Dietary Fish Oil Reduces O^6-Methylguanine DNA Adduct Levels in Rat Colon in Part by Increasing Apoptosis during Tumor Initiation[1]

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Abstract

There is epidemiological, clinical, and experimental evidence that dietary fish oil, containing n-3 polyunsaturated fatty acids, protects against colon tumor development. However, its effects on colonocytes *in vivo* remain poorly understood. Therefore, we investigated the ability of fish oil to modulate colonic methylation-induced DNA damage, repair, and deletion. Sprague Dawley rats were provided with complete diets containing either corn oil or fish oil (15% by weight). Animals were injected with azoxymethane, and the distal colon was removed 3, 6, 9, or 12 h later. Targeted apoptosis and DNA damage were assessed by cell position within the crypt using the terminal deoxynucleotidyl transferase-mediated nick end labeling assay and quantitative immunohistochemical analysis of O^6-methylguanine adducts, respectively. Localization and expression of the alkyl group acceptor, O^6-methylguanine-DNA-methyltransferase, was also determined. Lower levels of adducts were detected at 6, 9, and 12 h in fish oil- *versus* corn oil-fed animals ($P < 0.05$). In addition, fish oil supplementation had the greatest effect on apoptosis in the top one-third of the crypt, increasing the apoptotic index compared with corn oil-fed rats ($P < 0.05$). In the top one-third of the crypt, fish oil feeding caused an incremental stimulation of apoptosis as adduct level increased. In contrast, a negative correlation between apoptosis and adduct incidence occurred with corn oil feeding ($P < 0.05$). Diet had no main effect (all tertiles combined) on O^6-methylguanine-DNA-methyltransferase expression over the time frame of the experiment. The enhancement of targeted apoptosis combined with the reduced formation of O^6-methylguanine adducts may account, in part, for the observed protective effect of n-3 polyunsaturated fatty acids against experimentally induced colon cancer.

Introduction

It is estimated that dietary factors account for a significant proportion of colon malignancies, suggesting that the majority of cases of colon cancer are preventable (1). Among dietary factors, there are epidemiological, clinical, and experimental data indicating a protective effect of n-3 polyunsaturated fatty acids, *e.g.,* eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), with respect to colon cancer development (2–5). The protective effect appears to be exerted at both the initiation and postinitiation (promotional) stages of carcinogenesis (2). In contrast, the enhancing effect of dietary n-6 polyunsaturated fatty acids, *e.g.,* linoleic acid (18:2n-6), on colon tumorigenesis is mainly seen during the postinitiation phase (2). However, to date, few studies have investigated the mechanisms by which n-3 polyunsaturated fatty acids retard colon cancer initiation.

With respect to putative mechanisms of action, select dietary polyunsaturated fatty acids may modulate carcinogen activation (2, 6) and DNA repair enzymes (7, 8). It has been demonstrated recently that dietary fish oil, containing 20:5n-3 and 22:6n-3, reduces the incidence of activating G-to-A point mutations of K-*ras* in colonic mucosa of AOM[3]-treated rats (9, 10). This chemopreventive effect is consistent with the induction of MGMT (EC 2.1.1.63), an alkyl group acceptor that rapidly removes AOM-induced promutagenic O^6-methylguanine DNA adducts, thereby preventing oncogenic G-to-A mutations (11, 12). This is noteworthy because there is substantial evidence that select mutagenic and lethal lesions involve the O^6 position of guanine (13). Therefore, dietary fish oil may protect against colon carcinogenesis by either decreasing DNA adduct formation and/or enhancing DNA adduct removal (DNA repair).

Another mechanism to protect against colonic DNA damage is the induction of apoptosis (14, 15). Although apoptosis generally occurs with low frequency in the colon, *i.e.,* less than one apoptotic cell per crypt (15, 16), there is compelling evidence that it plays a central role in the regulation of cell number and the eradication of harmful cells (15–18). We have shown that dietary fish oil confers protection against experimental tumorigenesis during the promotion phase, in part by enhancing the deletion of cells through activation of apoptosis rather than decreasing cell proliferation (4, 15). However, the effect of diet on damage-induced cell suicide (targeted apoptosis) in the colon during tumor initiation has not been examined to date. Therefore, in this study, we determined the ability of fish oil feeding to simultaneously modulate O^6-methylguanine DNA adduct formation (DNA damage), removal (DNA repair), and deletion (apoptosis) during the initiation stage of colonic malignant transformation.

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[3] The abbreviations used are: AOM, azoxymethane; MGMT, O^6-methylguanine-DNA-methyltransferase.

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Table 1 Composition of experimental diets

Ingredient	g/100 g
Dextrose	51.06
Casein	22.35
D.L.-methionine	0.34
Salt mix, AIN-76	3.91
Vitamin mix, AIN-76	1.12
Choline bitartrate	0.22
Pectin	6.00
Fat	
Corn oil diet	
Corn oil	15.00
Fish oil diet	
Menhaden fish oil	11.50
Corn oil	3.50

Materials and Methods

Materials. AOM was purchased from Sigma (St. Louis, MO). Corn oil was kindly donated by Traco Labs (Seymour, IL). Vacuum-deodorized Menhaden fish oil was provided by the NIH Fish Oil Test Material Program, Southeast Center (Charleston, SC). High methoxylated pectin was purchased from Grinsted (Industrial Airport, KS). Rabbit anti-rat DNA alkyltransferase was generated as previously described (19), and mouse anti-O^6-methylguanine antibody was obtained from Dr. Christopher P. Wild, University of Leeds (Leeds, United Kingdom).

