypt labeling index, and a 19% fall in ϕ h in rectal biopsies incubated with ^3H -thymidine *in vitro* [18]. When the effect of calcium intake was related to the incidence rate for the development of colon cancer (as determined by Garland and co-workers), a maximum calcium effect could be calculated with a dietary calcium intake of about 1800 mg/day. As has been emphasized elsewhere [19], the epidemiologic data on an inverse relationship between calcium intake and colon cancer incidence is not uniform. Furthermore, dietary evaluation in populations is difficult particularly when accompanied by other accompanying dietary variations.

The potential importance of sources of vitamin D from the diet or from sunlight in influencing the incidence of colon cancer in the United States also has received attention. In 1980, Garland and Garland proposed that vitamin D is a protective factor against colon cancer [20]. This hypothesis was based upon the geographical distribution of colon cancer deaths in the United States, which showed that colon cancer mortality rates were highest in populations that were exposed to the lowest amounts of sunlight, such as major cities and rural areas in high latitudes. In contrast, colon cancer death rates adjusted for age were up to 40% lower in states in the Southwest and Western United States, where sunlight exposure was high. Garland and coworkers subsequently compared serum 25-hydroxyvitamin D levels with the subsequent risk of getting colon cancer in Washington County, Maryland [21]. They divided the serum levels of 25-OHD in their population into five quintiles and demonstrated a significant reduction in the relative odds ratio of developing colon cancer, particularly in the third and fourth quintile of the group. On the other hand, a more recent study did not find a correlation between serum 25-hydroxyvitamin D levels and colonic adenomas or colonic carcinomas in a large group of individuals who underwent colonoscopy because of occult blood in the stool or the presence of colonic polyps [22].

Epidemiologic studies in 600 residents of Stockholm, Sweden, with colorectal cancers sought an association between dietary intake of calcium and vitamin D and cancer risk. Increasing levels of dietary vitamin D were inversely associated with the risk of colorectal cancer. The association was more pronounced for cancers of the rectum (OR 0.5) than of cancers of the colon (OR 0.6) after adjustment for age, gender and total caloric and protein intake [23]. Dietary calcium was not associated with an adjusted risk of colon or rectal cancer in this study. It should be noted that the mean intake of calcium in this population was about 940 mg/day, significantly higher than that of many of the populations evaluated in the United States. In an important study of the relationship between vitamin and calcium supplement use and colon cancer from Washington state [24], the average daily intake of supplemental vitamins A, C, D, E, folic acid, calcium and multivitamins were associated with a reduced risk of colon cancer. Because almost all vitamin

D supplementation came from multivitamin pills, the association of vitamin D itself with colon cancer could not be distinguished from that of multivitamin use.

EPIDEMIOLOGIC EVIDENCE FOR THE POTENTIAL CHEMOPREVENTIVE EFFECTS OF DIETARY COMPONENTS OTHER THAN CALCIUM ON THE INCIDENCE OF COLON CANCER

The epidemiologic data showing major differences in colon cancer incidence rates throughout the world, as well as the studies of Japanese-born migrants to Hawaii, all suggested that the diet was a major factor in colon cancer risk. The excess intake of fat, and particularly meat and meat products, have long been suspected risk factors for colorectal cancer [25,26]. However, some of the epidemiologic evidence in the United States does not support the role of dietary fat.

Burkitt and others suggested that dietary fiber was an important protective factor for colon cancer [27]. The epidemiologic data on dietary intake of cereal fiber, fruits and vegetables do not, themselves, support a direct relationship. On the other hand, it is clear that the incidence of colon cancer among other cancers is very low in Seventh-Day Adventists, most of whom follow a vegetarian diet that includes milk and eggs and about 1400 mg of calcium intake daily. In the total group, the incidence of colon cancer appears to be one-half that of the general population of the areas in which they live in California [28]. In a recent double-blind phase II study, Alberts *et al.* failed to find significant effects upon rectal proliferation by adding up to 13.5 gm of wheat bran fiber and 1500 mg of calcium carbonate per day to a basal diet for nine months [29]. However, DeCosse and coworkers suggested that high fiber dietary supplementation (11 gm/day), plus vitamin C and E, reduced the incidence of rectal polyp recurrence following subtotal colectomy for familial adenomatous polyposis [30]. Although no beneficial effect was found by McKeown-Eyssen [31], some reduced polyp recurrence was recently observed by MacLennan [32]. Another population study suggested that increasing α -tocopherol level was associated with decreased occurrence of large (>1 cm) but not of small adenomas, and a strong trend was observed by using an α -tocopherol: γ -tocopherol ratio, which the authors thought was a very sensitive indicator of α -tocopherol intake [33]. A multi-institutional study of a group of antioxidant vitamins failed to find a change in the recurrence of colorectal adenomas [34]. An interesting observation made as a part of a randomized controlled trial of selenium supplementation to prevent cancers in patients with carcinomas of the skin suggested that the incidence of lung, colorectal and prostate cancer was reduced [35]. This observation will need to be followed with prospective studies specifically related to colon neoplasia.

EVIDENCE FOR A PREVENTIVE EFFECT OF VITAMIN D UPON THE DEVELOPMENT OF COLON NEOPLASIA

Epidemiologic data suggested an inverse relationship between the incidence of colon cancer and the amount of daily sunlight in the United States has been noted above. Other studies also imply that levels of 25-hydroxyvitamin D in the serum were inversely associated with rates of colon cancer [36]. It is important to emphasize that 25 hydroxyvitamin D levels found associated with subjects' increased risk were not in the deficiency range (<5 ng/mL) but ranged from 10 to 30 ng/mL. Several nutritional studies in the United States have shown that the dietary intake of vitamin D is quite low, particularly in lactose-intolerant individuals who avoid consuming more than small amounts of milk or milk-based foods. Older subjects have much lower levels of 25-hydroxyvitamin D than young controls [37,38], probably resulting mainly from a diet poor in vitamin D and lack of exposure to sunshine.

Although several studies in rodents have demonstrated the potential role of vitamin D supplementation in reducing colon cancer development [39,40], no human studies of the effect of vitamin D supplementation on either advanced-intermediate biomarkers of colon cancer risk (polyp formation) or early-intermediate biomarkers of risk (proliferation and differentiation) have been reported. The author is aware of one study, that is due to be analyzed shortly, that was designed to specifically ask whether vitamin D might beneficially alter colonic early-intermediate biomarkers of risk. One question that could be asked was whether responsiveness to vitamin D might differ depending upon the pattern of vitamin D gene polymorphism that exists in individual subjects. Differences in calcium absorption are not related to individual haplotypes of vitamin D receptors [41]. Other studies have suggested that the responsiveness of osteoporotic bones to vitamin D and calcium may also be modified by the specific vitamin D receptors haplotype in individual patients [42,43].

