

Review

Possible Mechanisms Relating Diet and Risk of Colon Cancer¹

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Abstract

Two recent developments in cancer epidemiology and experimental carcinogenesis provide the basis for two possible mechanisms relating diet and colon cancer risk. The first development is the accumulating epidemiological evidence for an association between insulin resistance and colonic adenomas and cancers. This evidence suggests the following mechanism: the consumption of excess dietary energy results in the development of insulin resistance with increased circulating levels of insulin, triglycerides, and non-esterified fatty acids. These circulating factors subject colonic epithelial cells to a proliferative stimulus and also expose them to reactive oxygen intermediates. These long-term exposures result in the promotion of colon cancer. The second development is the continuing identification of agents that significantly inhibit experimental colon carcinogenesis. These observations suggest the following mechanism: focal loss of epithelial barrier function resulting from a failure of terminal differentiation results in the “leak” of a presently undefined toxin and a focal inflammatory response characterized by evidence of the activation of the COX-2 enzyme and an oxidative stress with the release of reactive oxygen intermediates. The resulting focal proliferation and mutagenesis give rise to aberrant crypt foci and adenomas. The process is inhibited by: (a) demulcents confined to the colonic lumen that “repair” the surface; (b) anti-inflammatory agents; or (c) anti-oxidants. The two mechanisms, *i.e.*, insulin resistance acting throughout the body and focal epithelial barrier failure acting locally, can describe most of the known relationships between diet and colon cancer risk.

Introduction

Risk of colon cancer is closely related to diet and other lifestyle factors in ecological, case-control, and cohort studies and has been extensively reviewed (1–4). In international studies, risk is associated with an increased intake of dietary fat and a decreased intake of cereal grains and dietary fiber. With ana-

lytical studies, risk is associated with a deficiency of vegetables and fruits and a sedentary lifestyle (1, 2) and, perhaps less consistently, with increased dietary energy, meat, cooked meat, sugar, and obesity. These dietary associations with colon cancer are characterized as typical of the Western diet, and, indeed, countries adopting a more Western diet are noting an increase in colon cancer risk. Our problem is to explain the association of colon cancer with such a disparate group of dietary and lifestyle factors.

There have been many reviews of hypotheses that have been developed to explain the relationship between diet and risk of colon cancer (5–7). Pyrolysis products in cooked food can initiate colon cancer (8), and products of fat digestion can be toxic and could act as promoters (9). Fruits and vegetables contain dietary fiber and phytochemicals that can act as anti-oxidants and inhibit colon carcinogenesis (10). There appears to be no shortage of ideas regarding ways in which these dietary factors might work. Why another attempt to consider possible mechanisms at this time? Because there have been two major recent developments in the study of colon carcinogenesis that have implications in our understanding of relevant mechanisms. Neither development has been sufficiently noted, nor have their implications been fully appreciated, though the mechanisms suggested by these two developments can relate most, though certainly not all, of the dietary factors with colon cancer risk.

First, are the epidemiological studies that demonstrate a close association between colon cancer risk and evidence of insulin resistance. Insulin resistance is a condition in which higher levels of insulin are needed to dispose of plasma glucose, and it is associated with an increased risk of type 2 diabetes. These studies, together with experimental studies, suggest a mechanism with widespread effects. The mechanism begins with the excess dietary energy provided by the high-risk diet. This excess energy elevates the intravascular levels of insulin and energy substrates. The excess hormone exposure and excess energy available to epithelial cells stimulate cell signaling pathways to increase the proliferation, presumably favoring cells with defective cell cycle control. The effects are thus widespread throughout the colon and elsewhere in the body.

Second, are the recent carcinogenesis studies that have found a large number of diverse chemicals, including demulcents, anti-inflammatory agents, and antioxidants, which can markedly inhibit the development of colon cancer in mice and rats. These studies suggest a focal mechanism in which high-risk diets stimulate the development of colon cancer precursor lesions. The proposed mechanism involves the focal loss of normal epithelial cell barrier function. In some unknown way, this loss induces both a focal inflammatory response and a local release of ROIs³. These increase proliferation and mutation in normal as well as in precursor lesions, in ACF, and in adenomas

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³ The abbreviations used are: ROI, reactive oxygen intermediate; ACF, aberrant crypt foci; NEFA, non-esterified fatty acid; NSAID, non-steroidal anti-inflammatory drug; PEG 8000, polyethylene glycol MW8000.

