

Fish Oil Contaminated with Persistent Organic Pollutants Induces Colonic Aberrant Crypt Foci Formation and Reduces Antioxidant Enzyme Gene Expression in Rats

Mee Young Hong,¹ Eunha Hoh,² Brian Kang,¹ Rebecca DeHamer,¹ Jin Young Kim,³ and Jan Lumibao¹

¹School of Exercise and Nutritional Sciences and ²Graduate School of Public Health, San Diego State University, San Diego, CA; and ³Department of Food Science and Engineering, Ewha Womans University, Seoul, South Korea

Abstract

Background: Epidemiologic, clinical, and experimental studies have suggested that fish oil (FO), a rich source of n-3 (ω -3) polyunsaturated fatty acids, protects against colon cancer. However, this message is confounded by the FDA's warning that the consumption of certain types of fish should be restricted because of contamination with persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides.

Objective: We examined FO contaminated with POPs (PCBs, dichlorodiphenyltrichloroethane, and chlordane) compared with unmodified FO on the risk factors of colon cancer development.

Methods: Male Sprague-Dawley rats aged 28 d ($n = 30$) were allocated into 3 groups and fed 15% corn oil (CO), FO, or POP-contaminated FO for 9 wk with a subcutaneous injection of colon carcinogen azoxymethane at weeks 3 and 4. Colonic aberrant crypt foci (ACF) and cell proliferation were enumerated, and the gene expression of inflammation, antioxidant enzymes, and repair enzymes were determined with the use of real-time quantitative polymerase chain reaction analysis.

Results: FO-fed rats had a lower number of ACF (mean \pm SE: 29 ± 4.0 for FO compared with 53 ± 8.4 for CO and 44 ± 4.6 for POP FO) and higher-multiplicity ACF than the CO and POP FO groups (4.7 ± 0.9 for FO compared with 11 ± 1.5 for CO and 9.6 ± 1.8 for POP FO) ($P < 0.05$). FO feeding lowered the proliferation index compared with the CO and POP FO feeding groups ($18\% \pm 1.1\%$ for FO compared with $25\% \pm 1.6\%$ for CO and $23\% \pm 0.7\%$ for POP FO) ($P = 0.009$). Superoxide dismutase [2.4 ± 0.6 relative quantification (RQ) for FO compared with 1.2 ± 0.2 RQ for CO and 1.3 ± 0.3 RQ for POP FO] and catalase gene expression (10 ± 2.0 RQ for FO compared with 5.4 ± 1.1 RQ for CO and 6.6 ± 1.5 RQ for POP FO) were higher in the FO group than in the CO and POP FO groups ($P < 0.05$). There were no differences between CO and POP FO on the variables.

Conclusion: These results indicate that POPs in FO reduce the preventive effects of FO on colon carcinogenesis by increasing preneoplastic lesion formation through the downregulation of antioxidant enzyme expression and increasing cell proliferation in rats. *J Nutr* 2017;147:1524–30.

Keywords: fish oil, persistent organic pollutants, aberrant crypt foci, colon, antioxidant enzyme

Introduction

Cancer is the second leading cause of death in the United States, and colorectal cancer (CRC) is the third most common type (1). Fatty fish and fish oil (FO) rich in n-3 FAs, specifically EPA (20:5n-3) and DHA (22:6n-3), have been documented in numerous studies to have chemopreventive and chemosensitizing

effects on CRC (2–5). The pathogenesis of CRC is initiated through DNA damage followed by the mutation of oncogenes and tumor suppressor genes involved in vital processes, including cell proliferation and apoptosis, thus disturbing the homeostatic

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Address correspondence to MYH (email: mhong2@mail.sdsu.edu).

Abbreviations used: AC, apoptotic cell; ACF, aberrant crypt foci; CO, corn oil; Cox-2, cyclooxygenase 2; CRC, colorectal cancer; FO, fish oil; HMACF, high-multiplicity aberrant crypt foci; *Mgmt*, O⁶-methylguanine DNA methyltransferase; *Ogg1*, 8-oxoguanine glycosylase; PCB, polychlorinated biphenyl; PI, proliferation index; POP, persistent organic pollutant; *Ptgs2*, PG endoperoxide synthase 2; PZ, proliferative zone; *Rela*, component of NF- κ B; ROS, reactive oxygen species; *Sod*, superoxide dismutase; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

balance between cell production at the base and cell death at the surface of colonic crypts (2, 3).

