Fat/fiber interactions on colonic cytokinetics: Relationship to colon cancer

JR Lupton PhD, WCL Chang PhD, MY Hong MS and RS Chapkin PhD

Faculty of Nutrition, Texas A & M University, College Station, Texas, USA

Colon cancer is the second leading cause of death from cancer in the USA today and diet is known to play an important role in both its prevention and promotion. The dietary factors most associated with colon cancer are fat and fiber. Diets high in fish oil (containing n-3 fatty acids) are considered more protective against colon tumor development than are corn oil diets (containing n-6 fatty acids). The fiber/experimental colon cancer literature shows that less fermentable fibers (such as wheat bran and cellulose) are more protective than are highly fermentable fibers such as oat bran, pectin and guar. It was therefore hypothesized that the combination of a fish oil diet with a poorly fermentable fiber (cellulose) should confer maximum protection from tumor development. To test that hypothesis, a $2 \times 2 \times 2$ factorial design study was conducted using male Sprague Dawley rats (2 fibers: pectin or cellulose; 2 lipids: fish oil or corn oil; 2 injection protocols: carcinogen or saline). Surprisingly, the fish oil/pectin diet was most protective against tumor development. Further experiments showed a synergistic effect of fish oil/pectin which was related to this combination's ability to initiate apoptosis (programmed cell death) both during the promotion and initiation stages. Also, fish oil supplementation decreases cyclooxygenase-2 (Cox-2) expression in rat colonocytes. From these results, it was concluded that fish oil down-regulates Cox-2 expression which then provides a permissive environment for butyrate-induced apoptosis to occur.

Key words: fish oil, pectin, colon cancer, apoptosis, cox-2.

Introduction

Colon cancer is the second leading cause of death from cancer in the USA today and its development is highly responsive to diet.[1] The two diet components thought to have the most significant effect on colon tumor development are fat and fiber. Specifically, n-3 polyunsaturated fatty acids (n-3 PUFA) appear protective against colon cancer in epidemiological, clinical and experimental studies.[2–4] These fatty acids are found in high concentration in fish and fish oils.

The type of dietary fiber that is the most protective against colon tumor development remains the subject of debate. Butyrate, a short chain fatty acid derived in the colon from microbial fermentation, promotes differentiation and apoptosis in a variety of colon tumor cell lines.[5] These *in vitro* findings have resulted in the hypothesis that the more fermentable fibers should be the most protective against colon cancer since they produce the highest amounts of butyrate. However, there is some discrepancy between *in vitro* and *in vivo* studies regarding the efficacy of butyrate.[6]

For example, we recently showed that wheat bran was more protective against experimentally induced colon cancer than was oat bran, despite higher luminal levels of butyrate produced from the oat bran supplemented diet compared with the wheat bran supplemented diet.[7] Further, rats fed less fermentable fibers, such as wheat bran or cellulose, and injected with the experimental colon carcinogens azoxymethane (AOM) or dimethylhydrazine (DMH) generally have fewer colon tumors than those fed more fermentable fibers such as oat bran or pectin.[6] Whether or not fiber is protective against colon cancer due to its fermentation to butyrate is an issue clearly in need of resolution.

In attempting to understand why n-3 fatty acids are more protective than n-6 fatty acids and less fermentable fibers are more protective than highly fermentable fibers in the rat AOM or DMH model, several mechanisms are proposed. The fatty acid composition of the diet may alter both the concentration and the speciation of individual bile acids, some of which may be more promotive than others.[8] Colonic epithelial cell membranes may be modified by dietary fat, which in turn may supply different precursors for prostaglandin synthesis.[9] Fatty acids also differentially affect signal transduction processes, including protein kinase C.[10]

