Dietary long-chain n−3 fatty acids for the prevention of cancer: a review of potential mechanisms[1–3]

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ABSTRACT

Increasing evidence from animal and in vitro studies indicates that n−3 fatty acids, especially the long-chain polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid, present in fatty fish and fish oils inhibit carcinogenesis. The epidemiologic data on the association between fish consumption, as a surrogate marker for n−3 fatty acid intake, and cancer risk are, however, somewhat less consistent. This review highlights current knowledge of the potential mechanisms of the anticarcinogenic actions of n−3 fatty acids. Moreover, a possible explanation of why some epidemiologic studies failed to find an association between n−3 fatty acid intake and cancer risk is provided. Several molecular mechanisms whereby n−3 fatty acids may modify the carcinogenic process have been proposed. These include suppression of arachidonic acid–derived eicosanoid biosynthesis; influences on transcription factor activity, gene expression, and signal transduction pathways; alteration of estrogen metabolism; increased or decreased production of free radicals and reactive oxygen species; and mechanisms involving insulin sensitivity and membrane fluidity. Further studies are needed to evaluate and verify these mechanisms in humans to gain more understanding of the effects of n−3 fatty acid intake on cancer risk. *Am J Clin Nutr* 2004;79:935–45.

KEY WORDS n−3 Fatty acids, eicosapentaenoic acid, docosahexaenoic acid, α-linolenic acid, arachidonic acid, carcinogenesis, eicosanoids, gene expression, epidemiology

INTRODUCTION

We recently reviewed epidemiologic studies on the relation between intakes of fish and marine fatty acids and the risks of breast and prostate cancers and of other hormone-related cancers (1). In brief, ecologic studies have shown that high per capita fish consumption is correlated with a lower incidence of cancer in the population (2–5). Additionally, the decreased consumption of fish and increased intake of vegetable oils rich in n−6 fatty acids among Japanese women during the past decades have been accompanied by increased breast cancer rates (6). Nevertheless, analytic epidemiologic studies having a case-control or cohort design have not yielded clear conclusions concerning the protective effect of fish consumption or n−3 fatty acid intake against cancer; although some studies showed an inverse association between the intake of n−3 fatty acids (7, 8) or fish (9–16) and cancer risk, most did not (17–25).

The role that the long-chain, marine n−3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA, 20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3), which are present in fatty cold-water fish and fish oils, play in the etiology of cancer has been highlighted by animal experiments and in vitro studies showing that these PUFAs suppress the development of major cancers (26–31). These experimental findings are supported by results from clinical studies showing a reduction in intestinal hyperproliferation after consumption of fish oil–derived n−3 PUFAs in subjects at elevated risk of colon cancer due to sporadic colonic adenomas (32, 33). Although a few previous reviews have described some selected actions through which long-chain n−3 fatty acids may play a role in carcinogenesis, such as biosynthesis of eicosanoids (34, 35), lipid peroxidation (36–38), and some signal transduction pathways (34, 36), to our knowledge, no comprehensive review that puts all these pieces and further evidence together is available.

The present review focuses on several putative mechanisms whereby long-chain n−3 fatty acids may modulate the carcinogenic process. Furthermore, a potential explanation of why several case-control studies and large cohort studies failed to confirm a protective effect of long-chain n−3 fatty acids against cancer development is briefly discussed. Moreover, we discuss how knowledge of the mechanisms of action of PUFAs should be taken into account in epidemiologic analyses.

MECHANISMS OF POTENTIAL CHEMOPREVENTIVE EFFECTS OF n−3 FATTY ACIDS ON CARCINOGENESIS

Mounting evidence shows that dietary n−3 PUFAs inhibit the promotion and progression stages of carcinogenesis. Several molecular mechanisms whereby n−3 PUFAs potentially affect carcinogenesis have been proposed. These mechanisms include *1*) suppression of arachidonic acid (AA, 20:4n−6)–derived eicosanoid biosynthesis, which results in altered immune response to cancer cells and modulation of inflammation, cell proliferation, apoptosis, metastasis, and angiogenesis; *2*) influences on transcription factor activity, gene expression, and signal transduction, which leads to changes in metabolism, cell growth, and differentiation; *3*) alteration of estrogen metabolism, which leads

