VI. LETTERS OF EXPERT OPINION

Strang
Cancer Research Laboratory

Martin Lipkin, MD
Director of Clinical Research
Professor of Medicine
Cornell University Medical College,
Graduate School of Medical Sciences

Telephone:
212-734-0567 x206, x208
212-879-9371
Fax: 212-570-6995
e-mail:lipkin@rockvax.rockefeller.edu

May 3, 2004

Lisa Dispensa, MS, RD
Scientific Affairs
Wyeth Consumer Healthcare
Five Giralda Farms
Madison, NJ 07490

Dear Dr. Dispensa,

This letter is written to provide my expert opinion on how, based on scientific evidence, I would rank the use of calcium for the purpose of inhibiting colonic polyps. Based on the evidence described below my opinion is that calcium helps to prevent recurrent colonic polyps which are a major risk factor and cause of colon cancer. The use of increased dietary calcium for this purpose is unique because calcium is a natural substance and not a pharmaceutical agent, with great safety in the effective amounts of calcium shown to prevent recurrent colonic polyps in humans.

It is most important to note there is much greater evidence for calcium's ability to inhibit colonic polyps and other tumors than any other preventive drug treatment. This evidence has been widely developed both in clinical studies carried out in human subjects and in extensive preclinical studies. Much of this clinical as well as preclinical evidence is summarized below. The effect of calcium is further aided by vitamin D, and evidence as well as mechanisms of vitamin D activity are also described below. Recent articles of ours on this subject, and pages from our original textbook on calcium and colon cancer prevention also are enclosed.

Early human studies of calcium as a chemopreventive agent for colon cancer

In earlier work, we carried out the first study using supplemental dietary calcium as a candidate chemopreventive agent to inhibit colon cancer. That

study demonstrated the concept of calcium's inhibition of colonic cancer with normalization of a colonic proliferative biomarker in the study subjects (Lipkin and Newmark, 1985) (Table 1); this was later validated by other larger randomized clinical trials in which calcium had the same effect (summarized in Lipkin, 1999 and Lipkin and Newmark, 1993).

Following our initial study of calcium supplementation in human subjects, several large clinical trials were begun attempting to inhibit colonic adenoma recurrence, and those studies have now demonstrated significant inhibition of colonic adenoma recurrence in association with increasing dietary calcium intake (Hofstad *et al.*, 1998; Baron *et al.*, 1999; Bonithon-Kopp *et al.*, 2000). The first randomized adenoma clinical trial reported was that of Hofstad *et al.* (1998), which showed a significant reduction of adenoma recurrence in patients with single adenomas, and a reduction in adenoma growth. In that trial, 1600 mg of calcium daily plus several vitamins were administered. The second clinical trial reported was that of Baron *et al.* (1999), increasing dietary calcium alone, also resulting in a significant reduction in adenoma recurrence. A third study by Bonithon-Kopp *et al.* (2000) showed a trend towards decreased adenoma recurrence after increasing dietary calcium intake. A recent human study has also demonstrated a significant contribution of vitamin D to calcium's antiproliferative effects in the human colon (Holt *et al.*, 2000).

Earlier studies had indicated a basis for increasing calcium intake to inhibit colonic lesions (Newmark *et al.*, 1984) with preclinical supporting observations (Lipkin *et al.*, 1991; Lipkin and Newmark, 1995). Recent studies in rodent models have further shown a contribution of calcium in decreasing colonic tumors when the tumors were induced by targeted mutations that lead to colonic and intestinal tumors (Yang *et al.*, 1998) or by dietary factors (Newmark *et al.*, 2001). These studies and many others in rodent models have shown increased ability of dietary calcium to reduce colonic tumor development.

Table 1 Calcium effects on colonic proliferation, differentiation and cytotoxicity in human subjects

In vivo	References
Decreased hyperproliferation	Lipkin *et al.*, 1985
Decreased hyperproliferation	Lipkin *et al.*, 1989
Decreased hyperproliferation	Rozen *et al.*, 1989
Decreased proliferation	Lynch *et al.*, 1991
Decreased proliferation	Berger *et al.*, 1991

Decreased proliferation	Wargovich *et al.*, 1992
Decreased proliferation	Barsoum *et al.*, 1992
Decreased proliferation	O'Sullivan *et al.*, 1993
Decreased proliferation	Bostick *et al.*, 1995
Unchanged proliferation	Gregoire *et al.*, 1989
Unchanged proliferation	Cats *et al.*, 1985
Decreased ODC	Lans *et al.*, 1991
Increased differentiation	Yang *et al.*, 1991
Decreased cytoxicity of fecal water	Govers *et al.*, 1996
Decreased proliferation, increased differentiation	Holt *et al.*, 1996
Decreased adenoma recurrence	Hofstad *et al.*, 1998
Decreased adenoma recurrence	Baron *et al.*, 1999
Trend to decreased adenomas	Bonithon-Kopp *et al.*, 2000

Mechanisms of calcium's inhibition of colonic tumor development

The mechanisms of calcium's inhibition of colonic tumors are important. Calcium is an important micronutrient that controls a large number of intracellular and extracellular processes. Intracellular calcium is a pervasive second messenger that acts on many cellular functions, such as fertilization, secretion, muscle contraction, growth and memory. The tight regulation of calcium entry into cells and intracellular release from the endoplasmic reticulum results in a complex control of calcium action in term of amplitude, duration, and spatio-temporal execution; local and global responses to calcium are controlled in the eukaryotic cells with high precision and plasticity. All calcium in bodily fluids, whether in blood, in interstitial spaces or within the cell cytosol or organelles, originates from the diet.

