

Dietary Fat and Fiber Alter Rat Colonic Protein Kinase C Isozyme Expression^{1,2}

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ABSTRACT To better understand the biochemical mechanisms by which select fats and fibers modulate colonic cell proliferation, we determined the profile of protein kinase C (PKC) isozymes and cell proliferation in rat proximal and distal colonic mucosa following diet manipulation, because enhanced cell proliferation has been correlated with colon cancer incidence. Rats were assigned to one of four diets (each with 15 g fat + 6 g fiber/100 g diet) for 3 wk: fiber-free fish oil (FF), fiber-free corn oil (FC), cellulose + corn oil (CC), or pectin + corn oil (PC). Steady-state levels of colonic mucosal cytosolic and membrane PKC isozymes were determined. In vivo cell proliferation was determined by bromodeoxyuridine incorporation into DNA. In addition, viable exfoliated colonic epithelial cells were isolated from feces using Percoll-bovine serum albumin gradients. We found that 1) proximal and distal colonic mucosa possessed different steady-state levels and relative proportions of PKC isozymes; 2) PKC α and δ expression were significantly greater in distal membrane of the PC-fed group compared with the other dietary groups; 3) the number of exfoliated cells per 4-h fecal collection generally was proportional to the diet-induced changes in cell proliferation (PC>FC>CC>FF). These data demonstrate that dietary treatment altered colonic PKC isozyme expression, with animals fed the fiber-containing diets generally expressing higher steady-state levels of PKC α and δ . *J. Nutr.* 125: 49-56, 1995.

INDEXING KEY WORDS:

- rats • colonic mucosa • fiber
- protein kinase C isozymes • fat

Of all the cancers, colon cancer seems to be the most affected by diet, with certain fats believed to be promotive and some dietary fibers considered protective (Hilbrun et al. 1989, Willet 1989, Willet et al. 1990). However, the mechanisms by which dietary fibers and fats exert their biological effects are still unclear. It is known that less fermentable fibers, such as cellulose, increase the bulk of colon contents, speed transit time and bind carcinogens, all of which decrease exposure of the colonic mucosa to car-

cinogens (Kritchevsky 1988). In contrast, fermentable fibers, such as pectin, may actually enhance colon carcinogenesis (Jacobs and Lupton 1984). Certain fatty acids have been shown to affect colon carcinogenesis: (*n*-6) polyunsaturated fatty acids (found in corn oil) enhance colon tumorigenesis, whereas (*n*-3) polyunsaturated fatty acids (found in fish oil) cause no tumor promotion in animal models (Minoura et al. 1988, Reddy and Sugie 1988). Dietary fibers and fats also influence colonic cell proliferation (Lee et al. 1993a). This is noteworthy, because elevated cell proliferation is associated with enhanced colon cancer incidence both in humans and in animal models (Lupkin 1988).

Experimental results on colonic tissue indicate that the activation of protein kinase C (PKC) in part mediates colonic cell proliferation (Chapkin et al. 1993, Craven and DeRubertis 1987, Craven et al. 1987). Protein kinase C is a family of structurally similar yet distinct enzymes that modulate the biological activity of proteins via serine-threonine phosphorylation (Hug and Sarre 1993, Selbie et al. 1993). On the basis of biochemical and structural characteristics, the PKC family is divided into three groups: the classical isozymes (α , β _I, β _{II} and γ); the novel isozymes (δ , ϵ , η , μ and θ) and the atypical isozymes (λ , ζ and ι). The various isozymes have individual requirements for activation and different tissue and subcellular distributions. Therefore, examination of

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individual isozymes will help delineate the role of PKC on various cellular processes. We recently demonstrated that dietary fibers and fats alter the rat colon PKC membrane to cytosol enzyme activity ratio (Chapkin et al. 1993). This ratio and the total membrane PKC activity correlated with an expansion of the colonic epithelial proliferative zone. Therefore, overexpression and/or down-regulation of select PKC isozymes could modulate colonic cell growth patterns. However, the effect of diet on the steady-state levels of PKC isozymes has received little attention to date. To better understand the biochemical mechanisms by which dietary fibers and fats modulate colonic cell proliferation and carcinogenesis, the effect of these constituents on steady-state levels of PKC isozymes and cell proliferation in the rat colonic mucosa was investigated. In addition, we isolated viable, exfoliated epithelial cells from feces to characterize the effect of diet on the *in vivo* turnover of the colonic epithelium. We specifically determined the effect of dietary fiber and fat on steady-state levels of proximal and distal colonic PKC isozymes and on colonic mucosal cell proliferation. The results of this study offer insight into the potential influence of dietary fiber and fat on the signal transduction pathways regulating colonic mucosal proliferation.