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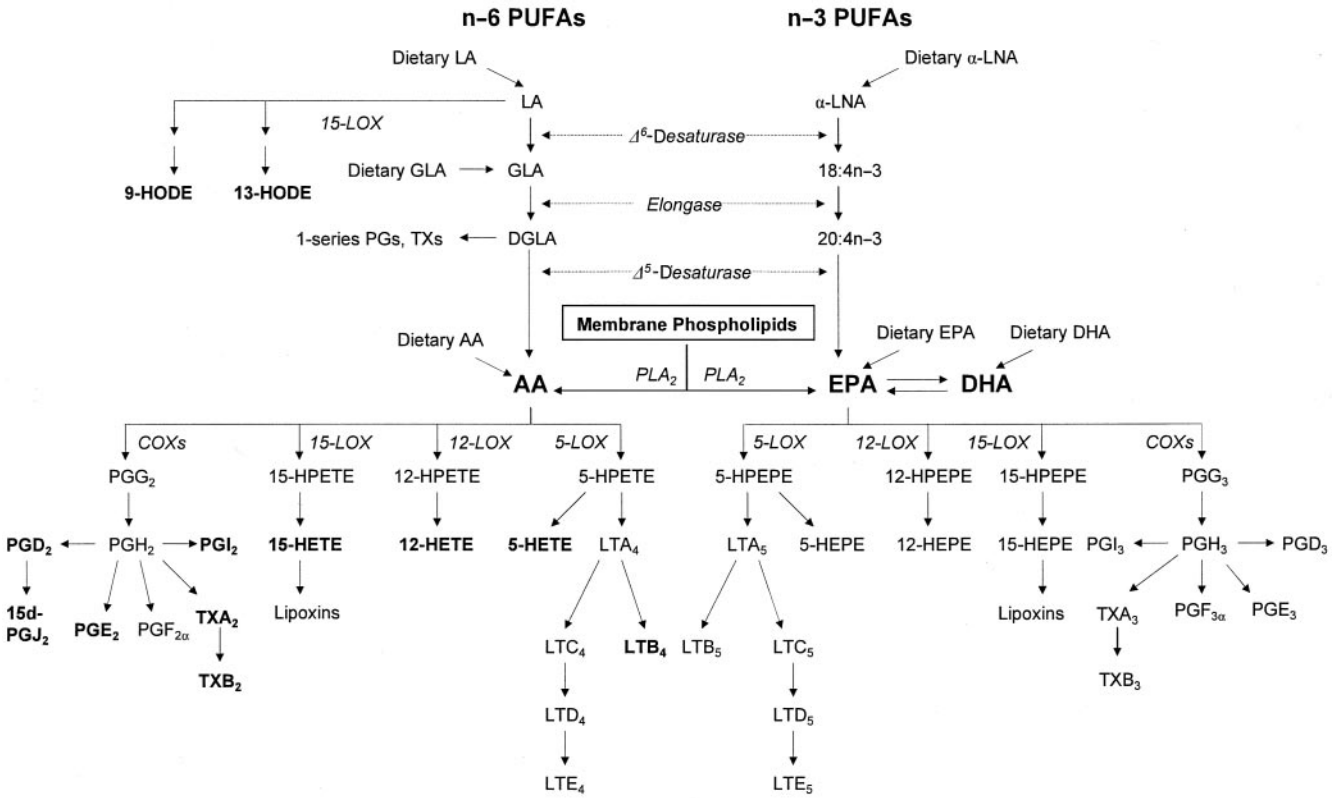

FIGURE 1. Overview of the metabolism of n−6 and n−3 polyunsaturated fatty acids (PUFAs) into eicosanoids involved in inflammation and carcinogenesis. The names of these eicosanoids are shown in bold. LA, linoleic acid (18:2n−6); α-LNA, α-linolenic acid (18:3n−3); GLA, γ-linolenic acid (18:3n−6); DGLA, dihomo-γ-linolenic acid (20:3n−6); AA, arachidonic acid (20:4n−6); EPA, eicosapentaenoic acid (20:5n−3); DHA, docosahexaenoic acid (22:6n−3); PLA_2, phospholipase A_2; LOX, lipoxygenase; COXs, cyclooxygenases (COX-1 and COX-2); 15-HETE, 15(*S*)-hydroxyeicosatetraenoic acid; 12-HETE, 12-hydroxyeicosatetraenoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HPEPE, hydroperoxyeicosapentaenoic acid; LT, leukotriene; HODE, hydroxyoctadecadienoic acid; PG, prostaglandin; TX, thromboxane.

to reduced estrogen-stimulated cell growth; *4*) increased or decreased production of free radicals and reactive oxygen species; and *5*) mechanisms involving insulin sensitivity and membrane fluidity.

Inhibition of arachidonic acid–derived eicosanoid biosynthesis

One of the more important functions of PUFAs (n−3 and n−6 fatty acids) is related to their enzymatic conversion into eicosanoids (**Figure 1**), which are short-lived, hormone-like lipids with chain lengths of 20 carbon atoms (eicosa = 20). Eicosanoids are biologically potent and have a wide array of activities: they modulate inflammatory and immune responses and play a critical role in platelet aggregation, cellular growth, and cell differentiation. The precursor fatty acids for the formation of eicosanoids are dihomo-γ-linolenic acid (DGLA, 20:3n−6), AA, and EPA. Linoleic acid (LA, 18:2n−6) and α-linolenic acid (α-LNA, 18:3n−3) are the predominant plant-derived dietary PUFAs and are the precursors of DGLA and AA and of EPA, respectively. The production of eicosanoids begins with the liberation of PUFAs from membrane phospholipids by the action of various phospholipases. Thereafter, these PUFAs serve as substrates for cyclooxygenases (COX-1, which is a constitutive enzyme, and COX-2, which is an inducible enzyme), lipoxygenases (5-, 12-, and 15-lipoxygenase), or cytochrome P450 monooxygenases. The cyclooxygenases give rise to prostaglandins and thromboxanes, whereas the lipoxygenases produce leukotrienes, hydroxy fatty acids, and lipoxins. Cytochrome P450 monooxygenase–mediated oxidation of PUFAs generates hydroxyfatty acids, dihydroxyfatty acids, and epoxy fatty acids. The relative proportions of PUFAs in cell membranes, as well as cell type, are the primary factors in regulating which eicosanoid will be generated. Hydrolytic release of PUFAs from phospholipids appears to occur indiscriminately with n−3 and n−6 PUFAs. Because the major PUFA in cell membranes is AA, most eicosanoids produced will be of the 2-series prostanoids (prostaglandins and thromboxanes) and the 4-series leukotrienes, with 2 and 4 double bonds, respectively, in the products. EPA is a substrate for 3-series prostanoids and 5-series leukotrienes. In general, AA-derived eicosanoids have proinflammatory effects (39–41)—although prostaglandin E_2 (PGE_2) has been suggested to also have antiinflammatory properties (42)—whereas EPA-derived eicosanoids have antiinflammatory effects. Eicosanoids generated from AA, such as PGE_2, leukotriene B_4, thromboxane A_2, and 12-hydroxyeicosatetraenoic acid, have been positively linked to carcinogenesis (34). For example, PGE_2 promotes tumor cell survival and is found at higher concentrations in cancer cells than in normal cells (43). The mechanisms whereby PGE_2 promotes tumor survival include inhibition of apoptosis and stimulation of cell proliferation (44–46). It has also been re-