There is a large amount of evidence to show that calcium has a direct growth-restraining, and differentiation and apoptosis-inducing action on normal and tumour cells, including cells of the gastrointestinal tract. An antiproliferative action of dietary calcium on intestinal cells also results from binding to bile and fatty acids, which might reduce the potential damaging, proliferative-inducing effects of these compounds on the intestinal mucosa. To gain a mechanistic insight into these chemopreventive actions of calcium, two basic questions must be addressed: how do cells sense subtle changes in extracellular calcium levels; and how does extracellular calcium convey its message to target cells, thereby setting in motion the appropriate cellular responses?

How cells sense calcium. Cells use different types of calcium-influx channel, which are defined on the basis of their activation mechanisms. Mounting evidence, however, indicates an alternative route for extracellular calcium to convey signals to cells. In this pathway, extracellular calcium acts on the cell membrane and, as a genuine first messenger, binds to a cognate calcium-sensing receptor (CaR). CaR was first cloned from bovine parathyroid cells.

CaR is expressed by a large variety of cells of diverse lineage that do not participate directly in systemic calcium homeostasis. A cation sensing "receptor" in intestinal cell lines was first predicted by Pazianas *et al* and CaR transcript and protein were found to be expressed in all regions of the gastrointestinal tract. Colon-crypt cells express CaR on the basolateral membrane; CaR is also present at the apical region of the crypts, indicating that this receptor functions to sense changes in dietary calcium concentrations. CaRs, which was first identified in human colon-cancer Caco-2 cells by Kallay *et al*, was found to be present in various human colon-cancer cell lines and in malignant cells of the human large intestine.

Further effects of calcium in tumor inhibition.

One of the consequences of a rise in extracellular calcium and CaR activation is an increase in the cytosolic level of calcium, which affects a wide variety of cell responses, some of which relate to the control of growth and differentiation of intestinal and colon cells.

Protein kinase C. Normal intestinal cells express diverse members of the protein kinase C (PKC) family, and their expression is markedly enhanced during differentiation. Modfied expression and activity of specific PKC isozymes have been observed in human and rodent colonic cancer cells. Early studies showed that PKC activity in the mucosa of human colon tumours was reduced compared with normal colon tissue, and the expression of several PKC isozymes was markedly downregulated or lost in adenomas of Apc mutant *min* mice. These observations indicate that decreased expression of PKC isozymes confers permissiveness for intestinal tumorigenesis. Cumulatively, these findings indicate that activation of PKC, through phospholipase C (PLC), might be an integral part of the molecular mechanisms involved in the differentiation of intestinal cells promoted by extracellular calcium. Although this view is attractive and mechanistically based on valid premises, it still awaits experimental evidence.

Interestingly, the chemopreventive action of calcium in colon

tumorigenesis also depends on the differentiation stage of transformed cells. Extracellular calcium at high concentration inhibits the proliferation of well-differentiated human colon-tumour cells, but had no effect on poorly differentiated cells. Shenin and collegues observed a molecular lesion that might contribute to the unresponsiveness of human malignant colon cells to calcium. They reported that the immunohistochemical expression of CaR in colon carcinomas was localized mostly to differentiated areas and was absent from poorly differentiated regions of the tumour. These findings have recently been confirmed by others. Notably, an intra-cellular calcium gradient along the colon-crypt axis, with the highest calcium concentration at the crypt surface, has been identified. This calcium gradient, which is dependent on vitamin D, seems to modulate the terminal differentiation and apoptosis of colon cells. An intriguing possibility, therefore, is that the colon-crypt calcium gradient is disturbed during colon carcinogenesis.

WAF1 and KIP1. PKC signaling is involved in a program of cell-cycle withdrawal in intestinal epithelium, which is associated with the downregulation of expression of cyclin D1 and the differential induction of the WAF1/CIP1 (p21) and KIP1 (p27) cyclin-dependent kinase (CDK) inhibitors. These investigators also reported that PKC-mediated cell-cycle arrest involves hyposphorylation of the retinoblastoma protein, thereby further impeding entry into the cell cycle.

