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# Chemopreventive Effects of Dietary Flaxseed Oil on Colon Tumor Development

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***Abstract:*** *Fatty acid composition of dietary fat, primarily the levels of ω-3 and ω-6 polyunsaturated fatty acids, has shown profound effect on colon tumor development in animal studies. Fats containing ω-6 fatty acids (for example, corn oil) enhanced and ω-3 fatty acids (for example, fish oil and mustard oil) reduced chemically induced colon tumors in rats. The purpose of this study was to investigate the effects of dietary flaxseed oil (containing α-linolenic acid, an ω-3 polyunsaturated fatty acid) on azoxymethane-induced colon tumor in rats and how it compared with the dietary corn oil–treated group. Male Fischer rats, separated into 2 groups of 30, were assigned to the AIN-93M diet, which was supplemented with either 15% corn oil or 15% flaxseed oil. Carcinogenesis was initiated with subcutaneous injections of azoxymethane (15 mg/kg) once a week for three consecutive weeks. Thirty-five weeks after initiation, the rats were sacrificed under ether anesthesia. Blood was collected by cardiac puncture. The gastrointestinal tract was isolated and flushed with ice-cold normal saline. The site, size, and number of tumors were recorded. The incidence and multiplicity of the tumors in the colon were determined. The fatty acid composition in the serum, colon, and tumors was estimated by using gas chromatography-flame ionization detection. Colon tumor incidence was found to be 100% and 54%, whereas multiplicity was found to be 3.1 and 0.7 tumors per rat in corn oil– and flaxseed oil–treated groups, respectively. Tumor size was significantly larger in the corn oil–treated group than in the flaxseed oil group. Colon and serum samples of the corn oil group showed an increase in the ω-6 fatty acid levels, whereas the flaxseed oil group exhibited an increase in the ω-3 fatty acid levels. The results indicate that dietary flaxseed oil, containing high levels of ω-3 fatty acids, is effective in preventing colon tumor development when compared with dietary corn oil containing ω-6 fatty acids in rats.*

## Introduction

Colorectal cancers are the third most prevalent type of cancer in the United States and account for 10% of cancer deaths (1). In fact, 104,950 new colon cases, 40,340 new rectal cases, and 56,290 colorectal deaths are estimated to occur in 2005 (1). Among the various risk factors, diet and nutrition have been implicated as important variables associated with colon carcinogenesis (2). The extended time duration in the conversion of an adenoma to an adenocarcinoma provides a window of opportunity for dietary intervention (3). Evidence for dietary fat as a possible colon cancer risk factor among the various dietary components was obtained from migrant and epidemiological studies. In epidemiological studies, diets high in red meat and animal fat were associated with an increased risk of colorectal cancer (4). Laboratory studies conducted in animal models revealed the influence of both the amount and type of fatty acids consumed in the form of triglycerides on the development of colon cancer. High-fat diets containing corn oil, safflower oil, beef fat, or lard increased chemically induced colon tumors in laboratory animals compared with low-fat diets. Diets containing high levels of coconut oil, olive oil, or trans fat did not exhibit tumor-enhancing effects. Hence, fatty acid composition, and not the total fat, is a determinant for colon cancer (5).

Dietary corn oil containing high levels of linoleic acid (an ω-6 fatty acid) enhanced and dietary fish and mustard oil, rich in ω-3 fatty acid, reduced azoxymethane-induced colon tumorigenesis in rats (6,7). Chemopreventive effects of dietary fish oil were found to be more effective in the postinitiation stage of colon carcinogenesis (8,9).

The chemopreventive effects of fish oil (containing ω-3 fatty acids) have been attributed to the inhibition of oxidative metabolism of arachidonic acid through the cyclooxygenase (COX) pathway (10,11). Overexpression of COX-2 in early stages of colon carcinogenesis and the inhibition of colon tumors by nonsteroidal anti-inflammatory drugs (NSAIDs) have suggested a tumor-promoting role for inflammatory prostaglandins in colon tumorigenesis (12,13). Flaxseed oil contains a higher percentage of α-linolenic acid (an ω-3 polyunsaturated fatty acid) than both mustard and fish oils. The purpose of the present investigation was to determine the chemopreventive effects of dietary flaxseed oil rich in ω-3 fatty acids on azoxymethane-induced colon tumor development and to compare them with the effects of dietary corn oil containing ω-6 fatty acids on colon tumor development.

