

# International Research Conference on Food, Nutrition & Cancer

## Omega-3 Fatty Acids to Augment Cancer Therapy<sup>1</sup>

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**ABSTRACT** The results of animal studies have demonstrated that the consumption of omega-3 fatty acids can slow the growth of cancer xenografts, increase the efficacy of chemotherapy and reduce the side effects of the chemotherapy or of the cancer. Molecular mechanisms postulated to contribute to the multiple benefits of omega-3 fatty acids include 1) suppressing the expression of cyclooxygenase-2 in tumors, thus decreasing proliferation of cancer cells and reducing angiogenesis in the tumor; 2) decreasing the expression of *AP-1* and *ras*, two oncogenes implicated in tumor promotion; 3) inducing differentiation of cancer cells; 4) suppressing nuclear factor- $\kappa$ B activation and *bcl-2* expression, thus allowing apoptosis of cancer cells; and 5) reducing cancer-induced cachexia. It seems reasonable to assume that after appropriate cancer therapy, consumption of omega-3 fatty acids might slow or stop the growth of metastatic cancer cells, increase longevity of cancer patients and improve their quality of life. J. Nutr. 132: 3508S–3512S, 2002.

**KEY WORDS:** • cancer • cancer survival • omega-3 fatty acids • nutrition

The results of animal studies have demonstrated that the consumption of omega-3 fatty acids can slow the growth of cancer xenografts, increase the efficacy of chemotherapy and reduce the side effects of the chemotherapy or of the cancer (1–6). Epidemiologic studies indicate that populations that consume high amounts of omega-3 (n-3) fatty acids have lower incidences of breast, prostate and colon cancers than do those that consume less n-3 fatty acids. Many of the mechanisms that are thought to slow or prevent the growth of cancers may also be postulated to slow or prevent the growth of metastatic or residual cancer cells. Thus increasing the consumption of n-3 fatty acids may be a nontoxic way to augment cancer therapy and to significantly increase life span. This summary is not intended to be a complete review of all the mechanisms of action of n-3 fatty acids but will highlight some of the important actions of n-3 fatty acids to inhibit cancer cell growth.

### Background

Fatty acids are hydrocarbon chains with a carboxyl group at one end. In saturated fatty acids, all of the carbons are connected by single bonds, whereas unsaturated fatty acids have some carbons connected by double bonds. The difference

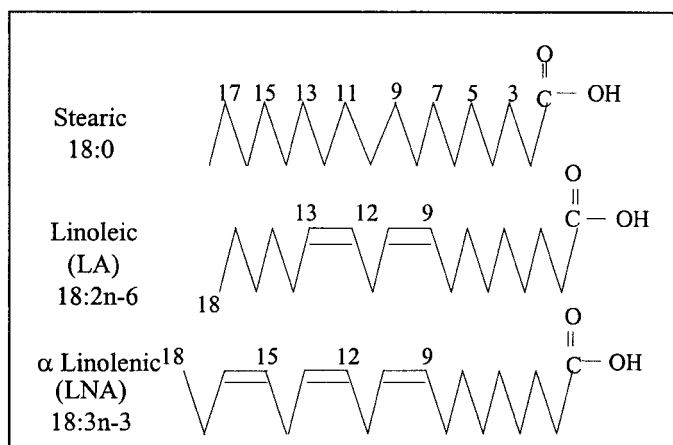
between n-3 and n-6 fatty acids is that n-3 fatty acids have the first double bond three carbons from the methyl end of the carbon chain and n-6 fatty acids have the first double bond six carbons from the methyl end (**Fig. 1**). Because humans cannot desaturate the n-3 or the n-6 bond, both n-3 and n-6 fatty acids are essential fatty acids (EFAs)<sup>3</sup> that must be obtained from dietary sources. Most n-6 fatty acid is consumed as linoleic acid (LA) [18 carbons, two double bonds, n-6 or 18:2(n-6)] primarily from vegetable oils, especially corn, safflower and soybean oils, and meat, but some arachidonic acid (AA) [20:4(n-6)] is also obtained from meats (7). n-3 fatty acids may be found in vegetable oils, especially canola and soybean oil, and green leafy vegetables as  $\alpha$ -linolenic acid (LNA) [18:3(n-3)] and in larger amounts in fatty cold-water fish as eicosapentaenoic acid (EPA) [20:5(n-3)] or docosahexaenoic acid (DHA) [22:6n-3]. Both n-3 and n-6 fatty acids may be incorporated into cell membrane phospholipids as consumed in the diet or may be elongated and desaturated to longer-chain fatty acids of the same series (8), but n-3 and n-6 fatty acids cannot be interconverted. The same enzymes use both n-3 and n-6 fatty acids as substrate for subsequent production of various cytokines. All three major n-3 fatty acids—LNA, EPA and DHA—suppress the production of AA from LA by competing more successfully than LA for the activity of the  $\Delta 5$  and  $\Delta 6$  desaturases (9).