A direct mechanistic link between PKC-induced negative regulation of cell cycle progression and cell differentiation is provided by the observation that WAF1 and KIP1 are potent inducers of differentiation in epithelial cells, including intestinal cells. Consistent with these observations, WAF1 and KIP1 are expressed by colon cells as they exit the crypt proliferative zones and their expression is lost during the early stages of colon tumorigenesis; inactivation of WAF1 enhances tumorigenesis induced by mutant *Apc* and the tumour-promoting activity of a WD.

MAPKs Functional cross-talk between CaR and the mitogen-activated protein kinase (MAPK) pathway was first observed in rat fibroblasts, and has since been reported in parathyroid cells and in CaR-transfected cells. The possibility of this cross-talk is intriguing as several studies have proposed an inportant role for p38 MAPK in the induction of the differentiation program in cells of disparate lineage, including intestinal cells. Phosphorylated and active forms of p38 MAPK are present mainly in the nuclei of differentiated intestinal cells. It is pertinent to note that p38 MAPK was found to interact with and positively regulate the caudal-related homeobox CDX2 transcription factor, which is

involved in the control of intestinal-cell differentiation; indeed, loss of the mouse CDX2 gene results in the development of polyps in the ileum and colon. On the strength of these observations, it might be argued that the differentiation-inducing action of extracellular calcium on transformed colon cells relies, at least in part, on a cascade of events leading to activation of p38 MAPK.

APC pathway and E-cadherin expression. Recently, Chakrabarty and co-workers have observed that exposure of human colon tumor cells to extracellular calcium suppressed β-catenin transcriptional activation and promoted E-cadherin expression. These findings are of great interest as the inappropriate expression of β-catenin is a hallmark of colon cancer.

In normal cells, the wild-type APC protein controls the steady state levels of β-catenin and has an important role in targeting the protein for degradation. Loss of wild-type APC function results in the translocation of β-catenin to the nucleus, where it interacts with TCF- family transcription factors and activates the transcription of several genes, including those encoding cyclin D1, *c*-MYC and the anti-apoptotic protein survivin, which is over expressed by colorectal tumors and in *Apc* 1638N mice. Moreover, the homotypic cell-cell adhesion molecule E-cadherin functions as a tumor suppressor, and upregulation of E-cadherin expression is associated with the induction of differentiation.

c-myc. Kallay *at al.* observed that human colon-cancer cells in culture rendered quiescent by exposure to calcium showed a marked reduction in expression of c-MYC, and they proposed that the decreased level of expression of this oncogene was responsible for the suppression of cell proliferation. This is an interesting and plausible proposition, as we know now that upregulation of c-MYC expression is one of the molecular consequences of inappriopriate β-catenin transcriptional activity in colon-tumor cells.

SERCA. Refilling of calcium stores in the endoplasmic reticulum by active, ATP-driven ion transport is controlled by sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) proteins. Convincing findings have shown that the expression of SERCA proteins is associated with the acquisition of the differentiation program in several cell types. Gelebart *et al* have recently observed that normal colon epithelial cells express the SERCA3 isoenzyme at a high level, but that the expression of this ATP-driven calcium pump is lost in colon-carcinoma cells. These observations are of great interest as they indicate that the loss of differentiation of colon cells during transformation depends, at least in part, on the silencing of a crucial component of the calcium machinery

involved in the cascade of intracellular responses to extracellular calcium.

Apoptosis. Several studies have indicated that changes in intracellular-calcium homeostasis have an important role in the modulation of apoptosis. Many stimuli for cell death, including growth factor withdrawal, hormone stimulation and drug administration, increase the concentration of intracellular calcium and affect the storage of calcium in intracellular organelles. Compounds that directly increase the intracellular concentration of calcium or calcium entry into cells, such as calcium ionophores, have been shown to induce apoptosis in various cell types. In normal mice, an increase in dietary calcium enhances apoptosis of colon epithelium.

In summary, the available evidence indicates that extracellular calcium can activate many signaling pathways through CaR. Much evidence is available to show that several nuclear proteins, such as the transcription factor cAMP-responsive element-binding protein (CREB) are directly influenced by calcium so this 'great signaller' might also influence colon-cell differentiation and impede colon carcinogenesis by acting directly on the nuclear transcription machinery. We surmise that CaR is an important molecular target for dietary calcium in its chemopreventive effect on colon tumorigenesis. The observation that transformed colon cells remain sensitive to calcium until the neoplastic process subverts the growth-restraining and differentiation-inducing signals of calcium indicates that therapy with dietary calcium will be most beneficial during the early stages of colon carcinogenesis, which is of obvious relevance to the design of preventive protocols for the human disease.

Mechanisms of vitamin D inhibition of tumor development

In addition to its well-known role in mineral and skeletal homeostasis, it is widely recognized that the physiologically most active molecular form of Vitamin D, 1α ,25-dihydroxyvitamin D3 [1,25 $[OH]_2$ D_3], also restrains cell proliferation and induces differentiation and apoptosis in a large variety of normal and tumor cells, including cells of the large intestine.