Perhaps the single most important attribute of poorly fermented fibers is their ability to dilute colonic luminal constituents. We showed a number of years ago, in establishing the rat/fiber/carcinogen model in our laboratory, that wheat bran was the best *in vivo* dilutor of the various fibers tested (pectin, guar, oat bran, cellulose and a fiber free diet).[11] This is important because substances such as carcinogens, pro-carcinogens and promoters (e.g. bile acids, ammonia, and long chain fatty acids) would have less access to the colonic mucosa in a bulky stool. In addition, less fermentable fibers form lower amounts of short chain fatty acids (SCFA) and SCFA have been shown to stimulate colonic epithelial

Correspondence address: Dr Joanne R Lupton, Texas A & M University, 218 Kleberg, College Station, TX 77843–2471, USA.
Tel: 1 409 845 2142; Fax: 1 409 862 2378
Email: JIupton@cvm.tamu.edu

cell proliferation whether infused into the rat colon[12] or produced from fiber fermentation.[13]

Methods

The experimental colon cancer literature suggests that diets high in n-3 fatty acids are more protective than those high in n-6 fatty acids, while diets high in poorly fermentable fibers are more protective than those rich in highly fermentable fibers. We therefore hypothesized that a combination of fish oil and cellulose should be more protective than a combination of corn oil and pectin. To test this hypothesis we conducted a $2 \times 2 \times 2$ factorial design study with two types of fat (fish oil or corn oil), two fibers (pectin or cellulose) and two injection protocols (AOM or saline).[14] To our surprise, the most protective diet against tumor development was the fish oil/pectin diet (Fig. 1).

Since changes in colonic cytokinetics are known to both precede and accompany colon tumor development, we next determined the effect of the fish/pectin diet on cell proliferation and programmed cell death (apoptosis). The fish oil/pectin diet had a greater effect on increasing apoptosis than it did on decreasing cell proliferation (Fig. 2).[14,15] This is significant because reduced apoptotic ability may imply increased cancer risk.[16] Next, we determined whether or not the combination of fish oil and pectin might increase apoptosis compared with corn oil/pectin at the very earliest stages of the tumorigenic process. Animals were acclimated to the facility for 1 week and then provided with experimental diets for 2 weeks. The diets contained 6% pectin by weight and either 15% fish oil or corn oil. After 2 weeks, animals were injected with AOM and terminated 3, 6, 9 and 12 h post injection. Time zero (no injection) was used as the control. Colons were immediately resected, divided into proximal and distal colon, fixed, embedded in paraffin, and sectioned for immunohistochemical analysis. DNA damage was assessed using quantitative immunohistochemical analysis of O^6-methylguanine adducts. Apoptosis was measured by the *in situ* terminal deoxynucleotidyl transferase assay (TUNEL) method.

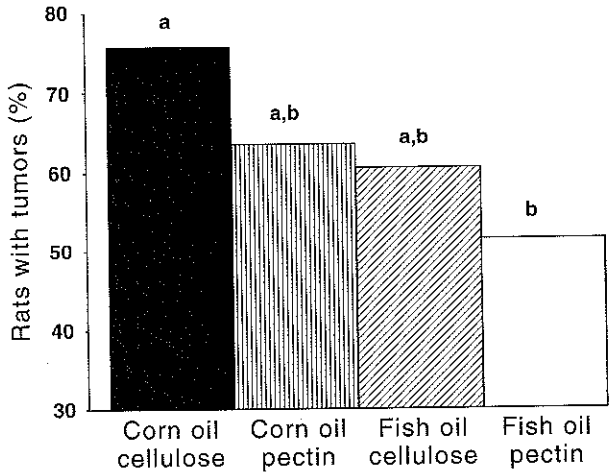

Figure 1. The combination of fish oil/pectin resulted in a lower proportion of rats with colon adenocarcinomas than did the combination of corn oil/cellulose. Bars not sharing a common letter are significantly different at $P < 0.05$. Figure adapted from data in Chang *et al.*, 1997.[14]