Animals. The animal use protocol was approved by the University Animal Care Committee of Texas A&M University and conformed to the NIH guidelines. Thirty male weanling Sprague Dawley rats (Harlan, Houston, TX) were provided with diets differing only in the type of fat (corn oil or fish oil). The rats were acclimated for 1 week before receiving the defined diets, and then stratified by body weight so that mean initial body weights did not differ between groups. Animals were provided with the defined diets for 2 weeks before AOM injection and throughout the entire duration of the study, and had free access to food and water at all times. Forty-eight-h food intakes were measured after 1 week of receiving the diets. Body weights were recorded weekly throughout the study.

Diets. The two defined diets (Table 1) differed only in the type of fat (corn oil or fish oil) as previously described (20). The major differences between the fatty acid composition of the two lipid sources were significantly higher amounts of 14:0; 16:1n-7; 20:5n-3; and 22:6n-3 in the fish oil compared with the corn oil diet, and higher amounts of 18:2n-6 and 18:1n-9 in the corn oil diet. Dietary fat was provided at 15 g/100 g of diet. The fish oil diet contained 3.5 g of corn oil/100 g of diet to ensure that essential fatty acid requirements were met. Animals were provided with fresh diet daily. The fish oil also contained 1 g/kg of α-tocopherol and 1.5 g/kg of γ-tocopherol and 0.025 g/100 g of tertiary butylhydroquinone as antioxidants. Corn oil was supplemented with α- and γ-tocopherol and tertiary butylhydroquinone to obtain antioxidant levels equivalent to that in fish oil. All diets contained pectin, a fermentable fiber, at 6 g/100 g of diet. These dietary ingredients were selected based on our previous studies in which dietary fish oil protected against AOM-induced colon tumorigenesis compared with corn oil (15). Pectin was selected because the protective effect of fish oil is enhanced when a highly fermentable fiber source is in the diet (15).

Carcinogen Treatment. After 2 weeks of receiving the experimental diets, AOM was injected s.c. (15 mg/kg of body weight) precisely at 9:00 a.m., and each animal (three rats per diet per time point) was killed 3, 6, 9, or 12 h after injection. Time zero represents a negative control because no injection was performed.

***In Vivo* Measurement of O^6-Methylguanine.** Rats were euthanized by CO_2 exposure, the entire colon was removed and rinsed with PBS, and the distal colon was subsequently isolated. Distal colonic sections were fixed in ethanol for an *in vivo* measurement of O^6-methylguanine adducts as previously described (21, 22) using mouse monoclonal anti-O^6-methylguanine. The specificity of this monoclonal antibody has been previously demonstrated (21–23). Liver O^6-methylguanine DNA adducts in AOM-injected animals were used as a positive control (24). Omission of primary antibody and preadsorption with ligand were used as negative controls (22). At least 20 crypt columns/animal that met architectural criteria were chosen for analysis. The staining intensity was assessed by cell position within the crypt as previously described (22, 25). Images of colonic crypts were captured on a MICROSTAR IV Reichert microscope networked to a Sony DXC-970 MD 3CCD camera and a Power Macintosh computer. Images were processed using NIH Image, version 1.61. Captured images were adjusted using offset and gain to optimize the image brightness and contrast so that the greatest difference between light and dark-stained pixels was achieved. Offset and gain were determined by pre-analysis of multiple darkly and lightly stained tissues to expand the scale in the necessary region to enhance detection. Once established, the settings remained constant for all samples. Representative photomicrographs have been published (22).

***In Situ* Apoptosis Measurement.** This method is based on the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling method (26) using a kit from Oncor (Gaithersburg, MD). Paraformaldehyde-fixed, paraffin-embedded distal colon sections were prepared (15). Positive control slides were treated with DNase I (Ambion, Austin, TX) at 37°C. Negative control slides were incubated without terminal deoxynucleotidyl transferase enzyme. The antibody-antigen complex was visualized by incubation with diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO; Refs. 15 and 22). Apoptotic cells were identified based on a combination of positive staining and morphological criteria as described by Kerr *et al.* (27). Crypt height in number of cells and the number and location of apoptotic cells were recorded, with 20 crypts analyzed per animal. The apoptotic index was 100 times the mean of the number of apoptotic cells per crypt column divided by the mean of the total number of cells per crypt column.

***In Vivo* Measurement of Repair Enzyme.** Colonic MGMT expression was determined using rabbit anti-rat alkyltransferase antibody and a tyramide signal amplification system (NEN Life Science Products, Boston, MA) as we have previously described (22). The specificity of this antibody has been documented (26). Endogenous peroxidase activity was quenched by immersing tissue sections in 3% H_2O_2 in methanol. Antigen was retrieved by microwave treatment with 0.1 M sodium citrate solution (pH 6.0). To block nonspecific background staining, tissue sections were incubated with avidin, biotin, and TNB buffer [0.1 M Tris-HCl (pH 7.5), 0.15 M NaCl, and 0.5% blocking reagent from the NEN kit]. Omission of primary antibody was used as a negative control. At least 20 crypt columns/animal were randomly chosen. The staining intensity was assessed by cell position within the crypt using an image analysis system (NIH Image, version 1.61; Ref. 22). Epithelial cells on the left side of the crypt were selected, the image was digitized, and the staining intensity was plotted. Background staining intensity was determined on 10 randomly obtained

