COMMENTARY

Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers

Helmut Bartsch1, Jagadeesan Nair and Robert Wyn Owen

Division of Toxicology and Cancer Risk Factors, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

The hypothesis that a high-fat diet promotes the development of postmenopausal breast cancer is supported by international data showing a strong correlation between fat intake and breast cancer rates and a modest positive association with high-fat diet in case-control studies. Dietary fat intake was found to be unrelated to the risk of breast cancer in cohort studies. In view of these conflicting findings it has been difficult to make nutritional recommendations for the prevention of breast cancer. Studies in animal models and recent observations in humans, however, have provided evidence that a high intake of ω-3 polyunsaturated fatty acids (PUFAs), stimulates several stages in the development of mammary and colon cancer, from an increase in oxidative DNA damage to effects on cell proliferation, free estrogen levels to hormonal catabolism. In contrast, fish oil-derived ω-3 fatty acids seem to prevent cancer by influencing the activity of enzymes and proteins related to intracellular signalling and, ultimately, cell proliferation. In this commentary, current evidence from experimental and human studies is summarized that implicates a high intake of ω-6 PUFAs in cancer of the breast, colon and, possibly, prostate and which indicates that ω-3 PUFAs and monounsaturated fatty acids such as oleic acid (ω-9) are protective. Plausible mechanisms for modulation of steps in the multistage carcinogenesis process by fats are summarized briefly in order to provide guidance for the design of epidemiological studies that include mechanistically relevant biomarkers. Owing to limitations of space, the literature cited is not exhaustive and the reader is referred to earlier reviews (4–8).

Composition of dietary fats and geographical variation in intake

Most human diets contain a variety of saturated fatty acids of different chain lengths (Figure 1). The major saturated fatty acid in the diet is palmitic acid (C16:0), followed by stearic acid (C18:0), myristic (C14:0) and lauric (C12:0) acids. Short and medium chain fatty acids (C4:0–10:0), which occur mainly in dairy fat, palm kernel oils and coconut oils, also contribute to the total intake of saturated fatty acids. Palmitic acid is found in all edible fats and oils, but olive and rapeseed oils are particularly rich in linoleic acid. Oleic acid is present in all edible fats and oils, and is particularly abundant in palm oil and in butter, milk, cheese and meats. Stearic acid is found predominantly in cocoa butter, used in chocolate, and in fats from cattle and sheep. Lauric oils such as coconut and palm kernel oils, used in confectionery, and dairy fats contain relatively large amounts of lauric and myristic acids. The major monounsaturated fatty acid in human diets is oleic acid (cis-C18:1 ω-9) and the principal polyunsaturated fatty acid is linoleic acid (cis, cis-C18:2 ω-6). Vegetable oils such as those from soybeans, corn and sunflowers are rich in linoleic acid. Oleic acid is present in all edible fats and oils, but olive and rapeseed oils are particularly rich sources.

Trans isomers of monounsaturated fatty acids (trans-C18:1) are produced during industrial hydrogenation of polyunsaturated vegetable oils. Major sources of trans-unsaturated fatty acids are partially hydrogenated fats such as frying fats used in industrial food preparation and in fast-food restaurants and hard margarines. Conflicting results have been reported on the

Abbreviations: COX, cyclooxygenase; CYP, cytochrome P450; NSAID, non-steroidal anti-inflammatory drug; PUFA, polyunsaturated fatty acid.

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role of trans fatty acids in the risk for breast cancer (9,10), which are not discussed further.

Both ω-6 and ω-3 fatty acids are essential and as such must be provided in the diet. Linoleic acid (ω-6) is found in vegetable seeds and oils such as those from safflower, sunflowers, soybeans and corn. α-Linolenic acid (ω-3) is found in dark green leafy plants and in linseed, rapeseed, walnut and blackcurrant oils. Deep cold-water fatty fish are rich sources of ω-3 fatty acids (eicosapentaenoic and docosahexaenoic acid) and the marine food chain is based on ω-3 fatty acids, which are present in the plankton and algae on which fish feed.