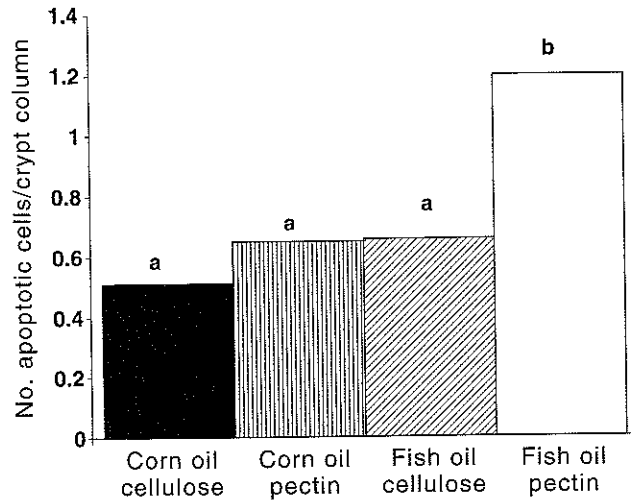

Figure 2. The combination of fish oil/pectin results in a greater number of apoptotic cells in the proximal colon of rats than do the other three diet combinations. Bars not sharing a common letter are significantly different at $P < 0.05$. Figure adapted from data in Chang *et al.*, 1998.[15]

Results and discussion

The combination of fish oil and pectin decreased O^6-methylguanine adduct levels throughout the colonic crypt compared

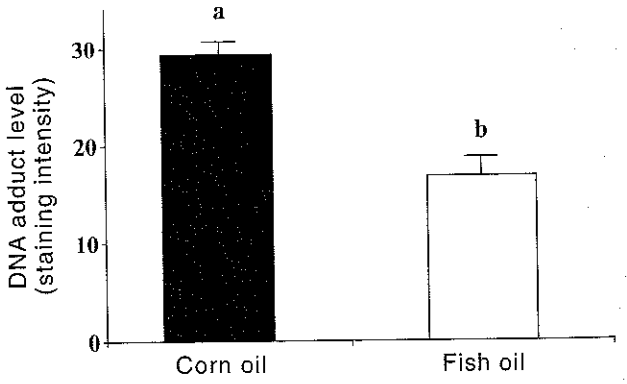

Figure 3. The fish oil/pectin diet resulted in a lower level of DNA adducts throughout the colonic crypt than did the corn oil/pectin supplemented diet ($n = 15$ rats/group). Bars not sharing a common letter are significantly different at $P < 0.001$.

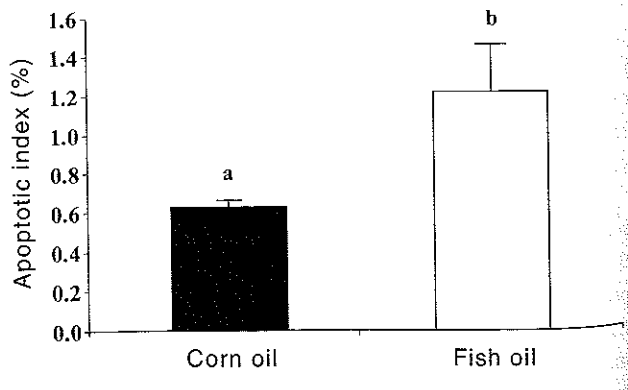

Figure 4. The fish oil/pectin diet resulted in a greater apoptotic index throughout the colonic crypt than did the corn oil/pectin diet ($n = 15$ rats/group). Bars not sharing a common letter are significantly different at $P < 0.05$.