Some information about the synthesis of eicosanoids (i.e., cell-signaling molecules derived from 20-carbon fatty acids) is also important to understanding the activity of n-3 and n-6 fatty acids. The 20-carbon fatty acids (EPA and AA) are

<sup>1</sup> Presented as part of a symposium, "International Research Conference on Food, Nutrition & Cancer," given by the American Institute for Cancer Research and the World Cancer Research Fund International in Washington, D.C., July 11–12, 2002. This conference was sponsored by BASF Aktiengesellschaft; California Dried Plum Board; The Campbell Soup Company; Danisco Cultor; Galileo Laboratories, Inc.; Mead Johnson Nutritionals; Roche Vitamins, Inc.; and Yamanochi/Shaklee/INOBYs. Guest editors for this symposium were Helen Norman and Ritva Butrum, American Institute for Cancer Research, Washington, D.C. 20009.

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<sup>3</sup> Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; LNA, linolenic acid; LOX, lipoxigenase; NF- $\kappa$ B, nuclear factor- $\kappa$ B; PG, prostaglandin.



**FIGURE 1** Structure and nomenclature of fatty acids. Stearic acid is an 18-carbon saturated fat. Linoleic acid (LA) is an 18-carbon fatty acid with two unsaturated bonds and with an unsaturation in the n-6 position; thus the abbreviation is 18:2n-6. Alpha-linolenic acid (LNA) is the 18-carbon n-3 fatty acid.

cleaved from cell membrane phospholipids by phospholipase  $A_2$  and can be used to produce eicosanoids. Cyclooxygenase (COX) activity on EPA or AA results in the production of prostaglandins or thromboxanes; lipoxygenase (LOX) activity results in the production of leukotrienes. COX or LOX activity on AA produces eicosanoid products that tend to be proinflammatory and proliferative in most tissues. COX or LOX activity on EPA produces a different series of eicosanoid products that tend to be less inflammatory and less promotional to proliferation in most tissues. COX has two isozymes: COX-1 and COX-2. COX-1 is constitutively produced by many cell types. COX-2 is induced in response to inflammation and is not detectable in most normal, noninflamed tissues. However, COX-2 is increased in a variety of human cancers, including epidermal (10), hepatocellular (11), cervical (12) and pancreatic (13) cancer; squamous carcinoma of the esophagus (14); bladder transitional cell carcinoma (15); colon cancer (16); and breast cancer (17,18). There is interest in the use of COX-2-specific inhibitors as a strategy for cancer prevention and in combination with cancer chemotherapy (19–22).

#### How might n-3 fatty acids improve survival of cancer victims?

Many mechanisms seem to be operational in n-3 fatty acid suppression of tumor growth. Most experimental studies have been conducted on primary cancers, but it seems reasonable that these same mechanisms might suppress the growth of metastatic cancer cells.

**Changes in eicosanoid metabolism.** n-3 fatty acids can inhibit the induction of COX-2 (23,24). **Figure 2** illustrates the suppression of COX-2 in MDA-MB 231 human breast cancer xenografts in nude mice with n-3 fatty acids in their diet. COX inhibitors have been used to suppress the growth of colon cancers (25,26), demonstrating that suppression of COX-2 may be a useful strategy to slow growth of metastatic tumors.

