Dietary Modulation of Colon Cancer Risk¹,²
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Abstract
Colon cancer remains a significant global health concern. The impact of specific dietary components on colon tissue likely depends on a host of genomic processes that influence the growth, development, and differentiation of the epithelial cells at the colon crypt surface, where the balance between proliferation and differentiation is maintained possibly through the Wnt (β-catenin/T-cell factor) signaling pathway. A loss of balance caused by either genetic mutations or environmental factors such as dietary habits can modulate the risk for the formation of aberrant crypt foci and ultimately the development of colon cancer. Evidence exists that butyrate reduces the number and the size of aberrant crypt foci in the colon. Butyrate is a natural histone deacetylase inhibitor as well as a molecule involved with enhanced TGF-β-induced SMAD3 phosphorylation, increased IFN-γ-mediated apoptosis, and altered expression of the intestinal muc2 gene that is responsible for mucin synthesis. Other dietary components, such as vitamin D and (n-3) fatty acids, may regulate proliferative properties of colon progenitor cells as well as the differentiation of subcellular lineages. Although these findings are intriguing, there are uncertainties that remain to be resolved including the optimal exposure needed to bring about an effect, the appropriate timing of administration, and if nutrient-nutrient and nutrient-gene interactions determine the overall response. The expanded use of high-throughput technologies, knowledge about the expression of genes and protein fingerprints, and metabolomic profiling will assist in addressing these issues and ultimately in determining the physiological significance of bioactive food components as cancer protectants. J. Nutr. 137: 2576S–2579S, 2007.

Introduction
Colon cancer is one of the most common types of cancer in the United States and accounts yearly for ~11% of all cancer deaths (1). Several epidemiological and preclinical studies reveal that the increased intake of selected bioactive food components including fiber, (n-3) fatty acids (n-3 FAs)⁴, selenium, curcumin, resveratrol, and vitamin D, which are associated with a reduction in conditions such as adenomatous polyposis and ulcerative colitis, may modulate colon cancer risk (Table 1). However, the specific molecular targets for the bioactive food components and the quantity needed to bring about the antitumorigenic effects remain largely unresolved.

Although human colorectal cancers are considered to be a consequence of the accumulation of multiple genetic alterations involved with a variety of cancer processes, the Wnt signaling pathway appears to have a critical role in the etiology of this disease (2). For example, either somatic or germline mutations in the adenomatous polyposis coli (APC) gene in this pathway directly target the proto-oncogene, c-myc, by stimulating β-catenin/T-cell factor (TCF) in the nucleus and thus foster the neoplastic process (3). The transformation further proceeds to adenomas and adenocarcinomas along with changes in gene expression such as k-ras, smad, and p53. Each of these steps has been reported to be influenced by several dietary components (4,5).

Aberrant crypt foci formation in colon
Among the earliest detectable neoplastic lesions in the colon are the aberrant crypt foci (ACF) (6). These are clusters of mucosal cells with an enlarged and thicker layer of epithelia than the surrounding normal crypt cells. Although the progression of the ACF to polyp, adenoma, and adenocarcinoma parallels the accumulation of several genetic and biochemical alterations, only a small fraction of ACF evolve to colon cancer. Currently, it is not clear which crypts develop into tumors and which do not. However, many studies support the concept that the formation of ACF precedes the development of colon cancer (6).

According to the model proposed by van de Wetering (7), the progenitor cells accumulate nuclear β-catenin and express β-catenin/TCF target genes at the bottom third of the crypt. As the cells reach the midcrypt region, β-catenin/TCF activity is down-regulated, which results in cell cycle arrest and differentiation. In this pathway, APC destabilizes oncogenic β-catenin and thus acts as a tumor suppressor gene. When APC or β-catenin is

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⁴ Abbreviations used: ACF, aberrant crypt foci; APC, adenomatous polyposis coli; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDAC, histone deacetylase; n-3 FAs, (n-3) fatty acids; TCF, T-cell factor; TGF, transforming growth factor; VDR, vitamin D receptor.
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Dietary prevention of colon cancer

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### TABLE 1 Dietary components linked to reduced colon cancer risk