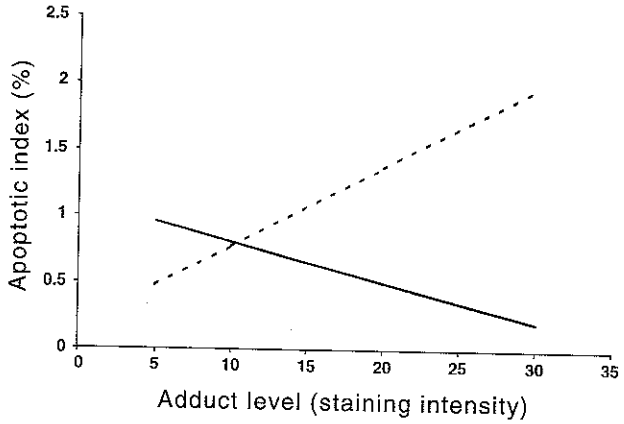

Figure 5. As adduct level increased, the fish oil/pectin supplemented animals (---) responded by increasing the apoptotic index. In contrast, as adduct level increased, the corn oil/pectin supplemented animals (—) responded by decreasing the apoptotic index. $n = 15$ rats/group; $P < 0.03$.

with corn oil and pectin (Fig. 3). In addition, the combination of fish oil/pectin doubled the apoptotic index in the top one-third of the crypt compared with corn oil/pectin ($P < 0.035$) (Fig. 4). Combining the DNA damage data with apoptotic removal showed a dramatic difference in the response of fish oil versus corn oil supplemented animals to DNA damage. As shown in Fig. 5, as adduct level increased, apoptotic index decreased with corn oil supplementation. The fish oil supplemented animals had the opposite response. As adduct level increased, fish oil animals increased the apoptotic response. This suggests that fish oil results in an apoptotic response targeted to DNA damaged cells.

Our data, taken collectively, suggest that whether or not a high-butyrate producing fiber (pectin) compared with a low-butyrate producing fiber (cellulose) is protective against colon carcinogenesis depends on the source of dietary lipid. In addition, the protective mechanism appears to be an up-regulation of apoptosis, rather than a down-regulation of cell proliferation. We next explored a possible mechanism for this fat/fiber interaction on apoptosis. Our working hypothesis was that the observed enhancement of apoptosis with fish oil

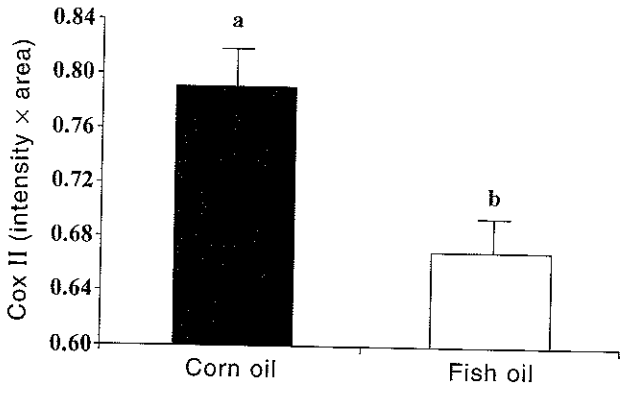

Figure 6. Rats provided with fish oil and injected with azoxymethane had a lower level of Cox II expression in colonic mucosa at 18 weeks post carcinogen injection than did their corn oil supplemented counterparts ($P < 0.03$). There were 10 animals in each group and Cox-2 expression was determined by western blotting. Bars not sharing a common letter are significantly different at $P < 0.05$.

supplementation is due to down-regulation of prostaglandin H synthase-2 (Cox-2) expression providing a permissive environment for butyrate-induced apoptosis. Support for this hypothesis was from several sources. Cox-2 expression is known to be induced by growth factors, oncogenes, and tumor promoters.[17,18] Cox-2 mRNA levels are markedly increased in 86% of human colorectal adenocarcinomas,[19] and tumors have a high level of expression of Cox-2 protein.[19–21] Cox-2 is also elevated in most colonic tumors in azoxymethane-treated rats.[22] Inhibition of Cox-2 by a specific Cox-2 inhibitor (Celecoxib), suppressed the overall colon tumor burden in a rat AOM model by more than 87%.[23]

Evidence is accumulating that Cox-2 may inhibit apoptosis and thus inhibitors of Cox-2 are apoptosis enhancing.[24] In a recent report,[25] rat intestinal epithelial (RIE) cells were stably transfected with a Cox-2 expression vector orientated in the sense (RIE-S) or antisense (RIE-AS) direction. The RIE-S cells expressed elevated Cox-2 protein and were resistant to butyrate-induced apoptosis. The resistance to apoptosis was reversed by administration of sulindac sulfide (a Cox-2 inhibitor). These *in vitro* data support the concept that inhibition of Cox-2 expression up-regulates apoptosis, and that if Cox-2 is over-expressed in rat intestinal epithelial cells, butyrate does not induce apoptosis.