The onset of CRC is promoted and exacerbated largely by chronic inflammation (3, 4, 6). This inflammation is associated with the gene expression of cyclooxygenase enzymes that regulate the synthesis of arachidonic acid-derived eicosanoids (3, 4). The competitive inhibition of cyclooxygenase 2 (Cox-2) by n-3 FAs leads not only to a decrease in inflammatory products but may also lead to the activation of apoptosis (3). Cox-2 also serves as a mediator for activating NF- κ B, which can repress the transcription of the phosphatase and tensin homolog gene (2, 4). Previous studies have linked increased n-3 FA status to the decreased activity of NF- κ B in mice (6). Thus, the mechanism by which n-3 FAs are chemopreventive in CRC is multifactorial and involves the suppression of cell proliferation, activation of colonocyte apoptosis, and suppression of chronic inflammation.

Because mammals are deficient for the gene that encodes n-3 desaturase, the availability of n-3 FAs depends primarily on dietary consumption (3). This consumption, however, allows anthropogenic persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and organochlorine pesticides that biomagnify in the marine food chain, to enter the human food chain (7-9). Clinical manifestations of PCB and organochlorine toxicity include endocrine, neurobehavioral, and developmental disruption, and PCB poses a considerable carcinogenic threat (9-11). A case-control study by Howsam et al. (11) showed that higher serum concentrations of mono-ortho PCB congeners were associated with elevated CRC risk. Although the risks and benefits of fish and FO consumption have been examined in several studies (12, 13), to our knowledge, the effects of FO contaminated with POPs on CRC have not been examined.

Given these concerns, we sought to investigate the effects of POP exposure through FO consumption on the anticarcinogenic properties of FO with the use of the azoxymethane-induced rat model of CRC. We investigated the effects of POP contamination in FO on colonocyte proliferation and apoptosis; gene expression of inflammatory biomarkers, DNA-repair enzymes, and antioxidant enzymes; and the number and multiplicity of aberrant crypt foci (ACF), which are considered the earliest neoplastic lesions in CRC progression and reliable biomarkers for colonic carcinogenesis (14-16). In addition, 8-hydroxy-2'-deoxyguanosine (8-OHdG) DNA adducts in serum were determined as a biomarker of DNA damage. We hypothesized that FO contaminated with POPs would reduce the protective capacity of FO against CRC by decreasing the expression of antioxidant enzymes, increasing the number and multiplicity of ACF, modulating 8-OHdG via expression of inflammation and repair enzymes, increasing proliferation and decreasing apoptosis in colon tissue of rats fed POP FO compared with rats fed uncontaminated FO.

Methods

Animals, diets, and study design

Thirty male Sprague-Dawley rats aged 28 d were purchased from Harlan and individually housed in a temperature- and humidity-controlled environment with a 12-h light-dark cycle. The animal use protocol was approved by the San Diego State University Institutional Animal Care and Use Committee.

Rats were randomly assigned to 1 of 3 groups ($n = 10$ /group) that received a diet of either corn oil (CO) as the control, FO, or FO contaminated with POPs. Diet composition was based on an AIN 93G

diet with some modification of fat content and POP contaminants. Each diet contained 54% carbohydrates, 20% protein, and 15% fat by weight (17). CO contained 15% CO, FO contained 11.5% by weight of fat from unmodified FO with 3.5% CO, and POP FO contained 11.5% by weight of fat from FO contaminated with POPs and 3.5% CO.

Rats had ad libitum access to drinking water and food. Body weights were measured weekly, and 48-h food and water intake were measured throughout the study.