MATERIALS AND METHODS

Materials. Rabbit antibodies to PKC α , β , γ , δ , ϵ and ζ as well as the peptides against which the antibodies were prepared were from Gibco BRL (Gaithersburg, MD). Protein kinase C η antiserum and peptide, as well as recombinant protein standards for all PKC isozymes, were from Oxford Biomedical Research (Oxford, MI). Affinity-purified mouse anti-rabbit IgG and alkaline phosphatase-conjugated goat anti-rabbit and goat anti-mouse IgG were from Jackson ImmunoResearch (West Grove, PA). Rabbit anti-human cytokeratin was from BioGenex (San Ramon, CA), and bovine albumin was from Biocell (Dominguez, CA). Precast SDS gradient mini-gels were from Novel Experimental Technology (San Diego, CA). Rainbow electrophoresis molecular weight markers were obtained from Amersham (Arlington Heights, IL) and PVDF membrane from Millipore (Bedford, MA). Nylon screens for cell filtering were from Small Parts, Inc. (Miami Lakes, FL), and Percoll was from Pharmacia (Piscataway, NJ). Cellulose (Avicel Microcrystalline cellulose, type PH 101) was purchased from FMC (Philadelphia, PA). Pectin (citrus pectin, polygalacturonic acid methyl ester) was purchased from U.S. Biochemical (Cleveland, OH). Corn oil was kindly provided by Traco Labs (Seymour, IL). Menhaden fish oil was provided by the NIH Fish Oil Test Material Program, Southeast Fisheries Center

(Charleston, SC). Protein assay reagent was from Bio-Rad (Richmond, CA). Histopaque 1119 and all other reagents were from Sigma Chemical (St. Louis, MO).

Animals and diets. All experimental procedures using laboratory animals were approved by the Institutional Animal Care and Use Committee of Texas A&M University. Sixty-eight male Sprague-Dawley rats (Harlan Sprague Dawley, Houston, TX) were randomly assigned to four dietary groups (17 rats per diet). Rats were fed four diets (fiber-free fish oil, fiber-free corn oil, cellulose + corn oil, pectin + corn oil) that differed only in fiber and fat concentration as previously described (Lee et al. 1993b) for 3 wk. The composition of the basal fiber-free experimental diet is shown in Table 1. Corn oil and fish oil contained identical levels of TBHQ (0.025%) and vitamin E (1.5 mg/g α -tocopherol and 1.0 mg/g γ -tocopherol).

Tissue preparation and protein extraction. At the end of the feeding period, rats were killed by asphyxiation, and the brain and colon were removed and placed in ice-cold PBS. All further procedures were conducted on ice or at 4°C. The colon was divided into two equal segments, designated proximal and distal, with colon segments from three animals pooled for each homogenate preparation. Colons were then slit open and rinsed with chilled PBS and the mucosa scraped off with a microscope slide. Tissues were weighed and then homogenized in five volumes of homogenization buffer using 10 strokes of a Teflon-in-glass homogenizer. Homogenization buffer, modified from Wetsel et al. (1992), consisted of 50 mmol/L Tris-HCl, pH 7.5, 0.25 mol/L sucrose, 2 mmol/L EDTA, 1 mmol/L EGTA, 25 mg/L each leupeptin, pepstatin and aprotinin, 1 mg/L soybean trypsin inhibitor, 50 μ mol/L sodium fluoride, 0.14 mmol/L 4-(2-aminoethyl)benzenesulfonyl fluoride and 10 mmol/L 2-mercaptoethanol. Following homogenization, samples were ultracentrifuged at 100,000 \times g

TABLE 1
Composition of the basal fiber-free experimental diets¹

Ingredient	Corn oil diet	Fish oil diet
	g/100 g diet	
Sucrose	52.6	52.6
Casein	23.6	23.6
DL-Methionine	0.4	0.4
Corn oil	15.0	3.5
Fish oil	0	11.5
Mineral mix ²	6.0	6.0
Vitamin mix ²	2.0	2.0
Choline bitartrate	0.4	0.4

¹Fiber-supplemented diets were produced by diluting the basal fiber-free diet with 6 g of pectin or cellulose per 100 g of diet. The corn oil/cellulose diet was considered the control diet.

²Refer to Lee et al. (1993b) for details.

for 30 min with the supernatant saved as the cytosolic extract. The pellet was resuspended in homogenization buffer plus Triton X-100 (final concentration 10 mL/L), placed on ice for 10 min, and centrifuged again, under the same conditions. The resultant membrane extract, as well as the cytosolic extract, was frozen in aliquots at -80°C until use. The protein concentration of each extract was determined, with bovine serum albumin as standard (Bradford 1976).