Table 1 Recent studies that have looked for an association between colonic polyps or cancers and insulin resistance or type 2 diabetes

Authors	Date	Subjects ^a	End points ^b	Design	Odds ratio ^c
C. L. Bird <i>et al.</i> (17)	1996	1,006 men, women from sigmoidoscopy clinic	Serum TG in patients with adenomas, controls	Case-control	1.5 (1.0–2.2)
G. E. Eyssen <i>et al.</i> (18)	1996	Colonoscopy clinic	Serum insulin, TG in patients with adenomas, controls	Case-control	3.2 ($P < 0.01$)
E. B. Hu <i>et al.</i> (19)	1999	118,072 nurses; 7,069 diabetics; 892 cancers	Incident colorectal cancer in subjects with diabetes, controls	Cohort	1.4 (1.1–1.9)
R. Kaaks <i>et al.</i> (20)	2000	New York University Women's Health study	Serum C-peptide and IGFbps	Cohort	2.9 (1.3–6.8)
S. Kono <i>et al.</i> (21)	1998	7,637 men; 821 with adenoma	Glucose tolerance in sigmoid adenoma patients, controls	Cohort	1.4 (1.0–2.0)
R. E. Schoen <i>et al.</i> (22)	1999	5,849 men; 102 incident adenomas	Glucose tolerance in colorectal cancer patients, controls	Cohort	2.4 (1.2–4.7)
J. C. Will <i>et al.</i> (23)	1999	>1,000,000; 15,487 diabetics; 3,218 cancers	Incident colorectal cancer in diabetics, controls	Cohort	1.3 (1.0–1.6)
K. Yamada <i>et al.</i> (24)	1998	83,272; 128 <i>in situ</i> cancers	Serum TG of patients with <i>in situ</i> cancers, controls	Case-control	3.0 (1.4–6.4)

^a Number of subjects in study and characteristics.

^b End-points measured. TG, triglyceride; IGFbps, insulin-like growth factor binding proteins.

^c Odds ratio, typical value from publication.

and give rise to the spectrum of oncogene activation and loss of suppressor gene function that is so characteristic of colon carcinogenesis.

The Insulin Resistance Mechanism. It has been known for many years, from animal carcinogenesis studies, that diet restriction and exercise markedly inhibit the development of colon cancers (11, 12), and that high-energy, high-fat diets generally promote carcinogenesis (13). As noted above, epidemiological studies have observed similar risk factors. McKeown-Eyssen (14) and Giovannucci (15) suggested a possible mechanism to explain these associations. They noted that the epidemiological risk factors for colon cancer are remarkably similar to those for insulin resistance. They suggested that lifestyle and dietary factors lead to both insulin resistance and to colon cancer promotion. The metabolic consequences of insulin resistance include hyperinsulinemia, hypertriglyceridemia, increased plasma NEFAs, and glucose intolerance (16). Both McKeown-Eyssen (14) and Giovannucci (15) suggested that hyperinsulinemia acts as a growth factor and tumor promoter. McKeown-Eyssen suggested further that hyperinsulinemia and hypertriglyceridemia increase epithelial cell energy and the growth of cancer cells (14). Accumulating evidence now supports the association of colon cancer risk with insulin resistance (Table 1). These include: (a) cohort studies of subjects that report a history of diabetes that subsequently report a higher rate of colon cancer (19, 23); (b) cohort studies of baseline measures for evidence of insulin resistance or diabetes that show an association with subsequent colon cancer (20–22); and (c) case-control studies of patients with colonic polyps and cancers that have shown these patients have elevated levels of fasting insulin, triglycerides, or VLDL, higher abdominal obesity, or abnormal glucose tolerance compared with age- and sex-matched controls (17, 18, 24).

A possible mechanism by which dietary energy excess could affect both insulin resistance and colon cancer promotion is illustrated in Fig. 1, as follows:

Excess dietary energy is a consequence of a mismatch between the dietary energy intake and the energy used for physiological functions including physical activity. Normally dietary energy intake is under remarkably fine control, preventing weight loss or obesity (25). However, the control depends on the content of the diet and the level of activity. Dietary fat, for instance, provides less satiety than dietary carbohydrate in

animal studies, whereas dietary protein provides slightly more (26). Increased fiber in the diet may also increase satiety (27). Thus, individuals consuming a highly palatable energy-dense diet that is high in fat and low in dietary fiber can consume in excess of requirements if they have a sedentary lifestyle (28).