Oils from anchovies and sardines were the sources of FO (Nordic Naturals, Inc.), which contained 1725 mg n-3 FAs in 5 g FO and was purified by molecular distillation to remove PCBs, heavy metals, and pesticides according to the manufacturer's instructions. The FO was analyzed for PCBs, dichlorodiphenyltrichloroethanes, and other organochlorine pesticides, and the concentrations were close to the detection limit or not detectable (PCBs: 0.0033 μ g/g; dichlorodiphenyltrichloroethanes: 0.0056 μ g/g; and chlordanes: <0.00001 μ g/g, respectively) (17). For the POP FO diet, the FO was added with standards of the 7 most common PCB congeners (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB180), dichlorodiphenyltrichloroethanes (*p,p'*-dichlorodiphenyldichloroethylene, *p,p'*-dichlorodiphenyldichloroethane, and *p,p'*-dichlorodiphenyltrichloroethane), and chlordanes (γ -chlordane, *trans*-nonachlor, and *cis*-nonachlor). Final concentrations of PCB, dichlorodiphenyltrichloroethane, and chlordane were 2.40, 1.89, and 1.91 μ g/g, respectively, in FO (17). The PCB concentration was within the range reported in the literature for fish (0.119-2.68 μ g lipid weight/g); the dichlorodiphenyltrichloroethane and chlordane concentrations were above the literature ranges (0.180-0.446 and 0.255-0.698 μ g lipid weight/g, respectively) (18-20).

All rats received the assigned diets for 9 wk. After 3 wk, all rats were subcutaneously injected with azoxymethane (15 mg body weight/kg) (Sigma-Aldrich) 1 time/wk at weeks 3 and 4. Six weeks after the second azoxymethane injection, rats were killed with the use of CO₂.

Sample collection

After the rats were killed, blood samples were collected, and the serum was separated and stored at -80°C. The entire colon was harvested, resected, and cleaned with RNase-free PBS solution. One centimeter of the most distal colon closest to the rectum was removed and fixed in 4% paraformaldehyde solution for histology. The remaining colon was divided into 2 longitudinal pieces. One half was fixed in 70% ethanol for ACF enumeration, and the other half was used for gene expression analysis.

ACF enumeration

For the ACF measurements, colon sections were stained with 0.5% methylene blue. ACF formations stand out as darker, thicker, and larger than normal crypts (21). The number of ACFs and high-multiplicity ACFs (HMACFs) [foci with ≥ 4 apoptotic cells (ACs)] were counted under a light microscope (Axioimager; Zeiss) at 50 \times magnification (21).

Serum 8-OHdG

8-OHdG DNA adducts in serum samples were measured with the use of an 8-OHdG ELISA kit (Trevigen) according to the manufacturer's protocol. Anti-8-OHdG monoclonal mouse antibody and HRP-conjugated antibody were used as primary and secondary antibodies, respectively. The TACS-Sapphire colorimetric substrate was added to visualize the formed complex, and the color absorbance was measured at 450 nm.

In vivo cell proliferation measurement

Colonic cell proliferation was determined by measuring Ki-67 with the use of immunohistochemical analysis. Mouse anti-human Ki-67 (BD Biosciences) and biotinylated rabbit anti-mouse IgG (Dako) were used as primary and secondary antibodies, respectively. The complex was visualized with the use of an avidin biotin complex (Vector Laboratories) followed by incubation with 3,3'-diaminobenzidine. At least 20 well-oriented crypt columns/rat were quantified. The proliferation index (PI) and proliferative zone (PZ) were calculated as previously described (21, 22). The crypt height in number of cells was divided into 3 compartments: bottom, middle, and top. The PI in each compartment was also calculated.

In vivo cell apoptosis measurement

A terminal deoxynucleotidyltransferase assay (EMD Millipore) was used to detect targeted cell apoptosis. Incubation with DNase I was used as a positive control, and the omission of the deoxynucleotidyltransferase enzyme was used as a negative control. Stained cells that exhibited apoptotic morphologic features were counted in 25 colonic crypt columns. A proportion of ACs in a crypt column was calculated (21, 22).

