

Review

Role of fatty acids in malignancy and visual impairment: Epidemiological evidence and experimental studies

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Summary. International variation in breast and colon cancer incidence is positively related to total fat intake. However, total fat consists of different fatty acid families, e.g., saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and n-3 and n-6 polyunsaturated fatty acids (PUFAs). Epidemiological evidence and experimental studies suggest that these fatty acid families have different effects on breast and colon carcinogenesis. Therefore the action of each fatty acid on carcinogenesis should be evaluated separately. Although it is difficult to establish firm conclusions on the effect of each fatty acid in human epidemiological studies, experimental studies on animals and cultured cells suggest that n-6 PUFAs (linoleic acid and arachidonic acid) may have a tumor promoting effect, while n-3 PUFAs (eicosapentaenoic acid, docosahexaenoic acid and α -linolenic acid) and conjugated fatty acids (CFAs; a mixture of positional and geometric isomers of PUFAs with conjugated double bonds) exert an inhibitory effect on tumor growth. SFAs such as palmitic acid and stearic acid show little or no tumor promoting effect, and the action of oleic acid, a MUFA, is inconclusive. In addition to regulation of abnormal cell growth seen in cancers, fatty acids also control cell loss seen in degenerative eye diseases, such as degeneration of lens material in cataract and degeneration of photoreceptor cells in retinitis pigmentosa. Experiments suggest that n-6 PUFAs cause deleterious effects, while n-3 PUFAs result in beneficial effects on the lens and retina. In particular, docosahexaenoic acid is known to be effective in rescuing photoreceptor cells from damage. Thus, understanding the function of each fatty acid is likely to

be important for making progress in treating these and other diseases.

Key words: Breast cancer, Cataract, Fatty acid, Colon cancer, Conjugated fatty acid, Retinitis pigmentosa

Introduction

Cancer is a main cause of mortality worldwide, but geographic differences in cancer incidence suggest an important role for environmental factors in the etiology of this disease. Among environmental factors, nutrition is the most relevant; it is estimated that at least one-third of all human cancers are associated with diet (Doll and Peto, 1981). International variation in the incidence and mortality of breast and colon cancer is related to fat intake (Bartsch et al., 1999; Kushi and Giovannucci, 2002). In retrospective case-control studies, an increase in fat intake correlates with increase in risk (Kushi and Giovannucci, 2002), whereas prospective cohort studies do not support such a strong association (Binukumar and Mathew 2005).

Human epidemiological studies of the effects of dietary fats have been inconclusive. It is becoming apparent that the type of fat (Fig. 1) should be considered, as well as total fat intake. The major saturated fatty acid (SFA) found in the human diet is palmitic acid (PA; 16 carbons: 0 double bonds (PA; 16:0)) followed by stearic acid (SA; 18:0) (Bartsch et al., 1999). In some studies, high intake of SFA correlated with increased risk of breast cancer in humans (Gonzalez, 2006), while SFA exerted little effect in rodents (Carroll, 1991).

The diet of Mediterranean populations is characterized by high consumption of olive oil with a high content of oleic acid (OA; 18:1n-9), a

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monounsaturated fatty acid (MUFA). It has been proposed to exert protective effects against breast and colon cancers (Trichopoulou et al., 2000). However, results obtained from animal and cell culture studies have been inconsistent (Chajes et al., 1995; Suzuki et al., 1997; Escrich et al., 2007). n-6 polyunsaturated fatty acids (PUFAs), especially linoleic acid (LA; 18:2n-6) and arachidonic acid (AA, 20:4n-6), show a strong association with mammary and colon carcinogenesis in rodents (Sakaguchi et al., 1984; Carroll, 1991; Escrich et al., 2007), while no such association has been seen in humans (Zock and Katan, 1998). In contrast, Inuit who consume meat from marine mammals and fish, and traditional Japanese who consume high amounts of fish and marine algae, both of which are diets high in n-3 PUFAs, such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), have a lower risk of developing breast and colon cancers. These findings have also been corroborated by animal studies (Bartsch et al., 1999). Perilla oil used in Asia for its medicinal properties contains an exceptionally high amount of n-3 α -linolenic acids (α -LN; 18:3n-3), which suppresses mammary and colon carcinogenesis in rodents (Hirose et al., 1990).

In humans, the amount and rate of intake of specific fatty acids are hard to measure. In contrast with experimental animals, for which the exact amount of purified fatty acid consumed can be monitored over a desired period, humans consume many different kinds of fatty acids in their diets, a mixture of which may exert opposite effects and contain highly variable amounts of crude fats with unknown purity. Moreover,

contamination of minor bioactive compounds in crude edible oils, such as squalene in olive oil (Newmark, 1997) and enterolantone in flaxseed oil (Danbara et al., 2005), may modify the effects. Thus, even in experimental studies, using crude seed oil or marine oil with inconsistent fatty acid composition may bias the results. On the contrary, experimental studies using purified fatty acids on laboratory animals or cell culture studies demonstrate more conclusive relationships between fatty acid and mammary and colon cancer risk, and may provide valuable information for humans (Tsubura et al., 2005a).

In contrast to cancer, which is characterized by autonomous and abnormal cell growth, degeneration is characterized by unusual loss of cell structure or function, such as that seen in diseases of the eye. Visual impairment due to degeneration of lens material and degeneration of retinal photoreceptor cells is an important public health problem. Retinitis pigmentosa is one of the most common causes of blindness for young individuals, and cataracts are a leading cause of low vision in the elderly (Buch et al., 2004). Cataract, defined as lens opacity, is caused by degeneration of lens material (Abraham et al., 2006). Lens opacity can cause a decrease in vision and may eventually lead to blindness. Many factors cause cataracts, and the most common type is idiopathic, and develops in older individuals. Retinitis pigmentosa is a group of inherited eye diseases that cause degeneration of photoreceptor cells due to apoptosis. Retinitis pigmentosa results in night blindness and initial loss of mid-peripheral visual field, and, eventually, loss of central vision (Hartong et