The relative contributions of various fatty acids to dietary intake differs widely among countries, as do the incidences of many cancers. In Italy and Spain, for example, where olive oil is a staple, the incidence of breast cancer is lower than that in North America and northern Europe (11), and Greek women, who consume 42% of their energy supply as fat, mainly from olive oil (ω-9, monounsaturated), have significantly lower rates of breast cancer than women in the USA, whose energy intake from fat is ~35%. Eskimos, who eat fish and meat from marine mammals rich in ω-3 fatty acids, and Japanese fishermen, who have the highest consumption of fish per capita in the world, have high blood levels of long chain ω-3 PUFAs such as eicosapentaenoic acid and low rates of cancers of the breast and colon, despite their overall high fat consumption.

Thus, the ratio of ω-6:ω-3 PUFAs in the diet is important, although an optimal ratio remains to be determined. The ratio seems to be increasing over time: while the diet of Mesolithic man had a ratio of 1–4:1, the European diet now contains 10–14:1. The present average ratio in Israel is ~22–26:1, as seen in the composition of subcutaneous fatty acids (12). This is one of the highest ratios of dietary polyunsaturated:saturated fat in the world, the consumption of ω-6 PUFAs being ~8% higher than in the USA and 10–12% higher than in most European countries. Although a diet high in ω-6 PUFAs was widely recommended until recently, its excess intake may have long-term side-effects, including tumourigenesis, hyperinsulinemia and atherosclerosis. In this article, we review recent evidence from experimental and human epidemiological and clinical studies to support this hypothesis, focusing on cancer risk modification.

**Effect of types and amounts of fat on tumour development in rodents**

Tannenbaum and Silverstone demonstrated that a high fat diet stimulates mammary tumour development in mice when compared with a low fat diet (13). In further studies, the effect was shown to be independent of caloric intake and the response to fat was non-linear, reaching a plateau. Subsequent studies have shown that many steps in the tumourigenic process, including initiation, promotion, latency, growth and metastasis, can be influenced by dietary fat. Within the last few decades, studies in rodents have shown that large amounts of dietary fat increase the incidence of cancers of the breast, colon and prostate. Moreover, diets that contain high levels of the ω-6 fatty acid linoleic acid enhance tumourigenesis. Thus, the essential linoleic acid appears to be pivotal in tumour induction and metastasis, whereas high levels of fats such as olive oil, which are rich in oleic acid (monounsaturated, ω-9) and fish oil (polyunsaturated, ω-3) do not promote tumour development in animal models and even have protective effects.

**Tumour-enhancing effects of ω-6 polyunsaturated fatty acids**

Fay et al. conducted a meta-analysis of data on mammary tumour incidence extracted from 97 reports of experiments involving over 12 800 mice and rats to study the effects of saturated and monounsaturated fats and ω-6 PUFAs (14). The results indicated that ω-6 PUFAs have a strong and saturated fats a weaker tumour-enhancing effect, whereas the ω-3 PUFAs have a small, statistically non-significant protective effect; monounsaturated fats had no significant effect. ω-6 PUFAs had a stronger tumour-enhancing effect when they represented <4% of total calories, but the effect was still stronger than that of saturated fat when they represented >4% of the caloric intake.

Hilakivi-Clarke et al. tested the hypothesis that consumption of a high fat diet during gestation increases the incidence of carcinoogen-induced mammary tumours in rats. Pregnant or virgin female Sprague–Dawley rats that had previously been treated with 7,12-dimethylbenz[a]anthracene were assigned to an isocaloric diet containing either 16 (low fat) or 43% calories from fat (high fat) throughout gestation (15). The fat source was corn oil, which is rich in ω-6 PUFAs (primarily linoleic acid). On gestation day 19, the serum oestradiol levels were ~2-fold higher in rats fed the high fat diet than in those fed the low fat diet and the number of rats developing mammary tumours was significantly higher (40% tumour-bearing animals) in the group given the high fat diet than in those given the low fat diet (10%). Thus, consumption of a high ω-6 PUFAs diet during gestation increased the risk of developing carcinoogen-induced mammary tumours, possibly by increasing the concentration of circulating oestrogens. These data raise the possibility that human breast cancer might be prevented by dietary manipulation during pregnancy, which has not been addressed in epidemiological studies.