Gene expression

Colonic mucosa was scraped and total RNA was extracted with the use of the Trizol method (Invitrogen). Reverse transcription was conducted with the use of Superscript III (Invitrogen) with oligo(dT)12–18 primers (21). RNA was quantified by measuring the optical density at 260 nm. The mRNA levels of prostaglandin endoperoxide synthase 2 (*Ptgs2*), component of NF- κ B (*Rela*), superoxide dismutase (*Sod*), catalase, O⁶-methylguanine DNA methyltransferase (*Mgmt*), and 8-oxoguanine glycosylase (*Ogg1*) were analyzed with the use of quantitative real-time PCR (ViiA7; Applied Biosystems). *Ptgs2* is involved in the production of PGs, the inflammatory processes, and CRC development. *Rela* is involved in NF- κ B heterodimer formation and activation (the dysregulation of which can lead to inflammation), aberrant cell proliferation, and protection against apoptosis. *Sod* and catalase are 2 major antioxidant enzymes responsible for the quenching of intracellular reactive oxygen species (ROS) and protection against CRC. *Mgmt* and *Ogg1* are DNA-repair enzymes necessary for maintaining genome stability. *Mgmt* catalyzes the conversion of mutagenic O⁶-methylguanine back to guanine, whereas *Ogg1* excises the mutagenic base 8-oxoguanine, a product of ROS-induced DNA damage. The gene expression was normalized to r18S expression.

Statistical analysis. Differences in ACF, cell proliferation, cell apoptosis, gene expression, and serum 8-OHdG between groups were determined with the use of 1-factor ANOVA followed by the Student-Newman-Kuels multiple-comparison procedure. Correlations between variables were tested with the use of Spearman's ρ . Levene tests were performed to account for unequal variance. If the variances were not equal between groups (*Ptgs2* and *Rela*), Kruskal-Wallis 1-factor ANOVA was used for analysis. All statistical analyses were performed with the use of SPSS (version 22; IBM). All data are expressed as means \pm SEs, and $\alpha < 0.05$ was considered statistically significant.

Results

Body weight and food intake. No significant differences were found in initial body weight, final body weight, body weight gain, and food intake between any of the experimental groups (data not shown).

ACF. Significant differences were found in the number of ACF for the FO group compared with the CO and POP FO groups. The FO group had 48% and 36% fewer ACFs (Figure 1A) and 56% and 51% fewer HMACFs (Figure 1B) than the CO and POP FO groups ($P < 0.05$), which did not differ from one another.

Serum 8-OHdG. Serum 8-OHdG concentrations were lower in the FO group than the CO and POP FO groups ($P < 0.05$) (Figure 2A). The FO group had 43.7% and 32.4% fewer 8-OHdG DNA adducts than the CO and POP FO groups, respectively. There was no significant difference in 8-OHdG concentrations between the CO and POP FO groups. There were strong correlations between 8-OHdG DNA adducts and ACF ($P = 0.001$) and HMACF ($P = 0.004$) (Figure 2B, C). Rats with greater DNA damage showed higher numbers of ACF and HMACF.

Cell proliferation and apoptosis. Rats fed FO had fewer proliferating cells per crypt column and a lower percentage of proliferating cells per crypt column than rats that consumed CO

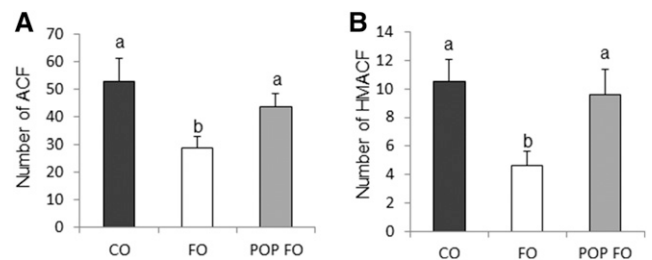


FIGURE 1 ACF (A) and HMACF (B) in male rats fed the CO, FO, or POP FO diet for 9 wk. Values are means \pm SEs ($n = 10$). Means without a common letter differ, $P < 0.05$. ACF, aberrant crypt foci; CO, corn oil; FO, fish oil; HMACF, high-multiplicity ACF; POP, persistent organic pollutant.

or POP FO diets ($P < 0.05$) (Figure 3A, B). CO and POP FO caused an expansion of the PZ that did not occur in the rats fed FO ($P < 0.05$) (Figure 3C). There were no significant differences between any of the experimental groups on apoptosis (data not shown).