ported that PGE_2 increases tumor progression by promoting tumor angiogenesis (47–49). 12-Hydroxyeicosatetraenoic acid has been shown to suppress apoptosis (50, 51) and promote tumor angiogenesis (52) and tumor cell adhesion to endothelial cells (53, 54); the latter is an essential and early event in the initiation of the metastatic cascade. Some lipoxygenase products generated from AA, such as leukotriene B_4 and 5-hydroxyeicosatetraenoic acid, also play a role in tumor cell adhesion (55) and thus may augment metastatic potential. Leukotriene B_4 further enhances generation of reactive oxygen species (40), which may attack DNA and lead to cancer initiation. AA-derived eicosanoids synthesized by the action of cytochrome P450 monooxygenase were recently shown to influence several biological processes, including cell proliferation, apoptosis, and inflammation (56). For example, 14,15-epoxyeicosatrienoic acid inhibits apoptosis (57) and increases cell proliferation (58). Although several AA-derived eicosanoids have been suggested to promote carcinogenesis, some of them, such as PGI_2 (59), 15d-PGJ_2 (metabolite of PGD_2) (60), and 15(*S*)-hydroxyeicosatetraenoic acid (61), as well as the LA-derived 13(*S*)-hydroxyoctadecadienoic acid (62, 63), have been found to suppress cell proliferation and induce apoptosis.

The most salient mechanism by which n−3 fatty acids may lower the risk of cancer is through their suppressing effect on the biosynthesis of AA-derived eicosanoids. This effect is achieved at several levels. First, high intakes of n−3 fatty acids result in their incorporation into membrane phospholipids, where they partially replace AA (64). By decreasing the availability of AA precursors, this substitution suppresses the biosynthesis of AA-derived eicosanoids in favor of EPA-derived 3-series prostanoids and 5-series leukotrienes. Second, n−3 PUFAs compete with n−6 PUFAs for desaturases and elongases, and n−3 PUFAs have greater affinities for the enzymes than do n−6 PUFAs. Thus, a higher intake of n−3 PUFAs reduces the desaturation and elongation of LA to AA (34) and thus the production of AA-derived eicosanoids. Third, n−3 fatty acids suppress COX-2 (65–67) and compete with n−6 fatty acids for cyclooxygenases to form eicosanoids (68–70). Compared with AA, EPA is the preferential substrate for lipoxygenase; hence an increased EPA intake leads to higher formation of EPA-derived lipoxygenase products at the expense of AA-derived lipoxygenase products when both fatty acids are simultaneously available (71). Dietary n−6 PUFAs, in contrast with n−3 PUFAs, have been reported to up-regulate the expression of rat COX-2 and, to some extent, COX-1 (72) and thus increase the production of prostanoids. Finally, n−3 PUFAs enhance eicosanoid catabolism, which is postulated to be mediated through induction of peroxisomal enzymes (73). The formation of AA-derived eicosanoids is decreased not only by n−3 PUFAs but also by eicosanoids derived from them, and some of these eicosanoids (eg, 15-hydroperoxyeicosapentaenoic acid) have an even more inhibitory effect than does EPA (74). Taken together, these effects at different levels dramatically reduce the AA-derived eicosanoids that are linked to inflammation and carcinogenesis.

Note that the potency of dietary EPA and DHA is estimated to be approximately five-fold that of α-LNA for the suppression of AA-derived eicosanoids (35). Similarly, the activities of Δ^5- and Δ^6-desaturase are considerably lower in rats fed a fish-oil (rich in EPA and DHA) diet than in those fed a flaxseed-oil (rich in α-LNA) diet (75, 76).

Influence on transcription factor activity, gene expression, and signal transduction

Dietary PUFAs and their metabolites may exert some of their antitumor effects by affecting gene expression or the activities of signal transduction molecules involved in the control of cell growth, differentiation apoptosis, angiogenesis, and metastasis.

