Highly Unsaturated (n-3) Fatty Acids, but Not α-Linolenic, Conjugated Linoleic or γ-Linolenic Acids, Reduce Tumorigenesis in *Apc*$^{Min/+}$ Mice[1]

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ABSTRACT We showed previously that dietary eicosapentaenoic acid [EPA, 20:5(n-3)] is antitumorigenic in the *Apc*$^{Min/+}$ mouse, a genetic model of intestinal tumorigenesis. Only a few studies have evaluated the effects of dietary fatty acids, including EPA and docosahexaenoic acid [DHA, 22:6(n-3)], in this animal model and none have evaluated the previously touted antitumorigenicity of α-linolenic acid [ALA, 18:3(n-3)], conjugated linoleic acid [CLA, 77% 18:2(n-7)], or γ-linolenic acid [GLA, 18:3(n-6)]. Stearidonic acid [SDA, 18:4(n-3)], the Δ6-desaturase product of ALA, which is readily metabolized to EPA, has not been evaluated previously for antitumorigenic efficacy. This study was undertaken to evaluate the antitumorigenicity of these dietary fatty acids (ALA, SDA, EPA, DHA, CLA and GLA) compared with oleic acid [OA, 18:1(n-9)] at a level of 3 g/100 g in the diets of *Apc*$^{Min/+}$ mice and to determine whether any alterations in tumorigenesis correspond to alterations in prostaglandin biosynthesis. Tumor multiplicity was significantly lower by ~50% in mice fed SDA or EPA compared with controls, whereas less pronounced effects were observed in mice fed DHA ($P = 0.15$). ALA, CLA and GLA were ineffective at the dose tested. Although lower tumor numbers coincided with significantly lower prostaglandin levels in SDA- and EPA-fed mice, ALA and DHA supplementation resulted in equally low prostaglandin levels, despite proving less efficacious with regard to tumor number. Prostaglandin levels did not differ significantly in the CLA and GLA groups compared with controls. These results suggest that SDA and EPA attenuate tumorigenesis in this model and that this effect may be related in part to alterations in prostaglandin biosynthesis. J. Nutr. 130: 2434–2443, 2000.

KEY WORDS: • *tumor* • *cancer* • *(n-3) fatty acids* • *intestine* • *Apc* • *mice*

Colorectal cancer is the second leading cause of cancer deaths in the United States with 56,503 deaths reported in 1997 (Hoyert et al. 1999). Several lines of evidence overwhelmingly implicate environmental factors, specifically dietary components, as major variables influencing colorectal cancer incidence. Western-style diets characterized by high intakes of energy, fat, meat, refined grains and sugar, combined with low intakes of fiber, calcium, and fruits and vegetables have been strongly linked to an increased risk of colorectal cancer (Giovannucci and Willett 1994, Lipkin et al. 1999, Slattery et al. 1999). Among these, the amount and type of dietary fat consumed is of particular importance (Caygill et al. 1996, Hansen Petrik et al. 2000, Reddy 1992, Willett et al. 1990).

Epidemiologic studies indicate that consumption of fish and fish oil correlates with a reduced risk of colorectal cancer (Caygill et al. 1996, Fernandez et al. 1999, Kato et al. 1997). Fish oil is rich in the (n-3) polyunsaturated fatty acids (PUFA)[3] eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)]. Studies in humans and in chemically induced colonic tumor animal models overwhelmingly indicate a protective effect of (n-3) PUFA (in the form of fish, fish oils or EPA ethyl ester), and the mechanism is largely thought to be related to interference with biosynthesis of 2-series prostaglandins from arachidonic acid [AA, 20:4(n-6)] (Anti et al. 1992, Chang et al. 1998, Latham et al. 1999, Minoura et al. 1988, Reddy 1992). We and others demonstrated recently that diets containing EPA, and possibly DHA, are also antitumorigenic in a murine model of human colorectal cancer, and this effect is related at least in part to interference with AA metabolism (Hansen Petrik et al. 2000, Oshima et al. 1995, Paulsen et al. 1997). α-Linolenic acid [ALA, 18:3(n-3)], the parent fatty acid of the (n-3) family, has been studied on a more limited basis, but with some promising results. Dietary perilla oil and flaxseed oil, both rich sources of

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[3] Abbreviations used: AA, arachidonic acid; ACF, aberrant crypt foci; ALA, α-linolenic acid; AOM, azoxymethane; *Apc*, murine adenomatous polyposis coli; *APC*, human adenomatous polyposis coli; *Apc*$^{\Delta 716}$, *Apc* knockout mouse with *Apc* truncation at codon 716; *Apc*$^{Min/+}$, multiple intestinal neoplasia mouse; CLA, conjugated linoleic acid; COX, cyclooxygenase; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DMBA, 7,12-dimethylbenz(α)anthracene; EP, prostaglandin E receptor; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; FAP, familial adenomatous polyposis; GLA, γ-linolenic acid; GSK-3β, glycogen synthase kinase-3β; 15-HETrE, 15-hydroxyeicosatrienoic acid; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; LA, linoleic acid; OA, oleic acid; NSAID, nonsteroidal anti-inflammatory drug; PGD_2, prostaglandin D_2; PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2; PKC, protein kinase C; PUFA, polyunsaturated fatty acids; SDA, stearidonic acid; TXB_2, thromboxane B_2.

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ALA, have been shown to decrease chemically induced colonic tumors and aberrant crypt foci (ACF) in a rat model (Hirose et al. 1990, Narisawa et al. 1994, Onogi et al. 1996, Serraino and Thompson 1992). These results have coincided with competitive exclusion of (n-6) PUFA from membrane phospholipids and associated reductions in prostaglandin E_2 (PGE_2) concentrations in colonic mucosa (Onogi et al. 1996). Stearidonic acid [SDA, 18:4(n-3)], the Δ6-desaturase product of ALA found naturally in blackcurrant (*Ribes nigrum*) seed oil and oils derived from some members of the *Boraginaceae* family (Phillips and Huang 1996), has not been studied previously as an antitumorigenic agent, although it has been shown to strongly inhibit growth of NIH-3T3 cells (Cantrill et al. 1993). The use of dietary SDA as an antitumorigenic agent is promising because it is not dependent on the Δ6-desaturase reaction, the rate-limiting step in de novo biosynthesis of long-chain PUFA. As such, SDA increases tissue levels of EPA more effectively than does ALA (Huang et al. 1991, Yamazaki et al. 1992). Furthermore, other studies suggest that SDA may reduce formation of AA-derived eicosanoids in vitro (Guichardant et al. 1993, Kockmann et al. 1989). This collective evidence, albeit limited, warrants investigation into the antitumorigenic potential of SDA.

Other fatty acids, including some (n-6) PUFA and their derivatives, have also been investigated for purported antitumorigenic effects. Conjugated linoleic acid (CLA) refers collectively to several positional and geometric isomers of linoleic acid [LA, 18:2(n-6)] in which the double bonds are in conjugation, typically at positions 9 and 11 or 10 and 12 (Ha et al. 1987). CLA, predominantly as 9(*Z*),11(*E*)-18:2(n-7), occurs naturally in small amounts in cooked meats, dairy products and ruminant meats (Chin et al. 1992), and is potently antitumorigenic in chemically induced rat mammary tumors and murine skin tumors (Belury et al. 1996, Ip et al. 1991). It has recently been suggested that CLA exerts its antitumorigenic effect by inhibiting metabolism of LA to AA, thereby decreasing biosynthesis of AA-derived prostaglandins (Banni et al. 1999, Belury and Kempa-Steczko 1997, Kavanaugh et al. 1999). Compared with studies investigating the efficacy of CLA on mammary tumorigenesis, evidence for protection against colorectal cancer is less definitive. To date, gavage treatment with CLA has been shown to result in fewer chemically induced colonic ACF and gastric neoplasia in mice (Ha et al. 1990, Liew et al. 1995), and CLA treatment reduced proliferation of human colon tumor cells in vitro (O'Shea et al. 1999, Shultz et al. 1992).

γ-Linolenic acid [GLA, 18:3(n-6)], the Δ6-desaturase product of LA, is found predominantly in only a few dietary sources, including evening primrose (*Oenothera biennis*) oil, borage (*Boraginaceae*) oil, blackcurrant seed oil and spirulina (Phillips and Huang 1996). By bypassing the rate-limiting Δ6-desaturase reaction, dietary GLA is rapidly metabolized to dihomo-γ-linolenic acid [DGLA, 20:3(n-6)]. As a 20-carbon PUFA, DGLA purportedly competes with AA for cyclooxygenase (COX) and 15-lipoxygenase activity to produce prostaglandin E_1 (PGE_1) and 15-hydroxyeicosatrienoic acid (15-HETrE), respectively, in some cell types, and may thereby attenuate formation of AA-derived metabolites (Borgeat et al. 1976, Fan et al. 1997, Johnson et al. 1997). A second proposed mechanism whereby GLA might elicit antitumorigenic effects is via increased lipid peroxidation (Devi and Das 1994, Kokura et al. 1997, Takeda et al. 1992). Nevertheless, the actual antitumorigenicity of GLA has been only narrowly investigated to date. GLA treatment has been shown to limit the metastatic potential of human colon cancer cell lines and block cell cycle progression in vitro (Jiang et al. 1995 and 1998). Accordingly, arterial GLA injections have been shown to inhibit growth of implanted hepatoma cells in rats (Kokura et al. 1997), and intratumoral injections of lithium-GLA resulted in smaller tumor volumes in mice implanted with pancreatic tumor cells (Ravichandran et al. 1998). However, dietary GLA has demonstrated potential efficacy as an antitumorigenic agent only in 7,12-dimethylbenz(α)anthracene (DMBA)-induced mammary tumors in rats, a study in which a diet containing evening primrose oil (20 g/100 g) resulted in a lower tumor incidence than did corn oil (Abou El-Ela et al. 1987), and in nude mice bearing breast carcinoma xenografts (Pritchard et al. 1989). A preventive role for dietary GLA in gastrointestinal tumorigenesis has yet to be established.