Colonic gene expression. The FO group had 56% lower *Ptgs2* transcription amounts than the CO group ($P = 0.042$) (Figure 4A). No significant differences in *Ptgs2* expression were found for the POP FO group compared with the CO and FO groups. Both the FO and POP FO groups resulted in lower *Rela* expression than CO (by 63% and 58%, respectively) ($P = 0.011$) (Figure 4B). The FO group upregulated *Mgmt* 2-fold ($P = 0.044$) and downregulated *Ogg1* by 64% ($P = 0.047$) compared with the CO group (Figure 4C, D). The POP FO group was not significantly different than the CO and FO groups for *Mgmt* and *Ogg1* expression. The FO group resulted in >1.5-elevated amounts of *Sod* and catalase compared with the CO and POP FO groups ($P < 0.05$), whereas no differences in *Sod* and catalase amounts between the POP FO and CO groups were observed (Figure 4E, F).

Correlation between endpoints. The correlations between outcome measures and ACF, HMACF, and serum 8-OHdG are shown in Table 1. ACF, HMACF, and serum 8-OHdG showed positive correlations with PI in the middle and top regions of the colonic crypts and the PZ ($P < 0.05$). A greater PI and extended PZ were correlated with a higher number of ACF and HMACF and a greater DNA adduct amount. Higher numbers of ACs were significantly correlated with serum DNA damage ($P = 0.026$). Positive trends were observed between AC and ACF ($P = 0.085$) and HMACF ($P = 0.137$). For *Sod* and catalase expression in the colon, there were inverse relations between ACF, HMACF, and serum 8-OHdG ($P < 0.05$). Rats with a higher gene expression of *Sod* and catalase had reduced ACF and HMACF formation and lower amounts of serum DNA damage. Inverse relations were also shown between the *Mgmt* repair enzyme and ACF ($P = 0.050$), HMACF ($P = 0.011$), and serum 8-OHdG concentrations ($P = 0.007$). A mild correlation was observed between the *Ogg1* repair enzyme and serum DNA damage ($P = 0.067$) but not with ACF nor HMACF.

Discussion

Studies have examined cancer risk related to POPs, including PCB, organochlorine, and mercury; however, there has been limited reporting on the risk factors of exposure to POPs through an FO diet and the beneficial efficiency of FO related to

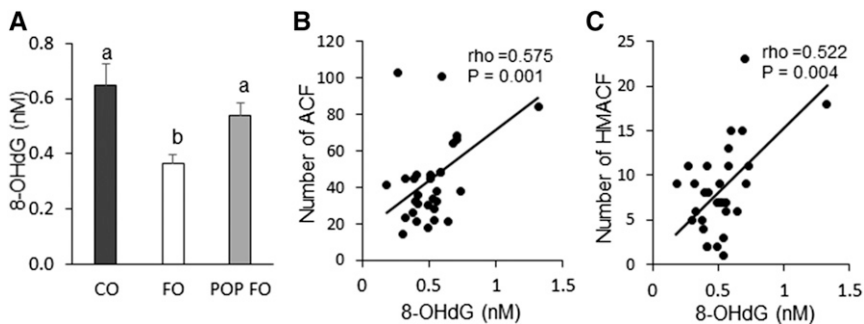


FIGURE 2 Serum 8-OHdG (A) and Spearman correlations between 8-OHdG and colon ACFs (B) and HMAcFs (C) in male rats fed the CO, FO, or POP FO diet for 9 wk. Values in panel A are means \pm SEs ($n = 10$). Means without a common letter differ, $P < 0.05$. ACF, aberrant crypt foci; CO, corn oil; FO, fish oil; HMAcF, high-multiplicity ACF; POP, persistent organic pollutant; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

CRC development. We investigated whether the anticarcinogenic effects of FO are attenuated when contaminated with POPs with the use of a rat model and treatment with azoxymethane to induce CRC. The POP doses administered were selected within the ranges found in fish and FO (18–20), which help strengthen the practical implications of our results. We found that ACF, neoplastic lesions found in early CRC progression and reliable biomarkers for colonic carcinogenesis (14–16), were suppressed by FO, but this protective effect was inhibited in the POP FO group. The oral administration of PCBs has been shown to decrease tight-junction protein (zonula occludens 1 and occludin) expression, which disrupts intestinal epithelial integrity and permeability (23). This mechanism may have contributed to the higher ACF found in the POP FO group.