Peroxisome proliferator-activated receptor

The first transcription factor that was identified as being regulated by fatty acids was the peroxisome proliferator-activated receptor-α (PPARα) (77), a member of the PPAR family, which also comprises PPARδ (also referred to as PPARβ) and PPARγ (3 isoforms: γ1, γ2, and γ3). These ligand-activated transcription factors were first found to be implicated in the regulation of lipid metabolism and homeostasis but have recently appeared to be involved in cell proliferation, cell differentiation, and inflammatory responses (78, 79). The preferred natural ligands of PPARγ are PUFAs, including LA, α-LNA, AA, and EPA (80, 81). Endogenous ligands include 15d-PGJ_2, 9(*S*)-hydroxyoctadecadienoic acid, 13(*S*)-hydroxyoctadecadienoic acid, and 15-hydroxyeicosatetraenoic acid (80, 82, 83). In addition to being a PPARγ agonist, EPA, but not other fatty acids (α-LNA, DHA, and n−6 PUFAs), has been shown to significantly increase PPARγ1 messenger RNA concentrations in isolated adipocytes (84). PPARα can be activated by fibrates (hypolipidemic drugs) (79) and by various saturated and unsaturated fatty acids, including palmitic acid, oleic acid, LA, AA (85), conjugated LA (86), and EPA (77). Known activators of PPARδ are DGLA, EPA, AA, palmitic acid, and the prostaglandins PGA_1 (derived from DGLA) and PGD_3 (80). PPARγ is expressed in several epithelial tissues that are important in human cancers (83). Agonists of PPARγ have been found to have antiproliferative effects both in vitro (87–92) and in vivo (93, 94). For instance, in a phase II clinical study in patients with advanced prostate cancer, the PPARγ agonist troglitazone blocked or reversed tumor progression, which led to a prolonged stabilization of or decrease in prostate-specific antigen in 50% of the patients (93, 95). Furthermore, reduced concentrations of 15-hydroxyeicosatetraenoic acid, an endogenous ligand for PPARγ in the prostate, contribute to increased proliferation of and reduced differentiation in prostate carcinoma (96). DHA was found to induce apoptosis in vascular smooth muscle cells by activation of PPARα, p38 mitogen-activated protein kinases, bax, and cytochrome *c* (94). Murata et al (97) reported that EPA decreases the activity of mitogen-activated protein kinase and inhibits cell proliferation in HepG2 cells. Both PPARα and PPARγ have antiinflammatory properties and may thereby contribute to suppression of carcinogenesis (79). PPARδ has been suggested to act as an inducer of cell proliferation and as a promoter of the progression of certain types of cancer (80). PPARδ antagonists may have a role in decreasing colon cancer risk (80) although this has not been conclusively shown.

Nuclear transcription factor κB

The nuclear transcription factor κB (NF-κB) family of transcription factors is involved in cytokine gene expression, cellular adhesion, cell cycle activation, apoptosis, and carcinogenesis (98). Constitutive NF-κB activation in cancer appears to play a role in tumor growth (98). In an experimental study, n−3 fatty acids significantly decreased NF-κB activation in murine

macrophages (99). Furthermore, cells treated with n−3 fatty acids showed a significant decrease in both messenger RNA and protein expression of tumor necrosis factor α (decreases of 47% and 46%, respectively) (99).

ras and protein kinase C

Collett et al (100) showed that, compared with LA, DHA lowers the activation of *ras* oncogenes, which are frequently activated in tumors, in mouse colon cells. *ras* activation by point mutation or overexpression is associated with elevated concentrations of cellular diacylglycerol and thus down-regulation of protein kinase C (PKC) (101). Feeding rats dietary fish oil has been shown to block the azoxymethane (a carcinogen)-induced decrease in steady-state concentrations of PKC Δ and λ-ζ isozymes, both of which have tumor suppressor functions (102). Unlike PKC Δ and λ-ζ, PKC β2, which is induced early during colon carcinogenesis (103), promotes colon cancer (104, 105). Murray et al (103) showed a significant decrease in the concentration of membrane-associated PKC β2 in the colonic epithelium of rats fed fish oil. Furthermore, the fish-oil diet blocks PKC β2–mediated hyperproliferation and enhances carcinogenesis in intestinal epithelial cells (103).

Ornithine decarboxylase

Ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, is intimately involved in normal cellular proliferation. Both ODC activity and polyamine content are significantly higher in most colorectal neoplasms than in normal, adjacent, healthy control tissues (106, 107). Rao and Reddy (108) investigated the modulating effect of high-fat diets rich in n−3, n−6, and n−9 fatty acids on ODC activity in the liver, colon, and small intestinal mucosa. The authors showed that high amounts of corn oil (rich in n−6 fatty acids) in the diet increase the activities of ODC and tyrosine-specific protein kinase in the colon and liver of male F344 rats, whereas high dietary amounts of fish oil and olive oil (rich in n−9 fatty acids) suppress these activities (108). These results were supported by those of Bartram et al (109), who showed that, compared with corn oil, dietary fish oil suppresses ODC activity in healthy humans.

3-Hydroxy-3-methylglutaryl coenzyme-A reductase

Several studies in rats showed that the long-chain n−3 fatty acids reduce the activity and concentration of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (110–113), which catalyzes the biosynthesis of mevalonate. In addition to being essential for the biosynthesis of cholesterol and coenzyme Q, mevalonate is required for DNA synthesis and cell proliferation (114). HMG-CoA reductase inhibitors (statins) have been shown to have antiangiogenic properties (115), which suggests that HMG-CoA is involved in angiogenesis. However, unlike statins, long-chain n−3 fatty acids have generally not been shown to decrease cholesterol concentrations in humans (116). Thus, a potential effect of long-chain fatty acids on HMG-CoA reductase activity in humans remains speculative.