Over the last several years, the $Apc^{Min/+}$ mouse model has been used to evaluate the effects of nutritional intervention on intestinal tumorigenesis because of its germline mutation in the murine adenomatous polyposis coli (*Apc*) gene (Hansen, Petrik et al. 2000, Oshima et al. 1995, Paulsen et al. 1997, Wasan et al. 1997). After somatic mutation of the wild-type allele, these mice spontaneously develop adenomas throughout the intestinal tract with preferred localization in the small intestine (Chiu et al. 1997). Development of colorectal cancer in humans from dysplastic crypts to metastatic carcinoma involves a series of genetic mutations, the earliest often involving *APC* (Kinzler and Vogelstein 1996, Powell et al. 1992). Individuals with familial adenomatous polyposis (FAP), like $Apc^{Min/+}$ mice, possess a germline mutation in *APC*, and mutational damage or loss of the wild type allele initiates intestinal tumor formation (Levy et al. 1994, Miyoshi et al. 1992). Although FAP accounts for <1% of all human colorectal cancer cases, somatic mutations resulting in loss of full length *APC* protein also occur early in spontaneous forms of the disease (Miyoshi et al. 1992, Powell et al. 1992), indicating that an *APC* defect is associated with a majority of human colorectal cancers (Jen et al. 1994, Smith et al. 1993). Therefore, in this study, we investigated effects of the dietary fatty acids ALA, SDA, EPA, DHA, CLA and GLA on intestinal tumorigenesis in $Apc^{Min/+}$ mice fed diets based on a typical Western diet.

MATERIALS AND METHODS

Animals. Male C57BL/6J $Apc^{Min/+}$ mice (n = 77; 37 d old) (Jackson Laboratories, Bar Harbor, ME) were randomly assigned to eight dietary groups (n = 9–10 mice/group) on arrival. They were housed in a temperature-controlled room with a 12-h light:dark cycle and given free access to food and water. The health of the mice was checked daily. Food was withheld overnight before they were killed. All animal procedures were approved by the University of Tennessee Animal Care and Use Committee and were in accordance with the NIH Guidelines (NRC 1985).

Diets. All diets were based on the composition of a typical Western diet with the following energy distribution: carbohydrate, 44.4%; fat, 35.4%; and protein, 21.2%. These diets were predicated on the "US17" diet formulated by Monsanto (St. Louis, MO) and Research Diets (New Brunswick, NJ) with input from the authors. The following fatty acid ethyl esters were added (31 g/kg) to the base diet at the expense of Trisun high oleic acid [OA, 18:1(n-9)] sunflower oil (Monsanto, St. Louis, MO): *1*) conjugated linoleic acid [CLA, 9,11–18:2(n-7)] isomers, 77%; [10,12–18:2(n-6)] isomers, 13%; mixed isomers, 5%, *2*) γ-linolenic acid [GLA, 18:3(n-6)], 95% pure, *3*) α-linolenic acid [ALA, 18:3(n-3)], 95% pure, *4*) stearidonic acid [SDA, 18:4(n-3)], 85% pure, eicosapentaenoic acid [EPA, 20:5(n-3)], 95% pure, or docosahexaenoic acid [DHA, 22:6(n-3)], 90% pure. The diet containing the nonsteroidal anti-inflammatory drug (NSAID) sulindac (320 mg/kg) was used as a positive control **(Table 1)**. Overall fatty acid compositions of the diets are shown in **Table 2**. All diets were divided into daily aliquots and stored under an atmo-

TABLE 1

Experimental diet composition[1]

Component	Dietary group							
					(n-3) fatty acid			
	Control	Sulindac	CLA	GLA	ALA	SDA	EPA	DHA
	g/kg							
Casein	229	229	229	229	229	229	229	229
L-Cystine	3	3	3	3	3	3	3	3
Cornstarch	274	274	274	274	274	274	274	274
Maltodextrin 10	86	86	86	86	86	86	86	86
Sucrose	114	114	114	114	114	114	114	114
Cellulose	57	57	57	57	57	57	57	57
Cocoa butter	43	43	43	43	43	43	43	43
Linseed oil	5	5	5	5	5	5	5	5
Palm oil	60	60	60	60	60	60	60	60
Safflower oil	33	33	33	33	33	33	33	33
Sunflower oil, Trisun[2]	31	31	0	0	0	0	0	0
9(*Z*), 11(*E*)-18:2(n-7)	0	0	31	0	0	0	0	0
18:3(n-6) ethyl ester	0	0	0	31	0	0	0	0
18:3(n-3) ethyl ester	0	0	0	0	31	0	0	0
18:4(n-3) ethyl ester	0	0	0	0	0	31	0	0
20:5(n-3) ethyl ester	0	0	0	0	0	0	31	0
22:6(n-3) ethyl ester	0	0	0	0	0	0	0	31
Sulindac	0	0.32	0	0	0	0	0	0
Salt mix RD-96[3]	11	11	11	11	11	11	11	11
Dicalcium phosphate	15	15	15	15	15	15	15	15
Calcium carbonate	6	6	6	6	6	6	6	6
Potassium citrate	19	19	19	19	19	19	19	19
Vitamin mix V13401[4]	11	11	11	11	11	11	11	11
Choline bitartrate	2	2	2	2	2	2	2	2
α-Vitamin E acetate	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
TBHQ	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

[1] The control diet is Monsanto "US17" diet (Monsanto, St. Louis, MO, and Research Diets, New Brunswick, NJ). Abbreviations: ALA, α-linolenic acid; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; SDA, stearidonic acid; TBHQ, tertiary butylhydroquinone.

[2] Trisun is a high oleic acid sunflower oil (Monsanto, St. Louis, MO).

[3] Supplied (per kilogram of salt mix): NaCl, 259 g; magnesium oxide, 41.9 g; $MgSO_4 \cdot 7H_2O$, 257.6 g; chromium potassium sulfate, 1.925 g; cupric carbonate, 1.05 g; NaF, 0.2 g; KI, 0.035 g; ferric citrate, 21.0 g; manganous carbonate, 12.25 g; ammonium molybdate $\cdot 4H_2O$, 0.3 g; sodium selenite, 0.035 g; zinc carbonate, 5.6 g; sucrose, 399.105 g (Research Diets, New Brunswick, NJ).

[4] Supplied (per kilogram of vitamin mix): all-*trans*-retinyl palmitate (500,000 IU/gram), 0.8 g; cholecalciferol (100,000 IU per gram), 1.0 g; *dl*-α-tocopheryl acetate (500 IU/gram), 10.0 g; menadione sodium bisulfite (62.5% menadione), 0.08 g; biotin (1.0%), 2.0 g; cyanocobalamin (0.1%), 1.0 g; folic acid, 0.2 g; nicotinic acid, 3.0 g; calcium pantothenate, 1.6 g; pyridoxine $\cdot$ HCl, 0.7 g; riboflavin, 0.6 g; thiamin $\cdot$ HCl, 0.6 g; sucrose, 978.42 g (Research Diets, New Brunswick, NJ).

sphere of nitrogen at −80°C to prevent oxidation. All mice were provided fresh food daily. Body weights were recorded weekly.

Experimental design. After randomization into dietary groups, all mice were fed the assigned diets for ~7 wk. At 87–89 d of age, mice were killed by cervical dislocation, and tumor number, size and location were determined as previously described (Chiu et al. 1997). Portions of normal-appearing intestine and several of the largest tumors were harvested for histologic examination based on previously reported features of intestinal development and dysplasia in this strain of mice. Phospholipid fatty acid composition and levels of PGE_2 and 6-keto-prostaglandin $F_{1\alpha}$(6-keto-$PGF_{1\alpha}$) were determined for each mouse from normal-appearing jejunal sections of the small intestine.

Fatty acid analysis. Fatty acid methyl esters (FAME) of the tissue phospholipids were prepared and analyzed as described previously (Whelan et al. 1992). Briefly, tissues were homogenized in ice-cold saline and lipids were extracted with chloroform/methanol (1:2, v/v), followed by extraction (×2) with chloroform. The pooled chloroform extracts were evaporated and resuspended in a small volume of chloroform, and the phospholipids were separated by TLC on silica gel 60 HP-TLC plates (Merck, Darmstadt, Germany) with chloroform/methanol (8:1, v/v) as the solvent system. Bands corresponding to the phospholipids were scraped, dissolved in toluene and saponified with KOH (0.5 mol/L) in methanol for 8 min at 86°C. After acidification with HCl in methanol (0.7 mol/L), the fatty acids were extracted twice with hexane, evaporated and methylated with ethereal diazomethane. The FAME were resuspended in hexane and analyzed using a Hewlett-Packard model 5890 series II gas chromatograph (Palo Alto, CA) equipped with flame ionization detector and a DB23 fused silica capillary column (0.25 mm i.d. × 30 m × 0.25-μm film; J&W Scientific, Folsom, CA). Separation was achieved by temperature programming from 160 to 250°C at 3.5°C/min with hydrogen as the carrier gas. The internal standard, pentadecaenoic acid (15:0) methyl ester (Avanti Polar Lipids, Alabaster, AL) was added to each sample before the saponification step. The FAME were identified by comparison of retention times with those of known standards (Nu-Chek-Prep, Elysian, MN). Various CLA isomers and elongation products of GLA and SDA were identified by comparison of retention times to purified FAME standards (Matreya, Pleasant Gap, PA).

