12-1-2007

Dietary Canola Oil Suppressed Growth of Implanted MDA-MB 231 Human Breast Tumors in Nude Mice

W. Elaine Hardman
Marshall University, hardmanw@marshall.edu

Recommended Citation
Hardman, W. Elaine. "Dietary canola oil suppressed growth of implanted MDA-MB 231 human breast tumors in nude mice." Nutrition and cancer 57.2 (2007): 177-183.

Dietary Canola Oil Suppressed Growth of Implanted MDA-MB 231Human Breast Tumors in Nude Mice

W. Elaine Hardman

Abstract: Long chain omega 3 (n-3) fatty acids, eicosapentaenoic (EPA) and/or docosahexaenoic acid (DHA), have been shown to suppress growth of most cancer cells. In vivo, alpha linolenic acid (ALA, 18:3n-3) can be converted to EPA or DHA. We hypothesized that substituting canola oil (10% ALA) for the corn oil (1% ALA) in the diet of cancer bearing mice would slow tumor growth by increasing n-3 fatty acids in the diet. Sixty nude mice received MDA-MB 231 human breast cancer cells and were fed a diet containing 8% w/w corn oil until the mean tumor volume was 60 mm^3. The dietary fat of half of the tumor bearing mice was then changed to 8% w/w canola oil. Compared to mice that consumed the corn oil containing diet, the mice that consumed the canola oil containing diet had significantly more EPA and DHA in both tumors and livers, and the mean tumor growth rate and cell proliferation in the tumor were significantly slower ($P < 0.05$). About 25 days after diet change, mice that consumed the corn oil diet stopped gaining weight, whereas the mice that consumed the canola oil diet continued normal weight gain. Use of canola oil instead of corn oil in the diet may be a reasonable means to increase consumption of n-3 fatty acids with potential significance for slowing growth of residual cancer cells in cancer survivors.

Introduction

Supplementing the diet of cancer bearing mice with fish oil has been shown to reduce the growth rate of multiple tumor types and to increase the efficacy of various cancer chemotherapies (briefly reviewed in [1]). Fish oil is high in long chain (20 and 22 carbons) omega 3 polyunsaturated fatty acids. Testing of individual fatty acids in cell culture indicates that overall the eicosapentaenoic (EPA, 20:5n-3) and/or docosahexaenoic (DHA 22:6n-3) fractions of fish oil are the fractions of fish oil that are directly active for suppressing cancer cell growth via multiple mechanisms (1).

The double bond at the omega 3 position is generated in plants but not in fish or mammals. The alpha linolenic acid (ALA, 18:3n-3) that is consumed by fish from plant or algae sources is desaturated and elongated to EPA and DHA for incorporation into cell membranes of fish. Thus, even though oils derived from fish are high in EPA and DHA, plant derived dietary ALA was the source of these longer chain fatty acids.

Humans and mice are also capable of desaturating and elongating 18 carbon fatty acids, of either the n-6 or n-3 derivation, to 20 or 22 carbon fatty acids. Linoleic acid (LA 18:3n-6) is converted to arachidonic acid (AA, 20:4n-6), whereas ALA is converted to EPA, docosapentaenoic (DPA, 22:5n-3), and DHA. The efficiency of conversion of ALA to longer chain fatty acids is varying and not completely known; however, there is some evidence that conversion of ALA to longer chain fatty acids in humans may be more efficient than previously thought (2). We hypothesized that incorporating ALA in the diet of mice, at levels that humans might reasonably consume would be beneficial for slowing the growth of an implanted cancer.

In dietary studies, it seems important to use a source of ALA that could be used routinely in the diets of humans. Flaxseed oil (linseed oil) contains 50 to 60% ALA and about 15 to 20% linoleic acid, is an excellent source of ALA and consumption of flaxseed has been shown to slow

growth of cancers implanted in mice (3). However, flaxseed oil is expensive (in the range of $15 per liter), is not readily available in grocery stores, has a somewhat bitter taste, and is not recommended for cooking uses. Canola oil (rapeseed oil) costs about the same as corn oil (about $2 per liter), is readily available in grocery stores, and can be used in cooking, baking, and salad dressings. It contains about 10% ALA and 20% LA, not nearly as much ALA as flaxseed, oil, but could be a realistic, cost-effective means of increasing of omega 3 fatty acids in the human diet. Since corn oil contains 55 to 60% LA and less than 1% ALA, the routine use of canola oil instead of corn oil would improve the omega 6 to omega 3 ratio in the diet.