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## Materials and Methods

### Materials

Mazola corn oil (Best Foods, Englewood Cliffs, NJ) was purchased from a local supermarket, and flaxseed oil was provided by Eversco, Ltd. (Rhinelander, WI). AIN-93M was purchased from Dyets, Inc. (Bethlehem, PA). Azoxymethane, chloroform, ether, and tertiary butyl-ammonium hydroxide were purchased from Sigma Chemical Company (St. Louis, MO).

### Animals

Male Fischer rats (10 wk old, Frederick Cancer Research and Development Center, National Cancer Institute, Frederick, MD) were used in this study. Rats were housed in the College of Pharmacy animal room facility (temperature 22±1°C, humidity 40–60%, and light from 6:00 AM to 6:00 PM) and provided with food and water ad libitum.

### Colon Carcinogenesis Protocol

Male Fischer rats (10 wk old) were divided into 2 groups of 30 each and placed on AIN-93M meal (Dyets, Inc.) supplemented with either corn or flaxseed oil. Group assignments were as follows:

Group 1: Corn oil (AIN-93M, containing 15% corn oil)
Group 2: Flaxseed oil (AIN-93M, containing 15% flaxseed oil)

Supplemented diets were prepared by mixing AIN-93M meal with appropriate oil in a mechanical mixer and then storing it in airtight containers at 4°C in a refrigerator. Diets were prepared twice a week. Peroxide content of the two diets did not change during the storage period (7).

The rats were fed the respective diets for a week. Then the colon cancer was initiated with subcutaneous injections of azoxymethane (15 mg/kg) once a week for three consecutive weeks. The rats were fed their respective diets ad libitum throughout the duration of the experiment. Bowls filled with the respective diets were placed in corresponding cages in the afternoon and replaced the following day.

On completion of 35 wk after initiation, the rats were sacrificed under ether anesthesia. Blood was collected by cardiac puncture. The gastrointestinal tract was removed and cleaned thoroughly with ice-cold normal saline. The site, size, and number of tumors were recorded. Serum was collected from the blood by centrifugation. The tumors were separated from the colon tissue (14). The serum, tumor, and colon samples were then analyzed for fatty acid composition.

### Fatty Acid Analysis

**Microsome preparation:** The colon and tumor samples were washed with 1.15% ice-cold KCl and blotted. The individual samples were minced and homogenized using an Omni GLH homogenizer (Omni International, Inc., Warrenton, VA). The homogenates were then centrifuged at 10,000 *g* for 15 min in a J2-21 centrifuge (Beckman Instruments, Inc., Fullerton, CA). The supernatant obtained was recentrifuged in an Optima LE-80K preparative ultracentrifuge (Beckman Instruments, Inc.) at 105,000 *g* for 60 min under refrigeration. The pellet was washed two or three times with ice-cold 1.15% KCl and suspended in 1.15% KCl, which was then used for extraction of fatty acids (15).

**Fatty acid extraction:** Corn or flaxseed oil (20 mg) combined with distilled water (1.6 ml), or samples of serum, colon, or tumor microsomes (1.6 ml), were extracted by the Bligh and Dyer method (16). Methanol (4 ml) and chloroform (2 ml) were added to the samples and vortexed for 1 min. Chloroform (2 ml) followed by water (2 ml) was added, and the mixture was vortexed for 1 min after each addition. The chloroform layer was separated and evaporated completely under nitrogen. The initial and the final ratios of water to methanol to chloroform were maintained at 0.8:2:1 and 1.8:2:2, respectively. The dried samples were combined with 3 ml of diethyl ether (ethanol-free) and 100 µl of tetramethylammonium hydroxide, vortexed for 1 min, and allowed to stand for 20 min. The samples were again vortexed with 5 ml water to remove any water-soluble constituents. The ether layer, collected and dried to 1 ml under nitrogen, was then used for the fatty acid analysis.