How cells sense vitamin D. .

Most of the pleiotropic, long-term actions of [1,25 $(OH)_2$ D_3] are mediated by binding to a high-affinity receptor (VDR) which is a member of the nuclear hormone-receptor superfamily. VDRs have been identified in human colon tumour cells, and several studies have addressed the question of whether

alterations in VDR expression occur in human during the development and progression of human colon cancer. Cumulatively, the findings indicate that, when compared with normal colon mucosa, VDR density is increased in hyperplastic polyps and in early stages of tumorigenesis, but declines in late stage neoplasia. A significant decrease in [1, 25 $(OH)_2D_3$] - binding sites in the colon of carcinogen-treated rats has been shown.

Vitamin D mediated repression or activation of specific proto-oncogenes or tumor suppressor genes that are related to proliferation and differentiation has been observed in a large variety of normal and tumor tissues, including the small and large intestine. Responsive genes include those that encode WAF1, KIP1, c-MYC, laminin, tenascin, fibronectin, cyclin C, c-FOS, c-JUN, PLCγ and members of the transforming growth factor β (TGF-β) family.

It should be borne in mind, however, that despite the large number of genes that are regulated by vitamin D, only relatively few of them – for example, the genes encoding WAF1 and CaR - contain demonstrable Vitamin D Response Elements (VDREs) in the promoter regions and thereby might be under the direct transcriptional control of vitamin D. This link between vitamin D and CaR is particularly intriguing. Does [1,25$(OH)_2D_3$] –mediated upregulation of expression of CaR further enhance the acquisition of a more benign phenotype by transformed colonic cells in response to extracellular calcium?

As most genes that are responsive to vitamin D do not contain the VDRE consensus sequence in their promoter regions, their regulation might be an indirect, albeit important, response to the cascade of events that is induced by [1,25$(OH)_2D_3$]. Ornithine decarboxylase (ODC), the rate-limiting enzyme in the polyamine biosynthetic pathway, is a pertinent example. Polyamines are important for normal and neoplastic growth. The *ODC* gene promoter contains sequence motifs that can mediate transactivation by c-MYC. ODC activity has been found to be markedly increased in normal proliferating cells and in tumor cells, including intestinal cells of carcinogen-treated rats and Apc mutant *min* mice. As the enhanced expression of ODC is inhibited by vitamin D, the negative action of [1,25 $(OH)_2D_3$] on ODC expression might be a consequence of its direct suppressive effect on *c*-MYC.

Recently, Makishima *et al* have reported that VDR has a role as an intestinal bile-acid sensor. They showed that VDR-activation by the potentially toxic lithocholic acid (LCA), which is known to promote colon carcinogenesis, induced the expression of CYP3A, a cytochrome P45O enzyme that detoxifies

LCA in the liver and intestine. Notably, the gene encoding CYP3A is a target of vitamin D in the intestine [77]. By binding to VDR, therefore, LCA might activate a feedback mechanism that results in its own degradation. The protection provided by VDR against LCA might be overridden when the detoxification pathway is saturated, for example, by increased levels of LCA provided by WDs that are rich in fats.

Pertinent findings relating to the central importance of activated VDR in translating the growth-restraining effects of vitamin D have been obtained in mice that are deficient for VDR. Surprisingly, no apparent histological abnormalities were detected in tissues other than bone that normally express VDR, including the intestine. However, Kallay *et al.*have presented convincing evidence of enhanced mitotic activity in the distal colon of VDR-deficient mice. These investigators also observed biochemical signs of oxidative stress and suggested that loss of responsiveness to [1, 25 $(OH)_2D_3$] might result in oxidative DNA damage.

[1,25 $(OH)_2D_3$] might also bind to a putative receptor located in the plasma membrane, and might therefore act on target tissues through a non-nuclear, non-genomic pathway. The response of the target cells is rapid, within seconds to minutes, and is linked to the activation of intracellular signaling pathways. The response of the target cells is rapid, within seconds to minutes, and is linked to the activation of intracellular signaling pathways. So far, however, no molecular details of the putative membrane-bound receptor are available. Norman *et al* have recently reported the presence of a specific [1,25(OH)2D3] - binding protein in an intestinal membrane fraction. Studies have also indicated the presence of a rapid, non-genomic pathway in rat intestinal cells, and in human colon-cancer-derived cell lines exposed to vitamin D. Recent studies, however, have indicated that nuclear VDR might mediate the non-genomic effects of vitamin D. A targeted disruption of the first zinc finger in the DNA binding domain of VDR abrogates both genomic and non-genomic effects of [1, 25$(OH)_2D_3$].

Further effects of vitamin D

The growth-restraining, anticancer effects of vitamin D on colon cells are conveyed through genomic and post-genomic pathways involving the following factors and processes.