Measurement of prostaglandins. After cervical dislocation, normal-appearing intestinal sections were quickly perfused with ice-cold saline (NaCl, 154 mmol/L), immediately snap-frozen in liquid nitrogen and stored at −80°C. For analysis, the tissues were homogenized using a Tenbroeck tissue grinder (Pyrex, England) in ice-cold Tris-HCl buffer (0.1 mol/L, pH 7.4) containing indomethacin (final

TABLE 2

Fatty acid composition of the experimental diets[1]

	Dietary group							
					(n-3) fatty acid			
	Control	Sulindac	CLA	GLA	ALA	SDA	EPA	DHA
	g/100 g							
Fatty acid								
12:0	0.36	0.41	0.36	0.37	0.39	0.41	0.42	0.47
14:0	0.56	0.56	0.54	0.55	0.56	0.60	0.60	0.66
16:0	24.91	23.37	23.90	23.93	23.50	25.38	24.28	25.38
16:1	0.15	0.15	ND	0.14	0.14	0.15	0.15	ND
17:0	0.13	0.11	ND	0.12	0.11	0.12	0.12	ND
18:0	13.71	12.38	13.27	12.68	12.24	13.45	12.63	13.45
18:1(n-9)	37.56	40.77	26.72	26.28	25.38	27.03	25.85	27.21
18:2(n-6)	19.37	18.99	18.89	18.65	18.32	18.72	17.97	18.62
9(*Z*), 11(*E*) 18:2(n-7)	ND	ND	11.05	ND	ND	ND	ND	ND
9(*Z*), 11(*Z*) 18:2(n-7)	ND	ND	2.84	ND	ND	ND	ND	ND
18:3(n-6)	ND	ND	ND	14.31	ND	ND	ND	ND
18:3(n-3)	1.86	1.83	1.81	1.93	18.38	1.77	1.69	1.74
18:4(n-3)	ND	ND	ND	ND	ND	11.31	ND	ND
20:0	0.67	0.60	0.62	0.59	0.56	0.62	0.59	0.57
20:1	0.16	0.16	ND	0.12	0.11	0.11	0.19	ND
20:4(n-6)	ND	ND	ND	ND	ND	ND	0.52	ND
20:5(n-3)	ND	ND	ND	ND	ND	ND	14.61	ND
22:0	0.38	0.43	ND	0.21	0.20	0.11	0.23	ND
22:6(n-3)	0.19	0.18	ND	0.12	0.11	0.23	0.16	11.91
Total saturated FA	40.72	37.86	38.69	38.45	37.56	40.70	38.87	40.52
Total MUFA	37.87	41.15	26.72	26.54	25.64	27.28	26.19	27.21
Total PUFA	21.41	20.99	34.58	35.01	36.80	32.02	34.94	32.27
Total (n-6)	19.37	18.99	18.89	32.96	18.32	18.72	18.48	18.62
Total (n-3)	2.05	2.01	1.81	2.05	18.49	13.30	16.46	13.65
P:S ratio	0.53	0.55	0.89	0.91	0.98	0.79	0.90	0.80
(n-3):(n-6) ratio	0.11	0.11	0.10	0.06	1.01	0.71	0.89	0.73

[1] The control diet is Monsanto "US17" diet (Monsanto, St. Louis, MO, and Research Diets, New Brunswick, NJ). Abbreviations: ALA, α-linolenic acid; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; GLA, γ-linolenic acid; MUFA, monounsaturated fatty acids; ND, none detected; P:S ratio, ratio of polyunsaturated fatty acids to saturated fatty acids; PUFA, polyunsaturated fatty acids; SDA, stearidonic acid.

concentration 1 mmol/L) to prevent ex vivo prostaglandin biosynthesis (basal levels of prostaglandins associated with the tissues). This was followed by methanolic acidification with 8.8% formic acid in 90% methanol (final pH 3.5). The resulting homogenate was vigorously mixed and centrifuged at 5000 × *g* for 5 min after which the supernatant was removed. The pellet was resuspended in a 20% solution of methanol in water (pH 3.5), vigorously mixed and centrifuged at 5000 × *g* for 5 min after which the supernatant was collected and pooled. Prostaglandins were isolated from pooled supernatants by solid-phase extraction using an octadecyl (C18) cartridge (Burdick & Jackson, Muskegon, MI) and eluted with 100% methanol. The recovery of prostaglandins was determined by adding 0.37 nBq [^{3}H]-prostaglandin D_2 (PGD_2) to the sample as an internal standard before processing. The methanol was evaporated under a stream of nitrogen gas and the extracts were resuspended in PBS (pH 7.4) containing gelatin (1.0 g/L). PGE_2 and 6-keto-$PGF_{1\alpha}$were measured by RIA as described previously using antiserum obtained from PerSeptive Diagnostics, (Cambridge, MA) (Whelan et al. 1993). Cross-reactivities at half maximal binding of various prostanoids with PGE_2 antiserum are as follows: PGE_2 (100%), PGE_3 (26%), 6-keto-$PGF_{1\alpha}$ (< 1%), $PGF_{1\alpha}$ (1%), thromboxane B_2 (TXB_2) (<1%), PGD_2 (< 1%) and $PGF_{2\alpha}$ (1%). Cross-reactivities with various prostanoids with 6-keto-$PGF_{1\alpha}$ antiserum are as follows: 6-keto-$PGF_{1\alpha}$ (100%), Δ-17, 6-keto-$PGF_{1\alpha}$ (14%), PGE_2 (<1%), $PGF_{2\alpha}$ (2%), $PGF_{1\alpha}$ (8%), PGD_2 (< 1%) and TXB_2 (<1%). All standards were purchased from Cayman Chemical (Ann Arbor, MI); [^{3}H]-PGE_2, [^{3}H]-PGD_2 and [^{3}H]-6-keto-$PGF_{1\alpha}$ were obtained from New England Nuclear (Boston, MA). An aliquot of the homogenate from each sample was used to determine protein concentration by the modified Lowry protocol (Markwell et al. 1981).

Statistical analyses. Values are expressed as means ± SEM. Levene's test was used to determine homogeneity of variance among groups. Differences in tumor number, tumor size and biochemical parameters were analyzed statistically by one-way ANOVA followed by Fisher's least significant difference multiple comparison method to determine differences among groups. For statistical analysis of tumor number, one extreme outlier (defined as a value > 3 interquartile ranges above the 75th percentile for each of the respective groups) was removed in each of the DHA and sulindac-supplemented groups. This did not affect the statistical results. By the same definition, one extreme outlier was removed from the EPA group for analysis of PGE_2 levels. This altered the statistical significance of PGE_2 levels in the EPA group compared with controls. Regression analysis was used to determine relationships among variables in the fatty acid–supplemented groups. The Statistical Analysis System (SAS Version 6.12, SAS Institute, Cary, NC) was used to evaluate the data. Repeated measures ANOVA was used to determine differences in body weight gain and dietary intake among groups (SPSS, Chicago, IL). Differences were considered significant at $P < 0.05$.

RESULTS

Food intake and body weights. Repeated measures ANOVA showed no significant difference in weekly body

TABLE 3

Effect of dietary supplementation with various fatty acid ethyl esters or sulindac for 7 wk on tumor number and size in the colon and small intestine of $Apc^{Min/+}$ mice[1]

	Dietary group							
					(n-3) fatty acid			
n	Control 10	Sulindac 8	CLA 9	GLA 9	ALA 10	SDA 10	EPA 10	DHA 9
Colon								
Tumors/group, *n*	13	1	10	11	8	2	9	14
Tumors/mouse, *n*	1.3 ± 0.6[a]	0.1 ± 0.1[b]	1.1 ± 0.4[ab]	1.2 ± 0.4[a]	0.8 ± 0.3[ab]	0.2 ± 0.1[b]	0.9 ± 0.3[ab]	1.6 ± 0.3[a]
Tumor size,[2] *mm*	2.96 ± 0.20[a]	2.00	3.03 ± 0.52[a]	2.57 ± 0.30[a]	2.75 ± 0.46[a]	1.50	2.48 ± 0.39[a]	3.09 ± 0.21[a]
Small intestine								
Tumors/group, *n*	337	18	409	421	364	185	176	208
Tumors/mouse, *n*	33.7 ± 4.5[ab]	2.3 ± 0.5[d]	45.4 ± 8.2[a]	46.8 ± 7.1[a]	36.4 ± 6.3[ab]	18.5 ± 1.9[c]	17.6 ± 2.2[c]	23.1 ± 3.0[bc]
Tumor size,[2] *mm*	1.32 ± 0.05[a]	1.29 ± 0.33[ab]	1.24 ± 0.03[ab]	1.30 ± 0.04[ab]	1.23 ± 0.04[ab]	1.07 ± 0.02[ab]	1.05 ± 0.03[b]	1.13 ± 0.04[ab]
Total								
Tumors/group, *n*	350	19	419	432	372	187	185	222
Tumors/mouse, *n*	35.0 ± 4.5[ab]	2.4 ± 0.5[d]	46.6 ± 8.3[a]	48.0 ± 7.4[a]	37.2 ± 6.6[ab]	18.7 ± 1.9[c]	18.5 ± 2.2[c]	24.7 ± 3.2[bc]
Tumor size,[2] *mm*	1.32 ± 0.04[ab]	1.04 ± 0.09[d]	1.28 ± 0.04[ab]	1.33 ± 0.04[a]	1.21 ± 0.03[bc]	1.08 ± 0.02[d]	1.11 ± 0.04[cd]	1.21 ± 0.04[bc]

[1] Values are means ± SEM. Different superscripts within each row indicate a significant difference among groups by Fisher's least significant difference multiple comparison method at $P < 0.05$. The control diet is Monsanto "US17" diet (Monsanto, St. Louis, MO, and Research Diets, New Brunswick, NJ). Abbreviations: ALA, α-linolenic acid; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; SDA, stearidonic acid.