Cyclooxygenase-2 and lipoxygenases

Several studies indicate that although n−6 PUFAs promote colon and mammary carcinogenesis by up-regulating expression of $p21^{ras}$ and COX-2, n−3 PUFAs may exert one of their antitumor effects by suppressing the expression of $p21^{ras}$ and COX-2 (65, 117, 118). COX-2 expression has been shown to down-regulate the apoptotic pathway (34). Overexpression of COX-2 has been detected in many types of cancer, including cancer of the breast, colon, and prostate (119, 120). Numerous epidemiologic studies found that long-term use of COX-2 inhibitors (nonsteroidal antiinflammatory drugs) is associated with a lower risk of colorectal cancer, adenomatous polyps, and perhaps other types of cancer (121). COX-2 catalyzes the conversion of procarcinogens to carcinogens, and significant amounts of xenobiotics could be oxidized to mutagens by COX-2. Moreover, metabolic turnover of AA is sufficient to produce mutagens. For example, malondialdehyde, a byproduct of the oxidation of AA, is highly reactive and forms adducts with DNA (122).

Nitric oxide

Nitric oxide (NO) and reactive products derived from it, such as reactive nitrogen species, are mutagenic and have the potential to produce nitration, nitrosation, and deamination reactions on DNA bases (123, 124). Excessive production of NO during chronic inflammation is believed to cause DNA damage and impaired DNA repair (eg, mutation of the p53 tumor suppressor gene) and, in the long term, cancer (124–126). Tumor-derived NO promotes tumor growth and metastasis by enhancing the invasive, angiogenic, and migratory abilities of tumor cells (124, 126, 127), which may also be triggered by activation of COX-2 (124). Another mechanism whereby NO may stimulate tumor growth is by increasing the production of PGE_2 (128), which is implicated in tumor progression. NO production in a macrophage cell line was found to be suppressed by the n−3 PUFAs α-LNA, EPA, and DHA in a dose-dependent fashion (129). Several other studies provide additional evidence for a suppressing effect of DHA on NO production (130–132).

Alteration of estrogen metabolism

It is well known that estrogen has proliferative effects on estrogen-sensitive tissues and that high estrogen concentrations may increase the risk of breast cancer and of some other hormone-dependent cancers. The AA-derived eicosanoid PGE_2 has been shown to stimulate the activity of aromatase P450, which converts 19-carbon steroids to estrogens (133). In contrast, PGE_3, a product of EPA metabolism, does not activate aromatase P450. Hence, an increased intake of EPA, which leads to increased production of PGE_3 and decreased production of PGE_2, is expected to decrease estrogen production and thus reduce estrogen-stimulated cell growth. Although a high intake of n−3 PUFAs relative to that of n−6 PUFAs may decrease endogenous estrogen production, no studies have yet directly examined this issue in humans.

Increased or decreased production of free radicals and reactive oxygen species

Free radicals and reactive oxygen species produced in cells may attack PUFAs to form lipid hydroperoxides, which decompose in chain reactions to form more free radicals and reactive aldehydes such as *trans*-4-hydroxy-2-nonenal and malondialdehyde. These metabolites potentially generate promutagenic exocyclic DNA adducts in human cells, which lead to cancer (134, 135). Generally, the autooxidizability of various fatty acids in an air atmosphere is roughly proportional to the number of double bounds in the molecule. The long-chain, highly unsaturated n−3