Measurement of cell proliferation. After the 3-wk feeding period, each animal was given an intraperitoneal injection of bromodeoxyuridine, 5 mg/kg body wt in PBS, pH 7.4, exactly 1 h before killing. In vivo cell proliferation was measured by the incorporation of bromodeoxyuridine into DNA (Lee et al. 1993a). The first 1 cm from the cecal-proximal colon junction was taken for proximal cell proliferation determination. Tissue for distal colon proliferation was taken 1 cm proximal to the rectum. Cell proliferation was measured as labeling index (the number of labeled cells divided by total cells in the crypt column, multiplied by 100) and proliferative zone (the position of the highest labeled cell divided by total cells in the crypt column, multiplied by 100).

Determination of steady-state levels of PKC isozymes. Rat brain cytosol and membrane homogenates were used as positive controls for all PKC isozymes in immunoblotting. Brain and colonic homogenates (20–100 μg) were treated with SDS sample buffer as per the method of Laemmli (1970) and subjected to SDS-PAGE in 4–12% mini-gels. Following electrophoresis, gels were soaked for 15 min in transfer buffer and electroblotted onto PVDF membranes using a Hoefer Mighty Small Transphor unit (San Francisco, CA) at 200 mA for 3.5 h. Following transfer, the membrane was processed by the method of Sheng and Schuster (1992) using antibodies to individual PKC isozymes. Dilution of primary antibody was titrated for each isozyme and the membrane incubated for 2 h at room temperature with rocking. Alkaline phosphatase-conjugated secondary antibody incubation was for 2 h at room temperature, followed by development to visualize PKC bands. In some cases, inhibitory peptide (1 mg/L) was added to the primary antibody solution to selectively block binding of antibody to antigen. Preliminary screening of proximal and distal cytosolic and membrane extracts with antibodies to seven PKC isozymes (α , β , γ , δ , ϵ , η and ζ) was performed. We were able to obtain quantifiable PKC signals for the α , δ and ζ isoforms. We also detected PKC β and ϵ in rat colonic mucosa but at levels too low to detect accurately. To increase sensitivity in the detection of PKC α , a tertiary antibody detection system was used.

For each isoform, a range of protein concentrations was loaded onto the gel to ensure that the ultimate visualization was within the detection range. Distri-

bution of PKC isozymes in the cytosolic and membrane fractions of brain and colon was quantified for each isoform present in significant amounts in the colon (α , δ and ζ). Blots were scanned on a BioImage Visage 110-2D imaging instrument (Kodak, Ann Arbor, MI), and results are expressed as band intensity (optical density of the specific PKC band multiplied by band area).

Isolation of exfoliated colonic cells. As a noninvasive method for collecting colonic epithelial cells, exfoliated cells from fecal material were isolated by a modification of the method developed by Lohani and Nair (Albaugh et al. 1992). These cells express characteristic colonic epithelial markers, notably cytokeratins and colon-specific antigen (Albaugh et al. 1992). Briefly, feces were weighed into ice-cold dispersing medium (Albaugh et al. 1992) at 50 mL/g feces, dispersed in a model 400 Stomacher (Tekmar, Cincinnati, OH) and sequentially filtered through nylon screens (149, 105 and 53 μm). Cells and particulate matter were pelleted and resuspended in dispersing solution (5 mL/g original material), and Histopaque 1119 (10 mL of Histopaque to 30 mL of homogenate) was gently layered under the cell suspension in a 50-mL conical tube. The tubes were centrifuged at $165 \times g$ for 30 min at room temperature, and the cell layer immediately above the Histopaque was carefully removed, diluted with 4 volumes of dispersing solution, and the cells repelleted. After resuspension, the suspension was layered onto a pre-formed Percoll gradient followed by centrifugation to form discrete layers within the gradient. The cell layer was removed and washed with dispersing solution. The final cell pellet was resuspended and checked for viability with trypan blue, and cells were enumerated. Exfoliated cell homogenates were probed for the presence of cytokeratin using immunoblotting as described above to confirm the colonic intestinal epithelium as their site of origin (data not shown).

Statistics. Data were analyzed by ANOVA followed by least squares means test (Ott 1988). Results were considered significant at $P < 0.05$.

RESULTS

Effects of dietary fiber and fat on cell proliferation. Both dietary fiber and fat had an influence on colonic cell proliferation. The pectin + corn oil diet and the fiber-free corn oil diet generally resulted in greater proliferation compared with the other diets (Fig. 1), as assessed by labeling index and proliferative zone. Cell proliferation tended to be lowest in the fish oil-fed animals.