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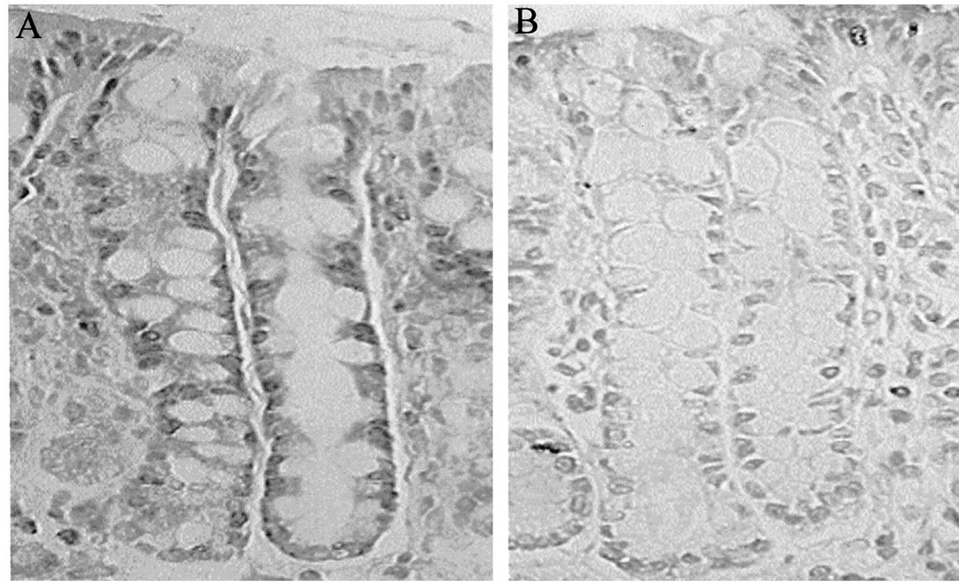

Fig. 1. Photomicrograph (×40) of colonic crypts stained for O^6-methylguanine adduct formation. *A,* representative corn oil-fed animal 12 h after AOM injection. *B,* fish oil-fed animal 12 h after AOM injection.

images on each slide outside of the tissue section and subtracted from the staining intensity of collected data.

Statistical Analyses. DNA adduct levels, apoptosis, and DNA repair enzyme expression were analyzed using two-way ANOVA to determine the effect of fat, time, and fat × time interaction. When *P*s for the interactions were <0.05, means of all diet groups were separated using the Student-Newman-Keuls multiple range test. When *P*s were <0.05 for the effects of fat, time, or carcinogen but not for the interactions, overall means for fat, time, or carcinogen treatment groups were separated by using the Student-Newman-Keuls multiple range test. Data on DNA adducts were also analyzed using PROC MIXED in SAS (SAS Institute Inc.). This mixed model approach simultaneously accounts for the between animal, within animal between crypts, and within crypt variation. Statistical inferences based on properly modeled variation were then used to evaluate the relationship between cell position within the crypt and adduct level and to determine the main effect of fat, time, carcinogen, and their interactions. The effects of diet on the regression of apoptotic index on adduct level, repair enzyme on adduct level, and repair enzyme on apoptosis were tested using linear regression. Differences between fish and corn oil within crypt tertiles were determined by one-way ANOVA. Comparisons among the different crypt compartments (bottom, middle, and upper tertiles) were made using paired *t* tests.

Results

Dietary Fish Oil Reduces Colonic DNA Adduct Levels in Carcinogen-injected Rats. Diet had no effect on body weight gain or food intake (data not shown). With regard to DNA adduct levels, substantial amounts of methylated purines are formed in the colon after the administration of alkylating carcinogens, such as 1,2-dimethylhydrazine or its derivatives (28, 29). Although 7-methylguanine is the major product of DNA alkylation in the colon, O^6-methylguanine has greater biological importance because of its ability to cause misincorporation mutations of relevant oncogenes (30). Therefore, we examined the effect of dietary lipid source on O^6-methylguanine adduct levels in the colon over time after AOM injection. Immunohistochemical staining was used to

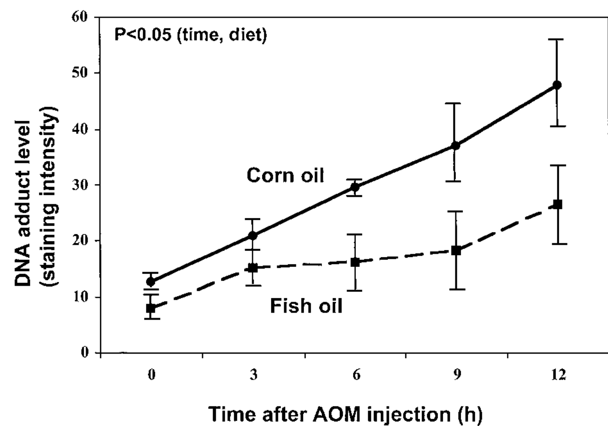

Fig. 2. Kinetics of dietary fat effect on DNA adduct level. Data are shown as means (n = 30). On average, over time, using two-way ANOVA, corn oil- *versus* fish oil-supplemented rats had a greater number of adducts ($P < 0.05$). Values for individual time points were significantly different using Fisher's least significant difference test at 6 (P = 0.027), 9 (P = 0.005), and 12 h (P = 0.002).