Colon cancer cells are characterized by uncontrolled cell proliferation via the perturbation of the cell cycle. Supplementation with FO protects against colon cancer development by reducing uncontrolled cell proliferation via the decreased expression of the Ras protein, which is involved in signaling pathways that regulate colonic epithelial growth, differentiation, and tumor formation (5). However, our results indicate that treatment with POP FO may contribute to uncontrolled cell proliferation by increasing preneoplastic lesions, as evidenced by substantially higher PI and PZ in the POP FO group, whereas the uncontaminated FO provided beneficial effects. Further research is needed to determine whether increased cell proliferation in POP FO was via the modulation of cell-cycle mediators or Ras signaling.

Colonic cell homeostasis depends on the equilibrium of cell proliferation and apoptosis. An imbalance can exacerbate colon cancer development. FO has been shown to suppress CRC pathogenesis by increasing apoptosis in colonocytes. The mechanism involves upregulating the expression of phosphatase and the tensin homolog, an important regulator in the apoptotic signaling pathway (2). It has also been shown that FO enhances apoptosis by reducing antiapoptotic B-cell lymphoma 2 mediator expression (24–27). FO treatment did not increase apoptosis, as has been shown previously. It has been shown that FO

with pectin or butyrate enhances apoptosis in the colon, whereas FO with cellulose did not increase apoptosis (28). Our study used cellulose as a fiber source, which may explain why there were no changes on apoptosis. Further investigations on the interactive effects of dietary fat and fiber are warranted. Our study did not find a significant difference in apoptosis between FO and POP FO treatments; however, higher serum DNA damage was correlated with increased apoptotic activity that removed damaged cells. Enhanced targeted apoptosis response to oxidative DNA damage was described in colon carcinogenesis models (27, 29).

Azoxymethane is metabolized by cytochrome P450 in the liver and leads to the formation of DNA adducts, of which the 8-OHdG DNA adduct is a major product of oxidative stress that has been used as a marker for DNA damage and inflammation (30). Elevated amounts of DNA adducts have been reported in gastric tumors compared with normal mucosa (31) and associated with poor survival in CRC patients (27). The use of azoxymethane induces highly mutagenic 8-OHdG DNA adducts that promote a GC \rightarrow TA transition, which is frequently found in tumor suppressor gene mutations (32, 33). Studies have shown that an increased concentration of 8-OHdG in the blood is associated with the development of colorectal adenoma and cancer (34, 35). In this study, FO treatment decreased the azoxymethane-induced 8-OHdG in serum, whereas POP FO did not. Wen et al. (36) found that a higher exposure to PCBs was associated with increased amounts of 8-OHdG DNA adducts in humans. POPs induce ROS, leading to increased oxidative DNA damage that can initiate DNA mutations associated with tumor development (37). Our study supported previous results that showed that 8-OHdG was positively correlated with azoxymethane-induced ACF formation (38). Therefore, in the POP FO group, elevated 8-OHdG concentrations might be associated with the higher ACF formation. Investigation into whether adding POP to the CO diet would increase 8-OHdG and the determination of colonic 8-OHdG concentrations are underway in our next study.

DNA damage can be removed by repair enzymes, which play key roles in genome stability and preventing the onset of CRC.