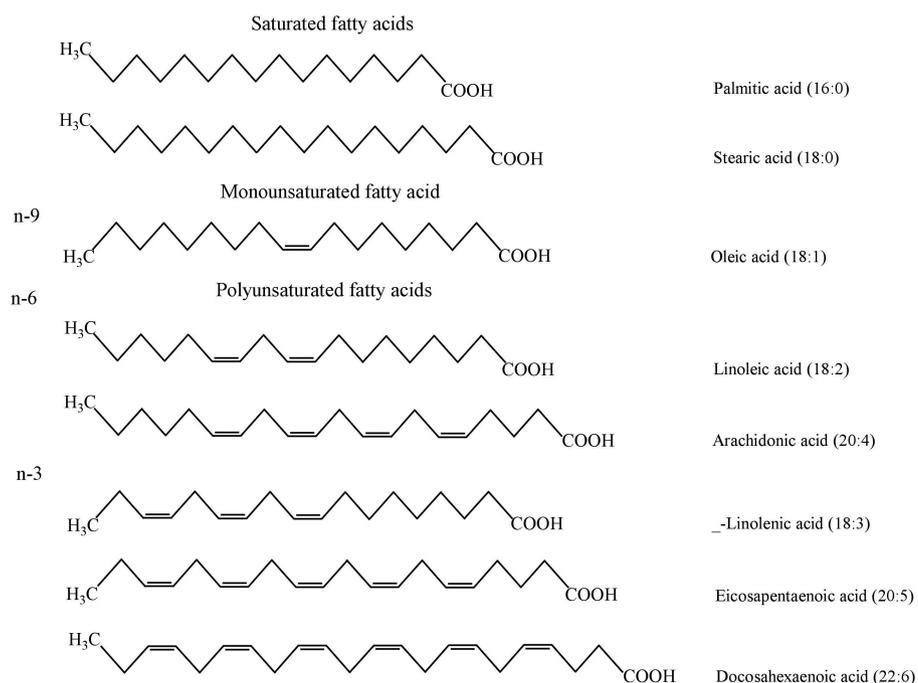


Fig. 1. Chemical structures of major saturated, monounsaturated, and polyunsaturated fatty acids. The number before the colon indicates the number of carbon atoms in the fatty acid chain, and the number after the colon indicates the number of double bonds. The conformation of the double bond is *cis* in configuration, and the first double bond located at 3rd, 6th, or 9th carbon from the terminal methyl group of a fatty acid is called n-3, n-6 and n-9 series fatty acids, respectively.

Role of fatty acids in cancer and vision

al., 2006). Compared with their role in carcinogenesis, little research has been conducted regarding the role of fatty acids in diseases of the eye.

Brain and eye are highly enriched in n-3 PUFAs; dietary supplementation of n-3 PUFAs may be essential for visual development and useful for treating pathologies of lens and retina. Epidemiologic studies show that higher dietary intakes of n-3 PUFAs decrease the incidence of cataract (Lu et al., 2005; Townend et al., 2007). Very high levels of the n-3 series of DHAs are present in the retina, specifically in the disk membranes of the outer segments of photoreceptor cells. DHA deficiency results in altered retinal function (Jeffrey et al., 2001), and dietary supplementation of DHA improves the condition of retinitis pigmentosa patients (Berson et al., 2004). In contrast to n-3 PUFAs, AA, a member of the n-6 PUFA family, has harmful effects in some nerve tissues (Macdonald et al., 1999). Thus, n-3 PUFAs may be useful therapeutic agents for pathologies of the retina and lens, while n-6 PUFAs may worsen the disease. The specific fatty acids may regulate not only abnormal cell proliferation, but also unusual cell loss. In this review, animal experiments and cell culture studies using purified fatty acids are presented for a better understanding of the role of fatty acids in malignancy and visual impairment.

Types of fatty acids that modify cancer risk

Historically, Tannenbaum and Silverstone (1953) first demonstrated that increased dietary fat intake enhances mammary carcinogenesis in rodents. Since then, animal studies have repeatedly shown that corn oil containing high levels of n-6 PUFAs, such as LA, enhances tumorigenesis, whereas fish oil, with a high proportion of n-3 PUFAs, such as EPA and DHA, reduces the risk of mammary and colon carcinogenesis (Bartsch et al., 1999). SFAs such as PA and SA do not show such tumor promoting activity (Carroll, 1991), and data regarding OA, a MUFA, are inconclusive (Escrich et al., 2007).

The fatty acid compositions of crude seed oil and marine oil have not been consistent. Therefore, to better clarify the relationship between the type of fat consumed and carcinogenesis, the same amounts of fatty acids or fatty acid ethyl esters of known purity should be administered to animals or cultured cells.

Human breast cancer cells transplanted into athymic mice rarely metastasize. However, MDA-MB-435 human breast cancer cells spontaneously and preferentially metastasize to the lung of athymic mice when transplanted orthotopically into the thoracic mammary fat pad. In this way growth of the primary tumor and the development of metastasis can be evaluated simultaneously. Using this system, it has been found that dietary LA enhances both primary tumor growth and lung metastasis, while EPA and DHA suppress them (Rose et al., 1996).

In humans, the initial sites of breast cancer

metastasis are the axillary lymph nodes. KPL-1 human breast cancer cells grow in athymic mice when inoculated orthotopically into the thoracic mammary fat pad, and spontaneously metastasize to axillary lymph nodes (Fig. 2) (Singh et al., 1997). Female athymic mice inoculated with KPL-1 cells were then fed a modified semipurified AIN-76 diet containing 10% fatty acid: 10% LA (LA diet), 9.5% EPA plus 0.5% LA (EPA diet), or 9.5% PA plus 0.5% LA (PA diet), starting 19 days before tumor cell inoculation and continuing until the end of the experiment (43 days after tumor cell inoculation) (Senzaki et al., 1998). Supplementation of 0.5% LA fulfills the essential fatty acid requirement in the EPA and PA diets. The LA diet stimulated but the EPA diet suppressed KPL-1 cell growth at the primary transplantation site compared with the PA diet (considered to be a control diet); final mean tumor weight in mice on the LA diet was 1254 ± 318 mg, on the PA diet 581 ± 437 mg, and on the EPA diet 107 ± 56 mg. The proliferation rate of the primary tumors in mice on