Serum concentrations of vitamin D often are low in Western societies particularly in the elderly [44]. Vitamin D metabolites are known to alter cellular metabolism of many cells including the colon. The vitamin also is known to regulate various gene products with important cellular functions including growth factors, polyamines, cell surface differentiation molecular, oncogenes and calcium-binding proteins. Many tissues, including the colon, contain vitamin D receptors. Colon cancer cell lines that contain vitamin D receptors, when treated with 1,25-dihydroxyvitamin D *in vitro* have exhibited decreased growth and morphologic changes suggesting increased levels of differentiation [45,46]. In the colon, more differentiated colon cancer cell lines show higher levels of vitamin D receptors than less differentiated cell lines [47]. The growth inhibitory properties of the vitamin appear to be related to the concentrations of vitamin D receptor on the cell surface rather than vitamin D

concentrations [47]. Vitamin D supplementation has been shown to inhibit the development of azoxymethane-induced colon cancer in rodents [36]. Subsequent studies by the same group of investigators headed by Brasitus showed that non-calcemic analogues of 1,25-dihydroxyvitamin D also would inhibit AOM-induced carcinogenesis in rodents [48]. They believe that vitamin D functions to alter colonocyte membranes [49] resulting in the release of phospholipase C [50,51] and via activation of cSrc [52]. Vitamin D metabolites may alter the synthesis and activity of HMG-CoA-reductase activity and cholesterol synthesis in cultured cells *in vitro* [53]. A very recent study examined the effects of vitamin D upon the distribution of calcium concentrations in isolated colonic crypts of mice kept on a diet deficient or sufficient in vitamin D [54]. The authors described a crypt base-mouth calcium gradient of 201 ± 79 nM which was abolished by vitamin D depletion. If confirmed, this observation implies a close relationship between this vitamin and exposure of epithelial cells in the crypt to extracellular calcium.

1,25-dihydroxyvitamin D also has been shown to inhibit proliferation of human colonic colorectal biopsy explants in organ culture using the sophisticated metaphase arrest technique [55]. Inhibition of proliferation by vitamin D has been confirmed in rectal biopsies from patients with familial adenomatous polyposis [56]. Subsequent studies by the same group of investigators confirmed the earlier studies and also demonstrated a different effect when the same biopsies were exposed to epidermal growth factor *in vitro*. Vitamin D also has been shown to inhibit the hyperproliferative state that occurs in the colorectum in patients with ulcerative colitis [57]. These combined data give study credence to a chemopreventive action of vitamin D against colonic neoplasia. Such an activity may be partly responsible for the beneficial effect of dairy foods.

HUMAN STUDIES OF CHEMOPREVENTION

Potential cancer preventing agents first are tested in animals. Testing of putative cancer preventive agents in humans cannot wait for cancer to develop as an end point. Therefore, possible effective preventive agents are studied using so-called "intermediate biomarkers of colon cancer risk" as end points. As a "late intermediate biomarker of risk" a reduction of adenoma recurrence is used in subjects who have had at least one adenomatous polyp removed and therefore are at increased risk for recurrence; in such subjects a reduction in the appearance of recurrent polyps is used. Changes in "early intermediate biomarkers" of risk in the flat colorectal mucosa of "at risk" subjects are also used.

Studies of reduced recurrence of colonic polyps in "at risk" populations rely upon the frequency of polyp recurrence. The National Colon Polyp Study found an overall recurrence rate of about 35% to 40% in three years [58]. Individual subgroups of

patients with larger or multiple initial adenomata had a higher recurrence rate. Evaluation of changes in colorectal polyps recurrence must include large cohorts of subjects and take a long time to complete. Studies also rely upon the accuracy of finding colon polyps at the time of follow-up colonoscopy. Recent studies indicate a "miss rate" of up to 25% of colon polyps in the hands of experienced endoscopists [59].

Hyman *et al.* [60] conducted a four-year multi-center randomized clinical trial of the effects of antioxidants for recurrent colorectal polyp prevention. The authors found no effect of their primary antioxidant intervention but did find a beneficial effect of an increased calcium intake. The relative risk, adjusted for caloric intake, showed a highly significant decrease in the number of adenomas with increasing calcium intake ($p = 0.005$). This association was particularly marked in the left colorectum. When the effect of dairy calcium was considered within the lowest and highest two quintiles of calorie-adjusted fat intake, there was a suggestion of a greater effect of calcium among individuals with a high dietary fat intake. Furthermore, there was a non-significant reduction in the number of recurrent adenomas among individuals who had greater than two servings of dairy foods per day *versus* those with fewer than 0.5 servings per day (calorie adjusted odds ratio of 0.74).

Early intermediate biomarkers of risk change much more rapidly in response to an effective chemopreventive agent. The most established biomarker of risk for colon neoplasia has been altered epithelial cell proliferation. The use of proliferative markers is based upon studies of the relative rate of proliferation and distribution of proliferating cells in the flat uninvolved colorectal mucosa of patients "at risk" for colon neoplasia, who have been treated for colon cancer, with familial adenomatous polyposis, ulcerative colitis, or sporadic adenomatous polyposis [61]. These subjects, as a group, show differences in proliferation kinetics from patients not "at risk" or at "low risk" (populations with a very low overall incidence of colon cancer such as Seventh-Day Adventists [62]). Such studies are based upon original observations demonstrating reversibility of several proliferative parameters by normal use of calcium supplementation [18]. Other markers, heretofore applied mainly in animal studies, involve changes that occur in cellular markers of differentiation such as lectin binding [63], the distribution of acidic mucins [64], or of cytokeratin AE1 [65] in colorectal crypt epithelial cells. Limited studies in humans have investigated the utility of ornithine decarboxylase concentrations as a biomarker of proliferation [66,67]. The potential of studying the number and distribution of apoptotic cells and the Bcl2 family of genes is presently being explored.

In animal studies, changes in proliferation were shown to result from exposure to both cancer-initiating agents as well as cancer-promoting agents. Again, the principle changes that have been reported, as in patients at risk for colon neoplasia, is an increase in proliferation rate and an upward shift of the proliferating compartment from the lower 60% into the upper 40% of colorectal crypts as measured by determining ϕh [68].

Studies of markers of differentiation such as acidic mucins and of the intermediate filament cytokeratin AE1 have been shown in rodents to be altered in experimental carcinogen-induced colon cancer. These differentiation markers also become abnormal following the administration of Western-style diets [69]. In addition, genetic animal models of colon cancer such as with knock-out or mutations in the FAP gene also show similar changes in proliferative markers in the colon [70]. These combined data are important ancillary observations that support the significance of both proliferative and differentiation marker changes in subjects at risk for colon cancer and their alteration toward normal with chemopreventive strategies.

MEASURING CHANGES IN PROLIFERATING CELLS IN COLORECTAL BIOPSIES *IN VITRO*

Both incubation techniques and methods that measure endogenous markers associated with DNA synthesis have been used. Most of the initial studies of colorectal proliferation utilized incubation with ^3H thymidine and autoradiographic

measurement of labeled nuclei. This technique has the advantage that proliferating cells can clearly be demonstrated by the presence of silver granules over the nuclei [71] (Table 1). The disadvantage of this technique is the need of disposal of ^3H material and a delay period for up to three to six weeks for exposure with a photosensitive film. Alternatively, incubation with bromodeoxyuridine (BrdU) has been used followed by immunostaining with anti-BrdU antibodies. Although the BrdU method has been validated [72], it is limited because tissues need to be fixed in ethanol and there is variation in the immunohistochemical staining and thus in reproducible interpretation of positive cells.