[2] Average tumor size was calculated as a weighted average for each size classification and expressed as mean ± SEM.

weights among the dietary groups throughout the study. Food intake was not significantly different among the groups at each time point by one-way ANOVA. However, repeated measures ANOVA revealed a significant difference in intake over time. This was most likely due to the decline in food intake in the GLA group during the final 2 wk, probably related to the increasing tumor burden toward the end of the study.

Effect of diet treatment on tumor frequency and size. As expected, the mice treated with sulindac (positive control) had 93% fewer overall tumors and significantly smaller tumors relative to the controls **(Table 3)**. Supplementation with CLA and GLA had no effect on tumor number or size. Of the (n-3) PUFA, dietary SDA and EPA supplementation resulted in the smallest number and size of tumors. ALA, the parent fatty acid in the (n-3) family, had no effect, whereas DHA-supplemented mice had 30% fewer tumors ($P = 0.15$). Interestingly, only SDA-supplemented mice had significantly fewer colonic tumors, which are typically resistant to chemotherapeutic intervention. In this regard, SDA was as effective as sulindac. There were no histologic distinctions between tumors harvested from any of the groups.

Intestinal fatty acid composition. Phospholipid fatty acid composition of normal-appearing small intestinal tissue appropriately reflected dietary fatty acid supplementation **(Table 4)**. Conjugated linoleic acid supplementation resulted in detection of CLA isomers in phospholipids at a level of ~2 mol/100 mol. Additionally, 11(Z),13(*E*)-20:2(n-7), the immediate elongation product of 9(Z),11(*E*)-18:2(n-7), was detected at low levels (0.02 mol/100 mol). However, we did not detect a conjugated AA derivative of CLA. Feeding CLA had little effect on long-chain PUFA composition of the phospholipids, whereas dietary GLA supplementation significantly enriched phospholipids with GLA, DGLA and AA at the expense of OA and LA. Inclusion of (n-3) PUFA in the diets progressively elevated concentrations of EPA and docosapentaenoic acid [22:5(n-3)] in phospholipids. In contrast, dietary SDA and EPA did not significantly alter phospholipid DHA levels, whereas ALA-supplemented mice had significantly higher levels of DHA than controls (by 0.6 mol/100 mol), and dietary DHA supplementation more than doubled the DHA content of phospholipids compared with controls. Elevations in levels of (n-3) PUFA were primarily at the expense of AA and, in most cases, coincided with elevations in LA content. SDA, the Δ6-desaturase product of ALA, was the fatty acid associated with the lowest AA content, and with the exception of dietary EPA, was associated with the highest tissue EPA content. Only minimal amounts of SDA were detected in intestinal phospholipids.

Prostaglandin levels in normal-appearing small intestine. GLA and CLA did not significantly alter basal prostaglandin levels (PGE_2 and 6-keto-$PGF_{1\alpha}$) in normal-appearing small intestinal tissue **(Table 5)**. In contrast, dietary (n-3) PUFA supplementation resulted in basal prostaglandin levels significantly lower than controls by ~50%, which coincided with similarly low levels of tissue AA. SDA and EPA were the dietary fatty acids associated with the lowest prostaglandin levels. Furthermore, treatment with sulindac, an inhibitor of cyclooxygenase, the committed step in prostaglandin biosynthesis, resulted in prostaglandin levels 56% lower than those observed in control mice.

DISCUSSION

This study clearly establishes the antitumorigenic properties of dietary (n-3) fatty acids in $Apc^{Min/+}$ mice. We previously reported that dietary EPA reduced tumor load by 50% and that this effect was due in part to antagonism of AA metabolism (Hansen Petrik et al. 2000). The current study expands on these earlier results by investigating the antitumorigenic effects of EPA and other (n-3) PUFA, viz. ALA, SDA and DHA, within the context of a typical Western diet. As observed previously, EPA-supplemented mice had ~50% fewer tumors, confirming EPA's antitumorigenic capacity in this animal model. Similarly, SDA-supplemented mice had an

TABLE 4

Fatty acid composition of mouse small intestinal phospholipids after consumption of diets supplemented with various fatty acid ethyl esters or sulindac for 7 wk[1]

	Dietary group							
					(n-3) fatty acid			
n	Control 10	Sulindac 9	CLA 9	GLA 9	ALA 10	SDA 10	EPA 10	DHA 10
	mol/100 mol total fatty acids							
Fatty Acid								
16:0	20.6 ± 0.3^{b}	20.9 ± 1.0^{b}	20.0 ± 0.3^{b}	20.8 ± 0.6^{b}	20.6 ± 0.4^{b}	21.5 ± 0.7^{ab}	20.6 ± 0.6^{b}	22.8 ± 0.2^{a}
18:0	21.7 ± 0.3^{a}	22.1 ± 0.7^{a}	21.6 ± 0.2^{ab}	21.7 ± 0.5^{a}	22.2 ± 0.5^{a}	22.2 ± 0.4^{a}	21.9 ± 0.3^{a}	20.5 ± 0.3^{b}
18:1(n-9)	7.2 ± 0.2^{b}	8.5 ± 0.4^{a}	6.3 ± 0.3^{c}	4.8 ± 0.2^{e}	6.0 ± 0.1^{cd}	6.3 ± 0.2^{c}	6.5 ± 0.2^{c}	5.6 ± 0.1^{d}
18:2(n-6)	21.2 ± 0.4^{e}	24.5 ± 0.6^{c}	22.6 ± 0.7^{d}	16.0 ± 0.4^{f}	28.2 ± 0.4^{b}	29.7 ± 0.4^{a}	21.0 ± 0.4^{e}	27.3 ± 0.4^{b}
9(*Z*), 11(*E*) 18:2(n-7)	ND^{b}	ND^{b}	1.6 ± 0.1^{a}	ND^{b}	ND^{b}	ND^{b}	ND^{b}	ND^{b}
9(*Z*), 11(*Z*) 18:2(n-7)	ND^{b}	ND^{b}	0.3 ± 0.1^{a}	ND^{b}	ND^{b}	ND^{b}	ND^{b}	ND^{b}
9(*E*), 11(*E*) 18:2(n-7)	ND^{b}	ND^{b}	<0.1^{a}	ND^{b}	ND^{b}	ND^{b}	ND^{b}	ND^{b}
18:3(n-6)	0.2 ± 0.1^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{b}	2.0 ± 0.3^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}
18:3(n-3)	<0.1^{b}	<0.1^{b}	<0.1^{b}	<0.1^{b}	1.1 ± 0.1^{a}	<0.1^{b}	<0.1^{b}	<0.1^{b}
18:4(n-3)	ND^{b}	ND^{b}	ND^{b}	ND^{b}	ND^{b}	0.3 ± 0.1^{a}	ND^{b}	ND^{b}
11(*Z*), 13(*E*) 20:2(n-7)	ND^{b}	ND^{b}	<0.1^{a}	ND^{b}	ND^{b}	ND^{b}	ND^{b}	ND^{b}
20:3(n-6)	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	2.7 ± 0.1^{a}	1.4 ± 0.0^{b}	1.2 ± 0.0^{b}	0.7 ± 0.0^{c}	0.7 ± 0.0^{c}
20:4(n-6)	19.8 ± 0.5^{b}	14.2 ± 1.2^{c}	18.4 ± 0.3^{b}	25.3 ± 0.7^{a}	9.2 ± 0.2^{d}	5.9 ± 0.1^{f}	7.6 ± 0.1^{e}	7.0 ± 0.2^{ef}
20:5(n-3)	0.3 ± 0.0^{e}	1.0 ± 0.1^{d}	0.4 ± 0.1^{de}	<0.1^{e}	3.2 ± 0.1^{c}	4.7 ± 0.2^{b}	12.9 ± 0.6^{a}	2.8 ± 0.1^{c}
22:4(n-6)	0.7 ± 0.0^{b}	0.8 ± 0.2^{ab}	0.7 ± 0.0^{b}	1.2 ± 0.4^{a}	0.1 ± 0.0^{c}	<0.1^{c}	<0.1^{c}	<0.1^{c}
22:5(n-6)	0.3 ± 0.0^{c}	0.2 ± 0.0^{d}	0.3 ± 0.0^{c}	0.7 ± 0.0^{b}	ND^{e}	ND^{e}	<0.1^{e}	1.3 ± 0.1^{a}
22:5(n-3)	0.3 ± 0.0^{e}	0.5 ± 0.1^{d}	0.3 ± 0.0^{e}	0.2 ± 0.0^{e}	1.0 ± 0.0^{c}	1.5 ± 0.1^{b}	2.8 ± 0.1^{a}	0.3 ± 0.0^{e}
22:6(n-3)	5.0 ± 0.2^{cd}	4.4 ± 0.5^{de}	5.0 ± 0.1^{bcd}	3.7 ± 0.2^{f}	5.6 ± 0.2^{b}	5.3 ± 0.2^{bc}	4.3 ± 0.2^{ef}	10.8 ± 0.1^{a}
Total (n-3)	5.5 ± 0.2^{d}	6.0 ± 0.6^{d}	5.7 ± 0.2^{d}	4.0 ± 0.2^{e}	10.9 ± 0.2^{c}	11.9 ± 0.5^{c}	20.1 ± 0.5^{a}	13.9 ± 0.2^{b}
Total (n-6)	43.8 ± 0.3^{b}	41.4 ± 1.5^{c}	45.4 ± 0.5^{b}	48.0 ± 0.5^{a}	39.2 ± 0.5^{d}	37.1 ± 0.4^{e}	29.6 ± 0.4^{f}	36.4 ± 0.4^{e}
(n-3)/(n-6) ratio	0.1 ± 0.0^{e}	0.1 ± 0.0^{e}	0.1 ± 0.0^{e}	0.1 ± 0.0^{f}	0.3 ± 0.0^{d}	0.3 ± 0.0^{c}	0.7 ± 0.0^{a}	0.4 ± 0.0^{b}