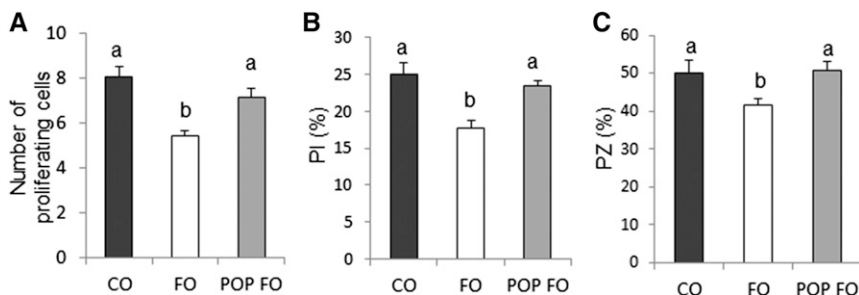
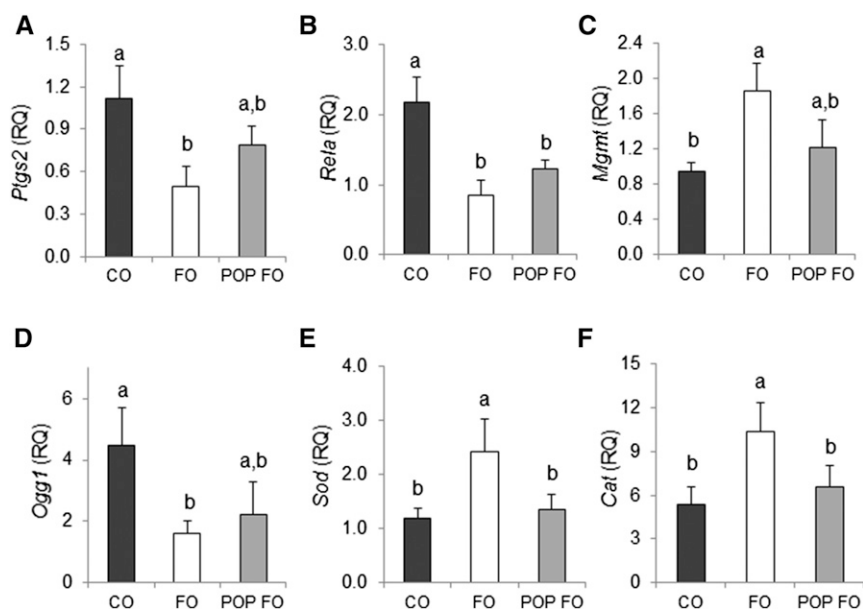


FIGURE 3 Number of proliferating cells (A), PI (B), and PZ (C) in male rats fed the CO, FO, or POP FO diet for 9 wk. Values are means \pm SEs ($n = 10$). Means without a common letter differ, $P < 0.05$. CO, corn oil; FO, fish oil; PI, proliferation index; POP, persistent organic pollutant; PZ, proliferative zone.

FIGURE 4 Colonic gene expression of *Ptgs2* (A), *Rela* (B), *Mgmt* (C), *Ogg1* (D), *Sod* (E), and *Cat* (F) in male rats fed the CO, FO, or POP FO diet for 9 wk. Values are means \pm SEs ($n = 10$). Means without a common letter differ, $P < 0.05$. *Cat*, catalase; CO, corn oil; FO, fish oil; *Mgmt*, O⁶-methylguanine DNA methyltransferase; *Ogg1*, 8-oxoguanine glycosylase; POP, persistent organic pollutant; *Ptgs2*, prostaglandin endoperoxide synthase 2; *Rela*, component of NF- κ B; RQ, relative quantification; *Sod*, superoxide dismutase.



The repair enzyme *Ogg1* is responsible for excising 8-OHdG DNA damage, and elevated amounts of *Ogg1* have been reported in colonic tumors compared with controls (31, 39). Another repair enzyme, *Mgmt*, converts azoxymethane-induced O⁶-methylguanine DNA adducts back to guanine (40). Reduced *Mgmt* expression was found in tumor tissues compared with normal tissue in CRC patients (41). Methylation of the *Mgmt* promoter was associated with a loss of *Mgmt* expression in >40% of CRC cases (40). This study found that FO treatment upregulated colonic gene expression of *Mgmt* and downregulated *Ogg1*. These results were consistent with earlier work that reported that FO was associated with higher *Mgmt* and lower *Ogg1* gene transcription, as well as reduced serum DNA damage (42, 43). However, these protective effects were not clearly observed in the POP FO group.