In situ methods involve immunohistochemical demonstration of the presence of proliferating cell nuclear antigen (PCNA), the auxiliary protein of DNA delta or the detection of Ki 67 (mib 1) which has a short half life and is expressed throughout the cell cycle. PCNA immunostaining usually requires fixation in alcohol not formalin and shows variation in staining intensity therefore requiring decision about which nuclei are to be considered positive [73]. Ki67 (mib 1) antibodies involve more standardized methods of fixation prior to immunohistochemistry [74]. It is clear from extensive studies that great

Table 1. Potential Biomarkers of Risk for the Study of Dairy Product-Inhibition of Colon Cancer

	Advantages	Disadvantages
Proliferation Markers		
^3H d Thd	Reading excellent Validated	Incubation gives variable results Radioactive material Slow
BrdU	Reading good Validated	Incubation results variable
PCNA	Simple to perform Not validated	Alcohol fixation Reading observer dependent
Ki67 (mib1)	Simple to perform Not validated Formalin fixation	Reproducible methodology important
Differentiation Markers		
Lectin binding	Formalin fixation	Not validated
Mucin distribution	Formalin fixation	Observer dependent
AE1 Cytokeratin	Alcohol fixation	Not validated
Nuclear morphometry		Requires special equipment Not validated
Apoptosis		
By light microscopy	Accepted histologic method	Not validated Laborious
By TUNEL	Simple to perform	Not validated Requires careful tissue handling
Bcl2 Genes		
By histochemistry	Method validated Reproducible	Not checked in flat mucosa
By Western blots		Not done
Other Methods		
Ornithine decarboxylase		Requires several biopsies Minimal studies performed Technique variable
Abberent crypt foci		Not studied

differences in proliferation rates are recorded if different methods are used.

The use of rectal biopsies to reflect increased risk for colon neoplasia is based upon the observations that such changes occur throughout the colon i.e. are present as a "field defect" in "at risk" individuals [75]. Whether proliferative kinetics also reflect dietary habits in addition to a cellular risk for colon neoplasia is presently uncertain [76,77]. An extensive study from Bostick and co-workers evaluated predictive factors and colorectal proliferation for the development of sporadic colonic adenomas in biopsies obtained from 150 subjects. Using an analysis of variance and multiple linear regression, the labeling index and ϕh were found to be 35% lower for the highest tertile of vegetable and fruit consumption, 36% lower for vitamin supplement use and 36% higher for the presence of a current incident polyp [78].

There clearly is some controversy regarding the effect of calcium supplements upon proliferation. The early studies of calcium administration showed a statistically reduced labeling indices (as well as reduction in ϕh [79,80]). The later negative studies of Baron [81] and Bostick [82] had initial labeling indices that were quite low (3.6 to 4.7) suggesting that a baseline elevated labeling index (or ϕh) may be necessary in order to see any positive effect (Table 2).

The late intermediate biomarker of risk that has been studied with calcium supplementation has been the recurrence of adenomatous polyps in individuals who have had such polyps removed at baseline. The study of Baron and coworkers, at this date presented in abstract form only [82], has suggested a 20%

to 25% reduction in the number of subjects who develop recurrent polyps or in the number of recurrent polyps with calcium supplements of 1500 mgs per day. This represents an important confirmation of the potential influence of calcium upon colonic neoplasia.

STUDIES OF FECAL COMPONENTS

Epidemiologic data that supports the concept that the Western-style diet is a cofactor for the development of colon cancer has focused on dietary fat, and particularly meat, as the most likely offending component. This concept led investigators to develop the hypothesis that fat in the colon is injurious and to study the fecal excretion of differing lipids. Since diets high in fat might also increase bile secretion, fecal bile acids were determined. Studies showed a progressive increase in fecal lipid output with increasing calcium administration consisting mainly of fatty acids [84] accompanied by relatively greater excretion of saturated fatty acid [85]. Fecal bile acid output in patients with colon cancer, with familial adenomatous polyposis and sporadic colonic adenomas, were reported to be increased in some studies, but most reported only a change in the composition of fecal bile acids [86-88]. Several reports showed an increase in secondary bile acids in the feces of patients with colon cancer and colonic adenomas. The concept that increased calcium in the colon might bind or precipitate soluble bile acids and fatty acids and thereby reduce their interaction with colonic mucosal epithelial cells led to the initial experimental animal studies of Wargovich and Newmark [89,90] and was followed by a hallmark study, which first showed a beneficial effect of feeding calcium to human volunteers upon colonic proliferation [18].

In 1990, van der Meer and his coworkers studied the fecal composition of 12 volunteers who were fed either their regular diet or their regular diet supplemented with 35 μM of inorganic calcium per day in a cross-over design [91]. Most of the added dietary calcium in this study was excreted in the stool, bile acid output increased by 35%, and there was a shift of the bile acid composition to an increased ratio of trihydroxy to dihydroxy bile acids. In a parallel study, using an *in vitro* hemolysis assay of cytotoxicity the same group of investigators demonstrated that fecal water showed significantly reduced cytolytic activity with calcium [92]. From these observations, they concluded that calcium also would reduce irritant damage to the colonic epithelium from fecal water. Subsequently, Van der Meer's group added low-fat dried milk powder to a Western-style diet in rodents and showed a similar reduction in the cytolytic activity of reconstituted fecal water [93]. Later they fed volunteers either 3 μM or 30 μM of dairy calcium in dried powdered milk daily. The high milk calcium diet significantly increased fecal pH, fecal calcium and phosphate, total fat, free fatty acids, and bile acids, indicating that these lipid components were

Table 2. Effects of Calcium Supplementation upon Total Labeling Index

Authors	Method	Change in Labeling Index (%)
Positive Results		
Lipkin and Newmark, 1985	[³ H]dThd	16.7 → 10.0
Buset <i>et al.</i> , 1986	[³ H]dThd	Responder 16.1 → 7.9 Non-Responder 7.4 → 6.9
Lipkin <i>et al.</i> , 1989	[³ H]dThd	Responder 14.1 → 8.7 Non-Responder 9.1 → 8.8
Rozen <i>et al.</i> , 1989	[³ H]dThd	6.6 → 4.4
Wargovich, 1992	[³ H]dThd	7.2 → 6.5
O'Sullivan <i>et al.</i> , 1993	BrdU	8.8 → 4.7
Negative Results		
Baron, 1995	PCNA	3.9 → 3.9
Bostick <i>et al.</i> , 1993	[³ H]dThd	4.7 → 5.3
Bostick <i>et al.</i> , 1995	PCNA	3.6 → 4.0*
Weisgerber <i>et al.</i> , 1996	BrdU	13.5 → 11.4
Alberts <i>et al.</i> , 1997	[³ H]dThd	7.4 → 6.7

Restricted to studies in patients post-polypectomy for adenomatous polyps with an intact colon and without evidence of inflammatory bowel disease or colon cancer. Also excluded are studies using measurements of crypt cell production rate.

* Significant shift in proliferating cells to crypt basal positions.

complexed with calcium and phosphate. Calcium also decreased the cytotoxicity of fecal water from 68% to 28% [94].

Recently, Glinghammer and coworkers performed a similar cross-over study in volunteers fed a dairy product-free diet containing no more than 372 mg of calcium per day, *versus* a dairy product diet which included sufficient dairy products to provide an additional 1100 mg of calcium per day [95]. A highly significant reduction in cytotoxicity of fecal water was shown in the subjects taking the high dairy product diet. No significant differences in genotoxicity of stools was found between the two groups using the "COMET" assay.

The original concept that excess calcium would precipitate bile acids and fatty acids in the colonic lumen was modified somewhat by studies that suggested that glycocholic acid precipitation is caused by combinations of calcium with phosphate [96] which then would alter fecal cytotoxicity. It should be pointed out that these conditions are, in fact, operative when subjects take a high dairy diet which provides both increased calcium and phosphate both of which appear in increased concentrations in the feces [97].