This study was designed to determine whether removing corn oil and substituting with canola oil in the diet would provide the cancer growth suppressive benefits linked to long chain omega 3 fatty acids.

Table 1. Diet Composition

	g/kg Diet	% of Total Energy	kcal/kg1 Dry Weight	Energy J/g2 Dry wt
Casein	200	19.3	716	3.00
Sucrose	470	45.3	1682.6	7.05
Corn starch	150	14.5	540	2.26
Alphacel	50	0	0	0
Choline bitartrate	2.0	0	0	0
DL-methionine	3.0	0	0	0
AIN-76 mineral mix	35.0	0.4	16.5	0.07
AIN-76A vitamin mix	10.0	1.0	39.2	0.16
Either corn or canola oil	80.0	19.4	720	3.01
Totals		99.9	3714.3	15.55

[1]Caloric density calculated from values supplied on Dyets, Inc., website: http://dyets.com/caloric.htm.
[2]Calculated 1kcal/g = 4.187 J/g.

Methods and Materials

Preparation of Cells for Injection

Cultured MDA-MB 231 cells (American Type Culture Collection, Rockville, MD) were harvested, rinsed, then suspended in serum-free, antibiotic-free L-15 base culture medium (American Type Culture Collection, Rockville, MD). Cells in suspension were counted using a hemocytometer, and the cell count was adjusted to 20 × 106/ml. The suspension was kept well mixed during the time of injection. MDA-MB 231 cells (1 × 106 cells in 0.05 ml of serum free media) were injected sc between the scapulae of each mouse.

Dietary Fats

Corn oil contains about 60% linoleic acid, 23% oleic acid, and 10% C16 fatty acids (>6 mg/g n-3 PUFAs). The composition of the canola oil is about 20% linoleic acid, 10% alpha lenolenic acid, and 50% oleic acid. Both corn oil and canola oil were obtained at a local grocery store and were without added antioxidant. Opened bottles of oil were stored at 4◦C.

Animals and Diet

Sixty female athymic nu/nu mice (Harlan Sprague Dawley, Inc., Madison, WI), 3 mo old, received tumor cells. The mice were housed 4/box under aseptic conditions in a temperature (24°C) and light-controlled (12 h/day) room. All animal use and handling was approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee.

The compositions of the diets are given in Table 1. All mice were fed the AIN-76A semipurified diet modified to contain 8% w/w corn oil from receipt until 3 wk after injection of cells to allow the tumors to become established. Forty-eight nude mice bearing growing MDA-MB-231 human breast carcinoma xenografts were then divided into 2 dietary groups such that the mean tumor size was not different between groups. Twenty-four mice were placed on the AIN-76A diet modified to contain 8% w/w canola oil. Diets were prepared monthly, daily portions were individually packaged and stored at −80°C in sealed containers to prevent lipid peroxidation. All mice were fed a measured amount of fresh food each day, and food remaining in the cage was discarded.

Tumor and Body Weight Measurements

Lengths, widths, and depths of the subcutaneous tumors and body weights were measured 3 times weekly using digital calipers (Fred W. Fowler Co., Newton, MA) and an electronic scale (Ohaus, Pinebrook, NJ), respectively. The instruments were connected to a laptop computer and all measurements were entered directly into an Excel spreadsheet by the instruments. The subcutaneous, mid-scapular location of the tumor allowed a good estimate of the depth of the tumor. Tumor sizes were calculated using the formula for the volume of a prolate spheriod: V = 4/3 * 3.14 * L/2 *W/2 * D/2.

Necropsy and Tissue Processing

Euthanasia was by isoflurane inhalation, then cervical dislocation. The tumor and liver were removed at necropsy. Portions of each tissue were fixed for histology, and the remaining portion was placed in an individually labeled vial and flash frozen in liquid nitrogen. At a later date, frozen livers or tumor were thawed and homogenized individually at 4°C using a Polytron homogenizer (Brinkmann Kinematica Polytron from Fisher Scientific, IL) then divided into aliquots for subsequent analyses.