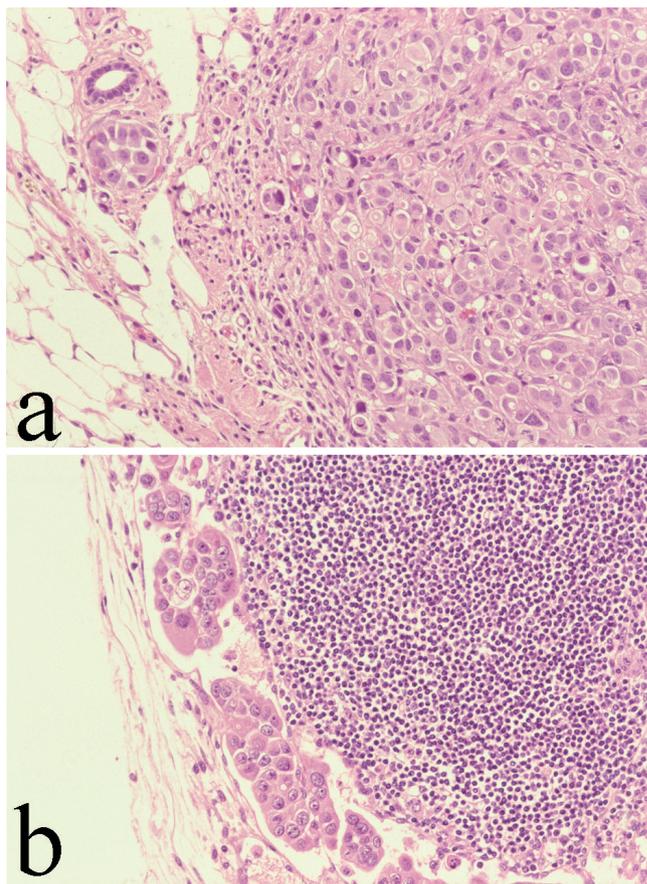


Fig. 2. KPL-1 human breast cancer cells in female athymic mice. **a.** Primary tumor growth within the thoracic mammary fat pad. Tumor embolus is seen within the lymph vessel adjacent to the primary tumor. **b.** Axillary lymph node metastasis. KPL-1 cells are trapped in marginal sinus. (original magnification x 200)

the LA diet was $59.0 \pm 3.4\%$, on the PA diet $39.6 \pm 0.7\%$, and on the EPA diet $24.7 \pm 1.2\%$. The apoptotic rate of the primary tumors in mice on the LA diet was $0.3 \pm 0.1\%$, on the PA diet $0.4 \pm 0.1\%$, and on the EPA diet $0.5 \pm 0.1\%$. The proliferation rate was highest in the LA group, followed by the PA group, then the EPA group (between-group differences were statistically significant), while the order of apoptotic rates was the opposite: highest in the EPA group followed by the PA group, then the LA group (between-group differences were not statistically significant). KPL-1 cells preferentially metastasized to the regional lymph node, and the incidence of metastasis was significantly higher in the LA group (36%, 4 of 11) compared with PA (0%, 0 of 12) and EPA groups (0%, 0 of 12).

To avoid the possibility that the primary tumor volume would influence metastasis, cancer cells were directly inoculated into the vein (artificial metastasis). Five-week-old male F344 rats were fed the same 10% fatty acid diets (LA, EPA, and PA), and ACL-15 cells (a 1,2-dimethylhydrazine (DMH)-induced F344 rat colon carcinoma cells) were inoculated into the superior mesenteric vein of 6-week-old rats. The rats were sacrificed at 9 weeks of age, and liver metastasis was evaluated (Iwamoto et al., 1998). ACL-15 cells preferentially metastasized to the liver in all treated rats. The number of metastatic liver foci per rat was significantly greater in the LA diet group (170 ± 47 foci) than in the EPA group (36 ± 11 foci), and the number of metastatic liver foci was intermediate in the PA group (70 ± 22 foci). The mean areas of the metastatic foci were significantly larger in the LA group ($3.4 \pm 0.3 \text{ mm}^2$) than in the EPA ($1.2 \pm 0.1 \text{ mm}^2$) and PA ($1.3 \pm 0.1 \text{ mm}^2$) groups. The cell proliferation rate (mitotic index) was significantly higher in the LA ($12.2 \pm 0.1\%$) and PA ($11.0 \pm 0.8\%$) groups compared with the EPA group ($6.0 \pm 0.1\%$). In contrast, the LA diet slightly decreased the apoptotic rate (10.4 ± 0.3) compared with the EPA group (12.3 ± 0.1), but not to a significant level. Thus, an EPA diet suppressed and a LA diet stimulated liver metastasis of ACL-15 colon carcinoma cells compared with the PA diet, mainly due to changes in cell proliferation rate.

In azoxymethane (AOM)-induced colon carcinogenesis and *N*-methyl-*N*-nitrosourea (MNU)-induced mammary carcinogenesis models in rats, semipurified diets containing 5% fatty acid, such as 5% LA (LA diet) or 4.7% SA plus 0.3% LA (SA diet) or 4.7% EPA plus 0.3% LA (EPA diet) modulated colon and mammary carcinogenesis. Colon cancer incidence and multiplicity was higher in rats fed the LA diet compared with those fed the SA diet, and colon and mammary cancer incidence and multiplicity were lower in rats fed the EPA diet compared with the LA diet (Sakaguchi et al., 1984; Minoura et al., 1988; Takata et al., 1990).