Effects of dietary fiber and fat on steady-state levels of PKC isozymes. Immunoblotting analysis showed that PKC α , δ and ζ were the major isozymes expressed in the rat colonic mucosa. Identity of

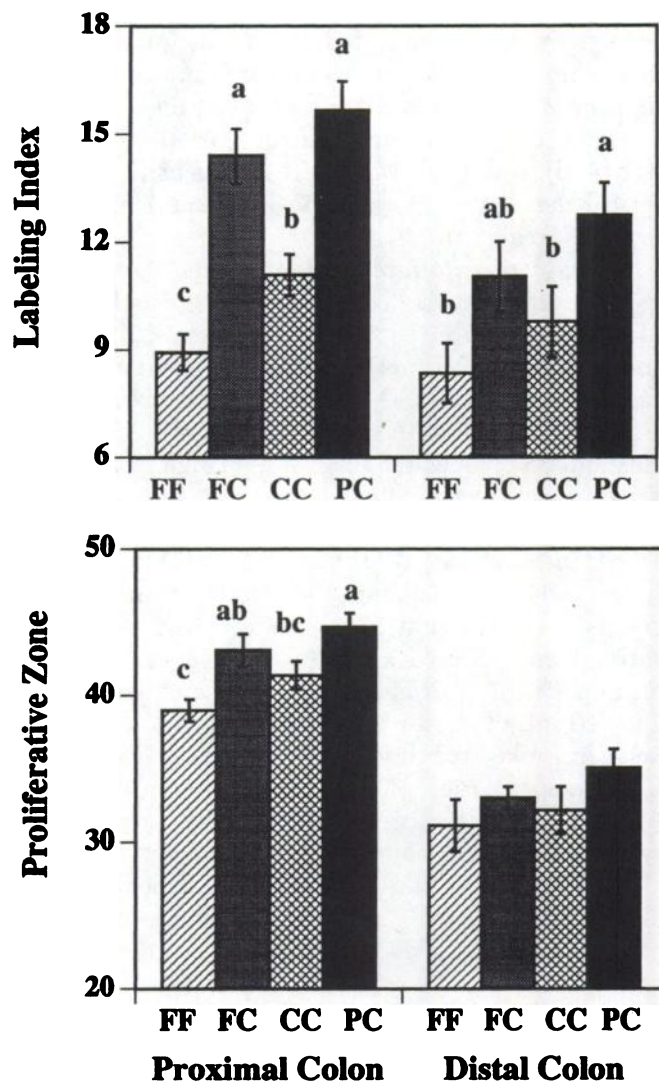


FIGURE 1 Effect of dietary fiber and fat on mucosal cell proliferation in proximal and distal colon of rats. Labeling index is the number of labeled cells divided by total cells in the crypt column multiplied by 100. Proliferative zone is the position of the highest labeled cell divided by the total cells in the crypt column multiplied by 100. Bars are means \pm SE, $n = 10$ rats/group. Bars with different letters are significantly different ($P < 0.05$). Diet abbreviations: FF, fiber-free fish oil diet; FC, fiber-free corn oil diet; CC, cellulose + corn oil diet; PC, pectin + corn oil diet.

specific PKC isozymes was confirmed by elimination of the immunoreactive band upon pre-incubation of the PKC isozyme-specific antibody with the corresponding PKC peptide (data not shown), as well as by comparison with individual PKC recombinant protein standards and brain homogenates. All seven isozymes were detected abundantly in rat brain homogenates. **Figure 2** shows representative blots for the three isozymes detected in significant quantities in rat colonic mucosa. A distinct cytosol to membrane ratio pattern can be seen for each isozyme. For example, PKC δ was localized to a greater extent in