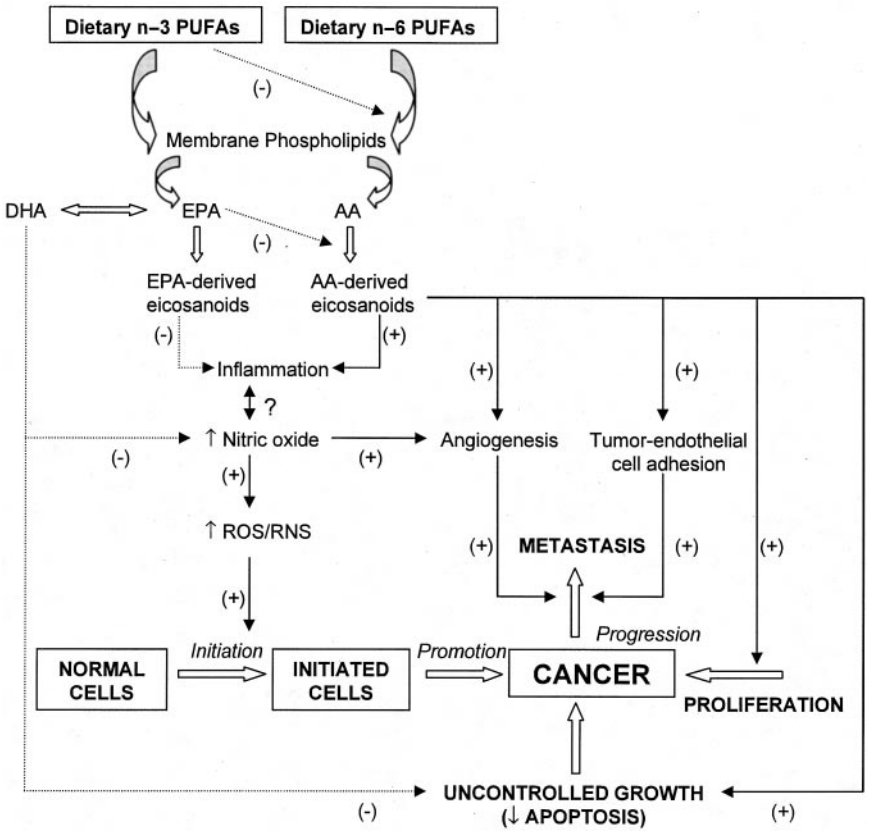

FIGURE 2. Hypothetical scheme showing potential mechanisms whereby n−6 polyunsaturated fatty acids (PUFAs) and n−3 PUFAs may promote and suppress carcinogenesis, respectively. In initiated tumor cells, phospolipase A_2, cyclooxygenase 2, and lipoxygenases are often overexpressed, which leads to overproduction of arachidonic acid (AA, 20:4n−6)–derived eicosanoids that augment inflammation. Nitric oxide, which is elevated in inflammation, is implicated in both the initiation and the progression stages of carcinogenesis. Nitric oxide may stimulate tumor growth and metastasis by enhancing the angiogenic and migratory abilities of tumor cells. Dietary n−3 PUFAs reduce the desaturation and elongation of linoleic acid (18:2n−6) to AA, the incorporation of AA into membranes, and the biosynthesis of AA-derived eicosanoids; suppress inflammation; stimulate apoptosis; up-regulate the expression of genes coding for antioxidant enzymes; and thus inhibit tumor growth and metastasis. + and solid arrows, stimulation; − and dashed arrows, suppression; ↑, increase. EPA, eicosapentaenoic acid (20:5n−3); DHA, docosahexaenoic acid (22:6n−3); ROS, reactive oxygen species; RNS, reactive nitrogen species.

fatty acids are therefore believed to promote lipid peroxidation and thus carcinogenesis. These assumptions are based on the results of investigations of the in vitro oxidation of unsaturated fatty acids in homogeneous systems (136). However, there is evidence that, compared with the intake of n−6 fatty acids, the intake of n−3 fatty acids suppresses so-called free radical diseases, such as cancer, ageing, and atherosclerosis, which suggests that lipid peroxidation in vivo may not correspond with that in vitro (35). For instance, several studies found that increasing the dietary intakes of EPA and DHA does not increase the oxidative susceptibility of LDL cholesterol (137–139). Moreover, Takahashi et al (140) reported that genes coding for some antioxidant enzymes (eg, glutathione transferases and manganese-superoxide dismutase) were up-regulated in mice fed a fish-oil diet, which suggests a protective effect against the production of reactive oxygen species and thus against cancer initiation. Studies in healthy humans also showed that consumption of a diet providing >2.3 g EPA plus DHA/d decreases superoxide production (40). Inflammation has been hypothesized to increase the production of free radicals and reactive oxygen species, which leads to carcinogenesis. Although n−6 fatty acids augment these events through the overproduction of AA-derived proinflammatory eicosanoids, the n−3 fatty acids suppress inflammation and thus the overproduction of free radicals and carcinogenesis (**Figure 2**).

In this context, note that some researchers found that the inhibitory effects of fish oil on the growth of tumors in vitro are abolished by the concurrent addition of vitamin E (37, 141–143) or vitamin C (143), which suggests that oxidized products of n−3 PUFAs suppress cell growth. Generation of oxygen radicals appears to be involved in the initiation of apoptosis and in the natural defense against transformed or foreign cells (144). Thus, the inhibitory effects of long-chain n−3 PUFAs on cell growth may, at least partly, be explained by their formation of oxidation products, which leads to apoptosis and cell growth arrest. To date, however, there is little direct evidence that n−3 PUFAs influence the carcinogenic process by alteration of free radical production in humans. Further studies on the role of lipid hydroperoxides in the modulation of tumor growth in vivo are needed to elucidate their role in carcinogenesis.

Other potential mechanisms

In addition to the potential mechanisms described above, dietary n−3 PUFAs may also modulate carcinogenesis through effects on insulin sensitivity and cell membrane fluidity, although these mechanisms have been less well studied. The n−3 fatty acid EPA has been found to improve insulin sensitivity in rats (145–147) and patients with type 2 diabetes (148). The effect has been proposed as being mediated through PPARα and