**Determination of fatty acids:** Fatty acid analysis of these diets, serum, colon, and tumor tissues was based on the procedure of Joseph and Ackman (17) with minor modifications (7). Fatty acid determination was carried out on a Hewlett-Packard 5890, Series II gas chromatograph with a flame ionization detector. The column used was a Supelco SP-2380 capillary column (30 m × 0.25 mm × 0.2 µm film). The temperature program was as follows: 50°C for 2 min, then 4°C per minute to 140°C, holding for 40 min, then raising the temperature from 140 to 210°C at 4°C per minute and holding for 8 min final time. The fatty acid peak assignments were made by comparing a standard fatty acid mixture (Nu-Chek-Prep standard mixture 461) with a quantitation performed by an area percent calculation. Results are reported as the percent mean of values obtained from at least five individual samples.

### Statistical Analysis

The Student's *t*-test and $\chi^2$ analysis were performed using INSTAT computer software (Graph Pad, San Diego, CA). The Student's *t*-test was used for the comparison of tumor multiplicity, fatty acid composition, and weight gain. $\chi^2$ analysis was used for the comparison of tumor incidence in corn and flaxseed oil–treated groups. Significance in all cases was considered at $P < 0.05$.

## Results

The effects of dietary corn and flaxseed oil on weight gain are given in Fig. 1. The average weights of the rats, in both the corn oil and the flaxseed oil groups, increased gradually over the 35-wk period. Weight gain was not significantly different between the corn and flaxseed oil groups.

The effects of dietary corn and flaxseed oil on tumor incidence are presented in Fig. 2. The percent incidence of colon tumors in the corn and flaxseed oil–treated groups were 100% and 54%, respectively. Tumor incidence in the flaxseed oil group was significantly ($P < 0.05$, $\chi^2$ test) lower than the corn oil–treated groups. The colon tumor incidence in the flaxseed oil group was almost half that of the corn oil group.

The effects of dietary corn and flaxseed oil on tumor multiplicity are shown in Fig. 3. The corn oil group had an average of 3.1 tumors per rat, whereas the flaxseed oil group had an average of 0.7 tumors per rat. The number of tumors per rat was significantly ($P < 0.05$, Student's *t*-test) higher in the corn oil–treated group than in the flaxseed oil–treated group.

The effects of dietary corn and flaxseed oil on tumor size are given in Fig. 4. The rats in the corn oil group had an average tumor size of 4.9 $cm^3$, whereas the flaxseed oil–treated group had an average tumor size of only 0.042 $cm^3$. The tumors in the corn oil group were significantly larger ($P < 0.05$, Student's *t*-test) than the tumors in the flaxseed oil–treated group.

The effects of dietary oils on serum fatty acid levels are given in Table 1. Linoleic and arachidonic acid levels were significantly ($P < 0.05$, Student's *t*-test) higher in the serum of the corn oil group than in the flaxseed oil group. α-Linolenic acid, eicosapentaenoic acid, and docosapentaenoic acid levels in the serum of the flaxseed oil group were significantly ($P < 0.05$, Student's *t*-test) higher than in the corn oil–treated group. The ratio of serum ω-6 (linoleic acid) and ω-3 (α-linolenic acid, eicosapentaenoic acid docosapentaenoic acid, and docosahexaenoic acid) fatty acids was approximately 7:1 in the corn oil–treated group. The ratio in the flaxseed oil group was approximately 1:1 for serum ω-6 and ω-3 fatty acids.

The effects of dietary oils on fatty acids in colon microsomal fractions are presented in Table 2. Linoleic and arachidonic acid levels in the colon microsomes from the corn oil group were significantly ($P < 0.05$) higher than the flaxseed oil group. α-Linolenic and eicosapentaenoic acid levels from the flaxseed oil group were significantly ($P < 0.05$) higher in the microsomes of the colon than the corn oil–treated group. The ratio of colon ω-6 (linoleic acid) and ω-3 fatty acids (α-linolenic acid, eicosapentaenoic acid,