Hilakivi-Clarke et al. also tested the hypothesis that feeding pregnant rats a high fat diet would increase both circulating 17α-oestradiol concentrations in the dams and the risk of
developing carcinogen-induced mammary tumours among their female offspring (16). Isocaloric diets in which 12–16% (low fat) or 43–46% (high fat) of the calories were derived from corn oil (primarily the ω-6 PUFA linoleic acid) were fed throughout gestation. The plasma concentrations of 17α-oestradiol were significantly higher in pregnant females fed the high fat diet. The female offspring of these rats were fed laboratory chow from birth onwards. When they were exposed to 7,12-dimethylbenz[a]anthracene, they had a significantly higher mammary tumour incidence (60 versus 30%) and a shorter latency for tumour appearance than the offspring of dams on the low fat diet; they also showed onset of puberty at a younger age and their mammary glands contained significantly higher numbers of the epithelial structures that are the targets for malignant transformation. (The term acceleration mass was used in the past for a very early onset of puberty and growth of breast tissue, hypothesized to be due to high protein intake.) If these findings can be extrapolated to humans, they may explain the link between diet and breast cancer risk, indicating that exposure in utero to a diet rich in ω-6 PUFA and/or oestrogenic stimuli may affect breast cancer risk later in life.

On the basis of earlier findings, Walker and Kurth conducted a multigeneration study on the effect of dietary fat on carcinogenesis in which the offspring of mice exposed to a high fat diet were maintained on a low fat diet during their own gestation (17). CD-1 mice received diets containing 2.6 or 29% fat from corn oil (by weight) during gestation and their female offspring were given a commercial control diet (10% fat), mated, continued on the control diet throughout gestation and observed for life. The total number of reproductive system tumours, pituitary tumours and metastases was increased in the offspring of dams exposed to a high dietary level of ω-6 PUFA.

Tumour-protective effects of ω-3 polyunsaturated fatty acids and monounsaturated fatty acids

By 1981, it was widely accepted that all types of PUFAs, ω-3 PUFAs present in fish oil (eicosapentaenoic acid, C20:5, ω-3, and docosahexaenoic acid, C22:6, ω-3) and ω-6 PUFAs present in vegetable oils (linoleic acid), had tumour-promoting activity in experimental models. Karmali et al. first reported a difference in the effects of ω-3 and ω-6 PUFAs on the growth of transplantable mammary tumours, in which the tumour-promoting activity of ω-6 PUFAs was abrogated by competitive inhibition by ω-3 PUFAs from the metabolism of arachidonic acid (18,19). Several studies subsequently showed that diets containing corn oil, with high levels of ω-6 PUFAs such as linoleic acid, enhance breast and colon tumourigenesis in rodents, whereas fish oil, which is rich in the ω-3 PUFAs eicosapentaenoic acid and docosahexaenoic acid, reduces carcinogenesis (5). There is now also evidence that consumption of ω-3 PUFAs is associated with reduced mortality from cardiovascular disease (20).