Gas Chromatography (GC)

The fatty acid compositions of cell membranes of liver and tumor were determined in 10 mice that consumed each diet. Samples were individually homogenized, and membranes were separated by ultracentrifugation. Chloroform:methanol was used for lipid extraction of each fraction; lipids were methylated and esterified in acetyl chloride-methanol as described (4). Gas chromatography was done using a Hewlett Packard 5890 Series 11 Gas Chromatograph (Palo Alto, CA) and conditions of: oven temperature ramp 1 at 150°C for 15 min, ramp 2 at 175°C for

15 min, then ramp 3 at 225°C for 7 min, injector temperature at 225°C, flame-ionization detector at 250°C, helium carrier gas at 42.3 psi. Fatty acid methyl ester standards (Nu-Chek-Prep, lysian, MN) were used for peak identification. The fatty acid methyl esters were reported as the percent of the total methylated fatty acids (area under the curve).

Immunohistochemistry for PCNA and COX-2

Immunohistochemical analyses for expression of proliferating cell nuclear antigen (PCNA) and cyclooxygenase 2 (COX-2) were performed on serial 4 μm thick histologic sections of the tumor. Standard immunohistochemical technique was employed using PC-10 (Biogenex, San Ramon, CA) as primary antibody for PCNA and PG27B (anti-PGHS 2, Oxford Biomedical Research, Oxford, MI) as primary antibody for COX-2. Secondary (anti-mouse or anti-rabbit IgG linked to biotin) and labeling (streptaviden-peroxidase) antibodies were from Biogenex (SuperSensitive Detection Kit, San Ramon, CA). Diaminobenzedine was used for the label. The number of PCNA positive cells in at least 500 cells in viable areas of 10 tumors were counted to determine the PCNA positive fraction of tumors from mice that consumed each diet. The presence of COX-2 was assessed on a scale of 0 to 4 with 0 being absent and 4 being heavily staining for COX-2. An observer blinded to the diet group assessed 5 fields in viable areas of each tumor using a 40× objective.

Statistical Analyses

PRISM™ (GraphPad Software, San Diego, CA) software was used for statistical analyses. Tests for normality (basic statistics) were used on each data set. A *t* -test was used to determine statistically significant ($P \leq 0.05$) differences in parametric parameters due to incorporation of corn or canola oil into the diet. A non-parametric Mann-Whitney test was used for differences in data (COX-2 expression) that did not meet the criteria for normality. PRISM™ was also used to generate a linear regression of linear portions (the latter half) of tumor growth and body weight curves. A *t* –test was used to test for differences between the slopes of the linear portions of the body weight and tumor growth curves.

Results

Effects due to increased omega 3 in the diet would not be expected to be seen until omega 3 fats had been incorporated into cell membrane phospholipids to be available for use as substrate for cell signaling molecules. Since the diet contained such a small amount of omega 3 fatty acid, it was expected that accumulation of significant amounts of EPA and DHA in the cell membranes would take 2 to 3 wk. Mouse weight and tumor growth rates began to diverge about 25 days after canola oil was incorporated into the diet of one group of mice. Thus day 25 was used as the cut point for calculation of effects of the diet on body weight and tumor growth.

Fatty Acid Content of Diet and of Tissues

Table 2. Polyunsaturated Fatty Acid Compositions of Oils Used in the Diets (% of total fatty acids)

	Corn Oil	Canola Oil
Linoleic acid (18-3:n-6)	57.00	21.00
Alpha linolenic acid (18-3:n-6)	1.000	11.00
Arachidonic acid (20-4:n-6)	0.080	0.16
Eicosapentaenoic acid (20-5:n-3)	0.170	0.22
Docosahexaenoic acid (22-6:n-3)	0.000	0.00

As expected, gas chromatography (Table 2) demonstrated that the fat in the diet containing 8% w/w corn oil was pre dominately omega 6 linoleic acid (57%) and that the corn oil diet contained only about 1% omega 3 fatty acids in the form of ALA. The fat in the diet containing 8% w/w canola oil, however, contained 21% omega 6 linoleic acid and about 11% ALA. The n-6 to n-3 ratio of the corn oil diet was 57:1 and of the canola oil diet was about 2:1. Neither the corn oil nor the canola oil contained appreciable amounts of either eicosapentaenoic (EPA) or docasahexaenoic (DHA). The polyunsaturated fatty acid composition of the tumor and liver changed significantly due to the diet (Figs. 1A and 1B). The EPA and DHA (omega 3 fatty acids) content of both tumor and liver were significantly ($P < 0.05$) higher and linoleic and arachidonic acid (omega 6 fatty acids) were significantly ($P < 0.05$) lower in mice that consumed the canola oil diet than in mice that consumed the corn oil diet. Since the diet did not contain EPA or DHA, the source of the increased EPA and DHA in mice that consumed the canola oil diet must have been conversion from dietary ALA.