Insulin resistance results from excess dietary energy. This occurs when the increased dietary energy increases intravascular energy as carbohydrate and lipid in the bloodstream (29, 30). If these energy sources are not required, particularly by muscle, as a source of mechanical energy, then muscle, liver, and adipocytes reduce their response to insulin. The exact mechanism responsible for “insulin resistance” is not known, but its effect is to reduce the “overnutrition” of these organs and tissues. The incipient increase in intravascular glucose stimulates the β -cells in the pancreas to increase insulin production. The result is that all cells, including colonic epithelial cells, are exposed to increased concentrations of insulin and energy substrates.

Increased proliferation and mutation result from insulin resistance. The increased levels of insulin and increased intracellular energy provide a proliferative stimulus through mechanisms we have suggested in a recent review (31). The proliferative stimulation would presumably have little effect on normal cells under normal crypt and cell cycle control but would favor the proliferation of cells with defective crypt and cell cycle control circuitry. Such cells would thus increase in number, and promotion of carcinogenesis would be observed. In addition, the increased intracellular energy would result in increased substrate oxidation and the formation of ROIs (32). The ROIs increase cellular damage, DNA oxidation, and mutation frequency. Initiation and promotion would be observed.

The insulin resistance mechanism rests in part on experimental observations and in part on hypotheses. As to the former, it is known from epidemiological studies that physical activity is protective, and obesity increases the risk of both insulin resistance and colon carcinogenesis; and it is known from the epidemiological and clinical studies cited in Table 1 that the development of colon cancer is associated with laboratory evidence of insulin resistance. It is known from animal carcinogenesis studies that insulin can promote the growth of ACF and colonic tumors (33, 34). It is also known that a high-energy (high-saturated fat, low-n-3 fatty acid, high-glycemic-index) diet increases insulin resistance rapidly before pro-

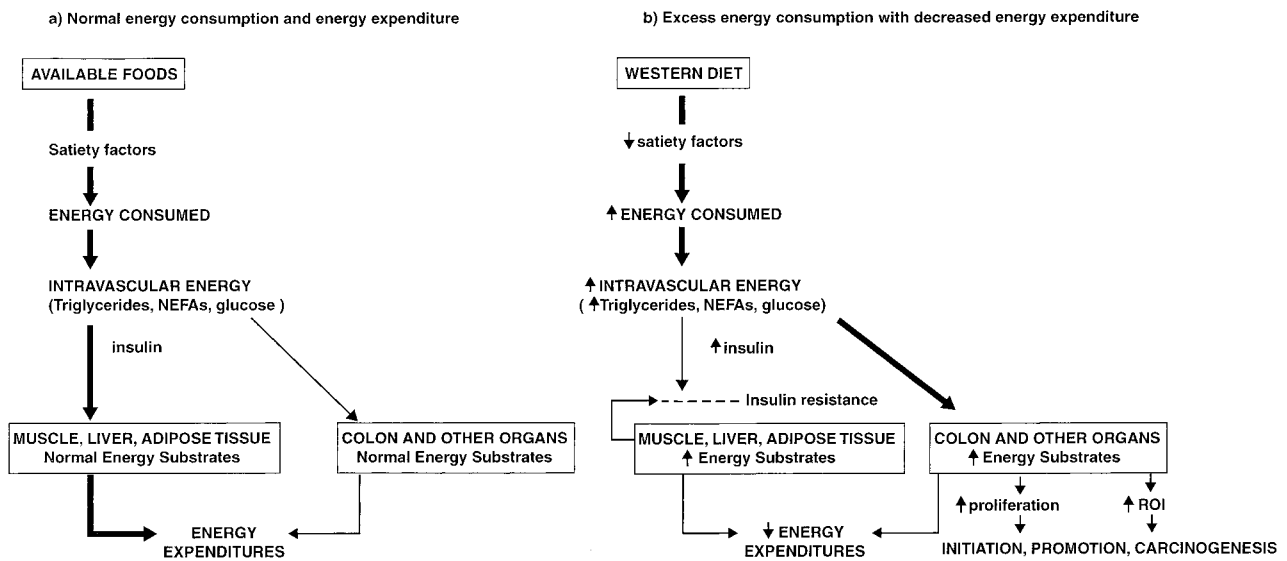


Fig. 1. A comparison of energy pathways from food to energy expenditure in the case of normal energy consumption and energy expenditure (a) and excess energy consumption and decreased energy expenditure (b) with a Western diet and a sedentary lifestyle. With an excess of energy substrates in muscle, liver, and adipose tissue, these organs show insulin resistance, and the colon and other organs are exposed to elevated levels of insulin and intracellular energy substrates. This may increase proliferation and oxidative stress and make the colon more susceptible to carcinogenesis.