[1] Values are means ± SEM. Different superscripts within each row indicate a significant difference among groups by Fisher's least significant difference multiple comparison method at $P < 0.05$. Abbreviations: ALA, α-linolenic acid; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; ND, none detected; SDA, stearidonic acid.

equally low number of intestinal tumors. In addition, it was the only dietary fatty acid associated with significantly fewer tumors in the colon. Tumor number and size were not lower, however, in ALA-supplemented mice. This is in contrast to previous studies that demonstrated a protective effect of perilla oil (as the source of ALA) on chemically induced colorectal tumors in a rat model (Hirose et al. 1990, Narisawa et al. 1994). This difference may be due to distinct mechanisms of tumorigenesis and/or contrasting dietary designs. In both of these studies, perilla oil replaced safflower oil, which is rich in the tumor-promoting PUFA LA (Reddy 1992). On the other hand, we designed our diets to control for variations in fatty acid content within the context of a typical Western diet, mimicking relative distributions of macronutrient and fatty

TABLE 5

Basal prostaglandin levels in normal-appearing small intestines of mice after consumption of diets supplemented with various fatty acid ethyl esters or sulindac for 7 wk[1]

	Dietary group							
					(n-3) fatty acid			
n	Control 10	Sulindac 9	CLA 9	GLA 9	ALA 10	SDA 10	EPA 9–10[2]	DHA 10
	ng/mg protein							
Prostaglandin								
PGE_2	37.4 ± 8.9^{a}	16.7 ± 4.4^{b}	31.5 ± 6.9^{ab}	30.7 ± 4.8^{ab}	20.3 ± 4.9^{b}	17.8 ± 3.1^{b}	16.6 ± 2.5^{b}	19.9 ± 3.0^{b}
6-keto-$PGF_{1\alpha}$	10.1 ± 1.5^{a}	4.4 ± 0.9^{c}	9.7 ± 1.2^{ab}	8.3 ± 0.7^{ab}	6.0 ± 0.7^{bc}	5.2 ± 0.7^{c}	4.6 ± 0.4^{c}	5.5 ± 0.7^{c}

[1] Values are means ± SEM. Different superscripts within each row indicate a significant difference among groups by Fisher's least significant difference multiple comparison method at $P < 0.05$. Abbreviations: ALA, α-linolenic acid; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; PGE_2, prostaglandin E_2; SDA, stearidonic acid.
[2] See Materials and Methods for explanation.

acid composition. Our experimental design used OA as the control fatty acid, thus maintaining constant levels of (n-6) fatty acids in our diets. We previously showed OA to be neutral with respect to prostaglandin biosynthesis and tumorigenesis in $Apc^{Min/+}$ mice (unpublished results). This unique dietary design allows us to avoid problems associated with substituting one dietary oil for another in that any biological or biochemical effects observed can be attributed only to the individual fatty acids supplemented to the diets.

ALA is the parent fatty acid of the (n-3) family of fatty acids and is metabolized to SDA via Δ6-desaturase, the rate-limiting step in this biosynthetic pathway (Guichardant et al. 1993). Our results suggest that desaturation of ALA to SDA via the Δ6-desaturase may be an important step involved in the antitumorigenic effect of (n-3) fatty acids. However, it is unclear whether these differences are related to the formation of down-stream metabolites, the ability to antagonize AA and its metabolism [highly unsaturated (n-3) PUFA are 2.5 to 5 times more effective than ALA] (Whelan et al. 1991) and/or whether the degree of unsaturation of the (n-3) fatty acids has an independent effect on cell signaling/function. Feeding SDA did not dramatically enrich tissue phospholipids with SDA; its ability to alter intestinal tumor load appears to be related more to its conversion to EPA than DHA given the fact that SDA had no effect on DHA levels and DHA-supplemented mice appeared to have only modestly fewer tumors. With the exception of EPA, dietary SDA had the greatest effect on elevating tissue EPA content, but was the most potent fatty acid in its ability to lower tissue AA levels. Similarly, supplementing rats with dietary SDA (1 g/100 g) resulted in significantly elevated levels of EPA, but not SDA, in hepatic phospholipid, triglyceride and free fatty acid fractions, whereas phospholipid AA levels were significantly lower compared with controls (Yamazaki et al. 1992). Rescuing tissue AA levels by supplementing AA to diets containing EPA effectively normalizes tumor load in $Apc^{Min/+}$ mice, highlighting the importance of AA in maintaining tumor integrity (Hansen Petrik et al. 2000).

The ability of dietary DHA to reduce intestinal tumorigenesis effectively is more equivocal than that of SDA or EPA. DHA-supplemented mice appeared to have fewer tumors, but these differences were not significant ($P = 0.15$). However, partial retroconversion of DHA to EPA and its subsequent effect on AA may have accounted for its less-pronounced efficacy compared with EPA itself. Although we cannot rule out any independent effects of DHA, there was no significant correlation between DHA levels in the tissue phospholipids and tumor number ($P = 0.19$). In the only other study examining the efficacy of dietary DHA in a similar animal model, DHA resulted in fewer tumors in female knockout mice with *Apc* truncation at codon 716 ($Apc^{\Delta 716}$), but not in their male counterparts (Oshima et al. 1995). Overall, these data suggest that the ability of SDA and EPA to lessen tumor multiplicity is not dependent upon their conversion to DHA.

The ability of dietary fatty acids to modify prostaglandin levels most likely contributes to their influence on tumorigenesis. Prostaglandin involvement in intestinal tumorigenesis was recently demonstrated in a study using prostaglandin E receptor (EP) knockout mice and an EP receptor antagonist (Watanabe et al. 1999). Currently, four EP receptors have been identified (EP1–EP4). Watanabe et al. (1999) treated C57BL/6J mice with the colon carcinogen azoxymethane (AOM) and observed significantly fewer early neoplastic lesions (ACF) in EP1 receptor knockout mice compared with wild-type controls. Similarly, AOM-treated wild-type mice developed fewer ACF in a dose-dependent manner after administration of ONO-8711, an EP1 receptor antagonist. They also showed that ONO-8711 treatment resulted in 44% fewer tumors in $Apc^{Min/+}$ mice (Watanabe et al. 1999), confirming the importance of EP1 and PGE to *Apc*-mediated tumorigenesis. The EP1 receptor acts through a phospholipase C-mediated signaling pathway resulting in the potential activation of protein kinase C (PKC) after the release of diacylglycerol. Overexpression of PKC β_{II} results in downregulation of glycogen synthase kinase 3β (GSK-3β), an elevation in cellular β-catenin levels and proliferation of colonic epithelium (Murray et al. 1999). The *Apc* gene product acts in concert with GSK-3β to regulate the wnt/β-catenin signaling pathway (Polakis 1999). Loss of full-length *Apc* protein, as occurs in the $Apc^{Min/+}$ mouse model, disables the cell's ability to downregulate β-catenin; as a result, free (not bound to E-cadherin) β-catenin increases in the cytoplasm and moves into the nucleus where it acts in conjunction with the nuclear transcription factors LEF/TCF to induce expression of target genes (Polakis 1999). Treatment with NSAID, inhibitors of prostaglandin biosynthesis, may reduce tumor loads in this model, at least in part, by attenuating β-catenin levels (Mahmoud et al. 1998, McEntee et al. 1999), and (n-3) PUFA may also modulate this signaling pathway via reductions in AA and PKC activation (Jiang et al. 1997).

In our study, prostaglandin levels were significantly lower in all (n-3) fatty acid–supplemented groups, with dietary EPA having the greatest effect. However, lower prostaglandin levels did not always equate to a lower tumor number. For example, ALA significantly attenuated prostaglandin levels, but did not alter tumor multiplicity, suggesting that prostaglandins play a partial role in tumorigenesis. Furthermore, regression analysis revealed a small, but significant relationship between prostaglandin levels and tumor number, with prostaglandins accounting for 7–17% of the variability in tumor number (**Fig. 1**A, *B*). It is possible that the degree of unsaturation associated with the (n-3) fatty acids could differentially affect the expression or, more importantly, the activity of COX (Laneuville et al. 1995), the committed step in prostaglandin biosynthesis (Hamid et al. 1999, Singh et al. 1997). Intestinal tumors in $Apc^{Min/+}$ mice express both isozymes of COX, i.e., COX-1, which is constitutively expressed, and COX-2, the inducible isoform (Boolbool et al. 1996, Hull et al. 1999, Williams et al. 1996). Dietary fish oils (rich in EPA and DHA) reduce COX-2 expression in an AOM-induced rat colonic tumor model (Singh et al. 1997) and immunoreactive COX-1 and -2 protein levels in DMBA-induced mammary tumors (Hamid et al. 1999), whereas (n-6) fatty acids increase COX-2 expression (Singh et al. 1997). The involvement of COX in this animal model is clearly established: *1*) inhibition of the COX isozymes by nonselective inhibitors results in 85–96% fewer tumors (Chiu et al. 1997 and 2000, Jacoby et al. 1996, Ritland and Gendler 1999); *2*) selective inhibition of COX-2 results in 52% fewer tumors (Nakatsugi et al. 1997); *3*) crossing COX-2 knockout mice with $Apc^{\Delta 716}$ knockout mice results in fewer tumors in a gene-dose–responsive manner (Oshima et al. 1996); and *4*) crossing COX-1 knockout mice with $Apc^{Min/+}$ mice results in 80–90% fewer tumors (Langenbach et al. 1999). Nevertheless, the precise mechanisms underlying the antitumorigenic effects of SDA and EPA remain unclear.