Mouse Body Weights

Figure 2 illustrates the mouse body weights. The mean body weights of the 2 groups of mice may began to diverge about day 12 but have certainly diverged by day 25. The initial body weights of the 2 groups of mice are not different, but at the end of the study, the final body weights were significantly different. The rates of gain for the last 32 days were also significantly different. The group that consumed canola oil continued to gain weight at about the same rate, 0.06 ± 0.02 g/day (mean ± SD) as during the first 25 days. However, linear regression from day 25 to day 57 indicated that the group that consumed the corn oil diet gained less weight. The rate of weight gain from day 25 to day 57 was 0.02 ± 0.02 g/day, a rate that was significantly ($P<0.05$) less than the rate of weight gain of the canola oil group.

Growth Rate of the Tumors

The growth of the tumor was not expected to be altered until omega 3 dietary fatty acids (especially EPA and DHA) had accumulated in cell membranes. The tumor growth curves (Fig. 3) show that perhaps by day 10 but certainly by day 25, the tumor growth rates of the 2 groups began to diverge indicating that sufficient omega 3 had accumulated for the tumor growth to be affected. The tumor growth rates for the first 25 days are not different and the complete tumor growth curves are obviously not linear if assessed from the first day of diet change until the end of the experiment. However, the growth curve is approximately linear (see Fig. 3) from day 25 until the end of the experiment and linear regression of tumor growth from day 25 to day 57 (the end of the experiment) was used to estimate the tumor growth rate. The mean (± SEM) tumor growth rate of the mice that consumed the corn oil diet was 7.5 ± 0.8 mm3/day, whereas the mean tumor growth rate of mice that consumed the canola oil diet was 3.2 ± 0.4 mm3/day. A *t* - test of growth rates from day 25 to day 57 indicated that the mean growth rates of the two groups were significantly different, $P < 0.001$.

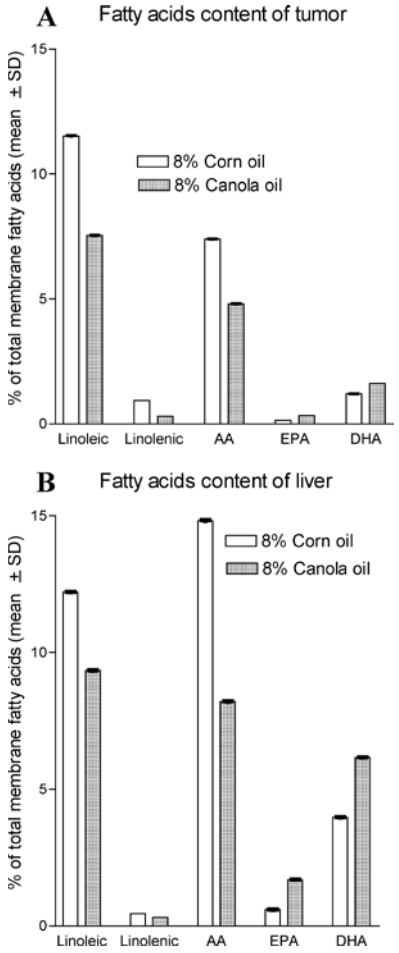

Figure 1. Fatty acid compositions of the tumor (A) and liver (B) of mice fed diets containing either 8% corn oil or 8% canola oil. The percent of the total membrane fatty acids (means ± SEM) of major omega 6 (linoleic and arachidonic) and omega 3 (linolenic, eicosapentaenoic, and docosahexaenoic) fatty acids are shown. Error bars are given, but are so small that they may disappear into the top of the column.

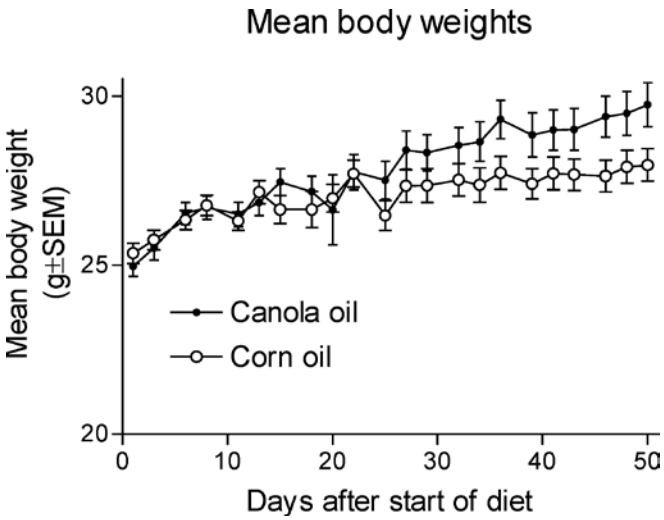

Figure 2. Mean body weights (g ± SEM) of tumor bearing mice that consumed diets containing 8% corn oil or 8% canola oil. Day 0 is the day of diet change.