motion, as assessed by the growth of ACF (35), and that this diet increases intravascular insulin, triglycerides, and NEFAs over much of the day almost 2-fold compared with the levels in a low-fat diet. These changes are associated with an increase in the levels of intracellular energy stores as triglyceride in the liver and muscle, as well as in spleen and colon cells (36). Caloric restriction, in contrast with caloric excess, reduces carcinogenesis and increases insulin sensitivity (37). Furthermore, it is known that boluses of both fat and carbohydrate increase the proliferation of colonic epithelial cells (38, 39), and that lipoproteins and insulin can stimulate the proliferation of epithelial cells in culture (40, 41). Finally, it is known that infusions of insulin or energy substrates can produce increased oxidation, resulting in reduced levels of antioxidants in the body (42–44).

The insulin resistance mechanism also rests on hypotheses. Thus, it is not known whether increased proliferation is a result of only insulin resistance. Energy excess itself could affect both insulin resistance and proliferation, and insulin resistance *per se* could be protective against the proliferative effects of insulin for cells of tissues such as muscle and liver that show insulin resistance. Energy excess could affect other pathways to increased proliferation. Insulin-like growth factors and their binding proteins could be involved in addition to excess intravascular energy and insulin (45). High glycemic loads with resulting high insulin fluxes could be particularly important. Signaling and metabolic pathways from the sensing of excess energy to proliferation and the formation of ROIs could also be important (46, 47).

The insulin resistance mechanism provides a coherent and attractive explanation for the relation between many of the dietary and lifestyle factors and risk of colon cancer. However, it does not explain all of the dietary factors associated with colon cancer risk or the focal nature of the disease.

The Focal Epithelial Defect Mechanism. Early carcinogenesis studies identified several compounds as colon carcinogens, including dimethyl hydrazine, azoxymethane, methyl nitro-

sourea and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. These compounds produce their effects through a series of early steps involving increased proliferation in crypts, epigenetic and genetic events resulting in the development of aberrant crypts, ACF, adenomas, and cancers (48, 49). More recently investigators initiated animals with these carcinogens and then attempted to accelerate or inhibit promotion by modifying the diet (50, 51) or by adding chemical agents to it. The results of the chemical cancer inhibition or chemoprevention studies have been remarkable. They have identified a wide range of compounds that inhibit the development of colon cancer and its precursors. In several cases the compounds inhibit carcinogenesis almost completely. Table 2 lists some of the agents that have been investigated together with the animals studied and the end points used.

The mechanisms responsible for the inhibition can be inferred to a degree by arranging the agents into groups. There are two agents that are thought to be confined to the lumen of the gastrointestinal tract (52, 61); one is the known demulcent PEG 8000, the other is *Bifidobacteria*. Another group are the NSAIDs (58–60). A third group of agents is antioxidants (53–56, 62–64). The first group could inhibit the development of colon cancer by reducing the irritation from the luminal surface, the second group by inhibiting the production of prostaglandins by COX-2, and the third group by reducing the quantities of ROIs. What is puzzling is that many of the agents in the different groups are each capable of almost completely inhibiting the carcinogenesis process. This implies that a reduction of surface irritation, or a reduction of products of the COX-2 enzyme, or a reduction of ROIs, can each inhibit carcinogenesis, but there is no obvious single pathway that connects these three processes.

We propose two possible pathways to explain these puzzling results. Briefly, in the first, a defect in the epithelial barrier results in a local irritation; the irritation produces a focal inflammatory response that activates COX-2 and generates prostaglandins from arachidonic acid. This activates inflamma-

Table 2 Recent carcinogenesis studies that demonstrate significant reduction (>~50%) of promotion of colonic aberrant crypt foci or colon cancer by various agents