Because n-3 PUFAs are effective in suppressing mammary and colon carcinogenesis, the ability of dietary n-3 PUFAs EPA and DHA to suppress mammary cancer was compared in the MNU-induced mammary

carcinogenesis rat model (Yuri et al., 2003). Rats were treated with MNU at 49 days of age and maintained on a modified semipurified AIN-76A diet containing 10% fatty acids: either 9.5% EPA plus 0.5% LA (EPA diet), 4.25% EPA plus 4.25% DHA plus 0.5% LA (EPA + DHA diet), or 9.5% DHA plus 0.5% LA (DHA diet) until the largest mammary tumor reached more than 1 cm in diameter, or until the end of the experiment (20 weeks after MNU treatment). The DHA diet tended to delay the development of mammary carcinomas greater than 1 cm in diameter, and was associated with significant suppression of the carcinogenic action of MNU compared with the EPA or the EPA + DHA diet: tumor incidence (percent of rats with mammary carcinomas at least 1 cm in diameter) decreased to 23% compared with 73% and 65%, and tumor multiplicity (when all sizes of mammary carcinomas were included) decreased to 0.2 compared with 1.7 and 1.6. The incidence and multiplicity were significantly lower in the DHA group; tumor latency in the DHA, EPA, and EPA + DHA groups was 119, 105, and 117 days, respectively (not significant between different dietary groups). Taken together, n-6 PUFA (LA) accelerated, while n-3 PUFAs (EPA and DHA) suppressed mammary and colon cancer cell growth compared with SFA (PA or SA). n-3 PUFAs at a dose of 5-10% in the diet suppressed mammary and colon carcinogenesis, and DHA suppressed more effectively than EPA. Half-doses of DHA in the EPA + DHA diet did not evoke an adverse effect.

In KPL-1 human mammary cancer and COLO 201 human colon cancer cells in culture, the 50% inhibitory concentration (IC₅₀) values for DHA were lower than for EPA (Yamamoto et al., 1999; Danbara et al., 2004b; Tsujita-Kyutoku et al., 2004). In addition, α -LN, another n-3 PUFA, suppressed mammary and colon carcinogenesis in rats (Hirose et al., 1990), as well as the growth of human breast cancer cells in culture (Chajes et al., 1995).

To clarify the role of OA, a MUFA, rats were fed a diet with olive oil, which is high in OA and low in LA. Although the number of mammary carcinomas per rat was low (Cohen et al., 2000), different olive oils contain varying levels of OA and LA, making it difficult to evaluate the OA and LA effects separately. Mice were fed a semipurified AIN-93M.c. diet containing 4% fat, in which soybean oil was replaced with coconut and rapeseed oil (60/40, w/w) to lower the LA content (Suzuki et al., 1997). These rats were then injected with highly metastatic Co26Lu mouse colon cancer cells and given 0.1 ml of OA, LA, EPA or DHA daily. DHA in particular, but also EPA and OA, significantly decreased lung metastasis compared with the control rats that did not receive fatty acid supplements (Suzuki et al., 1997).

Using the AOM-induced colon carcinogenesis model, α -LN more effectively reduced the number of aberrant colonic crypt foci than OA did (Komaki et al., 1996). Thus, the ability of OA to suppress cancer cell growth, if present, may be less than that of n-3 PUFAs.

In a cell culture study, OA showed no significant effect on estrogen receptor-negative human breast cancer cells, but significantly stimulated estrogen receptor-positive human breast cancer cells (Chajes et al., 1995). OA at high concentration inhibited growth of estrogen receptor-negative human breast cancer cells, but at low concentration stimulated it (Rose and Connolly, 1990). Thus, the role of OA in controlling cancer cell growth appears to be complex.

Changes in fatty acid composition in serum and mammary fat tissue

The fatty acid content of the sera of rats fed diets with varying fatty acid compositions is shown in Fig. 3. In rats fed the AIN-76A diet, the major fatty acids in rat plasma were PA, SA, OA, LA, and AA (Moriguchi et al., 2004). In general, differences in serum fatty acid composition reflected differences in composition of the respective diets compared with rats fed the standard AIN-76A diet (Moriguchi et al., 2003). Not only did serum levels of fatty acid increase within the respective diets, but AA levels increased in LA-fed rats, and decreased in EPA- and DHA-fed rats. The change in serum fatty acid levels occurred within a few days after diets were changed (Moriguchi et al., 2004).

The fatty acid composition of mammary fat tissues also reflected past qualitative dietary intake of fatty acids, and fatty acid composition of mammary fat tissue correlated with serum fatty acid composition (Yuri et al., 2003). Marked differences in fatty acid composition in serum and mammary fat tissues occurred after feeding on a diet containing single purified fatty acids. In contrast with these experimental conditions, the human diet contains varieties of fatty acids. As n-3 and n-6

PUFAs show opposite effects against breast and colon carcinogenesis, the balance of n-3 and n-6 intake may be more important to consider than evaluating mere intake of n-3 or n-6 PUFAs (Bougnoux et al., 2005). In general, the n-3/n-6 ratio is inversely related to breast cancer risk (Zhu et al., 1995, Maillard et al., 2002).

The n-3/n-6 ratio in mammary fat tissue reflects the hormonal milieu, even when animals are given the same diet (Liu et al., 2007). In pregnant mice, a significant increase in the n-3/n-6 ratio is seen when compared with virgin mice. Early full-term pregnancy decreases breast cancer risk in humans and rodents, but mechanisms underlying such parity protection largely remain unknown (Tsubura et al., 2008). Changes in the host hormonal environment and structural or functional alteration of the mammary glandular trees have been proposed to explain the mechanisms of parity protection, and several candidate genes have been isolated. However, as the pregnant mammary gland is accompanied by preferential accumulation of n-3 over n-6 PUFAs, the change in fat composition may partly explain the mechanism of parity protection against breast cancer.