Dietary supplementation of CLA and GLA did not alter tumorigenesis in this study. This is of particular interest because CLA has been shown to be a potent antitumorigenic agent in chemically induced mammary tumors in rats at levels ≤ 1 g/100 g diet regardless of the level or type of dietary fat (Ip et al. 1991 and 1996). It has recently been suggested that CLA may exert its antitumorigenic effect by inhibiting the conver-

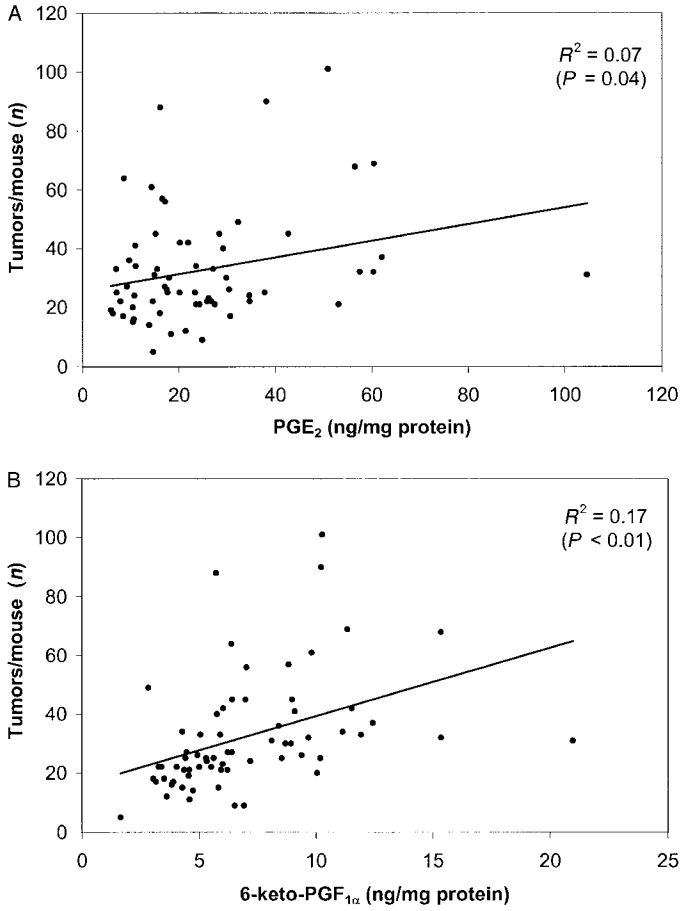

FIGURE 1 The relationship between total tumor numbers of male $Apc^{Min/+}$ mice fed various fatty acid ethyl esters for 7 wk and prostaglandin (PG) levels in samples of normal-appearing small intestinal tissue was determined by regression analysis. There was a significant correlation between PGE_2 and tumor number (*A*) and 6-keto-$PGF_{1\alpha}$ and tumor number (*B*).

sion of LA to AA and, ultimately, to eicosanoids (Banni et al. 1999, Belury and Kempa-Steczko 1997, Kavanaugh et al. 1999). Banni et al. (1999) showed that CLA at a level of 1 g/100 g diet maximally lowered GLA, DGLA and AA levels as a percentage of total lipid in normal mammary tissue. Others have detected conjugated AA derivatives of CLA in hepatic lipids of rats after intragastric CLA administration (Sébédio et al. 1999). However, we did not observe any alterations in the small intestinal phospholipid content of LA metabolites or detect conjugated AA; concomitantly, we did not observe any differences in tumor number or size. Earlier evidence suggested that CLA has antitumorigenic potential in the gastrointestinal tract by reducing chemically induced colonic ACF in rats and gastric neoplasia in mice after oral administration by gavage (Ha et al. 1990, Liew et al. 1995). In contrast, others reported that dietary CLA had no effect on carcinogen-DNA adduct formation in colons of rats treated with 2-amino-3-methylimidazo[4,5-*f*]quinoline or 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (Josyula and Schut 1998). The effectiveness of CLA on intestinal tumorigenesis could be related to the type of feeding regimen or the underlying mechanisms responsible for tumor initiation and progression. Nonetheless, our study is the first to investigate the effectiveness of dietary CLA on intestinal tumorigenesis in $Apc^{Min/+}$ mice and we observed no effect on tumor number or size.

To date, the efficacy of dietary GLA on intestinal tumorigenesis has not been determined. Therapeutic studies involving dietary GLA have largely utilized the DMBA-induced rat mammary tumor model or nude mouse mammary carcinoma xenograft model with encouraging results (Abou El-Ela et al. 1987, Pritchard et al. 1989). The use of GLA in this study, however, did not alter intestinal tumor load in $Apc^{Min/+}$ mice, although tissue DGLA levels were significantly higher in the phospholipid fraction of the intestines. DGLA can be metabolized to 15-HETrE and PGE_1. Both of these compounds reportedly have antiproliferative properties [as reviewed by Fan and Chapkin (1998)]. Like PGE_2, the actions of PGE_1 are mediated via the G-protein–coupled EP receptors (Boie et al. 1997, Fan and Chapkin. 1998, Funk et al. 1993). However, recent evidence indicates that antagonism, not agonism, of the EP1 receptor results in significantly fewer tumors in this mouse model (Watanabe et al. 1999). Furthermore, we found that feeding GLA resulted in 28% higher AA concentrations in intestinal phospholipids, although levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ were not significantly different from control values, possibly accounting for the lack of efficacy of GLA. We determined previously that elevating tissue AA levels and prostaglandin levels, for that matter, above control values had no effect on tumor number or size in this animal model (Chiu et al. 1997, Hansen Petrik et al. 2000).

In summary, dietary SDA and EPA supplementation resulted in ~50% fewer intestinal tumors in $Apc^{Min/+}$ mice, a genetic murine model of colorectal cancer based on a mutation of the *Apc* tumor suppressor gene. SDA was the most efficacious (n-3) fatty acid, resulting in significantly fewer tumors in both the colon and small intestine plus significantly smaller tumors in the small intestine. The antitumorigenic effects of both SDA and EPA are likely related to alterations in tissue AA content and prostaglandin levels (Hansen Petrik et al. 2000). The fact that dietary ALA had no effect on tumor multiplicity suggests the importance of its conversion to SDA via the Δ6-desaturase, whereas the ability of DHA to affect tumorigenesis is less clear. Like ALA, CLA and GLA had no effect on intestinal tumorigenesis in this model.

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LITERATURE CITED

Abou El-Ela, S. H., Prasse, K. W., Carroll, R. & Bunce, O. R. (1987) Effects of dietary primrose oil on mammary tumorigenesis induced by 7,12-dimethylbenz(α)anthracene. Lipids 22: 1041–1044.

Anti, M., Marra, G., Armelao, F., Bartoli, G. M., Ficarelli, R., Percesepe, A., De Vitis, I., Maria, G., Sofo, L., Rapaccini, G. L., Gentiloni, N., Piccioni, E. & Miggiano, G. (1992) Effect of ω-3 fatty acids on rectal mucosal cell proliferation in subjects at risk for colon cancer. Gastroenterology 103: 883–891.

Banni, S., Angioni, E., Casu, V., Melis, M. P., Carta, G., Corongiu, F. P., Thompson, H. & Ip, C. (1999) Decrease in linoleic acid metabolites as a potential mechanism in cancer risk reduction by conjugated linoleic acid. Carcinogenesis 20: 1019–1024.

Belury, M. A. & Kempa-Steczko, A. (1997) Conjugated linoleic acid modulates hepatic lipid composition in mice. Lipids 32: 199–204.

Belury, M. A., Nickel, K. P., Bird, C. E. & Wu, Y. (1996) Dietary conjugated linoleic acid modulation of phorbol ester skin tumor promotion. Nutr. Cancer 26: 149–157.

Boie, Y., Stocco, R., Sawyer, N., Slipetz, D. M., Ungrin, M. D., Neuschäfer-Rube, F., Püschel, G. P., Metters, K. M. & Abramovitz, M. (1997) Molecular cloning and characterization of the four rat prostaglandin E_2 prostanoid receptor subtypes. Eur. J. Pharmacol. 340: 227–241.