POSSIBLE MECHANISMS OF ACTION OF CALCIUM AND VITAMIN D UPON COLONIC NEOPLASIA

The initial hypothesis for the action of calcium upon colonic stool contents suggested that calcium worked by complexing with bile acids and fatty acids resulting in precipitation and, thus, reducing bile and fatty acid concentrations in the colonic aqueous phase [98]. Subsequent studies of Van der Meer and coworkers suggested that supplemental calcium stimulated formation of insoluble calcium phosphate in the intestinal lumen, thus increasing bile acid binding [99]. This caused a relative increase in the concentration of cholic acid and decrease in the concentrations of chenodeoxycholic and deoxycholic acids [92]. Such a shift in bile acid composition was believed to be beneficial since the latter are more toxic to intestinal cells than the former. The subsequent studies of the Van der Meer group of investigators directly demonstrated that fecal water from subjects who were administered either supplemental calcium phosphate or a diet high in dairy foods showed considerably less cytotoxicity using several *in vitro* assays [100–102].

These studies all suggested that the principal action of calcium was within the colonic lumen reducing the cellular toxicity of bile acids and fatty acids. Studies in laboratory animals supported this mechanism of action and similar beneficial effects were seen when calcium was added to human epithelial cells exposed to bile acids and fatty acids *in vitro* [103]. Other experimental studies on this subject are summarized in reference 104. It also is possible that calcium may alter the rate of apoptosis occurring in colonic epithelial cells which,

itself, could normalize a possible discrepancy between ratio of proliferation and apoptosis in preneoplastic flat mucosa [105].

In addition, calcium appears to have a wide range of actions upon proliferation in cancer cells *in vitro*. For many years calcium was known to participate in several steps during cellular proliferation [106]. Indeed, research on proliferation and increased differentiation has been observed following calcium addition to normal epidermal [107], esophageal [108], mammary [109,110], and colon cells [111].

POTENTIAL ADVERSE EFFECTS OF INCREASING CALCIUM AND/OR VITAMIN D INTAKE IN THE UNITED STATES POPULATION

Epidemiologic studies of the dietary intake in the United States (NHANES) have shown that the majority of individuals consume less calcium and vitamin D than is recommended. Indeed, the gap between actual and desirable intake of calcium recently has widened. National recommendations for calcium intake, in the young and the elderly, have been increased from the previous level of 800 mg/day to 1200 mg or more per day [112]. Thus, calcium and vitamin-fortification is not likely to be generally injurious to such a population.

The concerns raised about broad-based addition of calcium into the diet by food fortification, recommendations to increase low fat milk product intake or by calcium pill supplements include the hypothetical risk of hypercalcemia, of precipitation of renal stones and of interference with the absorption of other minerals. A common side-effect may be constipation.

None of the trials of calcium tablet supplementation that examined changes in rectal biopsy biomarkers of risk or changes in adenomatous polyp recurrence have reported serious side-effects. Constipation may occur, but its presence is readily managed by increasing dietary fiber or the administration of stool softeners. A prospective study evaluated the relationship between dietary calcium intake and the risk of symptomatic kidney stones in a cohort of over 45,000 men without a prior history of renal calculi (The Health Professional Follow-up Study) [113]. After adjustment for age, dietary calcium intake was inversely associated with the risk of kidney stones even after adjustment for other potential risk factors. This protective effect of a high calcium diet may be mediated through increased precipitation of calcium oxalate in the colonic lumen, decreased oxalate absorption with an increase in excretion in the stool. Whatever the mechanism, this study relieves some of the anxieties about the risk of kidney stones with added dietary calcium.

The metabolic effect of the slightly increased fecal fat excretion during administration of calcium, either in the form of inorganic calcium supplementation or increased dairy food ingestion, appears to be minor and unlikely to be clinically

relevant. It is of interest that some studies have shown increased fecal excretion of saturated fats with calcium supplementation which, indeed, might be beneficial. Most authorities, however, would recommend the addition of low-fat dairy products if an increased intake of dairy foods containing calcium is recommended in order to minimize hyperlipidemia and atheroma formation. Calcium supplementation can interfere with iron absorption [114,115], although whether this is clinically important has not been established [116].

The risks of the present degree of vitamin D fortification of foods, or the extensive over-the-counter use of vitamin D appear to be very small [117]. Vitamin D toxicity has been described following errors in food fortification [117], but is rare following multivitamin or administration of single-nutrients containing vitamin D. Individuals may be found to be extremely hypersensitive to vitamin D administration with sarcoidosis, granulomatous diseases, and several infectious diseases or may be idiopathic [118], but fortunately this is a very rare event. It is clear that the prevalence of significant side effects from the amount of supplemental calcium and vitamin D that has been recommended is very small.

DATA SUPPORTING THE CHEMOPREVENTIVE EFFECTS OF MILK PRODUCTS UPON THE RISK OF HUMAN COLORECTAL CANCER

The epidemiologic evidence supporting a potential role for milk consumption in reducing the risk for colon neoplasias has been discussed briefly above. It is well-accepted that studies seeking a correlation between per capita consumption of individual foods and, particularly for micronutrients, are subject to considerable ecological fallacy. Observational studies are hampered by the similarity of diets within particular populations, as well as the multitude of dietary variables and the lack of precision in dietary measures which are used in population studies. Furthermore, many of the older epidemiologic studies were seeking positive or negative effects of such items as fiber, meat and fat intake and only included dairy products as secondary items. The Melbourne Colorectal Cancer Study suggested that a low intake of milk drinks increased the risk of colorectal cancer in both males and females [119]. The Marseille Study [120] and the studies of Negri *et al.* in Italy [121] appear to show no protection at least for intakes of milk products providing between 0.5 and 1.5 gm of calcium per day. Jensen and coworkers [122] compared the diet of a rural community in Finland with a large urban city in Denmark which has a three-fold increased incidence of colon cancer. This study failed to show a significant influence of milk product ingestion, but, in contrast, later studies (from Scandinavia) from the same group [123] demonstrated a strong positive effect of milk intake. The most telling study focused upon the

positive role of milk products in lowering colon cancer is that of Garland and coworkers in Chicago described in detail before [37]. Stemmermann in Hawaii studied cancer rates migrants from Japan and found that a higher milk product intake in these individuals clearly was associated with a lower risk of the development of sigmoid cancers [124]. The epidemiologic studies of Rosen and coworkers in Sweden also suggested a strong preventive role for milk product ingestion [125], and that of Peters focused on the potential benefit of yogurt [126].

Subsequent studies directed to evaluating effects of dairy food ingestion in groups of individuals subjects used as endpoints of benefit the excretion of bile acids and fatty acids in the soluble phase of the fecal effluent. Fecal excretion of excess bile acids and fatty acids have been associated with changes in colorectal proliferation [89] and possibly in differentiation. Administration of calcium appears to reduce both altered proliferation and the concentrations of bile acids and fatty acids in fecal water. When the soluble fraction of feces then was exposed to erythrocytes or to colon cancer cells, reduced *in vivo* toxicity appeared to be paralleled by reduction in cytotoxicity *in vitro* [92].

In an animal study, Govers, Tremont and Van der Meer [93] fed Wistar rats a Western-style high-risk diet with and without the addition of lactase-treated whole milk powder and compared the effect of these two diets upon the fecal excretion of calcium, phosphorus, bile acids and fatty acids. At the same time they measured both the surfactant and the cytolytic activity of the excreted feces. The main purpose of this study was to define differences in the effect of feeding whole milk powder and inorganic calcium upon fecal composition and evidence for colonic epithelial damage. Calcium carbonate or calcium phosphate, each in a concentration of 150 $\mu\text{M}/\text{Kg}$ of diet, were added to two other groups of animals. All supplemental groups excreted large amounts of calcium in the feces, but phosphate increased significantly only in the animals fed calcium phosphate and the milk powder. However, soluble bile acids and soluble fatty acids were dramatically reduced in the three groups of animals that were fed increased calcium. There also was a reduction in epithelial cell damage (alkaline phosphatase activity in fecal water), and in epithelial proliferation (as judged by ^3H -thymidine incorporation into colonic DNA). These studies closely parallel human data. Furthermore, although serum gastrin increased reproducibility in the rats fed the three calcium-rich diets, there was no relationship between fasting serum gastrin concentrations and colonic proliferation rates. An earlier study of milk powder supplementation in dimethylhydrazine-treated rats demonstrated no reduction in tumor number, but a significant fall in tumor burden [127].