<table>
<thead>
<tr>
<th>Food group</th>
<th>Bioactive food components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Fiber (inulin/oligofructose)</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>n-6: Conjugated linoleic acid</td>
</tr>
<tr>
<td></td>
<td>n-3: Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA)</td>
</tr>
<tr>
<td>Phytochemicals</td>
<td>Curcumin, epigallocatechin-3-gallocate, luteolin, quercetin, resveratrol, sulforaphane</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Vitamin A, vitamin D, folate</td>
</tr>
<tr>
<td>Minerals</td>
<td>Calcium, selenium</td>
</tr>
</tbody>
</table>

mutated, as occurs in the majority of colorectal cancer. β-catenin cannot be degraded but accumulates and translocates into the nucleus, where it binds to TCF to activate Wnt target proliferative genes and oncogenes. Thus, these cells become independent of the physiological signals controlling oncogenic β-catenin/TCF activity. As a consequence, they continue to behave as crypt progenitor cells in the surface epithelium, giving rise to ACFs.

### Dietary components can modify ACF formation

The elucidation of molecular sites of action (targets) for dietary components is fundamental to the development of effective prevention strategies and approaches. Accumulating preclinical studies indicate that dietary constituents including those involved with differentiation/proliferation and inflammation can influence various genetic and epigenetic events associated with ACF formation. Evidence exists that 1,25-(OH)2D3 inhibits the accumulation of β-catenin by facilitating its degradation (8). The reduced β-catenin causes a decrease in its transcriptional activity that stimulates the expression of β-catenin/TCF4-responsive oncogenes such as c-myc. These findings are further supported by the data demonstrating that 1α,25-dihydroxyvitamin D significantly decreases the expression of c-myc gene in the nucleus of SW837 cells (9). Thus, it is likely that the active form of vitamin D suppresses the oncogenic function of c-myc and thus inhibits the formation of ACF.

Because vitamin D binding to the vitamin D receptor (VDR) magnifies its antitumorigenic effects, the genotype of VDR may influence the ability of vitamin D to suppress cell growth. The mutations at the key sites of the VDR could cause vitamin D deficiency even when vitamin D itself is supplemented adequately. Therefore, the genotyping of this gene in colorectal cancer patients may provide insights into the possibility that VDR can serve as a biomarker that predicts the susceptibility to colorectal cancer risk. Compared with individuals carrying the FF genotype, individuals with Ff genotype had a 51% increase in risk of colorectal cancer, and those with the ff genotype, an 84% increase in risk (P for trend = 0.01) (10). This effect of the VDR genotype on colorectal cancer risk appeared to be significant only among subjects with low calcium intake. Verification of these findings and the examination of other polymorphisms existing within the VDR will likely shed light on who will benefit most from food or light exposures which enhance vitamin D nutriture.

The relation between vitamin D and colorectal cancer risk is also suggested in the Women’s Health Initiative. The Women’s Health Initiative was a clinical trial examining the effect of a diet low in fat and high in fruit, vegetables, and grains in preventing breast and colorectal cancers and heart disease in healthy postmenopausal women. Findings from the nested case-control study revealed no significant interaction between serum 25-hydroxyvitamin D levels at baseline and treatment assignment (P = 0.54). However, analyses adjusting only for case-control matching revealed a significant inverse trend with lower baseline levels of serum 25-hydroxyvitamin D being associated with an increased risk of colorectal cancer (P for trend = 0.02) (11).

Links between inflammation and colorectal cancer have been demonstrated by the increased risk of developing colon cancer in patients with inflammatory bowel diseases as well as the effectiveness of antiinflammatory drugs to reduce intestinal tumors. Although it is obvious that chronic inflammation of the intestine is closely associated with the colorectal cancer incidence, the underlying molecular mechanisms accounting for this predisposition remain obscure. In any case, it is not terribly surprising that consumption of dietary components with antiinflammatory activity has been associated with the reduced risk of developing colorectal cancer. The inhibition of inflammatory processes by such diverse food components as butyrate, n-3 FAs, and curcumin is beginning to identify key events that are essential for the transformation of normal epithelial colonic cells into a neoplasm (12,13).