Author(s)	Date	Species	Initiation ^a	Protocol ^b	Agent ^c	Result ^{d,e}
D. E. Corpet <i>et al.</i> (52)	1999	Rat	AOM	Promotional	PEG	ACF/rat reduced 94% ^c ; large ACF reduced 99%
D. Guo <i>et al.</i> (53)	1995	Rat	PhIP	Promotional	Chlorophilin	ACF/rat reduced 51%; large ACF reduced 70%
T. Kawamori <i>et al.</i> (54)	1995	Rat	AOM	Promotional	MMTS	ACF/rat reduced 32%
T. Kawamori <i>et al.</i> (55)	1998	Rat	AOM	Promotional	Celecoxib	Tumors/rat reduced 97%; incidence reduced 92%
H. Mori <i>et al.</i> (56)	1999	Rat	AOM	Promotional	GABA rice germ	Tumors/rat reduced 60%; incidence reduced 53%
J. E. Paulsen <i>et al.</i> (57)	1998	Rat	AOM	Promotional	n-3 fatty acids	Large ACF reduced 64%
C. F. Quesada <i>et al.</i> (58)	1998	Mice	Apc 1309 gene knockout	Promotional	Piroxicam	Tumors/rat reduced 44%
B. S. Reddy <i>et al.</i> (59)	1987	Rat	AOM	Promotional	Piroxicam	Tumors/rat reduced 84%
S. R. Ritland <i>et al.</i> (60)	1999	Mice	Apc ^{Min}	Promotional	Piroxicam	Tumors/mouse reduced >90%
I. R. Rowland <i>et al.</i> (61)	1998	Rat	AOM	Promotional	<i>B. longum</i> , inulin	ACF/rat reduced 80%; large ACF reduced 59%
T. Tanaka <i>et al.</i> (62)	1995	Rat	AOM	Promotional	Astaxanthin	Tumors/rat reduced 82%; incidence 58%
				Promotional	Canthaxanthin	Tumors/rat reduced 69%; incidence 56%
T. Tanaka <i>et al.</i> (63)	1997	Rat	AOM	Promotional	Hesperidin	ACF/rat reduced 61%
H. Tsuda <i>et al.</i> (64)	1999	Rat	AOM	Promotional	EGMP	ACF/rat reduced 37%; large ACF 51%

^a Initiation: AOM, azoxymethane; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; Apc 1309 gene knockout, spontaneous initiation; Apc^{Min}, mice with spontaneous initiation.

^b Intervention begun after initiation agent exposure.

^c PEG, polyethylene glycol, MW8000; MMTS, S-methyl methane thiosulfonate; GABA (γ -amino butyric acid)-enriched rice germ; n-3 fatty acids; *Bifidobacterium longum* and inulin; EGMP, 3-(4'-geranyloxy-3'-methoxyphenyl)-2-propenoate.

^d ACF, assayed at ~100 days after dietary intervention was begun; large ACF, typically >4 aberrant crypt/ACF; tumors, typically assessed at ~40 wk after dietary intervention was begun; tumor incidence, fraction of animals with tumors.

^e All results quoted are reported as statistically significant.

tory cells; the inflammatory cells generate ROIs that are mutagenic and mitogenic and promote carcinogenesis. In the second pathway, a defect in the epithelial barrier results in an electrolyte imbalance, an efflux of potassium and an influx of sodium and calcium in epithelial cells. The electrolyte disturbance results in an oxidative stress and ROI generation as the epithelial crypt cells cope with restoring intracellular electrolytes to their normal values. The ROIs induce COX-2 and the formation of mitogenic prostaglandins that promote carcinogenesis. Both pathways may be blocked by agents that reduce defects in the epithelial barrier, or by antioxidants, or by NSAIDs. Both schemes are illustrated in Fig. 2, as follows:

A focal failure of the colon epithelial barrier is taken as the initial defect for both sequences. The colon epithelial cell barrier is remarkable in its capability, structure, and function. As a barrier, it separates the contents of the colonic lumen from the lamina propria and the vascular system. The fully differentiated surface columnar epithelial cells are covered with microvilli with an external phospholipid-rich bilayer, are closely connected together with tight junctions, and are involved in electrolyte and water transport. All cells have a complex mechanism for repairing surface injury (65), although the barrier provided by the cells of the gastrointestinal tract may not always remain intact (66). It could fail in several ways: physical agents such as fibers could penetrate the surface, or chemical agents in the colonic lumen could lead to a loss of surface cells (67). Perhaps most frequently, genetic or epigenetic alterations could result in a failure of terminal differentiation and a deficiency of mature differentiated cells. Indeed, ACF and adenomas can be recognized by the marked degree to which they are identified by vital stains. Their surface epithelial cells are immature and deficient in microvilli (68, 69). Adenomas have tight junctions that do not function effectively (70), and adenomas, especially villous adenomas, have long been known to "leak" potassium ions into the lumen (71). Both ACF and adenomas are also deficient in mucin as a result of a reduction in the number of mature mucous-producing goblet cells.