Two important studies have demonstrated that milk product ingestion results in major potential beneficial effects upon fecal cytotoxicity in human volunteers. Van der Meer's group performed parallel studies to those performed in animals on fecal cytotoxicity in volunteers provided a constant diet together with either a low or high intake of milk-based calcium. Examination of fecal contents and evaluation of cytotoxicity again

clearly showed that dairy-based calcium intake greatly reduced the cytotoxic potential of fecal water [94]. Glinghammer and coworkers recently published a cross-over study in humans who were fed either a lactovegetarian diet or a meat-based diet [95]. Analysis of the feces in these subjects again showed that the cytotoxicity of the soluble phase of feces was significantly reduced during the ingestion of the lactovegetarian diet.

Recently, our own group has completed a study of the effects of the ingestion of a diet rich in dairy foods upon markers of proliferation and differentiation in the rectum. Seventy subjects with a history of a recent polypectomy for an adenomatous polyp were entered into the study. Excluded were patients with histories of hereditary colon cancer, ulcerative colitis, gastrointestinal problems including lactose intolerance, diabetes mellitus or other serious diseases. No subject was entered if he or she was taking more than 200 mg of supplemental calcium or more than 1000 mg/day of calcium in the diet. Subjects were divided into two groups; one group which was maintained on its baseline diet and the other in which we attempted to increase the baseline diet with supplemental dairy foods totaling up to 1200 mg of calcium per day. In all of these subjects, two biopsies were taken at baseline in order to determine the variation in measuring endpoints, and then at six and 12 months of the study [128].

At baseline, the two groups of subjects were eating similar amounts of basic dietary components. It should be pointed out that the majority of subjects entered into the study were relatively health-conscious, eating no more than approximately 1700 kcal of energy per day which included only 50 to 60 gm of fat and about 75 gm of protein. Mean calcium intake averaged 630 mg, and vitamin D intake was about 4.8 $\mu\text{g}/\text{day}$. Although there were a total of 11 dropouts, difficulty with the diet was the cause in only two individuals. Analysis of the dietary intake in the control group of subjects indicated no significant changes in dietary components throughout the study. The dairy-treated subjects increased their total calcium intake to about 1500 mg/day of which dairy foods represented 82%. Their distribution of dairy intake consisted of milk (31%), yogurt and ice cream (11%) and cheeses (41%).

In control subjects, there were no significant difference in any of the proliferative kinetics that were evaluated. In contrast, there were significant changes in proliferative kinetics in the subjects who were taking the additional high dairy foods. There was a significant reduction in labeled cells per crypt and total crypt cell labeling index at 12 months ($p < 0.02$) and in the labeling index in crypt compartment five ($p < 0.05$). Three differentiation markers were analyzed in this study. None of these differed significantly in the control group over the course of the twelve months of study. In contrast, changes in acidic mucin distribution in the direction of normality were significantly associated with dairy food intake ($p < 0.02$) as were changes in cytokeratin AE1 ($p < 0.05$). The only change in nuclear morphometry that was determined with an increase in dairy intake was a modest reduction in nuclear size after six

months on the diet which no longer was significant by twelve months.

These combined data strongly suggest that the ingestion of dairy products, adding only about 850 mg of calcium per day, was accompanied by very significant changes in proliferation and two differentiation markers. Although previous studies using calcium supplements at doses as low as 850 mg/day have not been performed, in general the significant changes in proliferation previously studied with supplemental calcium used intakes of 1200 to 2000 mg per day. Thus, one may conclude that it is likely that dairy intake was accompanied by a greater improvement in these indices of risk for colon cancer than supplemental calcium.

REFERENCES

1. Distler P, Holt PR: Are right- and left-sided colon neoplasms distinct tumors? *Dig Dis* 15:302-311, 1997.
2. Haenszel W, Berg JW, Segi M *et al.*: Large bowel cancer in Hawaiian Japanese. *JNCI* 51:1765-1779, 1973.
3. Winawer SJ, Fletcher RH, Miller L *et al.*: Colorectal cancer screening: clinical guidelines and rationale. *Gastroenterology* 112:594-642, 1997.
4. Mandell JS, Bond JH, Church TR *et al.*: Reducing mortality from colorectal cancer by screening for fecal occult blood. *N Engl J Med* 328:1365-1371, 1993.
5. Winawer SJ, Zauber AG, Ho MN *et al.*: Prevention of colorectal cancer by colonoscopic polypectomy. *N Engl J Med* 329:1977-1981, 1993.
6. Muller AD, Sonnenberg A: Prevention of colorectal cancer by flexible endoscopy and polypectomy. A case-control study of 32,702 veterans. *Ann Intern Med* 123:904-910, 1995.
7. Boring CC, Squires TS, Tong T: Cancer statistics. *CA Cancer J Clin* 41:19-51, 1991.
8. Parker SL, Tong T, Bolden S, Wingo PA: Cancer statistics. *CA Cancer J Clin* 47:5-27, 1997.
9. Lipkin M: Application of intermediate biomarkers to studies of cancer prevention in the gastrointestinal tract: introduction and perspective. *Am J Clin Nutr* 54:188S-192S, 1991.
10. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Doody LA: Surrogate endpoint biomarkers for phase II cancer chemoprevention trials. *J Cell Biochem* 19:1-9, 1994.
11. Eaton SB, Shostak M, Konner M: "The Paleolithic Prescription." New York: Harper and Row, 1988.
12. Newmark H, Wargovich M, Bruce R: Colon cancer and dietary fat, phosphate and calcium—a hypothesis. *J Natl Canc Inst* 72: 1323-1325, 1984.
13. Potter JD, Slattery ML, Bostick RM, Gapstur SM: Colon cancer: a review of the epidemiology. *Epidemiol Rev* 15:499-545, 1993.
14. Garland C, Barrett-Connor E, Ross AH, Shekelle RB, Criqui MH, Paul O: Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* 307-309, 1985.
15. Slattery ML, Sorenson AW, Ford MH: Dietary calcium intake as a mitigating factor in colon cancer. *Am J Epidemiol* 128:504-514, 1988.