determine the level and localization of adducts within crypt epithelial cell nuclei (Fig. 1). Overall, corn oil-fed animals had more ($P < 0.05$) DNA adducts compared with fish oil-fed animals. These differences were detected at 6 h (P = 0.027) and were greatest at 12 h (P = 0.002; Fig. 2). Statistical likelihood ratio tests indicated that the relationship between DNA adduct levels and cell position within the crypts was parabolic in nature. Specifically, at each diet/time point, roughly 1800 data points (3 rats × 20 crypts × 30 cell positions) contributed to the parabolic fit (Fig. 3). These plots demonstrate the shape and nature of the distribution of the DNA adduct level measurements within the crypts, and they indicate a consistent suppressive effect of fish oil on adduct level throughout the crypt, regardless of the time after injection or cell position within the crypt.

Dietary Fish Oil Enhances Damage-induced Apoptosis. During the initiation of tumorigenesis after carcinogen treatment,

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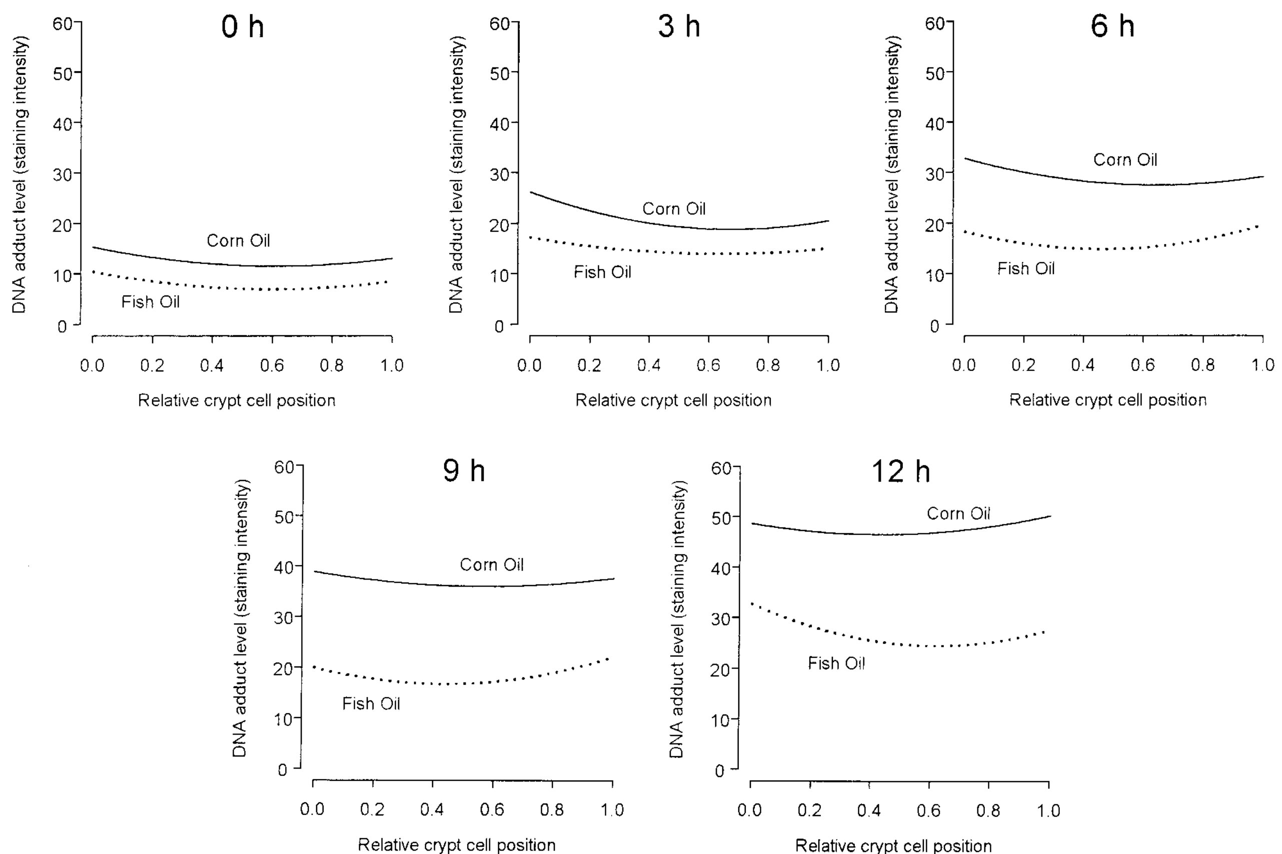

Fig. 3. The fitted DNA adduct frequencies at each position within the crypts is plotted (DNA adduct level, *Y, versus* relative cell position, *X*) for various times after treatment with AOM, using PROC MIXED in SAS. For relative cell position, 0 = the base of the crypt and 1.0 = the luminal surface. ———, corn oil-fed animals; ····, fish oil-fed animals.

there is a rapid apoptotic response to DNA adducts in colonic epithelium (31, 32). Therefore, we determined whether fish oil, containing 20:5n-3 and 22:6n-3, acts as an anticarcinogen by facilitating the removal of carcinogen adducted cells via deletion by apoptosis. As expected, there was a significant time effect ($P < 0.05$) with respect to the proportion of apoptotic cells in the colon (Fig. 4). Maximum apoptosis was achieved by 6–9 h after AOM injection. Most of the apoptosis was located toward the base of the crypt (Fig. 5), where the stem cells are located. In addition, there was a strong position and time effect (Fig. 5), indicating that cell death was dramatically increased ($P < 0.05$) in the base of the crypt at 6 h and continued through 12 h. Although there was no overall effect of lipid source on apoptosis (Fig. 4), fish oil feeding resulted in a significantly higher ($P = 0.035$) level of apoptosis in the upper one-third (upper tertile) of the crypt compared with corn oil treatment (Fig. 6).