DHA and EPA effectively compete with AA for COX activity, resulting in the production of three-series prostaglandins and five-series leukotrienes that tend to promote inflammation or proliferation less than the eicosanoids produced from AA (27). Thus n-3 fatty acids may be beneficial by

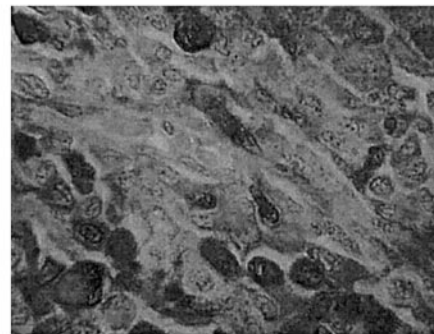
reducing production of COX-2 and by changing the type of product produced by COX-2.

However, there is also evidence that n-3 fatty acids suppress COX-2 expression by inhibiting nuclear factor- $\kappa$ B (NF- $\kappa$ B) (**Fig. 3**). NF- $\kappa$ B is the transcription factor that induces the expression of inflammatory response cytokines, including interleukins-1 and -6, COX-2 and tumor necrosis factor  $\alpha$  and of growth factors such as interleukin-2 and granulocyte colony stimulating factor (28). Constitutive activation of NF- $\kappa$ B in cancers seems to play a role in tumor growth and cancer cell survival (28). Interleukins-1 and -6 and tumor necrosis factor  $\alpha$  may play a role in the cachexia associated with many cancers (29,30). Thus suppression of the activation of NF- $\kappa$ B by n-3 fatty acids not only reduces the production of proliferative eicosanoids produced by COX-2 but also suppresses the production of other NF- $\kappa$ B-induced cytokines that promote cancer cell growth and that may be detrimental to the cancer victim.

**Suppression of mitosis.** Cancer cells must multiply for a tumor or metastatic site to grow. At least three mechanisms have been identified by which n-3 fatty acids suppress mitosis. LA and AA activate protein kinase C (31) and induce mitosis but EPA and DHA appear to reverse the protein kinase C activity changes associated with colon carcinogenesis (32,33). n-3 fatty acids decrease the activity of *ras* (34) and AP-1 (35) oncogenes that are frequently activated in cancers and that stimulate mitosis. The AA products of COX and LOX increase mitosis; EPA and DHA decrease mitosis and inhibit growth of breast and colon cancers (36–39).

**Restoring functional apoptotic pathways to help control cancer growth.** Apoptosis is programmed cell death. When

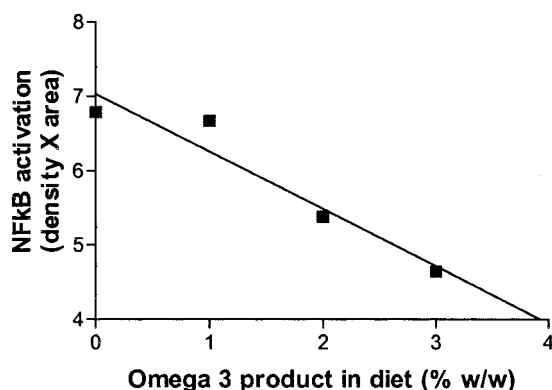
A.



B.



**FIGURE 2** Suppression of cyclooxygenase (COX)-2 expression by n-3 fatty acids: immunohistochemical localization of COX-2 (dark stain) in MDA-MB 231 xenografts grown in nude mice. (A) Tumor from a mouse fed a diet containing 5% by weight (wt/wt) corn oil. (B) Tumor from a mouse fed a diet containing 2% w/w corn oil and 3% w/w of an n-3 fatty acid product containing ~63% n-3 fatty acids.



**FIGURE 3** Suppression of the activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B) by n-3 fatty acids. NF- $\kappa$ B in the nuclear fraction is considered to be activated NF- $\kappa$ B. This figure shows the mean results of electrophoretic mobility shift assay of NF- $\kappa$ B in the nuclear fraction of livers of mice fed 8% w/w dietary fat. Part of the dietary fat was corn oil (8–5% in decreasing amounts); the remainder of the dietary fat was an n-3 fatty acid product (0–3% wt/wt in increasing amounts) containing 63% n-3 fatty acids. Equal amounts of protein were placed in each lane ( $n = 2$  or 3 per diet). The density of the bands on the gel shift was quantified using NIH Image software, and NF- $\kappa$ B was calculated as a product of the area of each band and the density of each pixel.