Boolbool, S. K., Dannenberg, A. J., Chadburn, A., Martucci, C., Guo, X.-J., Ramonetti, J. T., Abreu-Goris, M., Newmark, H. L., Lipkin, M. L., DeCosse, J. J. & Bertagnolli, M. M. (1996) Cyclooxygenase-2 overexpression and

tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res. 56: 2556–2560.
Borgeat, P., Hamberg, M. & Samuelsson, B. (1976) Transformation of arachidonic acid and homo-γ-linolenic acid by rabbit polymorphonuclear leukocytes. Monohydroxy acids from novel lipoxygenases. J. Biol. Chem. 251: 7816–7820.
Cantrill, R. C., Huang, Y.-S., Ells, G. W. & Horrobin, D. F. (1993) Comparison of the metabolism of α-linolenic acid and its Δ6 desaturation product, stearidonic acid, in cultured NIH-3T3 cells. Lipids 28: 163–166.
Caygill, C.P.J., Charlett, A. & Hill, M. J. (1996) Fat, fish, fish oil and cancer. Br. J. Cancer 74: 159–164.
Chang, W.-C.L., Chapkin, R. S. & Lupton, J. R. (1998) Fish oil blocks azoxymethane-induced rat colon tumorigenesis by increasing cell differentiation and apoptosis rather than decreasing cell proliferation. J. Nutr. 128: 491–497.
Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L. & Pariza, M. W. (1992) Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Compos. Anal. 5: 185–197.
Chiu, C.-H., McEntee, M. F. & Whelan, J. (1997) Sulindac causes rapid regression of preexisting tumors in Min/+ mice independent of prostaglandin biosynthesis. Cancer Res. 57: 4267–4273.
Chiu, C.-H., McEntee, M. F. & Whelan, J. (2000) Discordant effect of aspirin and indomethacin on intestinal tumor burden in *APCMin*/+ mice. Prostaglandins Leukot. Essent. Fatty Acids 62: 269–275.
Devi, A. M. & Das, N. P. (1994) Antiproliferative effect of polyunsaturated fatty acids and interleukin-2 on normal and abnormal human lymphocytes. Experientia 50: 489–492.
Fan, Y.-Y. & Chapkin, R. S. (1998) Importance of dietary γ-linolenic acid in human health and nutrition. J. Nutr. 128: 1411–1414.
Fan, Y.-Y., Ramos, K. S. & Chapkin, R. S. (1997) Dietary γ-linolenic acid enhances mouse macrophage-derived prostaglandin E_1 which inhibits vascular smooth muscle cell proliferation. J. Nutr. 127: 1765–1771.
Fernandez, E., Chatenoud, L., La Vecchia, C., Negri, E. & Franceschi, S. (1999) Fish consumption and cancer risk. Am. J. Clin. Nutr. 70: 85–90.
Funk, C. D., Furci, L., FitzGerald, G. A., Grygorczyk, R., Rochette, C., Bayne, M. A., Abramovitz, M., Adam, M. & Metters, K. M. (1993) Cloning and expression of cDNA for the human prostaglandin E receptor EP_1 subtype. J. Biol. Chem. 268: 26767–26772.
Giovannucci, E. & Willett, W. C. (1994) Dietary factors and risk of colon cancer. Ann. Med. 26: 443–452.
Guichardant, M., Traitler, H., Spielmann, D., Sprecher, H. & Finot, P.-A. (1993) Stearidonic acid, an inhibitor of the 5-lipoxygenase pathway. A comparison with timnodonic acid and dihomogammalinolenic acid. Lipids 28: 321–324.
Ha, Y.L., Grimm, N.K. & Pariza, M.W. (1987) Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis 8: 1881–1887.
Ha, Y.L., Storkson, J. & Pariza, M.W. (1990) Inhibition of benzo(α)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res. 50: 1097–1101.
Hamid, R., Singh, J., Reddy, B. S. & Cohen, L. A. (1999) Inhibition by dietary menhaden oil of cyclooxygenase-1 and 2 in *N*-nitrosomethylurea-induced rat mammary tumors. Int. J. Oncol. 14: 523–528.
Hansen Petrik, M. B., McEntee, M. F., Chiu, C.-H. & Whelan, J. (2000) Antagonism of arachidonic acid is linked to the antitumorigenic effect of dietary eicosapentaenoic acid in $Apc^{Min/+}$ mice. J. Nutr. 130: 1153–1158.
Hirose, M., Masuda, A., Ito, N., Kamano, K. & Okuyama, H. (1990) Effects of dietary perilla oil, soybean oil and safflower oil on 7,12-dimethylbenz(α)anthracene (DMBA) and 1,2-dimethyl-hydrazine (DMH)-induced mammary gland and colon carcinogenesis in female SD rats. Carcinogenesis 11: 731–735.
Hoyert, D. L., Kochanek, K. D. & Murphy, S. L. (1999) Deaths: Final Data for 1997. National Center for Health Statistics. Natl. Vital Stat. Rep. 47: 1–105.
Huang, Y.-S., Smith, R. S., Redden, P. R., Cantrill, R. C. & Horrobin, D. F. (1991) Modification of liver fatty acid metabolism in mice by n-3 and n-6 Δ6-desaturase substrates and products. Biochim. Biophys. Acta 1082: 319–327.
Hull, M. A., Booth, J. K., Tisbury, A., Scott, N., Bonifer, C., Markham, A. F. & Coletta, P. L. (1999) Cyclooxygenase 2 is up-regulated and localized to macrophages in the intestine of Min mice. Br. J. Cancer 79: 1399–1405.
Ip, C., Briggs, S. P., Haegele, A. D., Thompson, H. J., Storkson, J. & Scimeca, J. A. (1996) The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet. Carcinogenesis 17: 1045–1050.
Ip, C., Chin, S. F., Scimeca, J. A. & Pariza, M. W. (1991) Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res. 51: 6118–6124.
Jacoby, R. F., Marshall, D. J., Newton, M. A., Novakovic, K., Tutsch, K., Cole, C. E., Lubet, R. A., Kelloff, G. J., Verma, A., Moser, A. R. & Dove, W. F. (1996) Chemoprevention of spontaneous intestinal adenomas in the *Apc*Min mouse model by the nonsteroidal anti-inflammatory drug piroxicam. Cancer Res. 56: 710–714.
Jen, J., Powell, S. M., Papadopoulos, N., Smith, K. J., Hamilton, S. R., Vogelstein, B. & Kinzler, K. W. (1994) Molecular determinants of dysplasia in colorectal lesions. Cancer Res. 54: 5523–5526.
Jiang, W. G., Bryce, R. P., Horrobin, D. F. & Mansel, R. E. (1998) γ-Linolenic acid blocks cell cycle progression by regulating phosphorylation of $p27^{kip1}$ and $p57^{kip2}$ and their interactions with other cycle regulators in cancer cells. Int. J. Oncol. 13: 611–617.
Jiang, W. G., Hiscox, S., Hallett, M. B., Scott, C., Horrobin, D. F. & Puntis, M.C.A. (1995) Inhibition of hepatocyte growth factor-induced motility and *in vitro* invasion of human colon cancer cells by gamma-linolenic acid. Br. J. Cancer 71: 744–752.
Jiang, Y.-H., Lupton, J. R. & Chapkin, R. S. (1997) Dietary fish oil blocks carcinogen-induced down-regulation of colonic protein kinase C isozymes. Carcinogenesis 18: 351–357.
Johnson, M. M., Swan, D. D., Surette, M. E., Stegner, J., Chilton, T., Fonteh, A. N. & Chilton, F. H. (1997) Dietary supplementation with γ-linolenic acid alters fatty acid content and eicosanoid production in healthy humans. J. Nutr. 127: 1435–1444.
Josyula, S. & Schut, H.A.J. (1998) Effects of dietary conjugated linoleic acid on DNA adduct formation of PhIP and IQ after bolus administration to female F344 rats. Nutr. Cancer 32: 139–145.
Kato, I., Akhmedkhanov, A., Koenig, K., Toniolo, P. G., Shore, R. E. & Riboli, E. (1997) Prospective study of diet and female colorectal cancer: The New York University Women's Health Study. Nutr. Cancer 28: 276–281.
Kavanaugh, C. J., Liu, K.-L. & Belury, M. A. (1999) Effect of dietary conjugated linoleic acid on phorbol ester-induced PGE_2 production and hyperplasia in mouse epidermis. Nutr. Cancer 33: 132–138.
Kinzler, K. W. & Vogelstein, B. (1996) Lessons from hereditary colorectal cancer. Cell 87: 159–170.
Kockmann, V., Spielmann, D., Traitler, H. & Lagarde, M. (1989) Inhibitory effect of stearidonic acid (18:4 n-3) on platelet aggregation and arachidonate oxygenation. Lipids 24: 1004–1007.
Kokura, S., Yoshikawa, T., Kaneko, T., Iinuma, S., Nishimura, S., Matsuyama, K., Naito, Y., Yoshida, N. & Kondo, M. (1997) Efficacy of hyperthermia and polyunsaturated fatty acids on experimental carcinoma. Cancer Res. 57: 2200–2202.
Laneuville, O., Breuer, D. K., Xu, N., Huang, Z. H., Gage, D. A., Watson, J. T., Lagarde, M., DeWitt, D. L. & Smith, W. L. (1995) Fatty acid substrate specificities of human prostaglandin-endoperoxide H synthase-1 and -2. Formation of 12-hydroxy-(9*Z*, 13*E*/*Z*, 15*Z*)-octadecatrienoic acids from α-linolenic acid. J. Biol. Chem. 270: 19330–19336.
Langenbach, R., Loftin, C., Lee, C. & Tiano, H. (1999) Cyclooxygenase knockout mice: models for elucidating isoform-specific functions. Biochem. Pharmacol. 58: 1237–1246.
Latham, P., Lund, E. K. & Johnson, I. T. (1999) Dietary n-3 PUFA increases the apoptotic response to 1,2-dimethylhydrazine, reduces mitosis and suppresses the induction of carcinogenesis in the rat colon. Carcinogenesis 20: 645–650.
Levy, D. B., Smith, K. J., Beazer-Barclay, Y., Hamilton, S. R., Vogelstein, B. & Kinzler, K. W. (1994) Inactivation of both *APC* alleles in human and mouse tumors. Cancer Res. 54: 5953–5958.
Liew, C., Schut, H.A.J., Chin, S. F., Pariza, M. W. & Dashwood, R. H. (1995) Protection of conjugated linoleic acids against 2-amino-3-methylimidazo[4,5-*f*]quinoline-induced colon carcinogenesis in the F344 rat: a study of inhibitory mechanisms. Carcinogenesis 16: 3037–3043.
Lipkin, M., Reddy, B., Newmark, H. & Lamprecht, S. A. (1999) Dietary factors in human colorectal cancer. Annu. Rev. Nutr. 19: 545–586.
Mahmoud, N. N., Dannenberg, A. J., Mestre, J., Bilinski, R. T., Churchill, M. R., Martucci, C., Newmark, H. & Bertagnolli, M. M. (1998) Aspirin prevents tumors in a murine model of familial adenomatous polyposis. Surgery 124: 225–231.
Markwell, M.A.K., Haas, S. M., Tolbert, N. E. & Bieber, L. L. (1981) Protein determinations in membrane and lipoprotein samples: manual and automated procedures. Methods Enzymol. 72: 296–303.
McEntee, M. F., Chiu, C.-H. & Whelan, J. (1999) Relationship of β-catenin and Bcl-2 expression to sulindac-induced regression of intestinal tumors in Min mice. Carcinogenesis 20: 635–640.
Minoura, T., Takata, T., Sakaguchi, M., Takada, H., Yamamura, M., Hioki, K. & Yamamoto, M. (1988) Effect of dietary eicosapentaenoic acid on azoxymethane-induced colon carcinogenesis in rats. Cancer Res. 48: 4790–4794.
Miyoshi, Y., Nagase, H., Ando, H., Horii, A., Ichii, S., Nakatsuru, S., Aoki, T., Miki, Y., Mori, T. & Nakamura, Y. (1992) Somatic mutations of the *APC* gene in colorectal tumors: mutation cluster region in the *APC* gene. Hum. Mol. Genet. 1: 229–233.
Murray, N. R., Davidson, L. A., Chapkin, R. S., Gustafson, W. C., Schattenberg, D. G. & Fields, A. P. (1999) Overexpression of protein kinase C β_{II} induces colonic hyperproliferation and increased sensitivity to colon carcinogenesis. J. Cell Biol. 145: 699–711.
Nakatsugi, S., Fukutake, M., Takahashi, M., Fukuda, K., Isoi, T., Taniguchi, Y., Sugimura, T. & Wakabayashi, K. (1997) Suppression of intestinal polyp development by nimesulide, a selective cyclooxygenase-2 inhibitor, in Min mice. Jpn. J. Cancer Res. 88: 1117–1120.
Narisawa, T., Fukaura, Y., Yazawa, K., Ishikawa, C., Isoda, Y. & Nishizawa, Y. (1994) Colon cancer prevention with a small amount of dietary perilla oil high in alpha-linolenic acid in an animal model. Cancer 73: 2069–2075.
National Research Council (1985) Guide for the Care and Use of Laboratory Animals. Publication no. 85–23 (rev.). National Institutes of Health, Bethesda, MD.
Onogi, N., Okuno, M., Komaki, C., Moriwaki, H., Kawamori, T., Tanaka, T., Mori, H. & Muto, Y. (1996) Suppressing effect of perilla oil on azoxymethane-induced foci of colonic aberrant crypts in rats. Carcinogenesis 17: 1291–1296.
O'Shea, M., Stanton, C. & Devery, R. (1999) Antioxidant enzyme defence