Growth factors/cytokines synthesis and expression

Much evidence is available to show that [1, 25$(OH)_2D_3$] not only regulates the synthesis of growth factors and cytokines, but also modulates growth factor signaling. A salient example is the cross-talk between vitamin D and the TGFβ-SMAD signaling pathway which inhibits epithelial cell proliferation. VDREs have been identified in the promoter region of the genes encoding TGF-β2. Interestingly, studies have shown that SMAD3, a downstream protein component of the TGF-β signaling pathway, acts a co-activator of VDR and therefore positively regulates the vitamin D signaling pathways. Cross-talk between vitamin D and TGF-β1 in the growth inhibition of human colon-cancer derived cells has been reported.

Cell cycle. Part of the pleiotropic, antimitotic actions of vitamin D and synthetic analogues seems to be mediated by the induction of G1 cell-cycle arrest, as a result of the up-regulation of expression of WAF1 and KIP1. Cyclin D1, an important protein involved in control of the cell cycle, was found to be overexpressed by human colorectal tumours, in *Apc*-mutant *min* mice and in *Apc* 1638N mice kept on a WD. The aberrant expression of cyclin D1 in *Apc*-mutant mice and in human colorectal tumors conceivably results from the loss of negative control exerted by wild-type Apc on the activity of free *β*-catenin. So, the chemopreventive effect of dietary vitamin D on intestinal tumorigenesis might depend, at least in part, on the suppression of cyclin-D1 activity.

Interesting findings by Palmer *et al.* indicate that [1,25$(OH)_2D_3$] can also have a growth-restraining action at an early stage of colorectal carcinogenesis, at a time when the loss of APC function occurs. The investigators used the human colorectal cancer cell line SW480, which expresses a truncated form of APC, high levels of β-catenin, and is defective for E-cadherin expression. [1,25 $(OH)_2D_3$] increased transcription of the gene encoding E-cadherin and promoted the translocation of β~catenin from the nucleus to the plasma membrane. Activated VDR competed with TCF transcription factors for β-catenin binding and,therefore, interfered with inappropriate β-catenin-mediated transcriptional activity.

Apoptosis. There is a large amount of evidence indicating that, in addition to the inhibition of tumor growth and progression, [1, 25$(OH)_2 D_3$] exerts an anti-cancer action by inducing apoptosis in various transformed cells, including colon cancer cells. Vaderwalle *et al* first reported that [1, 25 $(OH)_2 D_3$]-induced apoptosis in the human adenocarcinoma cell line HT-29. Diaz and colleagues have shown that [1, 25$(OH)_2 D_3$] induces apoptosis in human colon adenoma and carcinoma cell lines; the death-inducing effect of vitamin D was associated

with up-regulation of the pro-apoptotic protein Bak.

Does this apoptosis-stimulating effect depend on the nuclear VDR pathway? If so, is the *Bak* gene promoter under the direct transcriptional control of vitamin D or is its regulation an indirect response to the cascade of events induced in colon cells after exposure to [1, $25(0H)_2D_3$]? In several types of cells, vitamin D also downregulates expression of the antiapoptotic protein Bcl-2, and upregulates expression of the pro-apototic protein BAX (BCL2-associated X protein). It has recently been shown that [1, $25(OH)_2D_3$] interferes with expression of the antiapoptotic proteins of the IAP (inhibitor of apoptosis) family in prostate cancer cell lines. Does vitamin D have a similar role in the inhibition of expression of survivin, an IAP protein that is normally downregulated by wild-type APC and that is over-expressed by colorectal cancer cells and by adenomas of *Apc* -knockout mice?

In summary, the growth inhibiting, and differentiation-and apoptosis-inducing effects of vitamin D on colon-tumor cells are well documented. Similar to calcium, these actions of [1,25 $(OH)_2$ D_3] depend on the differentiation status of the colon cells; the loss of response of colon cells to vitamin D with increasing malignancy has been attributed, at least partly, to loss of VDR expression. A tenable possibility is that genomic or post-translational alterations in co-activator proteins that are essential for the formation of transcriptionally competent VDR might also render transformed colonic cells poorly sensitive or refractory to the chemopreventive actions of vitamin D.

Martin Lipkin, MD

College of Physicians and Surgeons of Columbia University

Professor (Emeritus)
DEPARTMENT OF MEDICINE

PETER R. HOLT, M.D.
SENIOR ATTENDING
GASTROENTEROLOGY DIVSION
ST. LUKE'S/ROOSEVELT HOSPITAL CENTER
AMSTERDAM AVENUE AT 114TH STREET
NEW YORK, NY 10025
TEL: (212) 523 3679
FAX: (212) 523 3683

May 4, 2004

Lisa Dispensa
Nutrition Sciences Associate
Wyeth Consumer Healthcare
Five Giralda Farms
Madison, NJ 07940