16. Kune S, Kune GA, Watson LF: Case-control study of dietary etiological factors: the Melbourne colorectal cancer study. *Nutr Cancer* 9:21–42, 1987.
17. Sorenson AW, Slattery ML, Ford MH: Calcium and colon cancer: a review. *Nutr Cancer* 11:135–145, 1988.
18. Lipkin M, Newmark H: Effect of added dietary calcium on colonic epithelial-cell proliferation in subjects at high risk for familial colonic cancer. *N Engl J Med* 313:1381–1384, 1985.
19. Bostick RM: Human studies of calcium supplementation and colorectal epithelial cell proliferation. *Cancer Epidemiol Biomark Prev* 6:971–980, 1997.
20. Garland CF, Garland FC: Do sunlight and vitamin D reduce the likelihood of colon cancer? *Int J Epidemiol* 9:227–231, 1980.
21. Garland CF, Garland FC, Shaw EK, Comstock GS, Helsing KJ, Gorham ED: Serum 25-hydroxyvitamin D and colon cancer: Eight-year prospective study. *Lancet* 1176–1178, 1989.
22. Glass AR, Kikendall JW, Sobin LH, Bowen PE: Serum 25-hydroxyvitamin D concentrations in colonic neoplasia. *Horm Metab Res* 25:397–398, 1993.
23. Pritchard RS, Baron JA, Gerhardsson de Verdier M: Dietary calcium, vitamin D, and the risk of colorectal cancer in Stockholm, Sweden. *Cancer Epidemiol Biomark Prev* 5:897–900, 1996.
24. White E, Shannon JS, Patterson RE: Relationship between vitamin and calcium supplement use and colon cancer. *Cancer Epidemiol Biomark Prev* 6:769–774, 1997.
25. Willett W: The search for the causes of breast and colon cancer. *Nature* 338:389–394, 1989.
26. Weisburger JH: Causes, relevant mechanisms, and prevention of large bowel cancer. *Semin Oncol* 18:316–336, 1991.
27. Burkitt DP, Walker ARP, Painter NS: Effect of dietary fiber on stools and transit-times, and its role in the causation of disease. *Lancet* ii:1408–1411, 1972.
28. Phillips RL, Garfinkel L, Kuzma JW, Beeson WL, Lotz T, Brin B: Mortality among California Seventh-Day Adventists for selected cancer sites. *J Natl Cancer Inst* 65:1097–1107, 1980.
29. Alberts DS, Einspahr J, Ritenbaugh C *et al.*: The effect of wheat bran fiber and calcium supplementation on rectal mucosal proliferation rates in patients with resected adenomatous colorectal polyps. *Cancer Epidemiol Biomark Prev* 6:161–169, 1997.
30. de Cosse JJ, Miller HH, Lesser ML: Effective wheat fiber and vitamins C and E on rectal polyps in patients with familial adenomatous polyposis. *J Natl Cancer Inst* 81:1290–1297, 1989.
31. McKeown-Eyssen GE, Bright-See E: Dietary factors in colon cancer: international relationships. *Nutr Cancer* 6:160–170, 1984.
32. MacLennan R, McCrae F, Bain C *et al.*: The Australian Polyp Prevention Project. Randomized trial of fat, fiber and β carotene to prevent colorectal adenomas. *J Natl Cancer Inst* 87:1760–1767, 1995.
33. Ingles SA, Bird CL, Shikany JM, Frankl HD, Lee ER, Haile RW: Plasma tocopherol and prevalence of colorectal adenomas in a multiethnic population. *Cancer Res* 58:661–666, 1998.
34. Greenberg ER, Baron JA, Tosteson TD *et al.*: A clinical trial of antioxidant vitamins to prevent colorectal adenoma. *N Engl J Med* 331:141–147, 1994.
35. Clark LC, Combs G, Turnbull BW *et al.*: Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. *JAMA* 276:1957–1963, 1996.
36. Garland CF, Garland FC, Shaw EK, Comstock GW, Helsing KJ, Gorham ED: Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet* 1176–1178, 1989.
37. MacLennan WJ, Hamilton JC: Vitamin D supplements and 25-hydroxyvitamin D in the elderly. *Br Med J* ii:859–861, 1997.
38. Jacques PF, Felson DT, Tucker KL *et al.*: Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. *Am J Clin Nutr* 66:929–936, 1997.
39. Pence BC, Buddingh F: Inhibition of dietary fat-promoted colon carcinogenesis in rats by supplemental calcium or vitamin D₃. *Carcinogenesis* 9:187–190, 1988.
40. Sitrin MD, Halline AG, Abrahams C, Brasitus TA: Dietary calcium and vitamin D modulate 1,2-dimethylhydrazine-induced colonic carcinogenesis in the rat. *Cancer Res* 51:5608–5613, 1991.
41. Kinyamu HK, Gallagher JC, Knezetic JA, DeLuca HF, Prah JM, Lanspa SJ: Effect of vitamin D receptor genotypes on calcium absorption, duodenal vitamin D receptor concentration, and serum 1,25 dihydroxyvitamin D levels in normal women. *Calcif Tissue Int* 60:491–495, 1997.
42. Morrison NA, Qi JC, Tokita A *et al.*: Prediction of bone density from vitamin-D-receptor alleles. *Nature* 367:284–287, 1994.
43. Spector TD, Keen RW, Arden NK *et al.*: Influence of vitamin-D-receptor genotype on bone mineral density in postmenopausal women: a twin study in Britain. *Br Med J* 310:1357–1360, 1995.
44. Lore F, Di Cairano G, Di Perri G: Vitamin D status in the extreme age of life. *Ann Med Int* 137:209–211, 1986.
45. Brehier A and Tomassett M: Human colon cell line HT-29: characterization of 1,25-dihydroxyvitamin D₃ receptors in established human cancer cell lines in culture. *Cancer Res* 42:1116–1119, 1982.
46. Lointier P, Wargovich MJ, Saez S, Levin B, Wildrick D *et al.*: The role of vitamin D₃ in the proliferation of a human colon cancer cell line *in vitro*. *Anticancer Res* 7:817–822, 1987.
47. Shabahang M, Buras RR, Davoodi F, Schumaker LM, Nauta RJ, Evans SRT: 1,25-dihydroxyvitamin D₃ receptor as a marker of human colon carcinoma cell line differentiation and growth inhibition. *Cancer Res* 53:3712–3728, 1993.
48. Wali RK, Bissonnette M, Khare S, Hart J, Sitrin MD, Brasitus TA: 1 α ,15-dihydroxy-16-ene-23-yne-26,27-hexafluorocholecalciferol, a non-calcemic analogue of 1 α ,25-dihydroxyvitamin D₃, inhibits azoxymethane-induced colonic carcinogenesis. *Cancer Res* 55:3050–3054, 1995.
49. Wali RK, Baum CL, Sitrin MD, Bolt MJG, Dudeja PK, Brasitus TA: Effect of vitamin D status on the rapid actions of 1,25-dihydroxy-cholecalciferol in rat colonic membranes. *Am J Physiol* 262:945–953, 1992.
50. Bissonnette M, Tien X-Y, Niedziela SM *et al.*: 1,25(OH)₂ vitamin D₃ activates PKC- α in Caco-2 cells: a mechanism to limit secosteroid-induced rise in [Ca²⁺]_i. *Am J Physiol* 267:465–475, 1994.
51. Bolt MJG, Bissonnette BM, Wali RK, Hartmann SC, Brasitus TA, Sitrin MD: Characterization of phosphoinositide-specific phospholipase C in rat colonocyte membranes. *Biochem J* 292:271–276, 1993.
52. Khare S, Bolt MJG, Wali RK *et al.*: 1,25 dihydroxyvitamin D₃ stimulates phospholipase c in rat colonocytes: Role of c-Src in PLC-g activation. *J Clin Invest* 99(8):1831–1841, 1997.