Thus, the dietary ratio of ω-6:ω-3 appears to be crucial, since ω-3 fatty acids are competitive inhibitors of the effects of ω-6 fatty acids (21). Matsuba et al. examined the effects on animal tumourigenesis of three oils that differed in their ω-6:ω-3 ratio due to differences in the concentrations of linoleic acid and ω-6-linolenic acid. In comparison with safflower oil (70% linoleic acid and 0.1% ω-6-linolenic acid) and soybean oil (50% linoleic acid and 5% ω-6-linolenic acid), perilla oil (used in Japan, which contains 15% linoleic acid and 65% ω-3-linolenic acid) prevented tumours of the breast, colon and kidney in rats and of the breast in mice. Likewise, in comparison with a high ω-6-linolenic acid diet (5%) a diet rich in eicosapentaenoic acid (4%) and low in linoleic acid (0.3%) resulted in statistically significantly lower incidences of azoxymethane-induced colon cancers in rats, possibly due to lower concentrations of specific linoleic acid-derived prostaglandins (22).

Docosahexaenoic acid, the most abundant ω-3 fatty acid in human tissues, suppressed the formation and growth of aberrant crypt foci, which are presumed precursors of colon tumours, in carcinogen-treated rats (23). Hubbard et al. found that mice fed diets enriched in ω-3 fatty acids from fish oil had significantly smaller numbers of primary breast tumour and total metastatic load (24). Other studies suggest that mono-unsaturated fatty acids and ω-6 PUFAs have opposite effects on the risk for breast or colon cancer. The protective effects of a high intake of monounsaturated fatty acids derived mainly from olive oil and a low intake of ω-6 fatty acids were shown in animal models, in which Takeshita et al. found that the incidence of adenocarcinomas of the colon in 1,2-dimethylhydrazine-treated mice and of mammary tumours in N-methyl-N-nitrosourea-induced rats was significantly higher in animals fed a diet rich in linoleic acid than in animals fed a diet enriched with oleic acid or a low fat diet (25). These results have been confirmed in humans in recent studies on the association between dietary intake of monounsaturated and other types of fat and the risk of breast cancer (26,27).

Association between type of dietary fat and cancers of the human breast, colorectum and, possibly, prostate

Although lifestyle, genetic, reproductive and many other factors have been linked to the risk for breast cancer, much of the variation in breast cancer incidence across countries and cultures remains unexplained. The wide geographic variation spurred interest in the role of diet, leading to extensive research on fat intake, as critically reviewed by Wynder et al. and recently by Greenwald and Hunter (7,28,29). A meta-analysis of 12 case–control studies on breast cancer showed a modest positive association with dietary fat intake (30). In contrast, the findings from a pooled cohort study by Hunter et al. showed no role of dietary fat in breast cancer (31). Similarly, a large prospective cohort study in the USA gave no evidence that a lower intake of total fat or specific major types of fat was associated with a decreased risk of breast cancer (32). In the absence of data from dietary intervention trials, Wynder et al. argued that the weight of the evidence suggests that the type and amount of fat in the diet are related to the post-menopausal occurrence of breast cancer (7). Inability to detect associations within populations (cohort studies) is due to measurement errors and the relative homogeneity of the diets studied.

A meta-analysis was conducted of case–control and prospective cohort studies to determine whether a high intake of linoleic acid is related to cancer risk. None of the combined estimates indicated increased risks for cancers of the breast, colon or prostate, although a small increase could not be excluded (33). The serious limitations and confounders in most of these studies were discussed by the authors in an editorial and indicate the need for prospective molecular epidemiological studies on micronutrients, fat intake and cancer risk with biomarker measurements (34; see Lipid peroxidation and
oxidative DNA damage). Such a study is under way at the International Agency for Research on Cancer in Lyon (35).

In a population-based prospective cohort study on 61 471 women aged 40–76 years in Sweden with no previous history of cancer, 674 cases of invasive breast cancer occurred during an average follow-up of 4.2 years (26). Dietary intake, validated by a food frequency questionnaire, showed an inverse association with intake of monounsaturated fat and a positive association with intake of polyunsaturated fat, after mutual adjustment for different types of fat. The risk ratio for each 10 g increment in daily intake of monounsaturated fat was 0.45, whereas that for a 5 g increment in polyunsaturated fat was 1.7. Saturated fat was not associated with the risk for breast cancer.