Experimentally, the antitumorigenic properties of fiber, which may include nondigestible oligosaccharides such as inulin and oligofructose, appear to relate to butyrate formation by colonic bacteria (14). The preventive and antiinflammatory activities of butyrate have been associated with its ability to modulate transforming growth factor (TGF)-β signaling, IFN-γ-mediated apoptosis, and the expression of intestinal muc2 gene that is responsible for mucin synthesis in cell models. TGF-β is recognized as an antiinflammatory cytokine that is expressed in the gut epithelium and thus serves as an important negative regulator of the proliferation of colonocytes. This growth factor signals through its binding to a cell surface receptor complex, which subsequently phosphorylates Smad2 and Smad3 (15). The phosphorylated Smad2 or Smad3 forms a heterodimeric complex with Smad4. This complex translocates into nucleus and regulates transcription of tumor suppressor genes such as p27 or oncogenes such as c-myc.

Recently, Nguyen et al. (16) demonstrated that Na-butyrate selectively enhanced TGF-β-induced phosphorylation of Smad3 in gut epithelial cells. Smad3 protein is normally maintained in an unphosphorylated, inactive state in the cytoplasm, and thus, the response is to change the normal regulatory control. When TGF-β binds and activates its receptor complex, Smad3 is activated by phosphorylation, and this effect is further magnified by the supplementation of Na-butyrate. Therefore, the apoptotic effect of butyrate on colonocytes may be in part explained by the regulation of phosphorylation of Smad 3 in TGF-β signaling.

Butyrate is also shown to sensitize colon cancer cells to apoptosis induced by IFN-γ, a cytokine produced by activated T lymphocytes and natural killer cells. Preclinical study demonstrates the pretreatment of HT29 colon carcinoma cells with HDAC inhibitors including butyrate, trichostatin A, and suberoylanilide hydroxamic acid significantly enhanced IFN-γ-induced colon cancer cell death (17). This finding is consistent with the observation that the level of IFN-γ is extensively up-regulated in the intestinal mucosa from patients with inflammatory bowel disease.

Because intact STAT1 signaling is required for the expression of the majority of IFN target genes, it is logical that butyrate might influence IFN-γ-induced apoptosis through the inhibition of this signaling pathway. To examine this hypothesis, reporter transfected cells have been treated with HDAC inhibitors with or without IFN-γ. Treatment of colon cancer cells with IFN-γ induced STAT1-dependent gene activation ~150-fold, and this activation was reduced by HDAC inhibitors including butyrate. Impressively, the inhibition caused by butyrate was >90% (17).
Although these results suggest that butyrate indeed enhances IFN-γ-induced apoptosis through the inactivation of STAT1 transcription factor, it is possible that other pathways are being influenced simultaneously.

The ability of Na-butyrate to modulate the balance and localization of different cell lineages in the colon may also contribute to the regulation of intestinal homeostasis. Although the mechanisms responsible for establishing different intestinal cell lineages are largely unknown, the ability of butyrate to inhibit MUC2 gene expression may be important. MUC2 gene is a differentiation marker of the secretory goblet cell lineage that predominantly synthesizes mucins. In vitro studies demonstrate that the treatment of HT29 colon cancer cells with Na-butyrate induces a marked reduction in MUC2 mRNA levels as a function of time (18). Perturbations in goblet cell function via inactivation of the MUC2 gene may be associated with the decreased development of intestinal tumors (18).

Another bioactive food component that produces antiinflammatory effects is n-3 FAs. Epidemiological and preclinical studies suggest that the consumption of a diet high in n-3 FAs appears to be primarily attributed to its antiinflammatory functions. Inhibition of IL-1 and TNF-α synthesis is a common finding when n-3 FAs are provided. The n-3 FAs existing in foods include α-linolenic acid in plants such as olive, walnut, and canola as well as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) occurring in some fish. This article focuses on EPA and DHA because they are known to be more biologically potent than α-linolenic acid. A variety of fish such as salmon, herring, and sardine are good sources for EPA and DHA. Recommendations to consume ~500 mg/d of EPA and DHA to reduce the risk for cardiovascular disease are rather common worldwide. However, the amount needed to influence cancer risk or tumor behavior has been far more elusive. Regardless, it has been shown that supplementation of n-3 FAs to healthy humans suppresses the capacity of monocytes to synthesize TNF-α (19). It is likely that the n-3 FA-mediated decrease in TNF-α brings about increased activity of caspase 3 as well as a decreased activity of NFκB, which induces the apoptosis of colon cancer cells (3). However, the recent observations that n-3 FAs can suppress the expression of HER-2/neu transgenic mice suggest that these fatty acids may have multiple biological effects (20).