Lack of Dietary Effect on MGMT Expression. The major pathway for the repair of the premutagenic DNA adduct, O^6-methylguanine, is via the MGMT reaction (12). Because the alkyltransferase is inducible, quantitative immunohistochemical analysis can be used to estimate MGMT activity (19, 22, 33, 34). Therefore, we determined whether dietary fish oil reduced DNA adduct levels in part by enhancing MGMT expression within the colonic crypt. Fig. 7 shows that there was no main effect of either time or diet ($P > 0.05$) on alkyltransferase expression. Similar to previous reports (22, 34), expression was detected predominantly in the nuclei; however, some cytoplasmic staining was also observed. The expression of MGMT was localized primarily to the upper portion of the crypt. Interestingly, there was a strong diet × position interaction effect ($P = 0.004$) within the colonic crypt at 12 h after AOM injection

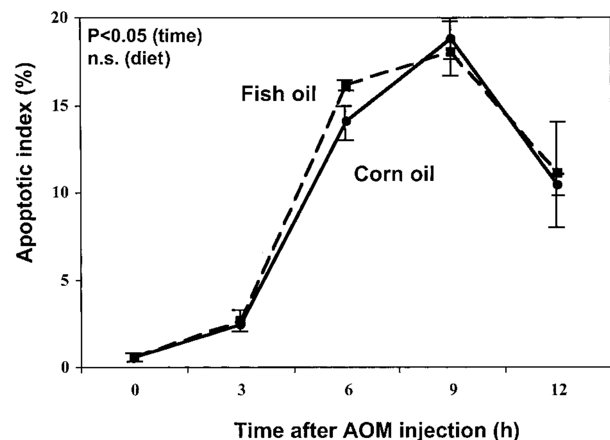

Fig. 4. Lack of overall effect of diet on apoptosis kinetics. Data are shown as means ($n = 30$ rats) by using two-way ANOVA. Maximum apoptosis was achieved by 6–9 h and decreased ($P < 0.05$) between 9 and 12 h after AOM injection. *n.s.,* not significantly different ($P < 0.05$).

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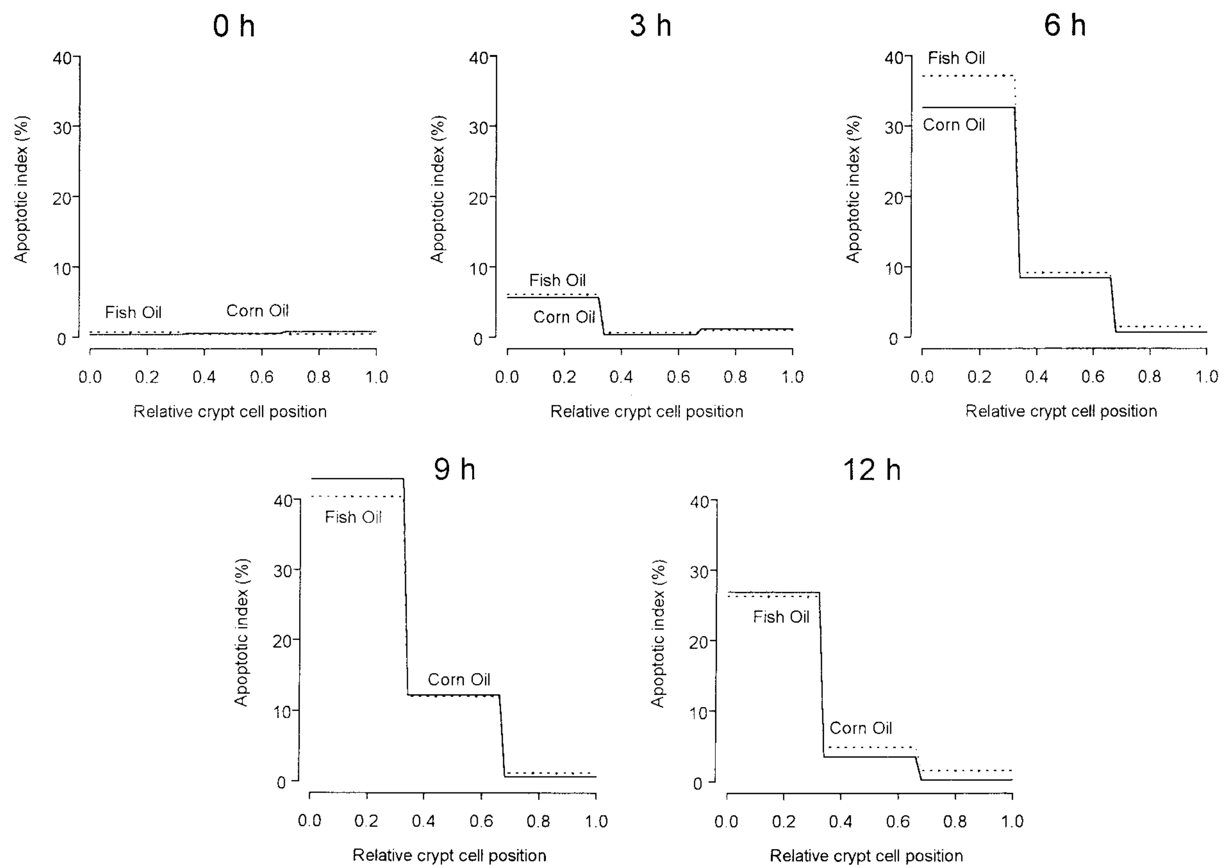