Chronic inflammation is a major risk factor for CRC development, with some studies suggesting that inflammatory cytokines are responsible for stimulating the uncontrolled cell growth of cancer cells (4, 44). Cyclooxygenase is involved in the production of PGs, which are related to inflammatory processes and colon cancer development (45). Dysregulation of the NF- κ B pathway through constitutive activation has been shown to cause abnormal cell proliferation and apoptosis in CRC cells (46). Supplementation with FO inhibits the growth of colon tumors by suppressing inflammation through decreases in NF- κ B (6) and *Cox-2* (47, 48). Our results are supported by previous studies that showed that FO treatment substantially lowered *Ptgs2* (*Cox-2*) and *Rela* expression, in which the latter was involved in NF- κ B heterodimer formation and activation. Interestingly, POP contamination did not alter the anti-inflammatory effect of FO, as evidenced by the considerably reduced *Rela* expression for both FO groups, suggesting that POP toxicity does not interfere with the anti-inflammatory properties of FO.

A considerable amount of evidence suggests that antioxidants play a critical role in protecting against inflammation and CRC (49). In this study, only uncontaminated FO substantially increased colonic gene expression of the antioxidant enzymes *Sod* and catalase. For *Sod* and catalase, there were strong inverse correlations between ACF, HMAF, and serum 8-OHdG. This suggests that POP contamination reduced the capacity of FO to

induce the expression of key protective enzymes. POPs exert their toxicity by binding to nuclear receptors, including the aryl hydrocarbon receptor. This binding increases intracellular ROS, which in turn can lead to the upregulation of NF- κ B and production of proinflammatory proteins (50). Because *Sod* and catalase play a critical role in removing ROS, the downregulation of *Sod* and catalase can contribute to ROS accumulation and exert inflammation and aberrant crypt formation. It has been shown that low *Sod* activity is associated with higher cell proliferation in the colon (51). Further mechanistic research that includes ROS as an indicator of inflammation is in progress.

Overall, POP contamination reduced the protective effect of FO on risk factors related to CRC development. Of particular note, measured variables for the POP FO group either fell between FO and CO or were similar to CO but were not worse than CO. Some studies have shown the importance of the interaction between POPs and different dietary lipids (i.e., n-3 compared with n-6) (52–54). Future studies would benefit by adding a POP CO group to investigate any exacerbating effects

TABLE 1 Spearman correlations between variables in male rats fed the CO, FO, or POP FO diet for 9 wk¹

	ACF		HMAF		8-oxo-dG	
	ρ	<i>P</i> value	ρ	<i>P</i> value	ρ	<i>P</i> value
PI	0.424	0.131	0.336	0.240	0.643	0.010
PI (bottom 1/3)	-0.077	0.794	-0.113	0.701	0.029	0.919
PI (middle 1/3)	0.499	0.035	0.402	0.077	0.621	0.013
PI (top 1/3)	0.595	0.025	0.626	0.017	0.706	0.003
PZ	0.520	0.027	0.455	0.029	0.645	0.002
AC	0.389	0.085	0.314	0.137	0.572	0.026
<i>Sod</i>	-0.391	0.040	-0.421	0.026	-0.399	0.043
Catalase	-0.338	0.053	-0.374	0.036	-0.344	0.040
<i>Mgmt</i>	-0.330	0.050	-0.492	0.011	-0.496	0.007
<i>Ogg1</i>	0.155	0.459	0.168	0.450	0.297	0.067

¹ AC, apoptotic cell; ACF, aberrant crypt foci; CO, corn oil; FO, fish oil; HMAF, high-multiplicity ACF; *Mgmt*, O⁶-methylguanine DNA methyltransferase; *Ogg1*, 8-oxoguanine glycosylase; PI, proliferating index; POP, persistent organic pollutant; PZ, proliferative zone; *Sod*, superoxide dismutase; 8-oxo-dG, 8-oxodeoxyguanosine.

of POPs in an n-6-rich environment. It should be noted that dioxin and dioxin-like PCBs, known carcinogens, were not tested in this study. Therefore, further research should also investigate the effects of the carcinogens and other POPs present in fish and FO on risk factors related to CRC development.

This study evaluated the integrative impact of n-3 FAs and POPs in FO. POP contamination in FO reduced the beneficial effects of FO on CRC development markers in rats. This was evidenced by multiple measures such as a substantially higher number of ACE, increased cell proliferation, and reduced antioxidant enzyme amounts. The results indicate the potential risk of exposure to POPs via fish or FO on colon cancer development. This study suggests that further investigations of the impact of POPs on the beneficial effects of FO are needed.

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