Proliferation and Tumor Volume

Proliferation was significantly (p = .0025) slower in tumors of the mice that consumed the canola oil diet. The PCNA positive fraction in viable areas of the tumors of mice that consumed the corn oil diet was 8.8 ± 2.7% (mean % ± SD) and in the tumors of mice that consumed the canola oil diet the fraction was 4.1 ± 2.2%.

At the time of euthanasia, the mean volume of the tumors of the group that consumed canola oil (126 mm^3) was less than 1⁄2 the mean volume of the tumors of the group that consumed corn oil (297 mm^3). This was not quite significantly (p = 0.12) different. The time of euthanasia was determined by the time at which several mice in the corn oil fed group had

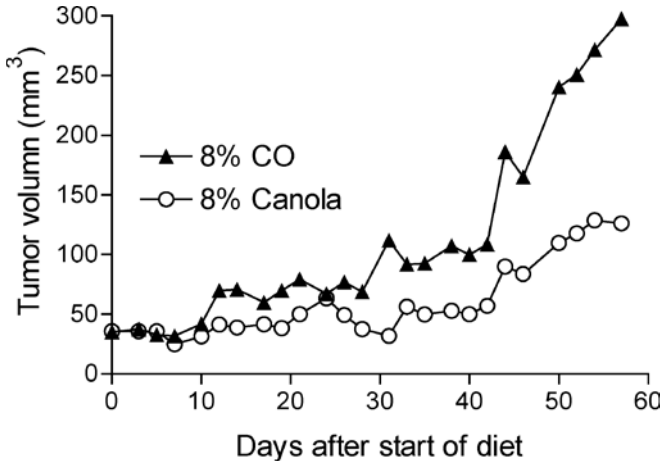

Figure 3. Mean tumor volume (mm^3) of mice that consumed diets containing 8% corn oil or 8% canola oil. Day 0 is the first day of the canola oil diet. The linear regression lines superimposed on the data illustrate the mean growth rate of the tumors of each group from day 25 until the end of the study. The rates of growth of the tumors of the mice that consumed corn oil is significantly (P <0.05 by t -test of the slopes of the linear regressions) greater than the rate of growth of the tumors of mice that consumed canola oil.

tumors so large that euthanasia was required. Given the large difference in tumor growth rates and in tumor cell proliferation between the two groups, had the experiment been continued, the final volumes would have been significantly different.

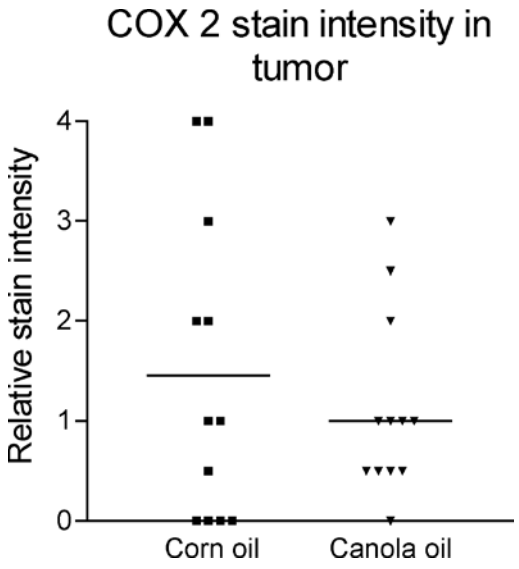

Figure 4. The mean COX-2 stain intensities in viable areas of the tumor were not significantly different due to the diet of the mice.

COX-2 in the Tumor

Long chain omega 3 fatty acids have been shown to suppress COX-2 expression in tumors. Immunohistochemistry was used for semi-quantitative assessment of the expression of COX-2 in the tumor (Fig. 4). A Mann-Whitney test showed that there was not a significant difference in the median stain due to the diet of the mice.