The reported inverse associations between the consumption of olive oil (rich in ω-9 monounsaturated fatty acids but low in ω-6 PUFAs) and the risk for breast cancer in case-control studies in Greece, Spain and Italy, where olive oil is a major source of mainly oleic acid, might be due to a protective effect of a high intake of monounsaturated fatty acids and a high intake of the antioxidants present in olive oil (27,36,37). In a study on the relationship between the fatty acid content of adipose tissue and breast cancer, a strong inverse association was found between stores of oleic acid and breast cancer in Spanish women (38). Similarly, the low rates of prostate cancer in Mediterranean countries have been attributed to a high consumption of olive oil (39). It is noteworthy that several chemopreventive agents are major components of olive oil, like α-tocopherol, squalene (40), a number of newly identified ingredients such as lignans and various classes of phenolics shown to be potent antioxidants (41,42). As foods containing large amounts of lignan precursors have been associated with decreased risks for cancers of the breast, colon and prostate, diets containing lignans as natural components may be important for cancer chemoprevention.

In a population study, an inverse relationship was found between a high consumption of fat from fish, rich in ω-3 PUFAs, and the development of colorectal cancer. Thus, data on mortality from colorectal cancer in 24 European countries between 1984 and 1987 were correlated with consumption of fish and fish oil currently and 10–23 years earlier (43). The study showed an inverse association between death from colorectal cancer and current fish intake (P = 0.036), a weaker correlation with fish consumption 10 years earlier (P = 0.042) and none with consumption 23 years earlier (P = 0.12) among males; the association was not significant for females. In a follow-up study (44), mortality from colorectal cancer correlated with the consumption of animal but not vegetable fat and an inverse correlation was observed with fish and fish oil consumption when expressed as a proportion of the total or animal fat (in countries with an animal fat consumption of 85 g/day). This correlation was significant for both males and females and for intakes currently or 10 or 23 years before death from cancer. The evidence from these two studies indicates that fish oil provides protection against the promotional effects of animal fat in colorectal carcinogenesis.

Case-control studies on colorectal cancer and diet have produced equivocal results in relation to the intake of fish, some showing no protective effect (45–49). In these studies, however, fish consumption was much lower than in those in which a protective effect was shown (50–53). Future studies should be conducted in areas where fish intake is habitually high and should be directed specifically to this food item.

Mechanisms in fat-related carcinogenesis

The existence of a causal relationship between a high dietary fat intake and increased cancer risk has been controversial for a long time, partly because of the lack of consensus on the mechanisms of action of dietary fat in mammalian cells. Dietary fats, specifically ω-6 and ω-3 PUFAs, affect a variety of steps in the multistage carcinogenesis process, adding further weight to a causal effect. The effects may be direct or indirect and include: (i) peroxidation of conjugated double bonds in PUFAs, leading to persistent oxidative stress and generation of reactive lipid peroxidation products (malondialdehyde, 4-hydroxynonenals), which can induce DNA damage; (ii) conversion of essential fatty acid to eicosanoids, short-lived hormone-like lipids derived primarily from dietary linoleic acid; (iii) interaction of fatty acids with signal transduction.
pathways leading to altered gene expression; (iv) in the case of breast cancer, effects on unbound oestrogenic hormone concentrations; (v) effects on membrane (lipid)-bound enzymes such as cytochrome P450 (CYP) that regulate xenobiotic and oestrogen metabolism; (vi) structural and functional changes in cell membranes resulting in alterations in hormone and growth factor receptors. In view of space limitations, we discuss below some recent relevant findings that may help to pinpoint specific biomarkers useful for designing molecular epidemiological studies on fat intake and cancer risk.