53. Gupta AK, Sexton RC, Rudney H: Effect of vitamin D3 derivatives on cholesterol synthesis and HMG-CoA reductase activity in cultured cells. *J Lipid Res* 30:379–386, 1989.
54. Brenner BM, Russell N, Albrecht S, Davies RJ: The effect of dietary vitamin D3 on the intracellular calcium gradient in mammalian colonic crypts. *Cancer Lett* 127:43–53, 1998.
55. Thomas MG, Tebbutt S, Williamson RN: Vitamin D and its metabolites inhibit cell proliferation in human rectal mucosa and a colon cancer cell line. *Gut* 33:1660–1663, 1992.
56. Thomas MG, Brown GR, Alison MR, Williamson RCN: Divergent effects of epidermal growth factor and calcipotriol on human rectal cell proliferation. *Gut* 35:1742–1746, 1994.
57. Thomas MG, Nugent KP, Forbes A, Williamson RCN: Calcipotriol inhibits rectal epithelial cell proliferation in ulcerative proctocolitis. *Gut* 35:1718–1720, 1994.
58. Winawer SJ, Zauber AG, O'Brien MJ *et al.*: Randomized comparison of surveillance intervals after colonoscopic removal of newly diagnosed adenomatous polyps. *N Engl J Med* 328:901–906, 1993.
59. Rex DK, Cutler CS, Lemel T *et al.*: Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. *Gastroenterology* 112:24–28, 1997.
60. Hyman J, Baron JA, Dain BJ *et al.*: Dietary and supplemental calcium and the recurrence of colorectal adenomas. *Canc Epidemiol Biomark Prev* 7:291–295, 1998.
61. Lipkin M: Biomarkers of increased susceptibility to gastrointestinal cancer: new application to studies of cancer prevention in human subjects. *Cancer Res* 48:235–245, 1988.
62. Lipkin M, Uehara K, Winawer S *et al.*: Seventh-Day Adventist vegetarians have a quiescent proliferative activity in colonic mucosa. *Cancer Lett* 26:139–144, 1985.
63. Yang K, Cohen L, Lipkin M: Lectin soybean agglutinin: measurements in colonic epithelial cells of human subjects following supplemental dietary calcium. *Cancer Lett* 56:65–69, 1991.
64. Filipe MI, Branfoot AC: Abnormal patterns of mucus secretion in apparently normal mucosa of large intestine with carcinoma. *Cancer* 34:282–290, 1974.
65. Garin-Chesa P, Rettig WJ, Melamed MR: Expression of cytokeratins in normal and neoplastic colonic epithelial cells. Implications for cellular differentiation and carcinogenesis. *Am J Surg Path* 10:829–835, 1986.
66. Moorehead RJ, Hoper M, McKelvey STD: Assessment of ornithine decarboxylase activity in rectal mucosa as a marker for colorectal adenomas and carcinomas. *Br J Surg* 74:364–365, 1987.
67. Lans JJ, Jaszewski R, Arlow FL, Tureaud J, Luk GD, Majumdar APN: Supplemental calcium suppresses colonic mucosal ornithine decarboxylase activity in elderly patients with adenomatous polyps. *Cancer Res* 51:3416–3419, 1991.
68. Wargovich ML, Allnut D, Palmer C, Anaya P, Stephens LC: Inhibition of the promotional phase of azoxymethane-induced colon carcinogenesis in the F344 rat by calcium lactate: effect of simulating two human nutrient density levels. *Cancer Lett* 53:17–25, 1990.
69. Yang K, Fan K, Newmark H *et al.*: Cytokeratin, lectin, and acidic mucin modulation in differentiating colonic epithelial cells of mice after feeding Western-style diets. *Cancer Res* 56:4644–4648, 1996.
70. Oshima H, Oshima M, Kobayashi M, Tsutsumi M, Taketo MM: Morphological and molecular processes of polyp formation in Apc^{Δ716}. *Cancer Res* 57:1644–1649, 1997.
71. Lipkin M, Enker WE, Winawer SJ: Tritiated-thymidine labeling of rectal epithelial cells in 'non-prep' biopsies of individuals at increased risk for colonic neoplasia. *Cancer Lett* 87:153–161, 1987.
72. Richter F, Richter A, Yang K, Lipkin M: Cell proliferation in rat colon measured with bromodeoxyuridine, proliferating cell nuclear antigen, and [³H]thymidine. *Cancer Epidemiol Biomark Prev* 1:561–566, 1992.
73. Bostick RM, Fosdick L, Tamera JL *et al.*: Methodological findings and considerations in measuring colorectal epithelial cell proliferation in humans. *Cancer Epidemiol Biomark Prev* 6:931–942, 1997.
74. Holt PR, Moss SF, Kapetanakis AM *et al.*: Is Ki-67 a better proliferative marker in the colon than proliferating cell nuclear antigen? *Cancer Epidemiol Biomark Prev* 6:131–135, 1997.
75. Terpstra OT, Blankenstein M, Dees J, Eilers GAM: Abnormal pattern of cell proliferation in the entire colonic mucosa of patients with colon adenoma or cancer. *Gastroenterology* 92:704–708, 1987.
76. Gregoire R, Yeung KS, Stadler J *et al.*: Effect of high fat and low-fibre meals on the cell proliferation activity of colorectal mucosa. *Nutr Cancer* 15:21–26, 1991.
77. Lieberman V, Nyska A, Kashtan H, Zajicek G, Lubin F and Rozen P: Differing proliferative responses in proximal and distal colons of growing rats fed food eaten by adenoma patients. *Dig Dis Sci* 41:1057–1064, 1996.
78. Bostick RM, Fosdick L, Grandits GA *et al.*: Colorectal epithelial cell proliferative kinetics and risk factors for colon cancer in sporadic adenoma patients. *Cancer Epidemiol Biomark Prev* 6:1011–1019, 1997.
79. Lipkin M, Friedman E, Winawer SJ, Newmark H: Colonic epithelial cell proliferation in responders and nonresponders to supplemental dietary calcium. *Cancer Res* 49:248–254, 1989.
80. Rozen P, Fireman A, Fine N, Wax Y, Ron E: Oral calcium suppresses increased rectal epithelial proliferation of persons at risk for colorectal cancer. *Gut* 30:650–655, 1989.
81. Baron JA, Tosteson TD, Wargovich MJ *et al.*: Calcium supplementation and rectal mucosal proliferation: a randomized controlled trial. *J Natl Cancer Inst* 87:1303–1307, 1995.
82. Bostick R, Potter JD, Fosdick L *et al.*: Calcium and colorectal epithelial cell proliferation: a preliminary randomized, double-blinded, placebo-controlled clinical trial. *J Natl Cancer Inst* 85:132–141, 1993.
83. Baron JA, Beach M: A randomized trial of calcium supplementation to prevent colorectal adenomas [abstract]. *Gastroenterology* 114:A563, 1998.
84. Welberg JWM, Monkelbaan JF, de Vries EGE *et al.*: Effects of supplemental dietary calcium on quantitative and qualitative fecal fat excretion in man. *Ann Nutr Metab* 38:285–291, 1994.
85. Denke MA, Fox MM, Schulte MC: Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. *J Nutr* 123:1047–1053, 1993.
86. Alder RJ, McKeown-Eyssen G, Bright-See E: Randomized trial of the effect of calcium supplementation of fecal risk factors for colorectal cancer. *Am J Epidemiol* 138:804–814, 1993.