As noted above, PEG 8000 is a known demulcent, or soothing agent. It is essentially confined to the colonic lumen (72) and belongs to a group of agents that have long been

known to protect cell membranes from mechanical damage (73). Natural foodstuffs may act in a similar manner. Ingestion of oligofructoses favors the growth of *Bifidobacteria* in the colon, and other factors favor the growth of *Lactobacilli* (74). Some strains of these species are known to bind to the apical surface of colonic epithelial cells in culture without injuring them (75), and it has been suggested that their protective action in gastrointestinal diseases is related to this selective binding (76, 77). Perhaps both PEG 8000 and *Bifidobacteria* protect the surface epithelium and thus facilitate repair and reduce irritation and epithelial permeability to electrolytes.

In the focal inflammatory response pathway, the failure of the colon epithelial cell barrier results in local irritation and an inflammatory response. The defect in the surface exposes the cells in the lamina propria to noxious agents such as a low pO₂, bile and fatty acids, and bacterial products, which may produce additional disruption of the membrane (78, 79). The subsequent inflammatory response includes the migration of granulocytes, macrophages, and lymphocytes into the lamina propria and the activation of COX-2. Products of this enzyme, including the J-series prostaglandins, stimulate the proliferation and reduce the apoptosis of developing epithelial cells (80). Selective cyclooxygenase inhibitors inhibit this mitogenesis (81). The accumulating inflammatory cells also expose the epithelial cells to ROIs. This leads to oxidative damage in the epithelial cells and to epigenetic and genetic effects (82–84) that could further affect the terminal differentiation and barrier function of epithelial cells and impair the function of cell and crypt cycle controls.

In the oxidative stress pathway, the focal failure of the surface colon epithelial cell membranes affects the maintenance of intracellular electrolytes, depleting the cells of potassium, exposing them to excess calcium and sodium, and resulting in oxidative stress and exposure to ROIs. This sequence is observed in membrane injury from electroporation, where the injury results in a rapid increase in cytoplasmic calcium and then by the generation of ROIs (85, 86), and also in membrane damage from fibers. As all cells in a crypt are connected together by gap junctions (87), a surface membrane injury would lead to oxidative stress and toxic and mutagenic effects

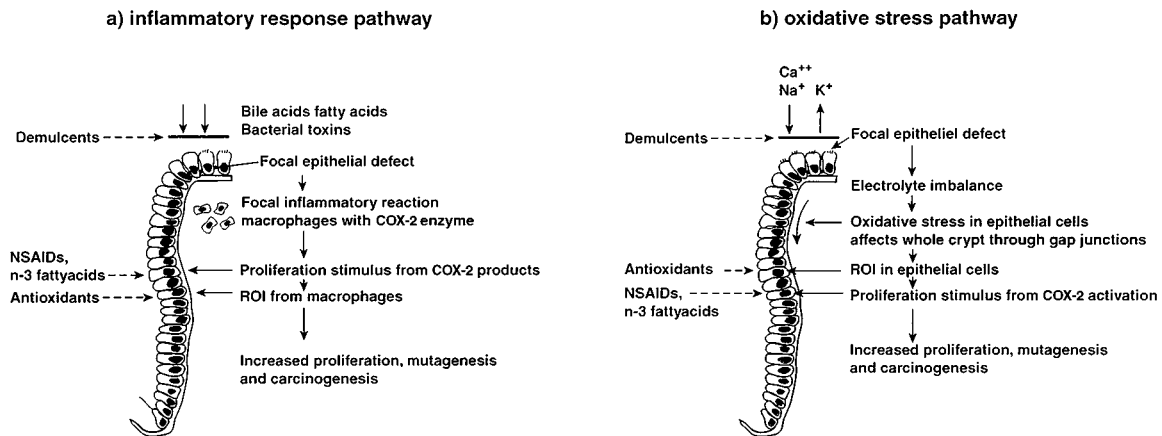


Fig. 2. Two pathways involved in the focal epithelial defect mechanism inferred from the inhibition of carcinogenesis by demulcents, NSAIDs, and antioxidants. In *a*, intraluminal toxic compounds pass through focal intracellular defects to produce a focal inflammatory reaction with the activation of the COX-2 enzyme in macrophages. Products of COX-2 stimulate proliferation in the epithelial cells, ROIs from macrophages lead to mutagenesis. In *b*, focal increased membrane permeability results in electrolyte imbalance and oxidative stress involving all of the cells of the crypt through gap junctions. The ROIs increase mutagenesis and activate COX-2 to produce a proliferative stimulus.

in the entire crypt. ROIs would also induce COX-2 in epithelial cells and stimulate proliferation (88). Thus, both sequences would expose the affected epithelial cells to proliferative stimulation and mutagenesis.