**Lipid peroxidation and oxidative DNA damage**

Persistent cellular oxidative stress and enhanced peroxidation of PUFA, leading to macromolecular damage and disruption of signalling pathways, are known to stimulate the development of human malignancies (56). Lipid peroxidation generates several reactive α,β-unsaturated aldehydes, such as trans-4-hydroxy-2-nonenal and malondialdehyde, which can form promutagenic exocyclic DNA adducts in human cells (Figure 2) and may thus contribute to diet-related cancers (57,58). *trans*-4-Hydroxy-2-nonenal, one of the major lipid peroxidation products, is formed by oxidation of linoleic or arachidonic acid (ω-6 PUFAs) and is readily oxidized by fatty acid peroxides to form 2,3-epoxy-4-hydroxynonal. This bifunctional alkylating agent can react with DNA to yield etheno and other base adducts. Etheno adducts are highly miscoding lesions in mammalian cells and are thought to initiate the carcinogenic process through specific point mutations, as shown with the known carcinogens vinyl chloride and urethane (59).

We used ultrasonic detection methods to analyse white blood cell DNA from volunteers in a carefully controlled dietary study and showed that a high intake of ω-6 PUFA moderately increased the frequency of malondialdehyde-derived DNA adducts, to a somewhat greater degree in women than in men (60). In contrast, the frequency of etheno–DNA adducts in white blood cells was 40 times greater in women and not increased in men, with a huge interindividual variation in lipid peroxidation-derived DNA damage. The results of this pilot study clearly indicated that the DNA adduction was due to consumption of a diet rich in ω-6 PUFA, which resulted in increased lipid peroxidation of membrane lipids. The marked interindividual difference in etheno adduct levels in some women on the same high PUFA diet is unexplained; it could be related to the menstrual cycle, resulting in synergism between high dietary ω-6 PUFA intake and oestrogen catabolism. In this scenario, 4-hydroxyoestradiol (discussed in Hormonal effects and interactions with oestrogen catabolism) could generate free radical-mediated DNA damage via redox cycling and trigger oxidation of ω-6 PUFAs to *trans*-4-hydroxy-2-nonenal, which is the precursor of etheno–DNA adducts. If exocyclic adducts are formed in the epithelium of the breast and colon via lipid peroxidation of ω-6 PUFA, these findings may offer new aetiological and mechanistic clues to tumour induction related to dietary fatty acids. Indeed, Wang *et al.*, using a 32P-post-labelling technique to quantify putative malondialdehyde–deoxygenosine and malondialdehyde–deoxygenosine adducts, found a 3-fold increase in the level of these lipid peroxidation-derived adducts in normal breast tissue from breast cancer patients in comparison with cancer-free controls (61). Transitional biomarker studies should now be conducted to address the following questions.

- Does a high dietary ω-6 PUFA intake (low ω-3:ω-6 ratio) by healthy women, as indicated from food frequency questionnaires or as measured by the fatty acid profile in breast adipose tissue, result in increased levels of exocyclic DNA adducts in breast epithelial cells and are the levels affected by the menstrual cycle?
- Is the positive correlation in the same individual between exocyclic adduct levels in peripheral lymphocytes and breast epithelial cells dependent on ω-6 PUFA intake?
- How do fat-soluble antioxidants in breast adipose tissue (vitamin E, carotinoids and retinoids) affect the adduct level in breast epithelium?
- Do the relative proportions of 2-, 4- and 16-hydroxylated metabolites of 17β-oestradiol in the plasma differ in relation to ω-6:ω-3 PUFA intake?
- What are the associations between these parameters and breast cancer risk when measured in age-matched patients with and without breast cancer?

Further data support the notion that lipid peroxidation-derived products and oxidative DNA base damage are associated with increased breast cancer risk. Lipid peroxidation, as measured from malondialdehyde in urine, in pre-menopausal women with mammographic dysplasia was approximately double that in women without these radiological changes (62). The presence of DNA damage in primary cultures of human mammary epithelial cells and the ability of extracts of human mammary cells to cause such damage were investigated with the Comet assay (63). Lipid extracts of breast tissue removed from healthy women undergoing reduction mammoplasty showed significant interindividual variation in DNA-damaging properties. The most active extracts tended to be those from donors whose mammary cells also contained the greatest amount of pre-existing DNA damage, indicating that mammary lipids can contain genotoxic substances which may be involved in breast carcinogenesis, although their nature and origin remain to be elucidated.