**NCI clinical trials for dietary colon cancer prevention**

Table 2 lists currently active NCI clinical trials involved with dietary colon cancer prevention. Most of these are phase II or III, which suggests that the safety/toxicity issue for these components has already been examined and resolved. More detailed information can be found at the web page http://www.cancer.gov.

NIH has sponsored 2 large colorectal cancer trials in which fiber is a variable. The Polyp Prevention Trial and the Wheat Bran Fiber Study were designed to determine the effects of a low-fat, high-fiber, and high-fruit/vegetable eating plan on the recurrence of precancerous polyps in the colon and rectum. The results provided no evidence that the particular dietary interventions were effective in preventing the recurrence of polyps. Questions remain if the fermentability, quantity, and duration of exposure were sufficient to lead to a change in cancer risk. Recent evidence from a wider range of exposure to fiber in the European Prospective Investigation into Cancer study suggests that fiber may have protective benefits (21). In any case, it is possible that dietary factors may influence critical cellular events well before but not after polyp formation, which is dictated mainly by genetics, and thus, fiber may offer protection in only a subset of society. Recent evidence in a rodent model suggests that genetics can influence the immune response to fermentable fibers (22). If this occurs in humans, the identification of the most vulnerable group becomes the real scientific challenge.

**Frontiers for nutrition and cancer prevention research**

The need for unraveling the interactions between the biological response to food components and human genetics can not be overstated. Because genetic and epigenetic differences are known to exist among individuals, it is not surprising that wide variations in the biological response to foods or their components occur in the scientific literature. It is obvious that various dietary components can modulate a number of key cancer processes, including those associated with cell proliferation/differentiation and inflammation. The response is dependent on genes that not only regulate the absorption, metabolism, and excretion of the bioactive food component but also determine the amount and activity of molecular targets where these agents function. Expanded research to determine which cellular process or processes dictate the response to foods or its components and the molecular target(s) accounting for the response will clarify who will benefit most from dietary intervention strategies. Greater attention to gene-diet interactions will undeniably assist in developing tailored approaches for cancer prevention. The

**TABLE 2** NCI colorectal cancer trials with dietary components (active)

<table>
<thead>
<tr>
<th>Bioactive food component</th>
<th>Protocol ID</th>
<th>Subjects (patients with)</th>
<th>Trial type</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin</td>
<td>NCT00319007</td>
<td>FAP*</td>
<td>Prevention</td>
<td>II</td>
</tr>
<tr>
<td>N-3 FAs</td>
<td>NCT0168987</td>
<td>Gastroenterological tumors</td>
<td>Supportive care</td>
<td>IV</td>
</tr>
<tr>
<td>Vitamin D/Ca</td>
<td>NCT0153816</td>
<td>Removed large bowel adenomas</td>
<td>Prevention</td>
<td>II / III</td>
</tr>
<tr>
<td>Selenium</td>
<td>NCT0078897</td>
<td>Adenomatous colorectal polyps</td>
<td>Prevention</td>
<td>III</td>
</tr>
<tr>
<td>Curcumin</td>
<td>NCT0295035</td>
<td>Metastatic colon cancer</td>
<td>Treatment</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>NCT0118989</td>
<td>Resected adenomatous colonic polyps</td>
<td>Prevention</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>NCT0248053</td>
<td>FAP</td>
<td>Prevention</td>
<td>II</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>NCT0256334</td>
<td>Colon cancer</td>
<td>Biomarker</td>
<td>I / II</td>
</tr>
</tbody>
</table>

* FAP, familial adenomatous polyposis; http://www.cancer.govclinicaltrials.
identification and validation of target genes that can be used to assess exposure to food components, evaluate one’s susceptibility, and monitor early signals of the biological response to bioactive food components are needed to create a roadmap for nutrition and health promotion, including that associated with cancer prevention.

**Literature Cited**