Conjugated fatty acids and cancer risk

Conjugated fatty acids (CFAs) are mixtures of positional and geometric isomers of PUFAs with conjugated double bonds (Fig. 4). The most widely studied CFA is conjugated LA (CLA), a collective term for isomers of the parent LA that have conjugated double bonds. The double bonds in CLA occur on adjacent carbons and are not separated by a methyl group. Surprisingly, although LA accelerates carcinogenesis, CLA exerts anticarcinogenic action in

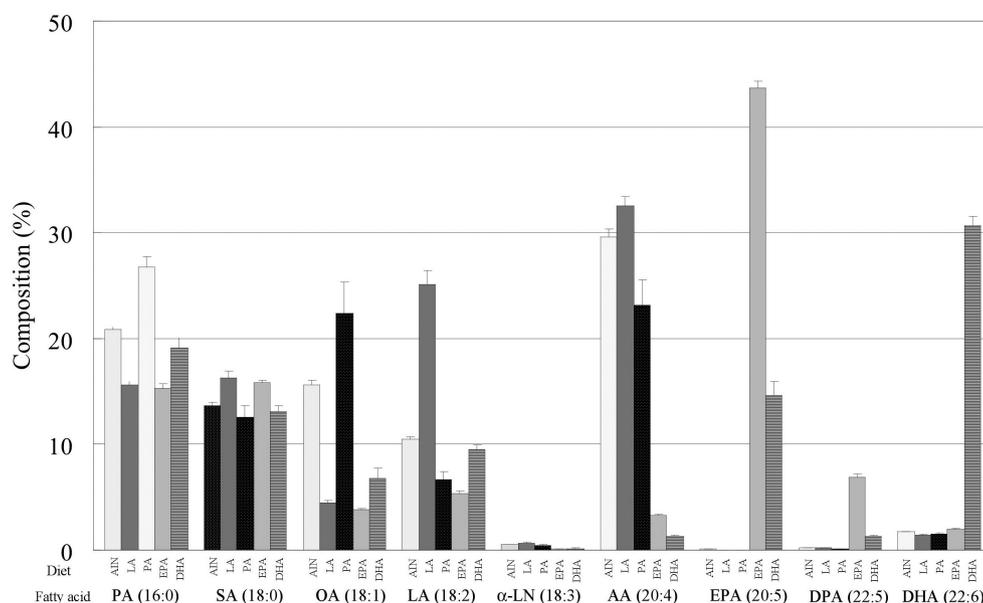


Fig. 3. Fatty acid composition (%) in plasma in rats after being fed on the AIN-76A diet or a diet containing purified 10% fatty acid. AIN: Female Sprague-Dawley rats were fed AIN-76A diet (containing 5% corn oil) for 3 weeks and sacrificed at 8 weeks of age; LA (10% linoleic acid), PA (9.5% palmitic acid plus 0.5% linoleic acid), EPA (9.5% eicosapentaenoic acid plus 0.5% linoleic acid), DHA (9.5% docosahexaenoic acid plus 0.5% linoleic acid): Female Lewis rats were fed a modified AIN-76A diet containing each 10% fatty acid combination for 20 weeks, and sacrificed at 27 weeks of age.

several organs in vivo and in various cell lines in vitro. Whereas suppression of chemically-induced mammary and colon cancer requires administration as 5-10% of the n-3 PUFAs, such as EPA, DHA and α -LN in the diet (Sakaguchi et al., 1984; Minoura et al., 1988; Takata et al., 1990; Hirose et al., 1990; Senzaki et al., 1998; Iwamoto et al., 1998; Yuri et al., 2003). CLA is a potent anticancer agent with an effective range when administered as 0.1-1.0% of the diet (Kelley et al., 2007). There are two important CLA isomers, 9cis(c),11trans(t)-CLA and 10t,12c-CLA. 9c,11t-CLA is a naturally existing isomer commonly found in ruminant meat and dairy products, and chemical isomerization of LA forms 9c,11t-CLA and 10t,12c-CLA in equal amounts (Badinga and Greene, 2006). To find out which isomer elicits higher anticarcinogenic activity, animal and cell culture studies with these purified isomers have been conducted. Overall, the growth inhibitory effects of 9c,11t-CLA and 10t,12c-CLA vary in different animal models and cancer cell lines used (Kelley et al., 2007).

Conjugated LN (CLN) is found naturally in certain seed oils, such as pomegranate seed oil (72%, 9c,11t,13c-CLN), tung seed oil (70%, 9c,11t,13t-CLN), bitter gourd oil (60%, 9c,11t,13t-CLN), pot marigold seed oil, (33%, 8t,10t,12c-CLN) and catalpa seed oil (31%, 9t,11t,13c-CLN) (Nagao and Yanagita, 2005). Characteristically, each of these naturally occurring seed oils contains one major isomer. Similarly to CLA, a dietary dose of 0.01-1.0% pomegranate seed oil (Kohno et al., 2004a) or bitter gourd oil (Kohno et al., 2004b) significantly suppresses AOM-induced colon carcinogenesis in male F344 rats, and a dietary dose of 0.1% CLN, synthesized from perilla oil rich in α -LN,

similarly suppresses 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine (PhIP)-induced rat mammary carcinogenesis in female Sprague-Dawley rats (Futakuchi et al., 2002). CLN synthetically prepared from α -LN shows cytotoxic effects on various human cancer cell lines, including the DLD-1 line of colon cancer origin and the MCF-7 line of mammary cancer origin (Igarashi and Miyazawa, 2000a). CLN is more cytotoxic to cancer cells than α -LA in vivo (Suzuki et al., 2001), and the cytotoxicity of the 9,11,13-CLN isomer is much stronger than that of the 8,10,12-CLN isomer, while the cis/trans configuration shows little effect (Suzuki et al., 2001).

Conjugated EPA (CEPA) is found in seaweeds, such as red algae (5c,7t,9t,14c,17c- and 5t,7t,9t,14c,17c- or 5c,8c,10t,12t,14c-CEPA) (Lopez and Gerwick, 1987; Burgess et al., 1991). Although the amount is extremely small, it can be prepared from EPA by alkaline isomerization (Igarashi and Miyazawa, 2000b). CEPA shows the strongest growth suppressive effect in DLD-1 colon cancer cell growth in athymic mice, followed by CLA, then EPA (Tsuzuki et al., 2004a). A similar tendency is seen in DLD-1 cells in culture, and CEPA isomers naturally present in the algae are more cytotoxic than CEPA prepared artificially and composed of a mixture of isomers (Tsuzuki et al., 2005).