Dear Ms. Dispensa,

On behalf of Wyeth Consumer Healthcare you have asked me to provide scientific opinion about the strength of the evidence that calcium may reduce the risk of recurrent colon polyps, a major risk factor for colon cancer. I believe that I am in a position to provide this opinion since I was chair of gastroenterology at St. Luke's/Roosevelt Hospital Center for 39 years, am Professor of Medicine Emeritus at Columbia University, am Senior Scientist at the Institute for Cancer Prevention, an Adjunct Professor at Rockefeller University, and have been a principal investigator in chemopreventive studies of colorectal cancer supported by the NCI and other agencies. In this letter I summarize the data that in my opinion strongly supports this claim. The information is based upon epidemiologic data, animal studies, studies of mechanism of action of calcium as well as human preventive studies. In addition, outside agencies such as the American Gastroenterology Association through its Practice Guidelines have evaluated chemopreventive approaches to colon cancer and have provided strong support for the role of calcium 3 grams of calcium carbonate (1200mg of elemental calcium) for the prevention of colorectal adenomas.

There are extensive, though not universal, studies that point to an inverse relationship between the dietary intake of calcium and the incidence or death rate from colorectal cancer. These studies include classical observation in the state of Utah as well as international observations focused upon colorectal cancer deaths in Europe and the United States. The weight of this epidemiologic evidence supports the effect of dietary calcium reducing the risk of colorectal neoplasia. A series of epidemiologic studies in the last half decade have given even greater strength to this evidence.

Animal studies have evaluated the effect of calcium upon the development of carcinogen -- induced colorectal cancer, a classical model for the evaluation of agents that might influence colorectal neoplasia. Such studies have almost consistently shown a beneficial effect. Other studies have used germline mutations of crucial genes that cause colon cancer in rodents and demonstrated lower rates of intestinal tumor formations with calcium administration. Furthermore, the important studies of Newmark and Lipkin using a Western-style diet in mice

and rats have demonstrated that calcium with vitamin D lowers several preneoplastic markers of risk for colorectal adenoma and carcinoma formation. Incidentally, these studies also show a change with such supplementation in similar pre-cancerous markers that occur in breast, pancreas and prostate.

There have been extensive studies of the possible mechanisms of action of calcium upon colorectal health and specifically the propensity to colorectal neoplasia. Such mechanistic studies derive from epidemiologic data indicating that a human Western-style diet high in fat, meat and calories and low in fiber was associated with an increased risk of colorectal neoplasia when compared to a diet low in fat, meat and calories and high in fiber. Follow-up observations showed that the fecal excretion of bile acids was increased with a Western-style diet and that if calcium was added to the diet the soluble fraction of colonic contents and feces contained lower levels of bile acids and fatty acids. Bile acids and fatty acids are believed to be damaging to the colorectal mucosa and cause proliferative changes that can lead to neoplasia. This mechanism of action also has been extensively studied in humans and support the contention that this is at least one important mechanism for the beneficial effect of calcium upon colorectal mucosal health.

Finally there have been three human prospective studies that have shown a reduction in colorectal adenoma (polyp) recurrence, a well-accepted precursor for cancer. The most important was that of Baron et al. published in the NEJM in which post-polypectomy patients were given either 1200 mg supplemented calcium or placebo in addition to regular diet. Calcium administration resulted in a 20-25% reduction in the incidence and/or number of polyps that recurred. Importantly, further analysis of the data in this study indicated that calcium had even a greater effect in so-called advanced adenomas, large in size or with more severe pre-cancerous features. In a small European study supplemental calcium 2 gm per day conferred a 25% reduction in polyp recurrence and an almost 50% reduction in those subjects whose dietary calcium intake was less than the median (960 mg per day). A study by Hofstad also showed a reduction of colonic polyp recurrence when calcium was provided with several vitamin supplements.

In my opinion, the weight of the scientific evidence described above thoroughly supports the contention that calcium lowers the recurrence of colorectal polyp (adenoma) formation as well as early markers of increased risk for cancer formation.

It is important to point out that the cited human studies examined the recurrence of colorectal adenomas in individuals who previously had had colorectal adenomas and therefore were at increased risk for recurrence. All human chemopreventive studies have used individuals at high risk for the formation of cancer in order to test chemopreventive approaches. Colonoscopic reevaluation only is justified ethically if adenomas were previously removed. It is reasonable to ask whether the data from such human studies apply to the general population to prevent first time occurrence of colorectal adenomas. In that regard, it is important to point out that colorectal cancer is an extremely common cancer in the United States and that the lifetime risk for colorectal cancer is approximately 6%. Furthermore, it has been estimated that as many as 50% of the United States population will develop a colorectal adenoma during their lifetime. Since colorectal adenoma formation is so common in the U.S. population and adenomatous polyps are established risk factors for colorectal cancer, a preventive approach in the U.S population as a

whole is justified. At the present time our proven approach to prevent colorectal cancer involves doing colonoscopies and removing adenomatous polyps at the time of colonoscopy. Colonoscopy has been proven to be effective in reducing the burden of colorectal cancer and is the standard of care in the U.S. population over age 50. However, the procedure is expensive in the utilization of healthcare professionals as well as in healthcare dollars. Furthermore, there is a small but significant risk in performing colonoscopies. If calcium were just to reduce the number of adenomas then this would clearly improve health and lower health care costs.