TABLE 1
Amounts of total fat (fatty acids), α-linolenic acid (α-LNA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA), and arachidonic acid (AA) and ratios of n−3 to n−6 fatty acids in selected species of fish and in meat[1]

		n−3 Fatty acids			n−6 Fatty acids		
	Total fat	α-LNA	EPA	DHA	LA	AA	n−3:n−6 Fatty acids
	g/100 g		*g/100 g*		*g/100 g*		
Fish							
Cod, Atlantic	0.7	Tr	0.06 (13.2)	0.17 (34.4)	Tr	0.02 (4.6)	11.11
Haddock	0.6	Tr	0.05 (12.2)	0.10 (24.4)	0.01 (2.4)	0.01 (2.4)	7.67
Herring, Baltic	9.3	0.29 (3.5)	0.56 (6.7)	0.83 (9.9)	0.54 (6.5)	0.03 (0.4)	2.94
Herring, Pacific	18.5	0.32 (1.9)	1.03 (6.2)	1.63 (9.8)	0.43 (2.6)	0.07 (0.4)	5.88
Mackerel, Atlantic	16.0	0.29 (2.0)	0.89 (6.2)	1.56 (10.8)	0.30 (2.1)	0.07 (0.5)	7.14
Perch, all varieties	1.3	0.01 (1.6)	0.08 (8.7)	0.19 (21.4)	0.02 (2.1)	0.05 (6.0)	4.00
Pike	0.7	0.01 (1.1)	0.04 (7.6)	0.16 (33.0)	0.01 (2.2)	0.02 (3.7)	7.14
Salmon, Atlantic	12.0	0.18 (1.7)	0.49 (4.5)	1.33 (12.3)	0.41 (3.8)	0.11 (1.0)	3.85
Salmon, Pacific	5.2	0.05 (1.1)	0.63 (13.5)	0.88 (18.9)	0.07 (1.6)	0.03 (0.7)	16.67
Sardines, in tomato sauce	14.8	0.22 (1.6)	1.24 (8.8)	1.77 (12.6)	0.22 (1.6)	0.06 (0.4)	11.11
Trout, rainbow	9.6	0.15 (1.7)	0.60 (7.0)	1.76 (20.4)	0.41 (4.8)	0.07 (0.8)	5.26
Tuna, in water	1.2	0.01 (1.6)	0.09 (11.3)	0.16 (19.4)	0.01 (1.6)	0.03 (3.2)	6.67
Meat							
Chicken, no skin	3.1	0.02 (0.9)	0.01 (0.3)	0.01 (0.6)	0.30 (12.2)	0.01 (0.5)	0.13
Beef, steak	8.8	0.03 (0.3)	Tr	Tr	0.18 (2.1)	0.03 (0.4)	0.14
Pork, fillet	1.6	0.01 (0.5)	Tr	0.01 (0.4)	0.12 (8.1)	0.01 (0.5)	0.25

[1] All values are $\bar{x}$; percentage of total fatty acids in parentheses. Tr, trace (≤0.005 g/100 g). The data for meat are from the Swedish National Food Administration Database (152).

PPARγ (149) but may also involve modification of the phospholipid components of skeletal muscle membranes (145, 150). Iigo et al (151) showed that treatment of colon carcinoma cells with DHA resulted in altered tumor cell membrane characteristics and a decreased ability to metastasize.

DISCUSSION

Substantial evidence from experimental and animal studies indicates that long-chain n−3 fatty acids in fish and fish oils inhibit carcinogenesis. Epidemiologic studies examining the associations of fish and marine n−3 fatty acids with the risk of development of cancer have, however, been inconclusive (1). About one-third to one-half of the studies that examined the relation between the intake of long-chain n−3 fatty acids or fish and cancers of the breast, prostate, endometrium, or ovary reported a statistically significant reduction in the risks of these cancers. The remaining studies either found an inverse association that was not statistically significant or failed to find any association.

There are several possible explanations for these null findings. First, in comparison with the consistent protective effect of long-chain n−3 fatty acids in animal and in vitro studies, the inconsistent associations observed in analytic epidemiologic studies may partly be due to the fact that the intake of long-chain n−3 fatty acids in some of the studied populations was too low to produce a protective effect. Other possible explanations include low within-population variability in the intake of fish or n−3 fatty acids (which limits the statistical power to detect an association) and nondifferential misclassification of estimated n−3 fatty acid intake. Although most of the potential mechanisms by which long-chain n−3 fatty acids may inhibit carcinogenesis are at the promotion and progression stages, the critical period for dietary n−3 fatty acid exposure may be during childhood or early adulthood. Thus, if exposure information is obtained at middle or old age when cancer is diagnosed, the association between n−3 fatty acid intake and cancer might be missed.

Another aspect that has to be taken into account when evaluating results from epidemiologic studies is that most studies examined the association between cancer risk and total fish intake rather than fatty fish intake, which may better mirror total intake of marine n−3 fatty acids. Total fat content in fish varies widely between species, from 0.6–0.7 g/100 g in halibut and cod to 16.0–18.5 g/100 g in mackerel and herring from the Pacific (**Table 1**). The composition of the fat depends on the geographic area in which the fish live, the fish's diet, and seasonal variations (34) and on environmental factors, such as temperature, salinity, and the depth at which the fish live, with the highest content of EPA and DHA in cold-water fish (152). In the future, the farming industry may also have important influences on the fat composition of the fish. The n−3 fatty acid α-LNA, which is found in dark green leafy vegetables, rapeseed oil (canola oil), flaxseed, some nuts (especially walnuts), and soybeans, may also bias results if only fish consumption is taken into account. Nevertheless, although humans can convert α-LNA to EPA, which can be further elongated and desaturated to DHA, this conversion is not very efficient. The extent of the conversion of α-LNA to EPA has not been fully characterized and may depend on intakes of total fat, α-LNA, EPA, DHA, and LA (153–157). It has been reported that when the intake of LA is held constant at 15 g/d, the total percentage of conversion of α-LNA to EPA and DHA is 11–18.5%, but when the intake of LA is increased from 15 to 30 g/d, this conversion is reduced to 5–11% (155). A recent study showed that 2.8% of the dietary α-LNA consumed was converted to EPA and that this conversion was down-regulated (2-fold) in subjects who consumed a diet high in EPA and DHA (154). Pawlosky et al (157) reported an even more limited conversion of α-LNA to EPA in humans: only ≈0.2% of plasma α-LNA was

converted to EPA. Low-fat diets result in increased Δ^5- and Δ^6-desaturation (156), which may increase the conversion of α-LNA to EPA.