apoptotic pathways are functional, cells with unreparable genetic damage should die. However, apoptotic pathways are frequently disrupted in cancers. Increased COX-2 expression alone has been shown to block apoptosis (40). In addition, NF- $\kappa$ B is frequently activated in cancer cells and NF- $\kappa$ B activation has been shown to block apoptosis (28). Thus blocking of NF- $\kappa$ B and COX-2 activation by n-3 fatty acids would be expected to contribute to restoration of apoptosis. When activated, genes of the *Bcl-2* family can block apoptosis. DHA has been reported to inactivate *Bcl-2* family genes and increase transcription of genes and transcription factors that induce apoptosis (41,42).

**Induction of differentiation.** Terminally differentiated cells do not multiply. n-3 fatty acids have been shown to induce differentiation of breast cancer cells (43).

**Suppression of angiogenesis.** Angiogenesis, the growth of new blood vessels, must occur for cancers to grow. The n-6 products of COX and LOX stimulate angiogenesis; the n-3 products of COX and LOX do not stimulate angiogenesis (33,44,45).

**Altering estrogen metabolism.** The promotion of breast cancer by estrogen is well known. Perhaps less well known is that prostate and colon cancers also exhibit estrogen receptors and that the growth of prostate and colon cancers can be promoted by estrogen (46,47). Prostaglandin (PG) $E_2$ , an AA product, activates P450 aromatase, increasing estrogen production (48). PGE $_3$ , an EPA product, does not activate P450 aromatase. Thus a decrease in PGE $_2$  and an increase in PGE $_3$  would be expected to decrease estrogen production and decrease stimulation of cell growth. A shift in estrogen metabolism toward 16 $\alpha$ -hydroxylation increased the formation of aberrant hyperproliferation in mammary explant cultures (49). In a clinical study, an n-3 dietary supplement decreased 16 $\alpha$ -hydroxylation (50) in human breast tissue and would be expected to decrease hyperproliferation in breast tissues.

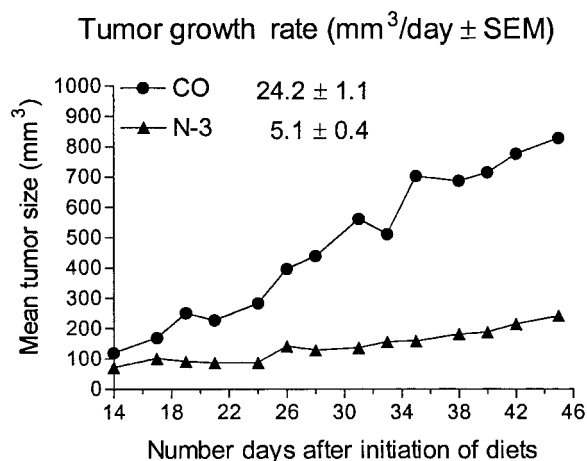
In summary, the results of experimental studies show that n-3 fatty acids may be detrimental to the growth of metastatic or residual cancer cells by altering eicosanoid metabolism, slowing cancer cell mitosis, increasing cancer cell death, in-

ducing differentiation, suppressing angiogenesis and altering estrogen metabolism. Numerous animal studies illustrate that incorporating n-3 fatty acids into the diet of mice suppressed the growth of chemically induced cancers or implanted human xenografts (36,51–63)., **Figure 4** illustrates the 75% suppression of the tumor growth rate seen when an n-3 fatty acid supplement was incorporated in the diet of mice bearing an MDA-MB 231 human breast cancer xenograft.