Fig. 5. The frequency of apoptosis for the lower, middle, and upper regions of the crypt is plotted (apoptotic index, *Y, versus* relative cell position, *X*) for various times after treatment with AOM. For relative cell position, 0 = the base of the crypt and 1.0 = the luminal surface. ———, corn oil-fed animals; ····, fish oil-fed animals.

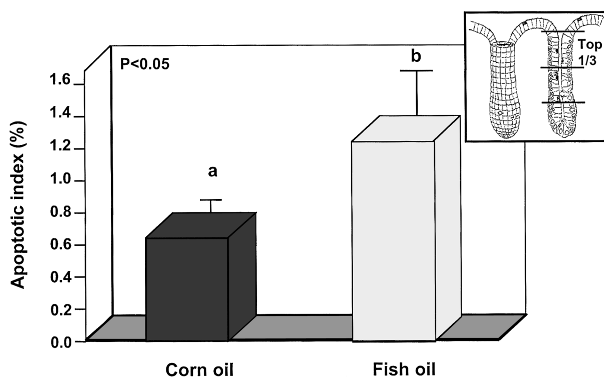

Fig. 6. Dietary fish oil enhances apoptosis in the upper tertile of the colonic crypt. Data representing all time points from the upper tertile (nearest the lumen) of the crypt were analyzed by using one-way ANOVA. *Bars,* mean ± SE (n = 15 rats/diet). Superscripts indicate a significant difference at $P < 0.05$.

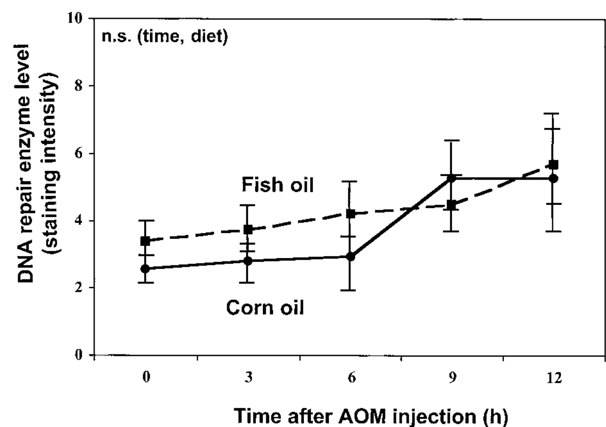

Fig. 7. Lack of dietary effect on MGMT expression on average over time. Data are shown as means (n = 30 rats). *n.s.,* not significantly different across time ($P >$ 0.05; two-way ANOVA).

(Fig. 8). Specifically, the steady-state level of MGMT for the fish oil fed animals was 4-fold higher ($P < 0.001$) in the upper *versus* lower region of the crypt, whereas there was no such effect for corn oil-fed animals.

Fish Oil-fed Animals Target DNA-damaged Cells for Deletion by Apoptosis. In the upper one-third of the crypt, the slopes for the DNA adduct level *versus* apoptotic index regressions differed significantly (P = 0.020) between fish and corn oil rats. In fact, the slopes were of opposite signs, *i.e.,* 0.06 for fish oil-fed animals and −0.03 for corn oil animals (Fig. 9). The slopes were not significantly different for the lower or middle tertiles ($P >$ 0.05). These data indicate that fish oil treatment selectively en-