Again, the focal epithelial defect mechanism rests in part on clinical and experimental observations and in part on hypotheses. Perhaps the strongest evidence is the association in recent clinical studies of fecal granulocyte marker protein, a direct measure of mucosal inflammation, with the presence of polyps and cancers (89–91). Similar markers appear in experimental animals with the development of ACF, polyps, and cancers (92). As noted above, it is known that agents with demulcent, anti-inflammatory, and anti-oxidant properties can each reduce the promotional phase of colon carcinogenesis in animal studies (Table 2), and that the surface epithelial cells of ACF and adenoma are often immature and not fully differentiated. COX-2 protein has been observed both in adenoma in macrophages underlying the surface epithelium (93, 94) and in the epithelial cells (95) of affected crypts. Some epithelial cells of dysplastic ACF and adenomatous polyps, as well as some macrophages, can contain microscopic granules that fluoresce under near UV irradiation with excitation and emission spectra characteristic of lipofuscin or ceroid (96, 97), suggesting that these cells are under oxidative stress (98). Finally, it is known that both the proliferation (99) and the genetic instability of cells in ACF and adenoma are increased (100, 101).

The proposed mechanism of focal epithelial defect also rests on hypothesis. Although PEG 8000 is a demulcent and does protect epithelial surfaces, it is not known that PEG produces its large reduction in colon tumor growth in the rat through this mechanism. Not all agents classified as demulcents affect the growth of ACF (52). Although it is known that COX-2 protein has been observed in adenoma, it appears not to have been looked for in ACF. It is also not clear that the NSAIDs produce their effect only by interaction with COX-2 (80) or how the formation of ROIs and prostaglandins are related and whether it involves peroxisome proliferator activated receptor γ . There is no direct evidence that epithelial defects lead to electrolyte abnormalities and oxidative stress in epithelial cells, and there have been no direct measurements of increased DNA, protein, or lipid oxidation in ACF or adenoma.

Finally, the steps involved in the regression of ACF in the presence of any of the preventive agents have not been described.

Our present understanding of the control of crypt cell proliferation and of crypt fission is also limited (102). In the later stages of the disease process, after the appearance of ACF and polyps, increased proliferation is undoubtedly a consequence of mutations that lead to a failure of the cell cycle control circuitry of colonic cells. But in the long, earlier stages of the disease process, this may not be the case. Instead early increased proliferation may be a consequence of a focal failure of the epithelial barrier function and the resulting focal inflammation. The loss of the barrier function itself may be partly a consequence of mutations in the colonic crypt stem cells that lead to their senescence or to their failure to terminally differentiate into mature columnar epithelium. Thus, environmental and genetic factors may be intimately related during the process of colon carcinogenesis.

Discussion

Two very different mechanisms relating diet and colon cancer have been described. The first mechanism involves excess dietary energy, the development of insulin resistance, and presently undefined pathways from intracellular energy to proliferation and to exposure to ROIs. This mechanism could affect many tissues in the body because all are likely to be exposed to the metabolic effects of dietary excess, and it may account for the similarities in dietary risk factors for many tumor types. The second mechanism involves a focal loss of epithelial barrier function, an inflammatory response, an oxidant stress, and pathways to proliferation and additional mutation. This mechanism is local, acting initially on the progeny of one cell in an aberrant crypt, then ACF and adenoma. Its effect may vary with the local environment through the length of the colon and rectum. The generalized and the local mechanisms, however, will interact in several ways. For instance, the generalized energy excess will increase proliferation and increase levels of ROIs, sensitizing normal colonic crypt cells to spontaneous and carcinogen-induced mutations as well as to local injury. The generalized effects will also affect the development of precur-

sor lesions, driving the expansion of the initial aberrant crypt toward the adenoma and cancer.

The two proposed mechanisms are an amalgamation of many earlier suggestions with the results of recent observations. They are both complex and involve many factors in the diet. As a result of this origin, the mechanisms can readily "explain" many of the relationships between diet and colon cancer risk. The complexity of these relationships can be illustrated with two dietary factors: fat, and fruits and vegetables.