#### Reduction of cancer risk by consumption of n-3 fatty acids

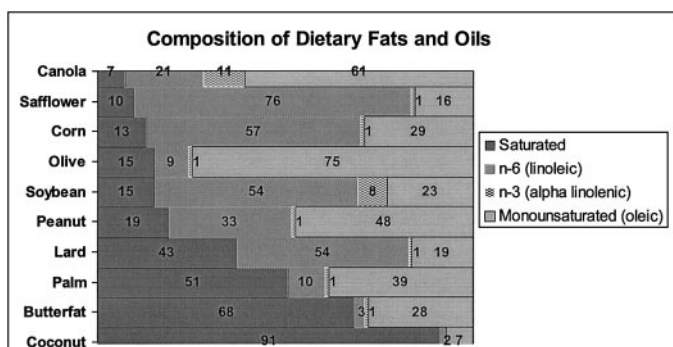
Evidence is accumulating that consumption of n-3 fatty acids can reduce cancer risk. In animal studies, the incidence of carcinogen-induced cancer has been reduced by supplementing the diet of the animal with n-3 fatty acids (36,55,58,61,64,65,66). Breast cancer incidence in Japanese women increases within one generation after migration to the United States (66). The incidence of breast, prostate and colon cancers is increasing in Japan (67) and in Alaskan natives (66) as the cultures adopt a Western diet, decrease fish intake and increase dietary intake of n-6 fatty acids.

Both animal and epidemiologic studies indicate that the ratio of n-3 to n-6 fatty acids in the diet is particularly important to the reduction of cancer risk. In animal studies the ratio of 1.2:1 has reduced cancer incidence (68). n-3 consumption was not significantly correlated to cancer incidence, but the ratio of n-3 to n-6 fatty acids in the diet was significantly inversely correlated to breast cancer incidence in three of four European countries examined in one study (69) and in France (70). In the French study, the odds ratio for breast cancer in the quartile with the highest ratio of n-3 to n-6 fatty acids consumed was 0.3 compared with an odds ratio of 1 for the quartile with the lowest the ratio of n-3 to n-6 fatty acids (70). Because these are epidemiologic studies there is no way to tell whether the reduced risk for cancer was due to reduced cell



**FIGURE 4** Mean growth rate of MDA-MB 231 xenografts in nude mice. After the tumors were ~5 mm in diameter, the diets of groups of tumor-bearing mice ( $n = 10$  per group) were changed to diets containing either 5% w/w corn oil or 2% w/w corn oil plus 3% w/w of an n-3 fatty acid product containing ~63% n-3 fatty acids. Tumor dimensions were measured 3 times weekly with calipers. Mean tumor size at each time is shown; the graph starts at day 14 after dietary change. Linear regression analysis using the individual data from each mouse was used to determine the tumor growth rates;  $t$  tests of the tumor growth rates showed that the growth rate of the tumors of mice fed the diet containing n-3 fatty acid was significantly less than the growth rate of the tumors of mice fed the corn oil diet ( $P < 0.05$ ). Data from Hardman et al. (4).





**FIGURE 5** Comparison of the fatty acid content of dietary fats and oils. Data from POS Pilot Plant Corporation, Saskatoon, Saskatchewan, Canada, June 1994 (72).

transformation or to death of the transformed cells before an overt cancer developed.

### Dietary reduction of cancer risk

The modern Western diet contains an excess of n-6 fatty acids compared with earlier diets (71) and with diets that appear to reduce risk for cancer. Because the ratio of n-3 to n-6 fatty acids in the diet affects the competitive advantage of n-3 fatty acids, it seems logical that n-6 fatty acid consumption should be reduced and that consumption of n-3 fats should be increased. As recommended by the American Institute for Cancer Research and by the U.S. Department of Agriculture, our diets should incorporate less meat and include more fruits, vegetables and whole grains to reduce total fat consumption. Such a change would also increase the beneficial antioxidants and phytochemicals contained in the fruits and vegetables.

To incorporate more heart-healthy fats in their diet, Americans have decreased their use of saturated fatty acids and have in large part substituted safflower, corn and soybean oils for frying and baking uses. These oils contain a very high amount of n-6 fatty acid (LA) and very little n-3 fatty acid (LNA) (Fig. 5). The use of canola or olive oil instead of safflower, corn or soybean oil for frying and baking would reduce the consumption of n-6 fatty acids. In addition, canola oil includes a significant amount of n-3 fatty acids. Consumption of one or more servings per week of fatty, cold-water fish and perhaps an n-3 supplement would add significant n-3 fatty acids to the diet.

We do not have definitive studies to demonstrate that n-3 fatty acids would reduce the growth of cancer metastasis or decrease the rate of cancer recurrence. However, the bulk of the available evidence indicates that increasing the amount of n-3 fatty acids in the diet will be beneficial to cancer survival.

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