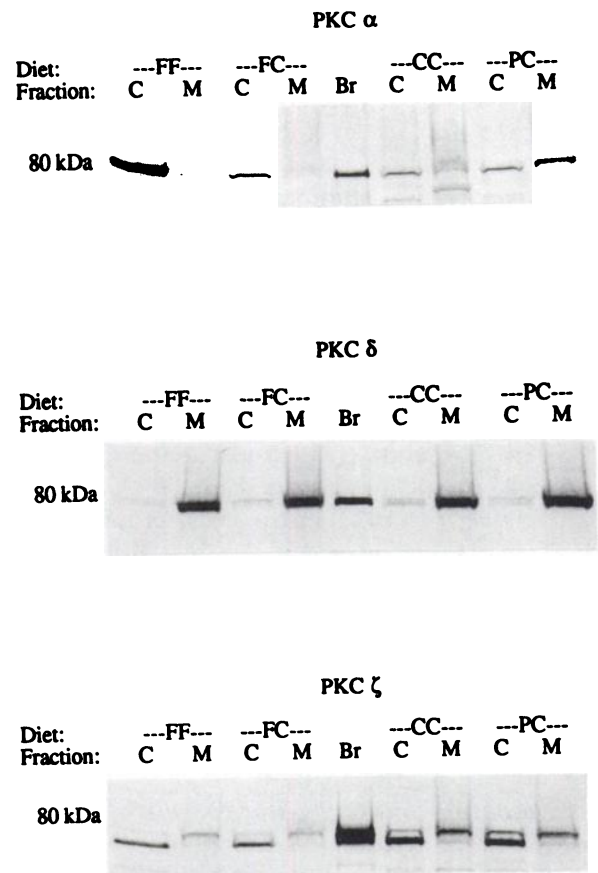


FIGURE 2 Effect of dietary fiber and fat on protein kinase C (PKC) isozyme expression in rat colonic mucosa as assessed by immunoblot analysis. Colonic cytosolic and membrane homogenates as well as brain homogenates were subjected to electrophoresis in 4–12% polyacrylamide gels, blotted onto PVDF membrane and processed with antibodies to specific PKC isozymes. Diet abbreviations: FF, fiber-free fish oil diet; FC, fiber-free corn oil diet; CC, cellulose + corn oil diet; PC, pectin + corn oil diet. C = cytosolic fraction, M = membrane fraction, Br = brain.

the membrane fraction than in the cytosolic fraction. In contrast, PKC α was primarily localized in the cytosol. Protein kinase C ζ , which produces a doublet possibly due to various phosphorylation states of the protein, was also localized to a greater extent in the cytosolic fraction.

Table 2 displays the extent to which the three PKC isozymes are membrane associated. Although each isozyme had a unique membrane to cytosol distribution, diet did not significantly alter this distribution. Protein kinase C α was ~30% membrane associated, PKC δ was 80% membrane bound, and PKC ζ was 20% membrane bound. Although there was no significant effect of diet on distribution of PKC between membrane and cytosolic fractions, diet did alter total PKC expression in the colon (**Fig. 3–5**). Rats consuming the pectin + corn oil diet had significantly higher steady-state levels of PKC α and δ in the distal colonic membrane fractions, and in the proximal

TABLE 2

Percentage of total protein kinase C (PKC) in the membrane fraction of colonic mucosa from rats fed various fats and fibers^{1,2}

Isozyme ⁴	Location	Dietary group ³			
		FF	FC	CC	PC
PKC α	Proximal	28.6 \pm 3.9	29.7 \pm 4.6	30.8 \pm 3.9	36.2 \pm 3.9
	Distal	22.1 \pm 4.7	28.4 \pm 4.7	34.9 \pm 3.6	40.0 \pm 5.0
PKC δ	Proximal	88.6 \pm 2.2	85.3 \pm 2.2	83.6 \pm 2.2	88.4 \pm 2.2
	Distal	77.9 \pm 6.1	84.1 \pm 6.1	66.6 \pm 6.1	75.0 \pm 6.1
PKC ζ	Proximal	9.7 \pm 8.3	22.7 \pm 6.8	15.1 \pm 10.6	12.9 \pm 8.2
	Distal	22.7 \pm 14.6	27.7 \pm 14.6	20.0 \pm 14.6	17.8 \pm 13.6

¹Values are means \pm SE of 3–4 analyses of tissue pooled from three animals.

²Statistical analyses were performed across diets within a single isozyme; values were not significantly different ($P > 0.05$).

³Abbreviations used: FF, fiber-free fish oil diet; FC, fiber-free corn oil diet; CC, cellulose + corn oil diet; PC, pectin + corn oil diet.

⁴PKC α , δ and ζ were detected in colonic mucosal cytosol and membrane extracts by immunoblotting with PKC isozyme-specific antibodies followed by densitometry to assess band intensity. Membrane PKC was expressed as percentage of total PKC (membrane plus cytosol).

membrane fraction for PKC α , relative to animals consuming the other experimental diets. Effects of diet on PKC ζ expression were confined to the proximal colon.

Differential steady-state levels of PKC isozymes in the proximal and distal colonic mucosa. As seen in Figure 6, the proximal and distal colon possessed

unique PKC isozyme distributions. Protein kinase C α was preferentially expressed in proximal colonic mucosa, whereas PKC δ and ζ were localized to a greater extent in the distal colonic mucosa. The extent of this proximal-to-distal variation was often significant.

Enumeration of exfoliated colonic epithelial cells.

The number of isolated exfoliated cells was consistent with the diet-induced changes in cell proliferation as measured by bromodeoxyuridine incorporation (Table 3). For example, pectin + corn oil-fed animals, which had the highest rates of colonic cell proliferation in vivo (Fig. 1), also had the highest number of exfoliated colonic epithelial cells relative to the other dietary groups. The animals consuming the fiber-free oil diet tended to produce the fewest number of exfoliated cells and to have the lowest rates of cell proliferation as assessed by labeling index.