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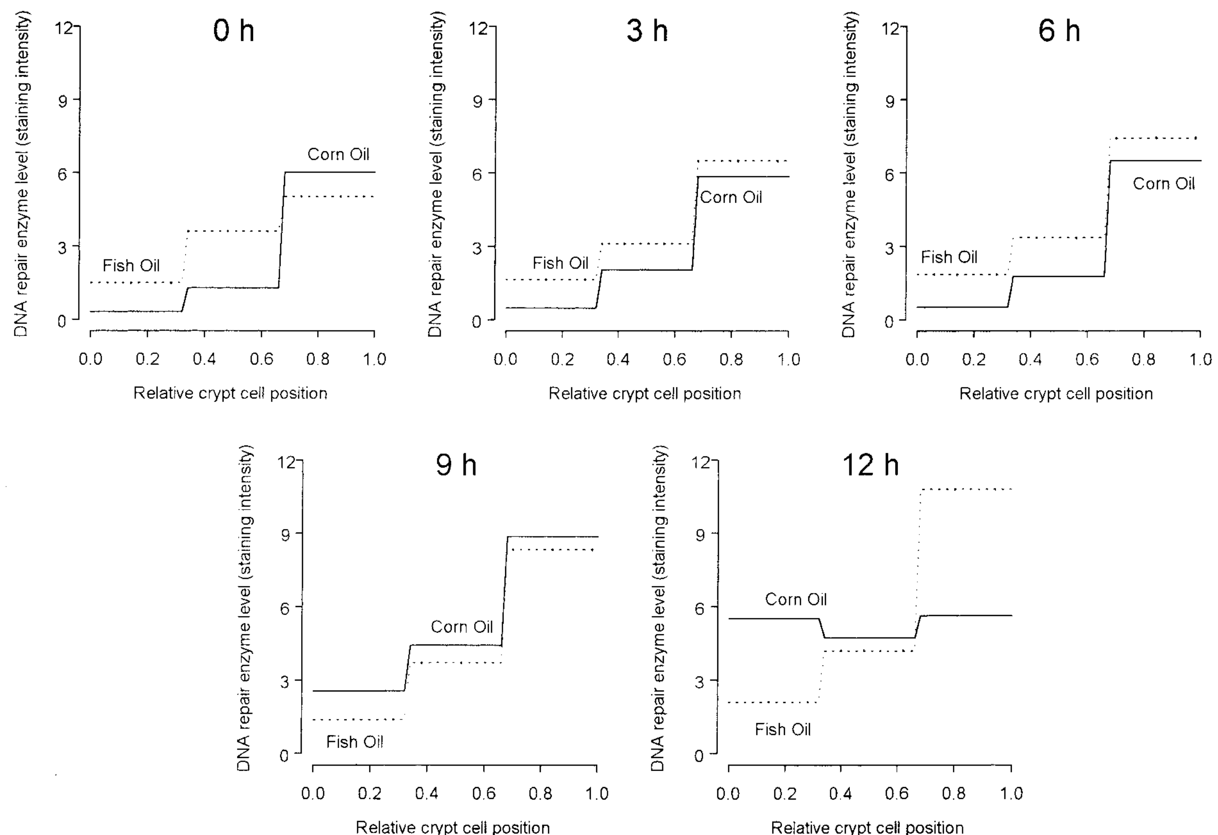

Fig. 8. The expression of MGMT at the lower, middle, and upper regions of the crypt is plotted (DNA repair enzyme level, *Y, versus* relative cell position, *X*) for various times after treatment with AOM. At 12 h, MGMT levels were significantly higher ($P < 0.001$) in the upper *versus* lower regions of the crypt in fish oil-supplemented rats only, using the paired *t* test. ———, corn oil-fed animals; ····, fish oil-fed animals. For relative cell position, 0 = the base of the crypt and 1.0 = the luminal surface.

hances the targeted removal of DNA adducts by increasing apoptosis in the top one-third of the crypt. Because polyps and tumors eventually develop from loss of growth control and retention of cells at the top of the crypt, the significant difference in fish oil *versus* corn oil on apoptosis targeted to this region may account, in part, for the observed protective effect of fish oil against experimentally induced colon tumorigenesis.

Discussion

The consumption of purified n-3 polyunsaturated fatty acids reduces colon cancer risk in humans and experimental animal models (3, 35–38). The average American normally consumes low levels (~100 mg/day) of 20:5n-3 and 22:6n-3 in the form of fish compared with 1000–3000 mg/day by the Japanese (39). In comparison, diets rich in n-6 polyunsaturated fatty acids, found in plant oils such as corn oil, enhance the development of colon tumors (2, 38, 40). This is particularly relevant because Americans consume >10 times the amount of this lipid source needed to meet minimal essential fatty acid requirements (41, 42). With respect to the putative mechanism(s) of action, we have demonstrated that the balance between colonic epithelial cell proliferation, differentiation, and apoptosis can be favorably modulated by dietary fish oil, conferring protection against carcinogenic agents (15). This is of significance because markers of apoptosis are better predictors of colon tumor outcome in tumorigenesis studies where diet is an experimental variable (15). In addition, it is now clearly established that the malignant transformation of colonic epithelium is associated with a progressive inhibition of apoptosis (16, 18, 43). These *in vivo* findings support our postulate that dietary n-3 polyunsaturated fatty acids act to facilitate the apoptotic removal of carcinogen adducted cells. In a continuation of our efforts to elucidate the mechanisms by which dietary 20:5n-3 and 22:6n-3 reduce colon tumor incidence, we determined how fish oil modulates methylation-induced DNA adduct levels in the colon during the initial stages of malignant transformation in the rat AOM carcinogenesis model.

AOM is metabolized to methylazoxymethane by p450-dependent multifunction oxidases in the liver. Subsequently, methylazoxymethane is converted to methylazoxyformaldehyde by alcohol dehydrogenase and eventually converted to methyldiazonium, the ultimate carcinogen (44, 45). The carcinogenicity of AOM can be inhibited using metabolic inhibitors (46). We have shown that colonic O^6-methylguanine adduct levels increase at a much faster rate in rats fed corn oil compared with fish oil. Although adduct levels were not different at the 0- and 3-h time points, the protective effect may be explained by the modulation of enzymology related to carcinogen activation, thereby altering the amounts and activities of oxidative (Phase 1) and conjugative (Phase 2) xenobiotic metabolizing enzymes (46, 47). Additional experiments are needed to address this hypothesis. It is unlikely that the protective effect of fish oil supplementation is related to an effect on MGMT-mediated DNA repair because overall, fish oil