We have previously shown that fish oil (compared with corn oil) results in lower levels of mucosal arachidonic-acid containing phospholipids[9,26] (arachidonate is the substrate for Cox-2). A recent report shows that arachidonic acid can initiate Cox-2 transcription in rat intestinal epithelial cells,[27] suggesting that a lower concentration of arachidonic acid could limit Cox-2 expression. We further showed that production of certain products of Cox-2 was also decreased with fish oil supplementation.[9] We now show that fish oil results in a lower expression of Cox-2 in rat colonocytes compared with rats supplemented with corn oil[28,29] (Fig. 6). This finding is supported by data from another laboratory which also found down-regulation of Cox-2 expression with fish oil versus corn oil 12 weeks after AOM injection.[30]

Conclusion

There is an interactive effect between fish oil and pectin on colon tumor development in that fish oil/pectin is more protective against colon tumors than is corn oil/cellulose.[14,15] The protective effect of fish oil/pectin is due to an enhancement of apoptosis, rather than to a decrease in cell proliferation.[15] Fish oil/pectin enhances apoptosis during the promotion, progression and initiation stages of tumorigenesis. Fish oil supplementation decreases Cox-2 expression in rat colonocytes. Pectin supplementation may be protective by enhancing butyrate production, which in turn stimulates apoptosis, but only when Cox-2 is down-regulated. If ongoing studies in our laboratory should verify this hypothesis, part of the dichotomy between *in vivo* and *in vitro* effects of butyrate may be explained. *In vivo*, the effect of butyrate would depend on the type of lipid in the diet.

Acknowledgements. The authors acknowledge the important contribution of Dr LA Davidson to immunohistochemical techniques development, and Dr ND Turner for statistical analysis and interpretation. We also acknowledge the support of NIH RO1 CA6175 and CA59034 and NIEHS 1P 30 ES09106.