Another drawback is that most epidemiologic studies largely analyzed the intake of n−3 PUFAs without taking into account the intake of n−6 PUFAs. Given the above-described mechanisms through which EPA and DHA may decrease the risk of cancer development, the ratio of n−3 to n−6 PUFAs seems to be more important than is the absolute intake of n−3 PUFAs. Indeed, ratios of n−3 to n−6 PUFAs, but not absolute concentrations of these fatty acids, in adipose tissue biopsy specimens were found to be inversely associated with breast cancer risk in a multinational epidemiologic study (158). Experimental data indicate that a ratio of n−3 to n−6 PUFAs of 1:1 or 1:2 is needed for protection against the development of cancer (159). In most Western countries, the ratio is ≈1:10–1:20 (159); hence, no effect on carcinogenesis would be expected. Although dietary LA intake of up to 2–3% of energy intake increases tissue AA concentrations, LA intake of >3% of energy intake is poorly correlated with tissue AA concentrations (160, 161). Because the average LA intake in the United States and Western Europe is 6–7% of energy intake (162), a moderate change in dietary LA intake would not be expected to modulate tissue AA concentrations. However, LA intakes of >12% of energy intake may actually decrease tissue AA concentrations because of inhibition of Δ^6-desaturase activity (156). On the other hand, dietary preformed AA, which is found in meat and fish (Table 1), is much more effective in enriching tissue phospholipid membranes than is LA (163). Thus, a low LA intake and a high n−3 fatty acid intake seems to be needed to suppress AA-derived eicosanoids, and such a diet is not very common in Western societies. Because tissue concentrations of AA, in contrast with those of LA, are strongly influenced by dietary intake, epidemiologic studies of the relation between the ratio of AA to n−3 PUFAs and cancer risk may be warranted.

The absence of an association between dietary long-chain fatty acids and cancer risk in some epidemiologic studies may not exclude the possibility of different effects in subgroups. The potential protective effect of dietary long-chain n−3 fatty acids may be modified by intakes of antioxidants, such as vitamins E and C; such modification has been observed in experimental studies but has not been taken into account in epidemiologic analyses to date.

An important issue of concern is that the fish oils and marine n−3 fatty acids used in experimental settings may differ from those normally consumed by humans in their content of contaminated substances. Thus, a possible beneficial effect of marine n−3 fatty acids may be offset by potential carcinogenic substances, such as some pesticides and heavy metals (eg, mercury), that accumulate in fatty fish. Furthermore, heterocyclic amines formed during the cooking of fish at high temperatures (164) have been shown to produce cancer in various organs in animals (165).

Another possible explanation for the discrepancy between animal and epidemiologic studies involves differences in doses and the stage of tumor development. In animal studies, large doses of n−3 PUFAs were usually used, and tumors were artificially induced. In addition, most of these studies did not address the initiation phase of carcinogenesis. Hence, high doses of n−3 PUFAs applied during the promotion and progression stages of tumor development may indeed inhibit carcinogenesis in animal models, whereas long-term exposures to relatively low doses of long-chain n−3 PUFAs may not be as effective against cancer development in humans. Alternatively, the inconsistencies in results between animal and epidemiologic studies may be due to publication bias. Small animal studies, which take relatively little effort and money, are more likely to suffer from publication bias than are large, well-designed epidemiologic studies. Consequently, the overall picture may be biased toward protective effects in animal studies.

In the light of the above-mentioned methodologic difficulties and limitations of observational epidemiologic studies, it is not surprising that the results reported from these studies to date on the association between long-chain n−3 fatty acids and cancer risk are inconsistent. Future epidemiologic studies have to take into account more aspects, as mentioned above, in the collection and analysis of data. In epidemiologic analyses, the biological interplay—observed in experimental studies—between n−3 and n−6 fatty acids and other factors (eg, vitamin E and antiinflammatory drugs) should be taken into account in appropriate statistical analyses to address these issues.

In summary, several mechanisms whereby n−3 fatty acids may modify the carcinogenic process were described. These fatty acids can suppress AA-derived eicosanoid biosynthesis; influence transcription factor activity, gene expression, and signal transduction pathways; modulate estrogen metabolism; increase or decrease the production of free radicals and reactive oxygen species; and influence insulin sensitivity and membrane fluidity. On the basis of these multiple mechanisms, n−3 PUFAs may have an important influence on carcinogenesis. Further studies are needed to identify new mechanisms and to evaluate and verify these mechanisms in humans to gain more understanding of the effects of marine n−3 fatty acid intake on cancer risk in real-life situations. Epidemiologic studies with more detailed information about n−3 and n−6 fatty acid exposures and improved analytic approaches that take into account the biological interplay between several nutritional factors in cancer development are needed.

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