Thus, the data for the United States indicates that the population as a whole (50%) are at risk for the development of adenoma and thus it is appropriate and reasonable to consider the use of calcium as a chemopreventive agent for the population as a whole. A health claim for calcium would provide a public health service to U.S. consumers. Furthermore, it is important to recognize that calcium is extremely safe. An NIH panel on calcium has concluded that taking up to 2.5 grams of calcium a day is very safe. Thus, in my opinion, the total publicly available scientific data strongly favors the benefits of this preventive measure for colorectal adenoma formation. Furthermore calcium is inexpensive and has other health benefits such as reducing the risk of osteoporosis in both men and women and potentially helping to reduce blood pressure.

Thus it is my opinion that the weight of the evidence strongly supports the use of supplemental calcium to benefit colorectal health and specifically the formation of colorectal adenomas (polyps) and therefore colorectal cancer.

If there is any further information you may need, please do not hesitate to contact me.

Yours truly,

Peter R. Holt, MD

PRH:smm

Desktop\SMM work\letters-memos\wyeth consumer healthcare.doc

UNIVERSITY OF FLORIDA

College of Medicine
Department of Medicine
Division of Gastroenterology,
Hepatology and Nutrition

1600 SW Archer Road
PO Box 100214
Gainesville, Florida 32610-0214
(352) 392-2877
Fax: (352) 392-3618

May 11, 2004

Lisa Dispensa
Wyeth Consumer
5 Giralda Farms
Madison, NJ 07940

Dear Ms. Dispensa,

It is a distinct pleasure for me to take this opportunity to convey my opinion about the importance of appropriate calcium intake to colorectal health and in particular the prevention of colon polyps and ultimately the prevention of colon cancer. This is a totally preventable cancer if prevention and appropriate screening measures are employed. I was president of the American Gastroenterological Association (AGA) in 1997-1998 and in that capacity aggressively attempted to educate physicians, patients, and legislators about the importance of this cancer. At the present time secondary prevention is being carried out by colonoscopy in patients throughout this country. It is clear to the AGA and to me that ultimate success in preventing this tumor depends not only secondary prevention but also in primary prevention that would include diet, calcium, folic acid, aspirin, and non-steroidal antinflamatory agents.

At the present time the most appropriate primary prevention modality appears to be calcium. The basic scientific findings of Dr. Martin Lipkin demonstrated quite clearly that calcium affected the hyperproliferation of colonic tissue in a very positive manner that would suggest that such calcium administration could prevent colon polyps and ultimately colon cancer. The basic science data were greatly amplified by impressive clinical studies that were well done and appropriately controlled (N Engl J MED 1999; 340: 101-107; J NAT Cancer Inst 94, 437-446). These kinds of studies prompted the American College of Gastroenterology under the leadership of Dr. John Bond to put forth the clinical guideline on management of colon polyps (American Journal of Gastroenterology, 2000; 95: 3053-3063). Dr. Bond and his colleagues suggested that 3g of Calcium Carbonate supplementation can reduce recurrence of colon polyps. Dr. Baron has put forth a number of studies which show that after resection of colon polyps fewer patients who took 3g of calcium carbonate (1.2g of calcium) a day over a number of years developed recurrent polyps. Just 1200mg of calcium per day affords that protection.

The data in regard to other agents for primary prevention is somewhat more cloudy. Regular short term use of aspirin is inversely associated with risk for colorectal polyps. However the greatest protective effect is evident at substantially higher doses (greater than 14 tablets per week) than those recommended for the prevention or cardiovascular disease (Annuals of Internal Medicine 2004; 140:157-166). Treatment of large numbers of healthy persons to prevent colorectal cancer in a small percentage requires a high standard of safety as well as efficacy. For example, the US Preventive Services Task Force recommends routine use of low dose aspirin as primary prophylaxes against myocardial infarction in those at high risk for coronary heart disease. The safety of calcium administration overwhelmingly favors the intake of calcium as a primary agent for prevention of colon polyps. The use of calcium to prevent polyp formation has strong basic science support in experimental animals and humans, solid evidence from clinical trials, and virtually no evidence of any side effects whatsoever with 1200mg of calcium per day.

I firmly believe it is high time that physicians and patients enthusiastically embrace calcium as a form of primary prevention. It is of interest that the same dose that has been shown to prevent the recurrence of colon polyps is also the dose recommended for the prevention and treatment of osteoporosis. I am employing this approach in my own practice and teaching it to our trainees.