Dietary fat provides energy at a high density with a low satiety for the calories consumed. Consequently, as we have noted, energy availability is increased and colon carcinogenesis is promoted. Dietary fat, however, is complex. Depot fat in animals composed primarily of saturated fat may have this effect, but unsaturated n-3 fatty acids may affect the disposition of fat in the body (103) and may increase the satiety effect of fat (34), thus reducing insulin resistance and promotion. n-3 fatty acids also inhibit the conversion of n-6 fatty acid to arachidonic acid (104) and possibly the focal events in colon carcinogenesis (57). Much of the n-3 and n-6 fatty acid is associated with phospholipids. These may have other effects that have only recently been fully appreciated. It has been known for some time that choline is a lipotrope that can inhibit experimental carcinogenesis (105). Recently, sphingosine content of the diet, as well as inositol and phytic acid, have been found to reduce colon cancer promotion (106). Cholesterol has a similar effect (107). Lipids can, of course, be involved in cell signaling, but they also make up a large part of the epithelial barrier membrane and their presence in the diet could facilitate the terminal differentiation of colonocytes. Dairy fats contain phospholipids including sphingomyelin (102). They also contain calcium that cannot only influence the stability of membranes but can also affect the solubility and digestibility of fat (108). Other processed fats are frequently stripped of their content of phospholipids to improve appearance, stability, and cooking properties (109). High-fat foods can also be heated more readily than low-fat foods, and, as a consequence, possible problems associated with pyrolysis, and perhaps thermolysis, of fats, proteins, and carbohydrates can be associated with high-fat diets. Possible problems associated with pyrolysis are the production of polycyclic aromatic hydrocarbons and aryl amines. Both groups of compounds can affect mutation frequency, the orderly differentiation of colonic cells, and the development of colon cancer.

Vegetables and fruits present similarly complex effects involving both the insulin resistance and the focal epithelial defect mechanisms. Both fruits and vegetables, of course, contain fiber, which will increase satiety (27). They will also reduce the glycemic index of the diet, possibly reducing the development of insulin resistance as well as decreasing colonic protein fermentation (110). Some vegetables contain fibers that support bacterial fermentation, which may protect the epithelial cell barrier (111). Other bacterial products may have deleterious effects on surface epithelial cells (112). In addition to their content of carbohydrate, protein, fat, and fiber, vegetables can also contain folic acid, which can affect nucleotide pool sizes, DNA methylation, cell proliferation, and mutation frequency. These effects might be most pronounced in ACF with their markedly increased proliferation rates. Finally, and perhaps most importantly, vegetables contain a diverse group of antioxidants (113). The properties of these antioxidants differ markedly in their differential solubility in water and nonhydrophilic environments (114), and it has been suggested that these compounds may well be more effective acting together as mixtures than are prototypic ascorbic acid and tocopherol in

reducing ROIs (115). Antioxidants could have effects through either mechanism by reducing proliferative stimulus in the normal epithelium associated with insulin resistance or by affecting the pathway to proliferation associated with the focal epithelial defect in ACF (116).

The complexity of these relationships may explain the disappointing results of recent intervention studies (*e.g.*, 117–119). That is, the interventions that have been used have frequently failed to significantly affect underlying mechanisms involved in colon carcinogenesis. Thus, dietary modifications used to reduce fat and increase fiber may not have reduced insulin resistance, insulin levels, or circulating energy. Diets increasing vegetables or antioxidants may not have reduced oxidative damage or inflammatory responses in the colon.

Future studies could be focused on the fundamental assumptions of the two hypotheses. The insulin resistance mechanism predicts that dietary and exercise interventions that reduce insulin resistance (as evidenced by reduced plasma insulin, triglycerides, and NEFAs) will reduce the rate of development of colon cancer. The interventions should involve more than only alterations in dietary fat and fiber. They might be developed in collaboration with other prevention studies, *e.g.*, for type 2 diabetes, cardiovascular disease, and other cancers. The focal epithelial defect mechanism predicts that dietary or pharmacological interventions that reduce colonic inflammation [as evidenced, for example, by reduced granulocyte marker protein in feces or morphological measurements in colonic mucosa (120)] will reduce the rate of development of colon cancer. The interventions should involve more than ascorbic acid and tocopherol or supplementary vegetables. They might be developed in collaboration with prevention studies for inflammatory bowel diseases. Such intervention studies should clarify the importance of these two mechanisms relating diet and colon cancer risk.

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