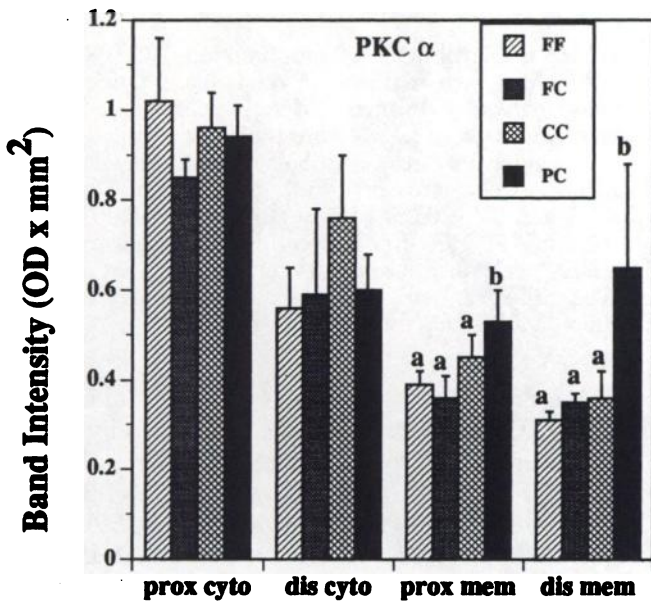


FIGURE 3 Effect of diet on expression of protein kinase C (PKC) α in proximal and distal colonic mucosal cytosol and membrane extracts as assessed by immunoblotting. Blotting was performed as described in Figure 2. Densitometry was used to assess band intensity (optical density multiplied by band area). Bars with different letters are significantly different ($P < 0.05$). Values are means \pm SE ($n = 4$), with tissue pooled from three animals to prepare homogenates. Diet abbreviations: FF, fiber-free fish oil diet; FC, fiber-free corn oil diet; CC, cellulose + corn oil diet; PC, pectin + corn oil diet.

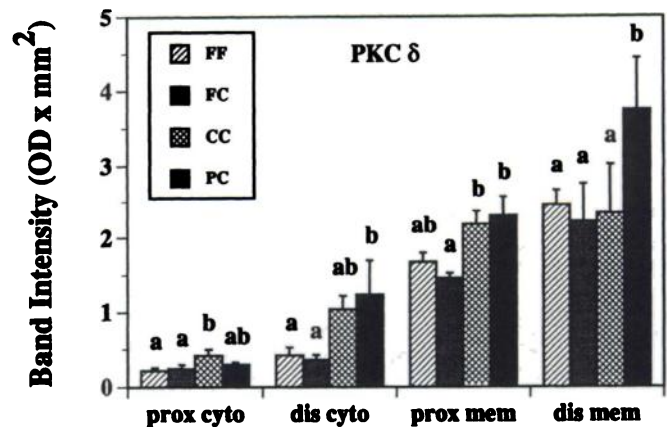


FIGURE 4 Effect of diet on expression of protein kinase C (PKC) δ in proximal (prox) and distal (dis) colonic mucosal cytosol (cyto) and membrane (mem) extracts as assessed by immunoblotting. Refer to Figure 3 for legend details.

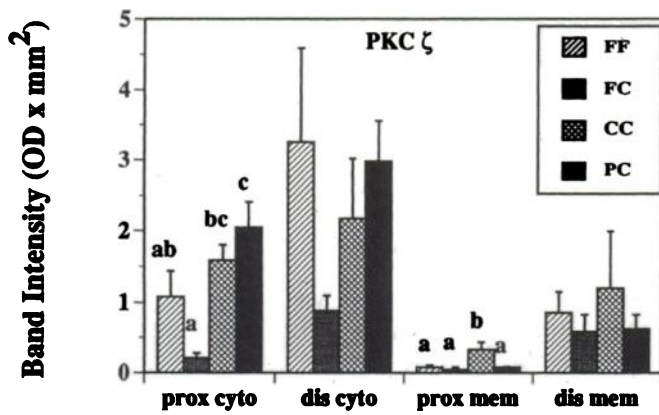


FIGURE 5 Effect of diet on expression of protein kinase C (PKC) ζ in proximal and distal colonic mucosal cytosol and membrane extracts as assessed by immunoblotting. Refer to Figure 3 for legend details.