Discussion

The composition and amount of dietary fat has been shown to alter the tumor growth rate in numerous animal studies (briefly reviewed in [1]). High amounts of dietary omega 6 fatty acids (especially linoleic acid) increase the growth rate of most tumor types compared to the growth rate of the tumor with lower amounts of omega 6 fatty acids in the diet. Consumption of long chain omega 3 fatty acids (i.e., fish oils containing EPA and DHA) has been shown to result in slower tumor growth than consumption of an equivalent amount of omega 6 fatty acids in the diet (5). Flaxseed at 10% w/w of the diet has been shown to slow growth of human breast cancers in animal models (3). The fat composition of that diet was not reported. However the fat composition of that diet can be approximated since flaxseed contains 54% fat. Flaxseed oil contains about 55% ALA thus about 30% w/w of the flaxseed or about 3% w/w of that diet was

ALA. In comparison about 0.8% of the test diet for this study was ALA. Humans do not consume 20% w/w of the diet as fish oil or as flaxseed, although a small amount of these sources of omega 3 fatty acids can be added to the diet.

We hypothesized that a dietary change that humans might reasonably make, that of using canola oil instead of corn oil in the diet, would make a difference in the growth rate of cancers. We selected an 8% w/w corn oil diet as one that would include an amount of linoleic acid that is a more reasonable estimate for human linoleic acid consumption than found in the 20% w/w corn oil diets used in many previous experiments. A value that has been used for the average American diet is that 6.6% of energy is from LA (2). The corn oil diet in this study contained 9.7% energy from LA, whereas the diet containing 20% w/w corn oil would derive about 24% of energy from linoleic acid. Substituting canola oil for the corn oil in the diet reduced the amount of LA and decreased the n-6 to n-3 ratio from about 57:1 to 2:1. To our knowledge, this is the first report indicating that substituting canola oil for the corn oil in the diet may have benefit for slowing the growth of cancers.

We expected that the ALA of the canola oil would have to be elongated and desaturated to EPA and DHA before there would be any effect on tumor growth. There was almost no EPA or DHA in the dietary fat. The data indicate that the ALA in the canola oil was elongated and desaturated for incorporation into the membrane phospholipids as EPA and DHA since the fatty acid compositions of both tumor and liver of mice that consumed the canola oil diet contained significantly more EPA and DHA than did the tumor and liver of mice that consumed the corn oil diet. Surprisingly, ALA was slightly decreased in the tissues of the group that consumed canola oil. This may indicate that most ALA was being converted to EPA and DHA rather than being stored as ALA. As expected, the LA and AA acid content of the tumor and liver of the group that consumed canola oil was significantly less than that of the group that consumed canola oil.

Consumption of long chain omega 3 fatty acids has been associated with decreased tumor associated cachexia (6–9). The graph of body weights (Fig. 2) indicates that the mean body weights of the 2 groups began to diverge about 25 days after the start of the dietary interventions. This is also about the same time as the tumor sizes began to diverge. Since tumors were larger in the group that consumed the corn oil containing diet, if the weight of the tumor had been subtracted, the differences between the mean body weights of the two groups would have been even more pronounced. We hypothesize, but do not know, that body weight gain continued in the group that consumed canola oil because the tumor size was smaller and there was less production of cytokines related to cachexia (9–13). This is an area to be investigated in the future.

Once omega 3 fatty acids were incorporated into the cell membranes of the mouse, the tumor growth rates began to diverge. During the second half of the project, the mean growth rate of the tumors of the mice that consumed canola oil was significantly less than the growth rate of the tumors of the mice that consumed corn oil. Our analyses of proliferation by PCNA immunohistochemistry indicated that a likely cause of the slower growth of the tumor was the decrease in tumor cell proliferation. The tumor volumes were not quite significantly different at the end of this study, however given the significant differences in growth rates and tumor cell proliferation, had the study continued for the longer time, the final tumor volumes would have been significantly different. Long chain omega 3 fatty acids have been shown to suppress proliferation in other studies (for example, [14]).

The expression of COX-2was slightly but not significantly suppressed in the tumor as assessed by semi-quantitative analyses of immunohistochemical staining. The proliferation

promoting eicosanoid, PGE_2 is a product of COX-2 action on AA, whereas proliferation suppressing PGE_3 is a product of COX-2 action on EPA. Production of PGE_2 or PGE_3 could not be assessed in this study; however, one does wonder if the combination of reduced COX-2 activity and decreased AA would have significantly reduced the amount of PGE_2 present in the tumor. SinceCOX-2 is at the beginning of a signaling cascade, further studies should be done to determine whether decreasedCOX-2 expression (even though not mathematically significant) could have biological significance for suppression of cytokines that promote cell proliferation.