Conjugated DHA (CDHA) is not present in nature but can be prepared from DHA by alkaline isomerization (Igarashi and Miyazawa, 2000b). CDHA prepared by alkaline isomerization was tested in a MNU-induced mammary carcinogenesis model. Female Sprague-Dawley rats were given 0, 0.2 or 1.0% CDHA in a semipurified AIN-76A diet, before or after MNU

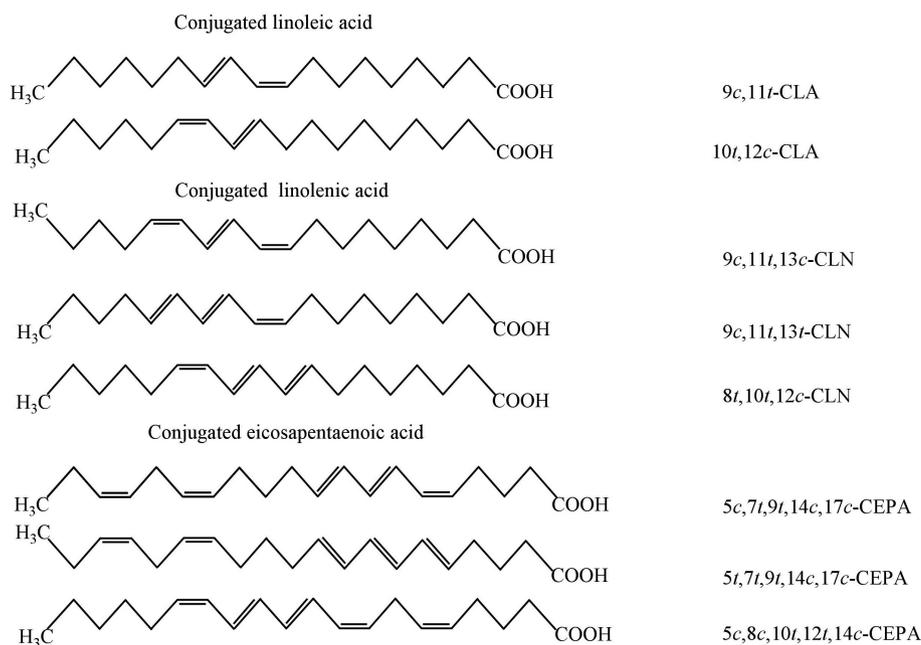


Fig. 4. Chemical structures of representative isomers of conjugated linoleic acid, conjugated linolenic acid and conjugated eicosapentaenoic acid. The conformation of the double bond can be either cis or trans.

treatment, and the development of mammary carcinomas was compared (Danbara et al., 2004a). In rats that received 0.2% and 1.0% CDHA after MNU treatment, the incidence and multiplicity of mammary carcinoma were similarly inhibited, and the latency was prolonged, whereas CDHA given before MNU treatment was ineffective in suppressing mammary cancer yield. When human cancer cells were transplanted into athymic mice, 1.0% dietary CDHA, but not 0.2%, significantly inhibited the growth of KPL-1 mammary and COLO 201 colon cancer cells (Tsujita-Kyutoku et al., 2004; Danbara et al., 2004b). CDHA also tended to suppress metastasis of KPL-1 cells to regional lymph nodes. Determining which isomer(s) of CDHA exhibit potent biological action requires further study.

Potent fatty acids that lower cancer risk

CFAs exhibit cytotoxic action against various human cancer cell lines and are more potent than their parent fatty acids (Igarashi and Miyazawa, 2000b; Suzuki et al., 2001; Danbara et al., 2004a; Tsujita-Kyutoku et al., 2004). In DLD-1 cells in culture, CEPA is more cytotoxic than conjugated AA (CAA), followed by CLA, then EPA (Tsuzuki et al., 2007). CLN is more cytotoxic than CLA (Igarashi and Miyazawa, 2000a; Tsuzuki et al., 2004b). In various human cells in culture, the cytotoxicity of CEPA and CDHA is almost equivalent (Igarashi and Miyazawa, 2000b). IC₅₀ measured under the same conditions in our laboratory are listed in Table 1. In various human breast cancer cell lines, the IC₅₀ of EPA after 72 h was in the range of 209-669 μ M. In KPL-1 breast cancer cells, the IC₅₀ of EPA, DHA and CDHA was 669 μ M, 270 μ M, and 97 μ M, respectively (Tsujita-Kyutoku et al., 2004). In COLO 201 colon cancer cells, the IC₅₀ of EPA, DHA, and CDHA after 72 h was 57 μ M, 47 μ M, and 32 μ M, respectively (Danbara et al., 2004b). Highly unsaturated n-3 fatty acids, such as EPA and DHA, showed cytotoxic effects on the cancer cell lines. CFAs exert stronger cytotoxicity than their parent fatty acids, and the cytotoxic action tends to be proportional to the number of double bonds.

Mechanisms of n-3 PUFAs and CFAs that suppress carcinogenesis

n-3 PUFAs and CFAs suppress tumor cell growth and metastasis. The mechanisms of action may include alteration of eicosanoid biosynthesis and/or accumulation of cytotoxic lipid peroxidation products, leading to reduction in cell proliferation, cell cycle arrest and induction of apoptosis. One of the important functions of PUFAs is related to their enzymatic conversion into eicosanoids. LA is the major source of AA, which is further metabolized to prostaglandin and thromboxane by cyclooxygenase (COX) and to leukotriene by lipoxygenase (LOX). AA-derived eicosanoids, such as prostaglandin, thromboxane and leukotriene, are short-lived hormone-like lipids and are biologically potent. These eicosanoids have a wide range of effects and have been positively linked to carcinogenesis due to their stimulation of cell proliferation and inhibition of apoptosis. In male F344 rats fed a modified semipurified AIN-74 diet containing 10% fatty acid, such as 10% LA (LA diet), 9.5% EPA plus 0.5% LA (EPA diet), or 9.5% PA plus 0.5% LA (PA diet) for 4 weeks, rats on the LA diet had a significantly higher serum AA composition (31.2 \pm 1.3%) compared with those on the EPA diet (8.2 \pm 0.4%) and PA diet (21.8 \pm 1.7%), and this elevated serum AA level is thought to lead to tumor growth (Iwamoto et al., 1998; see also Fig. 3). PUFAs are the basic constituents of membrane phospholipids, and the production of eicosanoids begins with the liberation of PUFA from membrane phospholipids. The major PUFA in the cell membrane is AA, and both n-3 PUFA and CFA may compete with AA to block its incorporation into membrane phospholipids or to inhibit the COX and/or LOX pathway, leading to a reduction in AA-derived eicosanoids. COX and LOX inhibitors efficiently block cell proliferation and induce apoptosis in breast and colon cancer cells (Ye et al., 2005; Hammamieh et al., 2007). Significant reductions in AA-derived prostaglandin and thromboxane are seen in MNU-induced mammary carcinomas in rats fed the EPA diet