Sincerely,

Phillip P Toskes MD

Phillip P. Toskes, MD
Professor of Medicine
Division of Gastroenterology, Hepatology and Nutrition

INSTITUTE FOR CANCER PREVENTION
PREVENTION IS THE BEST CURE
390 Fifth Avenue-3rd floor
New York, NY 10018
212-551-2516 tel./ 914-729-3382 fax

May 7, 2004

Lisa Dispensa, M.S., R.D
Nutrition Sciences Associate
Wyeth Consumer Healthcare
Five Giralda Farms
Madison, NJ 07940

Dear Ms. Dispensa:

With this letter I am responding to your written request of April 22, 2004 that I provide my "expert opinion as to the strength and quality of the science surrounding the claim that "calcium may reduce the risk of recurrent colon polyps, a major risk factor for colon cancer".

My qualifications for writing such an opinion include the following: Currently Professor of Medicine, and until three years ago Chief of the Nutrition Division at New York Presbyterian Hospital and Weill Medical College of Cornell University in charge of all education, research and patient care in nutrition. In addition, I continue as Principal Investigator, Clinical Nutrition Research Unit, one of only two center grant programs in the entire country funded by the National Cancer Institute dedicated exclusively to the study of nutrition and cancer prevention; extensive experience leading and conducting research programs, including calcium intake and colorectal health; service as President of the American Society for Clinical Nutrition, the leading academic organization for professional nutrition scientists; and Member, NIH Nutrition Study Section, the group of scientific experts charged with the responsibility of recommending to the federal government which research proposals are worthy of receiving financial support.

Three years ago I was appointed Senior Vice President for Medical Affairs, Institute for Cancer Prevention. My brief curriculum vitae listing education, awards, academic positions held, service for various agencies and original recent publications is attached for your interest. Should you wish to receive my complete curriculum vitae, listing all professional activities, I shall be happy to provide this as well.

It is my conclusion after a thorough review of the relevant medical literature that the scientific evidence to date overwhelmingly favors the recommendation of calcium on a daily basis for all adult Americans to help prevent colorectal adenomas. This recommendation assumes that the dietary intake of calcium from food sources of most individuals falls below that needed to prevent colorectal adenomas, and furthermore that such supplementation would be a safe practice even when dietary calcium intake is adequate.

At the present time very few adults consume calcium in the amounts that are believed needed (approximately 1200 mg elemental calcium) to achieve this level on a day in and day out basis to prevent colorectal adenomas. Furthermore, calcium is likely to have other benefits as well in both men and women, including reducing prevalence of osteoporosis and preventing and ameliorating some cases of hypertension. Thus, recommending calcium to adults would constitute a major public health service.

The scientific evidence in favor of calcium comes from a large number of independent reports, including the results of basic research studies in animals, and cell-free systems as well as clinical interventional. Of the recent interventional studies, the most significant is that of Baron et al published in the New England Journal of Medicine in 1999. In this report a major reduction in the recurrence of colonic adenomas was observed in subjects receiving 1200 mg/day of elemental calcium compared to controls receiving placebos. This study, as well as the impressive previously accumulated evidence, formed the basis for the official Practice Guidelines of the American Gastroenterological Association (AGA) to consume 1200 mg elemental calcium for the specific prevention of colorectal adenomas. In order for an academic society such as the AGA to issue Practice Guidelines the evidence must he considered to be overwhelming and ready to be applied to the patient population in its entirely.

The studies conducted to date among adults have largely been in individuals at genetic risk for adenomas or who have previously had an adenoma . These groups are "enriched" in the sense that they have a greater likelihood of developing an adenoma than does the general public. Studies are undertaken in these groups because they can be accomplished at much lower expense and in a shorter time period than in the population as a whole. In my opinion, the data obtained from these groups can be generalized to the overall adult population.

Calcium is not costly for individuals, is safe and highly effective.
There is a wide margin between effective doses and toxic doses of calcium. The benefit of calcium supplementation to the general public would be very high. The upper limit of calcium intake that would be safe for virtually all healthy persons is 2500 mg per day.

In summary, for all these reasons, I am of the strong opinion that calcium intake has a very firm relationship to supporting colorectal health, including colorectal cancer and polyps, the precursor lesion to colorectal cancer.

With respect to Vitamin D, it is now widely appreciated that calcium intake by itself cannot be adequately evaluated without considerations given to Vitamin D status. It has been known for many years that Vitamin D increases the extent of absorption of calcium from the intestinal tract. Vitamin D has a number of other effects as well that relate to its anticancer efficacy including promoting cellular differentiating and antimitotic actions and possibly immunological enhancement. Low serum levels of Vitamin D have been detected in patients with polyps. Thus, assuring adequacy of Vitamin D intake is also important in prevention of colon polyps.

Should you have any further questions, or require more information, don't hesistate to get in touch with me.

Very truly yours,

Richard S. Rivlin, M.D.
Senior Vice President for Medical Affairs
Naylor Dana Chair in Nutrition, IFCP
(Formerly)American Health Foundation
Professor of Medicine
Weill Medical College of Cornell University

RSR/lmc