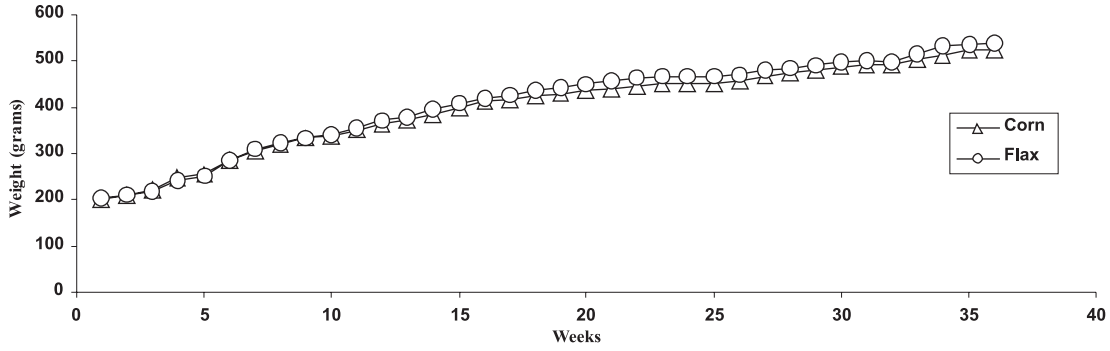


**Figure 1.** Effects of dietary oils on weight gain.

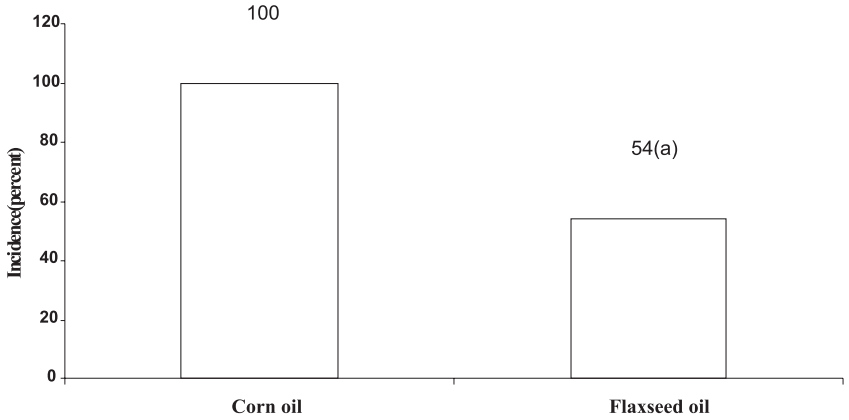


**Figure 2.** Effects of dietary oils on colon tumor incidence. The flaxseed oil group is significantly ($\chi^2$ test, $P < 0.05$) lower than the corn oil group.

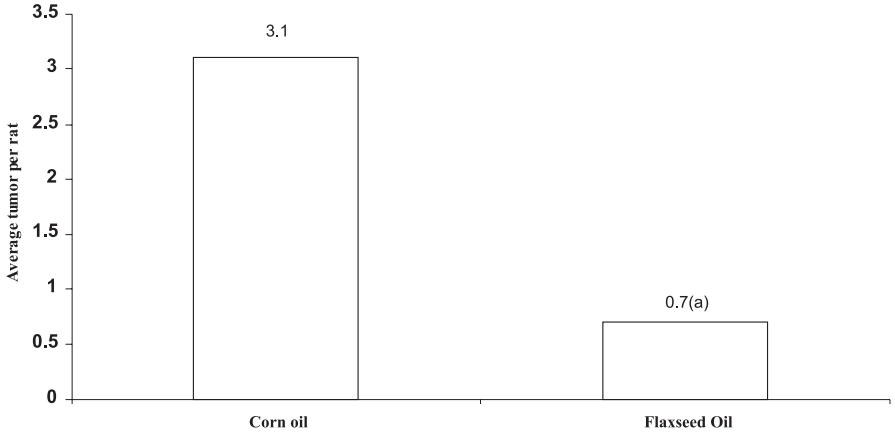


**Figure 3.** Effects of dietary oils on colon tumor multiplicity. The flaxseed oil group is significantly (Student's *t*-test, $P < 0.05$) lower than the corn oil group.