Table 1. 50% inhibitory concentration values for eicosapentaenoic acid, docosahexaenoic acid and conjugated docosahexaenoic acid with human breast and colon cancer cells for 72 h.

| Fatty acid | Breast cancer cells | | | Colon cancer cells | |
|---------------------------------|---------------------|-------------------|-----------------------------|--------------------|-----------------------------|
| | Cell line | Estrogen receptor | IC ₅₀ (μ M) | Cell line | IC ₅₀ (μ M) |
| Eicosapentaenoic acid | KPL-1 | + | 669 | COLO 201 | 57 |
| | MCF-7 | + | 562 | | |
| | MKL-F | - | 434 | | |
| | T47D | + | 234 | | |
| | MDA-MB-231 | - | 209 | | |
| Docosahexaenoic acid | KPL-1 | + | 270 | COLO 201 | 47 |
| Conjugated docosahexaenoic acid | KPL-1 | + | 97 | COLO 201 | 32 |

(Takata et al., 1990). Furthermore, decreases in levels of AA, AA-derived prostaglandin and thromboxane were observed in intestinal mucosa of rats fed the CLA diet (Park et al., 2001), indicating the involvement of eicosanoids in decreasing the incidence of chemically-induced mammary and colon cancer. CLA is involved in the COX and LOX pathways, but different CLA isomers regulate tumor growth through different mechanisms: 9c,11t-CLA through inhibition of the COX pathway, and 10t,12c-CLA through inhibition of the LOX pathway (Kelly et al., 2007). Low AA levels leading to suppression of AA-derived eicosanoid biosynthesis by n-3 PUFA and CFAs may modulate cell kinetics in a way that leads to a reduction in cancer cell growth.

The cancer-suppressing effects of n-3 PUFAs and CFAs are correlated with the extent of lipid peroxidation (Bartsch et al., 1999; Igarashi and Miyazawa 2000b; Tsuzuki et al., 2004a,b). The cytotoxic effects of n-3 PUFA and CFA on cancer cells in culture are abolished by the addition of antioxidant (Chajes et al., 1995; Igarashi and Miyazawa, 2000b; Suzuki et al., 2001). Generation of a lipid peroxidation product appears to be involved in the induction of apoptosis and arrest of cell growth. Thus, alteration of eicosanoid biosynthesis and/or production of a cytotoxic lipid by peroxidation may lead to a reduction in cell proliferation and to cell cycle arrest and apoptosis. In mammary cancer cell growth at the primary site (Senzaki et al., 1998) and colon carcinoma cell growth at the metastatic site (Iwamoto et al., 1998), LA increases and EPA decreases cell proliferation compared with PA-fed rats. In breast and colon cancer cells in culture, EPA, DHA, and CDHA halt cell cycle progression at the G1 phase by affecting G1 cyclin levels, and induce apoptosis via changes in protein levels of the Bcl-2 family (Tsujita-Kyutoku et al., 2004; Danbara et al., 2004b; Tsuzuki et al., 2007). It is likely that n-3 PUFA and CFA exert their cancer inhibitory properties via one or more of these mechanisms.

Combination effects of fatty acid with other compounds

Humans ingest a variety of compounds in their diets, so interactions between different food items may occur when consumed together in the same meal. Some dietary compounds may interact synergistically, while others interact antagonistically. Synergistic action by two or more chemicals is defined as a therapeutic effect that is greater than those expected from simple addition of the effects of the individual chemicals. Whether n-3 PUFAs and CFAs are more beneficial in combination with other chemicals is of interest.

Asian diets contain large amounts of soy products and fat derived from fish, which may be responsible for lower incidences of breast and colon cancer in Asia. According to median-effect analysis, EPA acts synergistically with genistein, an isoflavone abundant in

soy; EPA and genistein were added to estrogen receptor-positive MCF-7 breast cancer cells at concentrations of EPA > 210.9 μM and genistein > 93.2 μM , and to estrogen receptor-negative MDA-MD-231 cells at concentrations of EPA > 609.6 μM and genistein > 176.1 μM (Nakagawa et al., 2000). In a matrigel invasion assay, invasion capacity caused by EPA and DHA was more reduced by simultaneous addition of genistein than with EPA or DHA alone (Horia and Watkins, 2007). Thus, fish and soy in the same meal may synergistically suppress breast and colon cancer growth and metastasis.

Epidemiological studies have shown that diets containing fruits and vegetables rich in carotenoids and/or high levels of β -carotene are associated with a decreased incidence of various cancers (Riboli and Norat, 2003). Perilla oil rich in β -LN also shows synergistic action with β -carotene, reducing AOM-induced colonic aberrant crypt foci in male F344 rats (Komaki et al., 1996). Diallyl disulfide, an organosulfur compound found in garlic, shows anticarcinogenic action against human breast cancer cells (Nakagawa et al., 2001b). In MDA-MB-231 human breast cancer cells, EPA acts synergistically with diallyl disulfide at concentrations of EPA > 6.3×10^{-1} μM and of diallyl disulfide > 3×10^{-3} μM .

EPA also exerts synergistic action with angiogenesis inhibitor TNP-470 against human breast cancer cells (Yamamoto et al., 1999), and DHA synergistically enhances cytotoxicity of chemotherapeutic agents, such as taxanes and 5-fluorouracil against human breast and colon cancer cells, respectively (Calviello et al., 2005; Menendez et al., 2005). Thus n-3 PUFAs harmonize and act synergistically with other chemicals, but combination effects of CFAs with other chemicals have not been studied.