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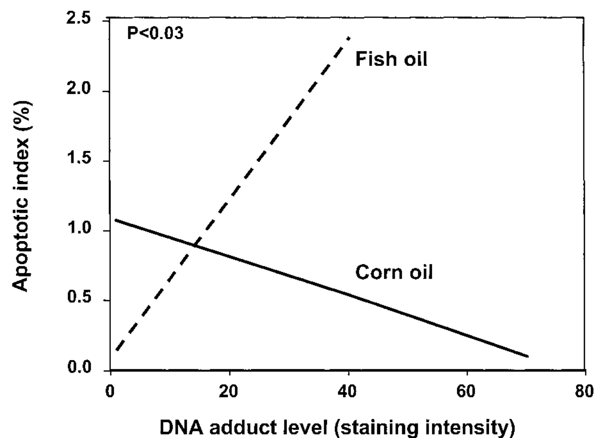

Fig. 9. Dietary fish oil enhances targeted apoptosis of DNA-damaged cells. A linear regression analysis of apoptosis on DNA adduct levels within the upper tertile (nearest the lumen) of the crypt (all time points were pooled). The slopes of the lines are significantly different ($P = 0.020$).

feeding had no significant effect on the induction of MGMT in the distal colonic epithelium during the time frame of this experiment. MGMT serves as the stoichiometric acceptor protein for O^6-methylguanine adducts, transferring the methyl group from DNA to the protein (12). This transfer inactivates MGMT and is irreversible. Therefore, a colonocyte's ability to withstand damage is in part related to the number of MGMT molecules it expresses and to the rate of *de novo* synthesis. Although not the focus of this study, it is possible that "back up" systems, such as DNA mismatch repair, which also recognize O^6-methylguanine adducts, may have been influenced by dietary lipid composition (48).

With regard to the deletion of colonocytes, in the rat distal colon, epithelial cells are derived from a stem cell population at the base of the crypt and migrate from a region of active cell proliferation in the bottom two-thirds of the crypt toward the top of the crypt, obtaining a differentiated or apoptotic phenotype. Cells at the lumenal surface are subsequently exfoliated into the fecal stream (18). Generally, apoptosis occurs after terminal differentiation (16). This form of cell deletion is predominant in the upper region of the crypt, indicating that "spontaneous" apoptosis is unlikely to effectively regulate stem cell number (16, 18). An important exception to this sequence of events occurs during the initiation of tumorigenesis when there is an immediate apoptotic response to DNA adduct formation (22, 31, 32). Previous reports suggest that this form of damage-induced cell deletion (targeted apoptosis) is primarily localized to the bottom two-thirds of the crypt, where actively proliferating cells reside (16, 18, 22). This is somewhat puzzling because cells with DNA adducts reside along the entire crypt axis immediately after carcinogen administration (Fig. 3). Our data also suggest that dietary fish oil enhances targeted apoptosis in the nonproliferating transit cells, *i.e.,* at higher cell positions. Specifically, in the upper one-third of the crypt, the region where polyps and tumors eventually develop, DNA adduct levels were positively correlated with apoptosis in the fish oil-fed animals (Figs. 6 and 9). In contrast, DNA adduct levels were negatively correlated with apoptosis after corn oil feeding, indicating the suppression of this protective response. Thus, targeted apoptosis should decrease DNA-adducted cells and reduce the possibility for clonal expansion into the colonic lumen (as a polyp) (49). Although the effect of dietary lipid on apoptosis is significant, it is numerically small and one might question its role in cancer prevention. In this regard, there is cogent evidence to indicate that apoptosis is a central component of cell number regulation in the colonic epithelium (15, 16, 18, 43). In addition, it is becoming increasingly apparent that small changes in the percentage (<1%) of apoptotic cells in the crypt can contribute to the development of colon tumors (4, 15, 16, 18).

The confinement of the apoptogenic effect of dietary fish oil to the upper one-third of the colonic crypt may indicate a modifying effect of cell differentiation. Interestingly, we have previously demonstrated that fish oil supplementation increases indices of cell differentiation in the rat colon (4). Unfortunately, the identity of the signaling pathways responsible for the transition from terminal differentiation to apoptosis remains to be determined. A second hypothesized mechanism by which fish oil may enhance apoptosis may be through its ability to influence cell-matrix contact. Because fatty acids are critical constituents of biological membranes and their composition can be altered by dietary lipids (50), it is possible that n-3 polyunsaturated fatty acids modulate cell viability by influencing crypt habitat/matrix adhesion (51, 52). The specific effects of diet on colonocyte matrix proteins and adhesion molecules require further study. Finally, our recent findings indicate that MGMT is also highly expressed toward the top of the crypt (22). Because the upper crypt region would have more direct exposure to lumenal carcinogens, this would suggest that cells on the lumenal surface are afforded inducible repair and deletion mechanisms.

Maximum levels of apoptosis occurred 9 h after AOM injection (Fig. 4). Although we did not measure colonic cell proliferation in this study, previous findings indicate that the elevation of methylation-induced apoptosis is associated with synchronous inhibition of mitosis in the colon (31, 32). This is consistent with the fact that O^6-methylguanine adducts signal S-phase arrest (49). It is generally presumed that the depression of cell proliferation occurs so that either repair or apoptosis can ensue.

In conclusion, dietary n-3 polyunsaturated fatty acids found in fish oil confer protection against experimental colon tumorigenesis in part by reducing the level of DNA adducts and by enhancing the deletion of cells through the activation of targeted apoptosis. These data support our hypothesis that 20:5n-3 and 22:6n-3 act as anticarcinogens.

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