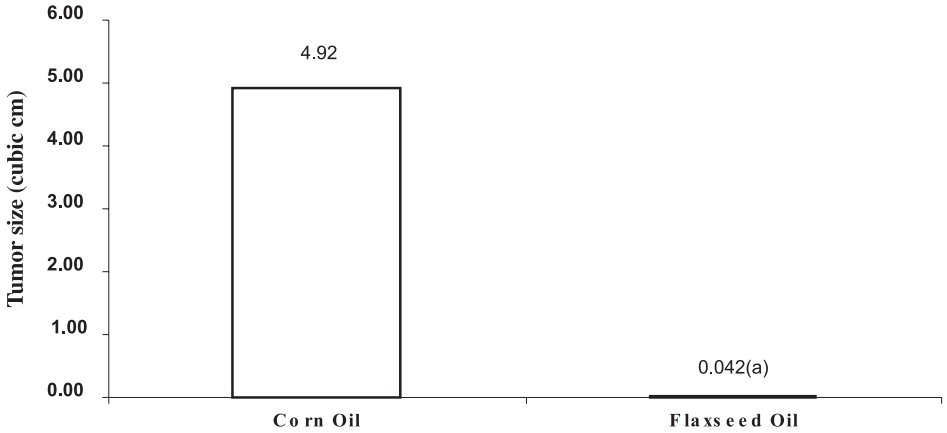


**Figure 4.** Effects of dietary oils on colon tumor size. The flaxseed oil group is significantly (Student's *t*-test, $P < 0.05$) lower than the corn oil group.

docosapentaenoic acid, and docosahexaenoic acid) was approximately 7:1 in the corn oil–treated group. The ratio of colon ω-6 and ω-3 fatty acids in the flaxseed oil group was approximately 1.25:1.

The effects of dietary oils on fatty acids in tumor microsomal fractions are given in Table 3. Linoleic and arachidonic acid levels in the corn oil group were significantly ($P < 0.05$) higher than the flaxseed oil group. α-Linolenic and docosapentaenoic acid levels in the flaxseed group were significantly ($P < 0.05$) higher than the corn oil–treated group. The ratio of ω-6 (linoleic acid) and ω-3 (α-linolenic and docosapentaenoic acid) fatty acid was approximately 206:1 in the corn oil–treated group. The ratio of ω-6 (linoleic acid) and ω-3 (α-linolenic and docosapentaenoic acid) fatty acids in the flaxseed oil group was approximately 3.2:1. Interestingly, the pattern of ω-6 and ω-3 fatty acids was similar in the tumor, and in the adjoining colonic mucosa, which appeared to be normal.

The fatty acid composition of corn and flaxseed oil used in the current study is given in Table 4. The corn oil contained 55.4% and 1.0% of linoleic and α-linolenic acids, respectively. Flaxseed oil was found to contain 14.9% and 53% of linoleic and α-linolenic acids, respectively.

## Discussion

Dietary corn and flaxseed oil produced no significant difference in the weight gain of the experimental animals (Fig. 1). Thus, food intake was not a factor in the development of colon tumor in this study.

The colon tumor incidence in the corn oil–treated group is significantly higher than the flaxseed oil–treated group. Tumors developed in only half of the rats in the flaxseed oil group, whereas in the corn oil–treated group the incidence was 100%. This shows that flaxseed oil is effective in preventing colon tumor incidence in azoxymethane-treated rats.

The average number of colon tumors per rat was about three in the corn oil–treated groups, whereas it was less than one in the flaxseed oil–treated group. This indicates that flax-

**Table 1.** Percentage Composition of Serum Fatty Acids[a]

| Fatty Acids | | Corn Oil Group | Flaxseed Oil Group |
|---|---|---|---|
| Palmitic acid | 16:0 | 20.9 | 17.6 |
| Palmitoleic acid | 16:1 | 0.3 | 0.9 |
| Stearic acid | 18:0 | 10.3 | 9.0 |
| Oleic acid | 18:1 | 14.0 | 14.5 |
| Linoleic acid | 18:2 (ω-6) | 33.4[b] | 21.3 |
| α–Linolenic acid | 18:3 (ω-3) | 0.5 | 17.3[c] |
| Arachidonic acid | 20:4 | 10.4[b] | 3.2 |
| Eicosapentaenoic acid | 20:5 (ω-3) | | 3.0[c] |
| Docosapentaenoic acid | 22:5 (ω-3) | | 2.2[c] |
| Docosahexaenoic acid | 22:6 (ω-3) | 1.4 | 1.7 |
| Other | | 9.0 | 9.0 |

*a:* Data represent mean derived from at least five samples.
*b:* Significantly higher in com oil group than flaxseed oil group ($P < 0.05$).
*c:* Significantly higher in flaxseed oil group than corn oil group ($P < 0.05$).