Identifying chemicals that negate the cancer promoting effects of n-6 PUFAs may likewise be important. Epidemiological studies have shown an inverse relationship between red wine consumption and cardiovascular disease, even in the French, who consume a diet relatively high in fat, a phenomenon labeled the French paradox. It has been suggested that resveratrol, a phytoalexin found in the skin of red grapes and in red wine, is the main cause of this effect. Resveratrol also possesses chemopreventative properties in breast and colon cancer cell lines (Nakagawa et al., 2001a; Wolter et al., 2001). Resveratrol at a concentration of 63 μM has been found to antagonize the effect of LA administered at a concentration of at least 3.2×10^{-2} μM , and was also found to suppress the growth of human breast cancer cells (Nakagawa et al., 2001a). Moreover, diallyl disulfide at a concentration of 1.8 μM antagonized the effect of LA administered at a concentration of 6.5×10^2 μM (Nakagawa et al., 2001b). Diallyl disulfide has also been found to suppress PhIP-induced mammary carcinogenesis in rats fed a diet high in corn oil (high LA) (Suzui et al., 1997). Thus, chemicals that mitigate the growth stimulatory effect of

n-6 PUFAs already exist.

Effects of fatty acids in prevention and treatment of visual impairments

Lens damage seen in cataracts can be reproduced *in vitro* (Iwig et al., 2004; Glanz et al., 2006). In organ-cultured bovine lenses, fatty acid cytotoxicity was strong with LA and AA, less strong with OA, and without any effect with SA (Glanz et al., 2006). In human lens epithelial cells in culture, cytotoxicity ranked in the following order: AA > LA = LN = OA, whereas SFA were much less effective (Iwig et al., 2004). n-6 PUFA seems to lead to significant damage of lens epithelial cells, while the effects of n-3 PUFA have not been tested *in vitro*. Animal models for cataract have been developed (Tsubura et al., 2005b), and the role of fatty acids on cataractogenesis should be further confirmed in animal studies.

Human retinitis pigmentosa has been characterized by photoreceptor cell loss due to apoptosis. Photoreceptor cell apoptosis can be reproduced in animals in a short period after a single intraperitoneal injection of MNU (Tsubura et al., 2003). A single intraperitoneal dose of 60 mg/kg MNU to 7-week-old female Sprague-Dawley rats causes damage to photoreceptor cells in all treated animals via an apoptotic mechanism, leading to loss of photoreceptor cells over the course of seven days. MNU-induced retinal damage is due to selective 7-methyldeoxyguanosine adduct formation in the photoreceptor cell nuclei. This is followed by apoptosis of photoreceptor cells, which involves up-regulation of Bax protein, down-modulation of Bcl-2 protein, and activation of caspase-3, -6, and -8 (Yoshizawa et al., 1999). In this model, short-term dietary supplementation of 9.5% DHA shortly before, during, or after MNU insult rescues photoreceptor cells by delaying the onset of apoptosis and slowing its progression (Moriguchi et al., 2004). A diet rich in DHA may enhance retinal function and influence photoreceptor cell survival in rats.

A single intraperitoneal dose of 50 mg/kg MNU to 7-week-old rats induced milder photoreceptor cell damage. Rats treated with 50 mg/kg MNU were switched to a modified semipurified AIN-76A diet containing 10% fatty acid: 10% linoleic acid (LA diet), 9.5% PA plus 0.5% LA (PA diet), 9.5% EPA plus 0.5% LA (EPA diet), 4.75% EPA plus 4.75% DHA plus 0.5% LA (EPA + DHA diet), or 9.5% DHA plus 0.5% LA (DHA diet). These rats were observed for 20 weeks after MNU injection, and incidence [(number of rat affected/total number of rats) x 100] and severity [indicated by retinal damage ratio: (length of retina with < 4 rows of photoreceptor cells/whole retinal length) x 100] were measured (Moriguchi et al., 2003). The incidence and severity of retinal damage in rats fed the respective diets were: LA diet, 88% and 60%; PA diet, 41% and 17%; EPA diet, 73% and 36%; EPA + DHA

diet, 53% and 23%; and DHA diet, 0% and 0%. Photoreceptor cell damage seems to be accelerated by the LA diet, but was completely prevented in rats fed 9.5% DHA, whereas 4.75% DHA was less effective.

In contrast to the effects of the DHA diet, it is noteworthy that CDHA did not rescue MNU-induced photoreceptor apoptosis (Tsubura et al., 2003). In rat retinal neurons in culture, DHA decreased photoreceptor cell death, but PA, OA and AA supplementation were not able to rescue photoreceptor cell death (Rotstein et al., 1996; Polit et al., 2001). Taken together, these experimental studies indicate that n-6 PUFAs worsen visual function, while n-3 PUFAs, particularly DHA, preserve it. However, the underlying mechanisms of the effects of fatty acids on vision should further be studied.

Concluding remarks

Experimental studies have confirmed beneficial and harmful effects of fatty acids against abnormal cell proliferation (seen in breast and colon cancers) and unusual cell loss (seen in lens and photoreceptor cell damage). In breast and colon cancer, animal experiments and cell culture studies have shown that n-3 PUFAs (DHA, EPA, and α -LN) and CFAs (CLA, CLN, CEPA, and CDHA) were beneficial, while n-6 PUFAs (LN and AA) were harmful; SFAs (PA and SA) were less effective or ineffective, and MUFA (OA) action was inconclusive. In particular, the dietary doses required for tumor suppression by CFAs were strikingly low ($\leq 1\%$) in comparison to the doses required to achieve tumor suppression with n-3-PUFAs (5-10%). Moreover, chemicals acting synergistically with n-3 PUFAs have been identified, and fortunately some chemicals negate the effects of n-6 PUFAs.

n-6 PUFAs tend to evoke deleterious effects on lens epithelial cells and retinal photoreceptor cells. In the retina, DHA (but not CDHA) rescued photoreceptor cell damage, while other fatty acids were ineffective. Thus, as specific fatty acids may regulate both abnormal cell proliferation and unusual cell loss, but do so differently, identification of the function of each fatty acid will be necessary to apply these findings to the control of human disease.

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