87. Van der Werf SDJ, Nagengast FM, Van Berg Henegouwen GP, Huijbregts AWN, Van Tongeren JHM: Colonic absorption of secondary bile acids in patients with adenomatous polyps and in matched controls. *Lancet* 1:759–762, 1982.
88. Moorehead RJ, Campbell GR, Donaldson JD, McKelvey STD: Relationship between duodenal bile acids and colorectal neoplasia. *Gut* 28:1454–1459, 1987.
89. Wargovich MJ, Eng VWS, Newmark HL, Bruce WR: Calcium ameliorates the toxic effect of deoxycholic acid on colonic epithelium. *Carcinogenesis* 4:1205–1207, 1983.
90. Wargovich MJ, Eng VWS, Newmark HL: Calcium inhibits the damaging and compensatory proliferative effects of fatty acids on mouse colon epithelium. *Cancer Lett* 23:253–258, 1984.
91. Van der Meer R, Welberg JWM, Kuipers F *et al.*: Effects of supplemental dietary calcium on the intestinal association of calcium, phosphate, and bile acids. *Gastroenterology* 99:1653–1659, 1990.
92. Lapre JA, De Vries HT, Termont DSML, Kleibeuker JH, De Vries EGE, Van der Meer R: Mechanism of the protective effect of supplemental dietary calcium on cytolytic activity of fecal water. *Cancer Res* 53:248–253, 1993.
93. Govers MJAP, Termont DSML, Van der Meer R: Mechanism of the antiproliferative effect of milk mineral and other calcium supplements on colonic epithelium. *Cancer Res* 54:95–100, 1994.
94. Govers MJAP, Termont DSML, Lapre JA, Kleibeuker JH, Vonk RJ, Van der Meer R: Calcium in milk products precipitates intestinal fatty acids and secondary bile acids and thus inhibits colonic cytotoxicity in humans. *Cancer Res* 56:3270–3275, 1996.
95. Glinghammar B, Venturi M, Rowland IR, Rafter JJ: Shift from a dairy product-rich to a dairy product-free diet: influence on cytotoxicity and genotoxicity of fecal water—potential risk factors for colon cancer. *Am J Clin Nutr* 66:1277–1282, 1997.
96. Govers MJAP, Van der Meer R: Effects of dietary calcium and phosphate on the intestinal interactions between calcium, phosphate, fatty acids and bile acids. *Gut* 34:365–370, 1993.
97. Newmark HL, Holt PR: Shift from a dairy product-rich to a dairy product-free diet: influence on cytotoxicity and genotoxicity of fecal water—potential risk factors for colon cancer. *Letter Am J Clin Nutr* (in press), 1998.
98. Van der Meer R, De Vries HT: Differential binding of glycine- and taurine-conjugated bile acids to insoluble calcium phosphate. *Biochem J* 229:265–268, 1985.
99. Van der Meer R, Termont DSML, De Vries HT: Differential effects of calcium ions and calcium phosphate on cytotoxicity of bile acids. *Am J Physiol* 260:G142–G147, 1991.
100. Lapre JA, Termont DSML, Groen AK *et al.*: Lytic effects of mixed micelles of fatty acids and bile acids. *Am J Physiol* 263:G333–G337, 1992.
101. Lapre JA, De Vries HT, Van der Meer R: Dietary calcium phosphate inhibits cytotoxicity of fecal water. *Am J Physiol* 261:G907–G912, 1991.
102. Welberg JWM, Kleibeuker JH, Van der Meer R *et al.*: Effects of oral calcium supplementation on intestinal bile acids and cytolytic activity of fecal water in patients with adenomatous polyps of the colon. *Eur J Clin Invest* 23:63–68, 1993.
103. Buset M, Lipkin M, Winawer S, Swaroop S, Friedman E: Inhibition of human colonic epithelial cell proliferation in vivo and in vitro by calcium. *Cancer Res* 46:5426–5430, 1986.
104. Scalmati A, Lipkin M, Newmark H: Calcium, vitamin D, and colon cancer. *Clin Appl Nutr* 2:67–74, 1992.
105. Chang W-CL, Chapkin RS, Lupton JR: Predictive value of proliferation, differentiation and apoptosis as intermediate markers for colon tumorigenesis. *Carcinogenesis* 18:721–730, 1997.
106. Whitfield JF, Boynton AL, MacManus JP *et al.*: The regulation of cell proliferation by calcium and cyclin AMP. *Mol Cell Biochem* 27:155, 1979.
107. Hennings H, Michael D, Cheng C *et al.*: Calcium regulation of growth and differentiation of mouse epidermal cells in culture. *Cell* 19:245, 1980.
108. Babcock MS, Marino MR, Gunning WE III *et al.*: Clonal growth and serial propagation of rat esophageal epithelial cells. *In Vitro* 19:403, 1983.
109. McGrath M, Soule HD: Calcium regulation of normal mammary epithelial cell growth in culture. *In Vitro* 20:652, 1984.
110. Soule HD, McGrath CM: A simplified method for passage and long-term growth of human mammary epithelial cell. *In Vitro* 22:6, 1985.
111. Boffa LC, Mariani MR, Newmark H *et al.*: Calcium as a modulator of nucleosomal histones acetylation in cultured cells. *Proc Am Assoc Cancer Res* 30:8, 1989.
112. Optimal calcium intake. NIH Consensus Statement 12:1–31, 1994.
113. Curhan GC, Willett WC, Rimm EB, Stampfer MJ: A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med* 328:833–838, 1993.
114. Cook JD, Dassenko SA, Whittaker P: Calcium supplementation: effect on iron absorption. *Am J Clin Nutr* 53:106–111, 1991.
115. Hallberg L, Brune M, Erlandsson M *et al.*: Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* 53:112–119, 1991.
116. Hallberg L: Does calcium interfere with iron absorption? *Am J Clin Nutr* 68:3–4, 1998.
117. Whiting SJ: Safety of some calcium supplements questioned. *Nutr Rev* 52:95–97, 1994.
118. Evron E, Golland S, von der Walde J, Schattner A, Stoeber ZM: Idiopathic calcitriol-induced hypercalcemia. A new disease entity? *Arch Intern Med* 157:2142–2145, 1997.
119. Kune S, Kune GA, Watson LF: Case-control study of dietary etiological factors: the Melbourne colorectal cancer study. *Nutr Cancer* 9:21–42, 1987.
120. Macquart-Moulin G, Riboli E, Cornee J, Charnay B, Berthezene P, Day N: Case-control study on colorectal cancer and diet in Marseilles. *Int J Cancer* 38:183–191, 1986.
121. Negri E, La Vecchia C, D'Avanzo B, Franceschi S: Calcium, dairy products, and colorectal cancer. *Nutr Cancer* 13:255–262, 1990.
122. Jensen OM, MacLennan R, Wahrendorf J: Diet, bowel function, fecal characteristics, and large bowel cancer in Denmark and Finland. *Nutr Cancer* 4:5–19, 1982.
123. Jensen OM, MacLennan R: Dietary factors and colorectal cancer in Scandinavia. *Israel J Med Sci* 15:329, 1979.
124. Stemmermann GN, Nomura A, Chyou P-H: The influence of dairy and nondairy calcium on subsite large-bowel cancer risk. *Dis Col & Rect* 33:190–194, 1990.

125. Rosen M, Nystrom L, Wall S: Diet and cancer mortality in the counties of Sweden. *Am J Epidemiol* 127:42–49, 1988.
126. Peters RK, Pike MC, Garabrant D, Mack TM: Diet and colon cancer in Los Angeles County, California. *Cancer Causes & Control* 3:457–473, 1992.
127. Nelson RL, Tanure JC, Andrianopoulos G: The effect of dietary milk and calcium on experimental colorectal carcinogenesis. *Dis Colon Rectum* 30:947–949, 1987.
128. Holt PR, Atillasoy EO, Gilman J, Guss J, Moss SF, Newmark H, Fan K, Yang K, Lipkin M: Modulation of abnormal colon epithelial cell proliferation and differentiation by low-fat dairy foods: a randomized controlled trial. *JAMA* 280(12):1074–1079, September 23/30, 1998.

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