**Table 2.** Percentage Composition of Fatty Acids in Colon Microsomal Fraction[a]

| Fatty acids | | Corn Oil Group | Flaxseed Oil Group |
|---|---|---|---|
| Palmitic acid | 16:0 | 21.5 | 18.1 |
| Palmitoleic acid | 16:1 | 0.6 | 1.0 |
| Stearic acid | 18:0 | 13.0 | 11.4 |
| Oleic acid | 18:2 | 17.1 | 17.8 |
| Linoleic acid | 18:2 (ω-6) | 20.0[b] | 14.1 |
| α-Linolenic acid | 18:3 (ω-3) | 0.2 | 7.3[c] |
| Arachidonic acid | 20:4 | 8.5[b] | 4.0 |
| Eicosapentaenoic acid | 20:5 (ω-3) | 2.1 | 1.4 |
| Docosapentaenoic acid | 22:5 (ω-3) | 0.1 | 2.0[c] |
| Docosahexaenoic acid | 22:6 (ω-3) | 0.3 | 0.5 |
| Other | | 17.0 | 22.0 |

*a:* Data represent mean derived from at least five samples.
*b:* Significantly higher in corn oil group than flaxseed oil group ($P < 0.05$).
*c:* Significantly higher in flaxseed oil group than corn oil group ($P < 0.05$).

**Table 3.** Percentage Composition of Fatty Acids in Tumor Microsomal Fraction[a]

| Fatty Acids | | Corn Oil Group | Flaxseed Oil Group |
|---|---|---|---|
| Palmitic acid | 16:0 | 25.1 | 20.9 |
| Palmitooleic acid | 16: 1 | 0.2 | 0.6 |
| Stearic acid | 18:0 | 17.3 | 14.5 |
| Oleic acid | 18:1 | 19.O | 16.8 |
| Linoleic acid | 18:2 (ω-6) | 20.6[b] | 12.5 |
| α-Linolenic acid | 18:3 (ω-3) | 0.1 | 3.6[c] |
| Arachidonic acid | 20:4 | 5.9[b] | 3.0 |
| Eicosapentaenoic acid | 20:5 (ω-3) | 0 | 0 |
| Docosapentaenoic acid | 22:5 (ω-3) | 0 | 0.3[c] |
| Docosahexaenoic acid | 22:6 (ω-3) | 0 | 0 |
| Others | | 11.8 | 27.8 |

*a:* Data represent mean derived from at least five samples.
*b:* Significantly higher in corn oil group than flaxseed oil group ($P < 0.05$).
*c:* Significantly higher in flaxseed oil group than corn oil group ($P < 0.03$).

**Table 4.** Percentage of Fatty Acid Composition in Dietary Oils[a]

| Fatty Acids | | Corn Oil | Flaxseed Oil |
|---|---|---|---|
| Palmitic acid | 16:0 | 10.5 | 26 |
| Palmitoleic acid | 16:1 | 0.1 | 0.1 |
| Stearic acid | 18:0 | 1.9 | 5.1 |
| Oleic acid | 18:1 | 28.9 | 21.1 |
| Linoleic acid | 18:2 (ω-6) | 55.4 | 14.9 |
| α-Linolenic acid | 18:3 (ω-3) | 1.00 | 53 |
| Arachidonic acid | 20:4 | | |
| Eicosapentaenoic acid | 20:5 (ω-3) | | |
| Docosapentaenoic acid | 22:5 (ω-3) | | |
| Docosahexaenoic acid | 22:6 (ω-3) | | |
| Other | | 2.2 | |

*a:* Data represent mean derived from at least five determinations.

seed oil not only prevents the incidence but also decreases the number of colon tumors.