There are 2 sets of reports of potentially detrimental effects of canola oil consumption that must be acknowledged and weighed with the evidence in this report. One set involves feeding canola (rapeseed) oil to stroke-prone spontaneously hypertensive rats (15–19). In these reports, canola oil consumption has been associated with reduced lifespan of the rats. These rats have a deficiency in membrane cholesterol that makes red cell membranes weak and fragile (17). Thus, the rats are very sensitive to reduced dietary cholesterol and the phytosterols in canola oil can inhibit the absorption of dietary cholesterol (17). Increase consumption of phytosterols was associated with early death of the rats in two reports (15,17). The renal injury seen in one report (18) is also consistent with fragile cells. Reduced dietary cholesterol is not usually a problem in humans, and one author commented that there was no direct evidence that the effects in rats related to human nutrition (19).

The other set of reports involves an increased risk for lung cancer among Chinese women who used unpurified canola oil (20–23) for stir frying. In these reports the increased risk was associated with excessive exposure to smoke from the high cooking temperatures. It is not clear if the carcinogens generated were from the meat being stir fried by generation of heterocyclic amines (22) or from carcinogens associated with the oil, though it was indicated that the risk seemed to increase with the use of canola oil, (21,23). It is unlikely that canola oil used in stir frying would effective suppress tumor growth, since the oil ingested would be highly oxidized and broken down to shorter chain fatty acids.

Conclusions

These data indicate that:

1. Substituting canola oil for the corn oil in the diet can serve as a source of omega 3 fatty acids for suppression of tumor growth. The mechanisms for growth suppression were not identified and remain for further study.

2. Gas chromatography indicated that the α-linolenic acid obtained from the canola oil was effectively converted to EPA and DHA for incorporation into tumor and host tissues.

The importance of this study is that increasing the consumption of canola oil, a source of omega 3 fatty acids, and reducing the consumption of corn oil is a dietary change that could easily be incorporated into the Western diet. It has been proposed that omega 3 fatty acids may be effective cancer preventive agents (24). Especially in conjunction with increased fish consumption, diet recommendations to include canola oil could reduce the risk for or slow the growth rate of cancer in future generations.

Acknowledgments and Notes

The author gratefully acknowledges the excellent technical assistance of Paige McCown and Kathryn Blalock. Sources of support: The American Institute for Cancer Research and an Institutional Grant from Pennington Biomedical Research Center. This work was accomplished at Pennington Biomedical Research Center, Baton Rouge, LA 70808. Address correspondence to W. Elaine Hardman is affiliated with the Department of Biochemistry and Microbiology, Marshall University School of Medicine 1542 Spring Valley Dr. Huntington, WV 25701. Phone: 304-696-7339, FAX: 304-696-7207. E-mail: hardmanw@marshall.edu

References

1. HardmanWE: (n-3) fatty acids and cancer therapy. *J Nutr* 134, 3427S–3430S, 2004.
2. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, and Kris-Etherton PM: Dietary α-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *J Nutr* 134, 2991–2997, 2004.
3. Chen J, Stavro PM, and Thompson LU:Dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epidermal growth factor receptor. *Nutr Cancer* 43, 187–192, 2002.
4. Ackman RG: Simplification of analyses of fatty acid in fish lipids and related lipid samples. *Acta Med Scand* 222, 99–103, 1987.
5. Connolly JM, Coleman M, and Rose DP: Effects of dietary fatty acids on DU145 human prostate cancer cell growth in athymic nude mice. *Nutr Cancer* 29, 114–119, 1997.
6. Dagnelie PC, Bell JD, Williams SC, Bates TE, Abel PD, and Foster CS: Effect of fish oil on cancer cachexia and host liver metabolism in rats with prostate tumors. *Lipids* 29, 195–203, 1994.
7. Moses AW, Slater C, Preston T, Barber MD, and Fearon KC: Reduced total energy expenditure and physical activity in cachexia patients with pancreatic cancer can be modulated by an energy and protein dense oral supplement enriched with n-3 fatty acids. *Br J Cancer* 90, 996–1002, 2004.
8. Tisdale MJ and Dhesi JK: Inhibition of weight loss by omega-3 fatty acids in an experimental cachexia model. *Cancer Res* 50, 5022–5026, 1994.
9. Tisdale MJ: The effects of eicosapentaenoic acid on tumour growth and cachexia in the MAC16 mouse colon cancer model. In: D.F. Horrobin (ed), *New approaches to cancer treatment: Unsaturated lipids and photodynamic therapy* pp 30–39, London: Churchill Livingston, 1995.
10. Beck SA and Tisdale MJ: Lipid mobilizing factors specifically associated with cancer cachexia. *Br J Cancer* 63, 846–850, 1991.
11. Guttridge DC,MayoMW, Madrid LV,Wang C-Y, and Baldwin AS; Jr.: NF-κB-induced loss of *MyoD* messenger RNA: possible role in muscle decay and cachexia. *Science* 289, 2363–2366, 2000.
12. Tisdale MJ: Mechanism of lipid mobilization associated with cancer cachexia: interaction between the polyunsaturated fatty acid, eicosapentanoic acid, and inhibitory guanine nucleotide-regulatory protein. *Prostaglandins Leukot Essent Fatty Acids* 48, 105–109, 1993.