DISCUSSION

Dietary fat and fiber independently and interactively modulate colonic cytokinetics (Lee et al. 1993a), which in turn alters the disposition of colonocytes to experimentally induced carcinogenesis (Lipkin 1988). We have previously shown a significant effect of diet on the ratio of colonic mucosal membrane to cytosolic PKC activity and a positive correlation between mucosal cell proliferation and membrane PKC activity (Chapkin et al. 1993). In addition, we have characterized diet effects on known lipid mediators of colonic PKC (phospholipids and diacylglycerols) (Lee et al. 1993b). However, the role of individual PKC isozymes in regulating colonic cytokinetics and their potential modulation by diet has not been examined to date. In this study, diets containing specific fats (corn oil and fish oil) and fibers (pectin, cellulose and no fiber) were fed to rats in order to

TABLE 3

Effect of dietary fats and fibers on the quantity of exfoliated colonic epithelial cells in rats^{1,2}

Dietary group	No. cells (total cells isolated/4 h fecal collection) $\times 10^{-6}$
Fiber-free fish oil	1.98 \pm 0.57 ^a
Fiber-free corn oil	3.76 \pm 0.57 ^a
Cellulose + corn oil	3.86 \pm 1.12 ^a
Pectin + corn oil	9.24 \pm 2.28 ^b

¹Values are means \pm SE, (n = 5). Values with different superscripts are significantly different ($P < 0.05$).

²Feces were collected and pooled over a 4-h period from 12 rats per diet group (n = 5). Exfoliated colonic cells were isolated as described in Materials and Methods and enumerated by hemocytometry.

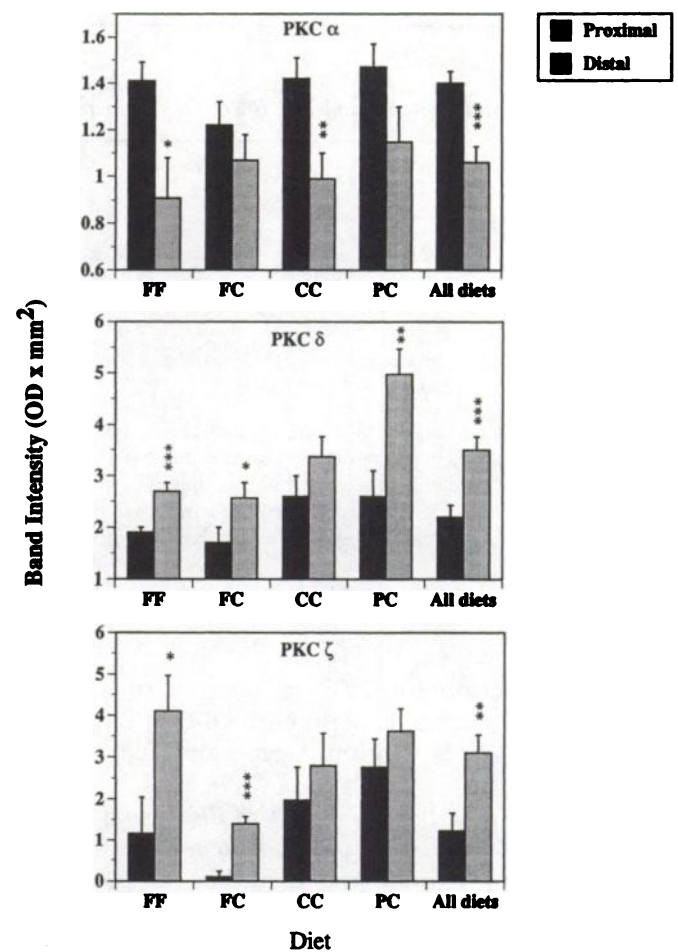


FIGURE 6 Distribution of protein kinase C (PKC) isozymes between proximal and distal colonic mucosa. Immunoblotting was performed as described in Figure 2. Densitometry was used to assess band intensity (optical density multiplied by band area). Symbols indicate level of significance between proximal and distal colonic mucosa within a diet: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Diet abbreviations: FF, fiber-free fish oil diet; FC, fiber-free corn oil diet; CC, cellulose + corn oil diet; PC, pectin + corn oil diet. The "all diets" column displays the average from all four diets. Values are means \pm SE (n = 3).

determine their effect on steady-state levels of colonic mucosal PKC isozymes and rates of cell proliferation.

The major PKC isozymes expressed in rat colon are α , δ and ζ (Fig. 2). We have shown that PKC β and ϵ are also present in rat colon (Davidson et al. 1994) but at levels too low to accurately measure. We did not detect PKC γ , which has been shown to be expressed only in the central nervous system (Hug and Sarre 1993, Nishizuka 1992), or PKC η . Craven and DeRubertis (1992) examined rat colonic mucosa for the presence of the classical PKC isozymes (α , β and γ) and detected only PKC α in normal tissue. Protein kinase C η may be present in a Triton-insoluble fraction in epithelial tissue (Osada et al. 1990) due to its association with cytoskeletal components (Osada et al. 1993). In this study we examined cytosolic and Triton-soluble membrane fractions and were unable to detect PKC η .