The average tumor size in the corn oil group is significantly larger when compared with the flaxseed oil group. This shows that the tumors in the flaxseed oil group, besides being few, were also very small in size when compared with the corn oil group.

The levels of the ω fatty acids in the serum are representative of the fatty acids present in the diets given. The serum of the corn oil group had high levels of ω-6 fatty acids with very low amounts of ω-3 fatty acid. The serum of the flaxseed oil group had ω-3 and ω-6 fatty acids in a ratio of 1:1. The ratio 1:1 of the ω-6 and ω-3 is suggested to be the optimal amount required (18). Hence, diet with flaxseed oil would provide the optimal amounts of both the ω-6 and ω-3 fatty acids.

The high levels of ω-6 fatty acids in the colon microsomal fractions of the corn oil–treated group and high levels of the ω-3 fatty acid in the flaxseed oil group indicated that the type of fatty acid present in the serum depends upon the type of fatty acid incorporated into the diet.

The tumor microsomal fractions of both the corn and flaxseed oil group had high levels of ω-6 fatty acids. The tumor microsomal fraction of the corn oil group had insignificant levels of ω-3 fatty acids. In the flaxseed oil group, the ω-6 fatty acid was about three times the ω-3 fatty acid level. This increase in the ω-6 fatty acid in the tumor microsomal fractions of both corn oil and flaxseed oil–treated groups is almost equal to both levels of ω fatty acids in the serum and colon microsomal fraction. This indicates that high levels of ω-6 fatty acids are associated with tumor development and may actually promote tumor development.

Arachidonic acid levels in the serum of the corn and flaxseed oil–treated groups indicate that about one third of the total linoleic acid in the diet is converted to arachidonic acid. The levels of arachidonic acid in serum depends upon the levels of linoleic acid in the diet. In the colon microsomal fraction, the arachidonic acid incorporated was almost equal to the serum levels in the corn oil–treated group; however, the flaxseed oil group's percentage of arachidonic acid incorporated was higher than that in the serum, indicating a preference of ω-6 over ω-3 for incorporation into cellular membranes. Higher levels of arachidonic acid were found in the tumor microsomal fractions of the corn oil group than of the flaxseed oil group. This shows that higher levels of arachidonic acid are associated with greater incidence and multiplicity of tumors.

High incidence and multiplicity of colon tumors and high levels of arachidonic acid in the colon and tumor microsomal samples of the corn oil group were observed when compared with the flaxseed oil group. These observations indicate higher incorporation of arachidonic acid in the cellular membranes of the corn oil group. The possible mechanism for enhanced tumor development in the corn oil group may have been due to the metabolism of the arachidonic acid to prostaglandins, which act as tumor promoters. Conversion of arachidonic acid to prostaglandins is mediated by the COX-2 enzyme. Hence, the enhanced tumor promotion can also be due to overexpression of the COX-2 enzyme (10–13). In the flaxseed oil group, there was higher incorporation of ω-3 fatty acids. The ω-3 fatty acids may then have been metabolized to trienoic series of prostaglandins and 5-series of leukotrienes. These have anti-inflammatory properties and, therefore, would have decreased tumor incidence and multiplicity (18). A competition among fatty acids for desaturation and chain elongation enzymes may also play a role.

Dietary oils (fish, mustard, and perilla) containing ω-3 fatty acids reduce colon tumor development (7–9,19) in experimental animals. Flaxseed has been shown to reduce colon cancer markers such as the number of aberrant crypts and aberrant crypt foci (ACF) in short term, size, and multiplicity of ACF over long term in feeding studies (20). Secoisolariciresinol diglycoside extracted from flaxseed also decreased ACF multiplicity (21). This study was performed using a moderate level (15%) of dietary flaxseed oil with 19% fat in the diet. Flaxseed oil has a high level (53%) of α-linolenic acid (an ω-3 fatty acid) and provides a high degree of protection against azoxymethane-induced colon tumor development in rats. Presumably, this is done by adjusting to the optimal ratio of ω-6 to ω-3 fatty acids. Moderate amounts of dietary flaxseed oil could be effective in reducing the risk of developing colon cancer. Further studies on the effects of dietary flaxseed oil on prostaglandin synthesis and COX expressions are needed to elucidate the mechanism of action.

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