13. Whitehouse AS, Smith HJ, Drake JL, and Tisdale MJ: Mechanism of attenuation of skeletal muscle protein catabolism in cancer cachexia by eicosapentaenoic acid. *Cancer Res* 61, 3604–3609, 2001.
14. Schley PD, Jijon HB, Robinson LE, and Field CJ: Mechanisms of omega-3 fatty acid-nduced growth inhibition inMDA-MB-231 human breast cancer cells. *Breast Cancer Res Treat* 92, 187–195, 2005.
15. Ratnayake WM, Plouffe L, Hollywood R, L'Abbe MR, Hidiroglou N, Sarwar G, and Mueller R: Influence of sources of dietary oils on the life span of stroke-prone spontaneously hypertensive rats. *Lipids* 35, 409–420, 2000.
16. Huang MZ, Naito Y, Watanabe S, Kobayashi T, Kanai S, Nagai H, and Okuyama H: Effect of rapeseed and dietary oil on the mean survival time of stroke-prone spontaneously hypertensive rats. *Biol Pharm Bull* 19, 554–557, 1996.
17. Ratnayake WMN., L'Abbe MR, Mueller R, Hayward S, Plouffe L, Hollywood R, and Trick K: Vegetable oils high in phytosterols make erythrocytes less deformable and shorten the life span of stroke-prone spontaneously hypertensive rats. *J Nutr* 130, 1166–1178, 2000.
18. Miyazaki M, Takemura N, Watanabe S, Hata N, Misawa Y, and Okuyama H: Dietary docosahexaenoic acid ameliorates, but rapeseed oil and safflower oil accelerate renal injury in stroke-prone spontaneously hypertensive rats as compared with soybean oil, which is associated with expression for renal transforming growth factor-beta, fibronectin and renin. *Biochim Biophys Acta* 1483, 101–110, 2000.
19. Tatematsu K, Fuma S-Y., Satoh J, Ichikawa Y, Fujii Y, and Okuyama H: Dietary canola and soybean oil fed to SHRSP rat dams differently affect the growth and survival of their male pups. *J Nutr* 134, 1347–1352, 2004.
20. Gao YT, Blot WJ, ZhengW, Ershow AG, Hsu CW, Levin LI, Zhang R, and Fraumeni JF, Jr.: Lung cancer among Chinese women. *Int J Cancer* 40, 604–609, 1987.
21. Metayer C,Wang Z, Kleinerman RA,Wang L, Brenner AV, Cui H, and Lubin JH: Cooking oil fumes and risk of lung cancer in women in rural Gansu, China. *Lung Cancer* 35, 111–117, 2002.
22. Seow A, Poh WT, Teh M, Eng P, Wang YT, Tan WC, Yu MC, and Lee HP: Fumes from meat cooking and lung cancer risk in Chinese women. *Cancer Epidemiol Biomarkers Prev* 9, 1215–1221, 2000.
23. Zhong L, Goldberg MS, Gao YT, and Jin F: Lung cancer and indoor air pollution arising from Chinese-style cooking among nonsmoking women living in Shanghai, China. *Epidemiology* 10, 488–494, 1999.
24. Rose DP and Connolly JM: Omega-3 fatty acids as cancer chemopreventive agents. P*harmacology & Therapeutics* 83, 217–244, 1999.