responses of human MCF-7 and SW480 cancer cells to conjugated linoleic acid. Anticancer Res. 19: 1953–1960.
Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Evans, J. F. & Taketo, M. M. (1996) Suppression of intestinal polyposis in $Apc^{\Delta 716}$ knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87: 803–809.
Oshima, M., Takahashi, M., Oshima, H., Tsutsumi, M., Yazawa, K., Sugimura, T., Nishimura, S., Wakabayashi, K. & Taketo, M.M. (1995) Effects of docosahexaenoic acid (DHA) on intestinal polyp development in $Apc^{\Delta 716}$ knockout mice. Carcinogenesis 16: 2605–2607.
Paulsen, J. E., Elvsaas, I.-K.Ø., Steffensen, I.-L. & Alexander, J. (1997) A fish oil derived concentrate enriched in eicosapentaenoic and docosahexaenoic acid as ethyl ester suppresses the formation and growth of intestinal polyps in the Min mouse. Carcinogenesis 18: 1905–1910.
Phillips, J. C. & Huang, Y.-S. (1996) Natural sources and biosynthesis of γ-linolenic acid: an overview. In: γ-Linolenic Acid: Metabolism and Its Roles in Nutrition and Medicine (Huang, Y.-S. & Mills, D.E., eds.), pp. 1–13. AOCS Press, Champaign, IL.
Polakis, P. (1999) The oncogenic activation of β-catenin. Curr. Opin. Genet. Dev. 9: 15–21.
Powell, S. M., Zilz, N., Beazer-Barclay, Y., Bryan, T. M., Hamilton, S. R., Thibodeau, S. N., Vogelstein, B. & Kinzler, K. W. (1992) *APC* mutations occur early during colorectal tumorigenesis. Nature (Lond.) 359: 235–237.
Pritchard, G. A., Jones, D. L. & Mansel, R. E. (1989) Lipids in breast carcinogenesis. Br. J. Surg. 76: 1069–1073.
Ravichandran, D., Cooper, A. & Johnson, C. D. (1998) Effect of lithium γ-linolenate on the growth of experimental human pancreatic carcinoma. Br. J. Surg. 85: 1201–1205.
Reddy, B. S. (1992) Dietary fat and colon cancer: animal model studies. Lipids 27: 807–813.
Ritland, S. R. & Gendler, S. J. (1999) Chemoprevention of intestinal adenomas in the Apc^{Min} mouse by piroxicam: kinetics, strain effects and resistance to chemosuppression. Carcinogenesis 20: 51–58.
Sébédio, J. L., Juanéda, P., Grégoire, S., Chardigny, J. M., Martin, J. C. & Ginies, C. (1999) Geometry of conjugated double bonds of CLA isomers in a commercial mixture and in their hepatic 20:4 metabolites. Lipids 34: 1319–1325.
Serraino, M. & Thompson, L. U. (1992) Flaxseed supplementation and early markers of colon carcinogenesis. Cancer Lett. 63: 159–165.
Shultz, T. D., Chew, B. P., Seaman, W. R. & Luedecke, L. O. (1992) Inhibitory effect of conjugated dienoic derivatives of linoleic acid and β-carotene on the in vitro growth of human cancer cells. Cancer Lett. 63: 125–133.
Singh, J., Hamid, R. & Reddy, B. S. (1997) Dietary fat and colon cancer: modulation of cyclooxygenase-2 by types and amount of dietary fat during the postinitiation stage of colon carcinogenesis. Cancer Res. 57: 3465–3470.
Slattery, M. L., Edwards, S. L., Boucher, K. M., Anderson, K. & Caan, B. J. (1999) Lifestyle and colon cancer: an assessment of factors associated with risk. Am. J. Epidemiol. 150: 869–877.
Smith, K. J., Johnson, K. A., Bryan, T. M., Hill, D. E., Markowitz, S., Willson, J.K.V., Paraskeva, C., Petersen, G. M., Hamilton, S. R., Vogelstein, B. & Kinzler, K. W. (1993) The *APC* gene product in normal and tumor cells. Proc. Natl. Acad. Sci. U.S.A. 90: 2846–2850.
Takeda, S., Horrobin, D. F., Manku, M., Sim, P. G., Ells, G. & Simmons, V. (1992) Lipid peroxidation in human breast cancer cells in response to gamma-linolenic acid and iron. Anticancer Res. 12: 329–334.
Wasan, H. S., Novelli, M., Bee, J. & Bodmer, W. F. (1997) Dietary fat influences on polyp phenotype in multiple intestinal neoplasia mice. Proc. Natl. Acad. Sci. U.S.A. 94: 3308–3313.
Watanabe, K., Kawamori, T., Nakatsugi, S., Ohta, T., Ohuchida, S., Yamamoto, H., Maruyama, T., Kondo, K., Ushikubi, F., Narumiya, S., Sugimura, T. & Wakabayashi, K. (1999) Role of the prostaglandin E receptor subtype EP_1 in colon carcinogenesis. Cancer Res. 59: 5093–5096.
Whelan, J., Broughton, K. S. & Kinsella, J. E. (1991) The comparative effects of dietary α-linolenic acid and fish oil on 4- and 5-series leukotriene formation *in vivo*. Lipids 26: 119–126.
Whelan, J., Broughton, K. S., Surette, M. E. & Kinsella, J. E. (1992) Dietary arachidonic and linoleic acids: comparative effects on tissue lipids. Lipids 27: 85–88.
Whelan, J., Surette, M. E., Hardardóttir, I., Lu, G., Golemboski, K. A., Larsen, E. & Kinsella, J. E. (1993) Dietary arachidonate enhances tissue arachidonate levels and eicosanoid production in Syrian hamsters. J. Nutr. 123: 2174–2185.
Willett, W. C., Stampfer, M. J., Colditz, G. A., Rosner, B. A. & Speizer, F. E. (1990) Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study of women. N. Engl. J. Med. 323: 1664–1672.
Williams, C. S., Luongo, C., Radhika, A., Zhang, T., Lamps, L. W., Nanney, L. B., Beauchamp, R. D. & DuBois, R. N. (1996) Elevated cyclooxygenase-2 levels in *Min* mouse adenomas. Gastroenterology 111: 1134–1140.
Yamazaki, K., Fujikawa, M., Hamazaki, T., Yano, S. & Shono, T. (1992) Comparison of the conversion rates of α-linolenic acid (18:3(n-3)) and stearidonic acid (18:4(n-3)) to longer polyunsaturated fatty acids in rats. Biochim. Biophys. Acta 1123: 18–26.