In this study we were interested in examining diet-induced changes in individual PKC isozyme levels, with respect to changes in cell proliferation. Although the results were not always statistically significant, fish oil-fed animals consistently had the lowest levels of colonic mucosal cell proliferation, as measured by labeling index and proliferative zone (Fig. 1). Rao and Reddy (1993) recently examined the effects of high fat corn oil or fish oil diets on colon carcinogenesis in rats. They found that fish oil consumption suppressed colonic ornithine decarboxylase and tyrosine-specific protein kinase as compared with corn oil consumption. Elevated ornithine decarboxylase and tyrosine-specific protein kinase have been linked to enhanced cell proliferation and tumor development (Cantley et al. 1991, Wade and Norred 1976). This is important because there is substantial evidence to indicate that fish oils inhibit colon cancer in a number of animal models, whereas diets containing (*n*-6) and (*n*-9) fatty acids, derived from animal and vegetable fats and oils, promote colon cancer (Reddy 1988). Human epidemiological data also imply a protective effect of marine oil on colon cancer (Willett 1989 and 1990). In addition, prompt improvement in the proliferative pattern of rectal mucosa occurs when the (*n*-3) polyunsaturated fatty acid (fish oil) content of the diet is increased in subjects at risk for colon cancer (Anti 1992). Therefore, fish oil seems to exert a rapid effect that may protect high risk subjects from colon cancer. The fiber-free corn oil and pectin + corn oil diets used in this study resulted in the highest levels of cell proliferation, with consumption of the cellulose + corn oil diet producing intermediate levels. Results from the isolation of exfoliated colonic cells from feces were similar to the proliferation results, with the largest number of cells recovered from the pectin-fed group and the fewest from the fish oil-fed animals. This confirms our previous observation that pectin, a highly fermentable fiber, stimulates cell proliferation in the rat colon as compared with the less fermentable cellulose (Lee et al. 1993a, Lupton et al. 1988).

Diet was shown to have no significant effect on the characteristic membrane to cytosol distribution of each isozyme. However, nonsignificant differences in the percent distribution of some isozymes in the membrane fraction were noted. Therefore, it may be premature to conclude that diet does not alter membrane vs. cytosolic distribution of PKC isozymes. Additional studies are required to address this point. Clarification of this observation is important because the membrane to cytosol ratio gives an indication of the activation state of the enzyme, because activation generally involves translocation of PKC from cytosol to membrane (Hug and Sarre 1993). Total PKC isozyme expression was altered by diet, with the pectin + corn oil diet typically resulting in increased colonic content of PKC α and δ . Diet may exert an effect at multiple levels, including transcription, translation and degradation. Additional studies are required to definitively characterize the mechanism

by which diet modulates PKC steady-state levels in the colon. Overall, these data are consistent with the concept that dietary constituents are capable of influencing signal transduction pathways that regulate cell proliferation (Chapkin et al. 1993, Fowler et al. 1993, Murray et al. 1993). Changes in isozyme expression in carcinogen-treated rats have been reported, with a dramatic decrease in PKC α expression and a corresponding increase in PKC β expression in colon adenocarcinomas as compared with normal surrounding tissue (Craven and DeRubertis 1992). Diet had a differential effect on PKC α , δ and ζ , indicating that each isozyme has its own specific effectors and kinetics and should be analyzed individually. These observations suggest that each PKC isozyme participates in distinct signal transduction pathways within the colon. Comparison of expression levels between different isozymes is not valid, because binding affinities of the antibodies to various epitopes are not uniform and have not been standardized.

Also of interest is the differential expression of PKC isozymes in proximal vs. distal colon (Fig. 6). For PKC δ and ζ , ~100% more enzyme was found in the distal colon than in the proximal colon. The opposite was observed for PKC α . This is an indication that the proximal and distal colon have distinct characteristics (Lee et al. 1993a) and that dietary fat and fiber modulate cell proliferation and PKC signal transduction in a site-specific manner. In fact, we have previously shown that whereas pectin stimulates colonic mucosal cell proliferation, the effect is confined to the proximal colon (Jacobs and Lupton 1984, Lupton et al. 1988). In addition, dietary fat exerts an effect on colonic cell proliferation in the distal colon (Lee et al. 1993a).

In conclusion, the results of this study provide evidence that dietary constituents can differentially influence steady-state levels of PKC isozymes in the colon. Further studies are needed to determine the role of specific PKC isozymes in regulating colonic cell proliferation and malignant transformation.

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