References

1. American Institute for Cancer Research, World Research Fund. Food, nutrition and the prevention of cancer: a global perspective. Washington DC: American Institute for Cancer Research, 1997: 85–96.
2. Caygill CP, Charlett A, Hill MJ. Fat, fish, fish oil and cancer. Br J Cancer 1996; 74: 159.
3. Lindner MA. A fish oil diet inhibits colon cancer in mice. Nutr Cancer 1991; 15: 1.
4. Nelson RL, Tanure JC, Andrianopoulos G, Souza G, Lands WEM. A comparison of dietary fish oil and corn oil in experimental colorectal carcinogenesis. Nutr Cancer 1988; 11: 215.
5. Smith JG, Yokoyama WH, German JB. Butyric acid from the diet: actions at the level of gene expression. Crit Rev Food Sci Nutr 1998; 38: 259.
6. Lupton JR. Butyrate and colonic cytokinetics: differences between in vitro and in vivo studies. Eur J Cancer Prev 1995; 4: 373.
7. Zoran DL, Turner ND, Taddeo SS, Chapkin RS, Lupton JR. Wheat bran reduces tumor incidence in a rat model of colon cancer independent of effects on distal luminal butyrate concentrations. J Nutr 1997; 127: 2217.
8. Reddy BS. Dietary fat and colon cancer: animal model studies. Lipids 1992; 27: 807.
9. Lee DYK, Lupton JR, Aukema HM, Chapkin RS. Dietary fat and fiber alter rat colonic mucosal lipid mediators and cell proliferation. J Nutr 1993; 123: 1808.
10. Davidson LA, Lupton JR, Jiang Y-H, Chang WC, Aukema HM, Chapkin RS. Diet induced alteration of rat colon protein kinase C isozyme expression. J Nutr 1995; 125: 49–56.
11. Gazzaniga JM, Lupton JR. Dilution effect of dietary fiber sources: an in vivo study in the rat. Nutr Res 1987; 7: 1261.
12. Ichikawa H, Sakata T. Stimulation of epithelial cell proliferation of isolated distal colon of rats by continuous colonic infusion of ammonia or short-chain fatty acids is nonadditive. J Nutr 1998; 128: 843.
13. Lupton JR, Kurtz P. Relationship between colonic luminal short chain fatty acids and pH to in vivo cell proliferation. J Nutr 1993; 123: 1522.
14. Chang WCL, Chapkin RS, Lupton JR. Predictive value of proliferation, differentiation and apoptosis as intermediate markers for colon tumorigenesis. Carcinogenesis 1997; 18: 721.
15. Chang WCL, Chapkin RS, Lupton JR. Fish oil blocks azoxymethane-induced rat colon tumorigenesis by increasing cell differentiation and apoptosis rather than decreasing cell proliferation. J Nutr 1998; 128: 491.
16. Garewal H, Bernstein H, Bernstein C, Sampliner R, Payne C. Reduced bile acid-induced apoptosis in 'normal' colorectal mucosa: a potential biological marker for cancer risk. Cancer Res 1996; 56: 1480.
17. Smith WL, Dewitt DL. Prostaglandin endoperoxide H synthases-1 and -2. Adv Immunol 1996; 62: 167.
18. Herschman HR. Regulation of prostaglandin synthase-1 and prostaglandin synthase-2. Cancer Metastasis Rev 1994; 13: 241.
19. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994; 107: 1183.
20. Sano H, Kawahito Y, Wilder RL, Hashiramoto A, Mukai S, Asai K, Kimura S, Kato H, Kondo M, Hela T. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res 1995; 55: 3785.
21. Kutchera W, Jones DA, Matsunami N, Groden J, McIntyre TM, Zimmerman GA, White RL, Prescott SM. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect. Proc Natl Acad Sci USA 1996; 93: 4816.
22. DuBois RN, Radhika A, Reddy BS, Entingh AJ. Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. Gastroenterology 1996; 110: 1259.
23. Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res 1998; 58: 409.
24. Pasricha PJ, Bedi A, O'Connor K, Rashid A, Akhtar AJ, Zahurak ML, Piantodosi S, Hamilton SR, Giardiello FM. The effects of sulindac on colorectal proliferation and apoptosis in familial adenomatous polyposis. Gastroenterology 1995; 109: 994.
25. Tsujii M, DuBois RN. Alterations in cellular adhesions and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995; 83: 493.
26. Lupton JR. Fat/fiber interactions: Effect on colon physiology and colonic cytokinetics. In: Kritchevsky D, Bonfield C, eds. Dietary fiber in health and disease. St Paul, MN: Eagan Press, 1995; 115–125.
27. Peri KG, Almazan G, Varma DR, Chemtob S. A role for protein kinase Cα in stimulation of prostaglandin G/H Synthase-2 transcription by 14,15-epoxyeicosatrienoic acid. Biochem Biophys Res Commun 1998; 244: 96.
28. Chang WCL, Turner ND, Davidson LA, Chapkin RS, Lupton JR. Fish oil depresses the carcinogen-induced expression of cox-2 in rat colonic mucosa. FASEB J 1998; 12: A564.
29. Chapkin RS, Lupton JR. Colonic cell proliferation and apoptosis in rodent species: modulation by diet. In: Colon Cancer Prevention: Dietary modulation of cellular and molecular mechanisms. Advances in Experimental Medicine and Biology. New York: Plenum Press (in press).
30. Singh J, Hamid R, Reddy BS. Dietary fat and colon cancer: modulation of cyclooxygenase-2 by types and amount of dietary fat during the post initiation stage of colon carcinogenesis. Cancer Res 1997; 57: 3465.