The effect of a low fat diet on levels of oxidative DNA damage in peripheral nucleated blood cells was studied in women at high risk for breast cancer who were randomly assigned to a normal or low fat diet (64). The concentration of oxidized thymine, specifically 5-hydroxymethyluracil, was 3-fold higher in the group given normal diet and there was a significant, linear relationship between daily total fat intake and 5-hydroxymethyluracil level. These results suggest that oxidative DNA damage is a mechanistic link between diet and...
cancer risk. This was confirmed by Frenkel et al., who found that serum autoantibodies that recognize 5-hydroxymethyl-2'-deoxouridine, an oxidized DNA base, can serve as markers for breast and colorectal cancer risk in women (65). Women who were healthy at the time of blood donation but who were later found to have breast or colorectal cancer had significantly higher titres of autoantibodies against 5-hydroxymethyl-2'-deoxouridine than age-matched controls. These titres may be an early sign of cancer risk, since they were significantly increased in otherwise healthy women who had a family history of breast cancer, in women who had benign breast disease or benign gastrointestinal tract disease and, most importantly, in women who developed breast or colorectal cancer 0.5–6 years after the initial blood donation.

Progression of human breast cancers to the metastatic state has been linked to hydroxyl radical-induced DNA damage (66). Oxidative stress has been implicated as an important factor in metastasis, notably because it results in loss of cell adhesion, which is the prerequisite for cellular detachment and invasion of host tissues. A >2-fold increase in oxidative base damage was found in DNA from metastatic breast tumours compared with that from non-metastatic tumours, suggesting that the hydroxyl radical generates DNA phenotypes with various metastatic potentials, which probably contribute to the heterogeneity of metastatic cell populations and to a poorer prognosis.

**Hormonal effects and interactions with oestrogen catabolism**

The risk of developing breast cancer is closely linked to reproductive events, implying a role of endogenous oestrogens in breast cancer development. It has been proposed that a high fat intake raises the concentrations of circulating sex hormones such as oestrogen. Sex hormone-binding globulin is an important regulator of plasma sex steroids and decreased globulin levels have been reported in high risk groups, resulting in increased concentrations of unbound ‘free’ oestrogen. Similarly, high fat intake may affect the bioavailability of oestrogen (bound versus free) by increasing the concentration of free fatty acids in serum, which, in turn, displace oestrogen from serum albumin, making it free for uptake by oestrogen receptors. Although epidemiological studies in women have provided inconsistent results on the role of increased circulating oestrogens (free or bound) in breast cancer, studies in rats have revealed that consumption of a high fat diet (67), in particular a high ω-6 PUFA diet (51), during gestation increases the risk of developing chemically induced mammary tumours.

The relative ease with which dietary fat alters cellular lipid profiles has important implications for membrane-bound enzyme systems. As CYP-dependent, mixed function oxidases play a key role in the biotransformation of chemical carcinogens, drugs and steroids and dietary fat can affect their activity, the risk for breast cancer has been associated with altered oestrogen catabolism. According to Bradlow et al., the conversion of oestrone to the catechol oestrogen 2-hydroxyoestradiol decreases the risk of breast cancer, whereas conversion to 4-hydroxyoestradiol generates free radicals through metabolic redox cycling and the chemically reactive oestrogen semi-quinone/quinone intermediates (68; Figure 3). These metabolic intermediates can damage DNA, induce cell transformation and initiate tumourigenesis (reviewed in ref. 69). 4-Hydroxyoestradiol is a strong carcinogen in hamster kidney under conditions in which 2-hydroxyoestradiol is not carcinogenic. The ability of 4-hydroxyoestradiol and oestrone-3,4-quinone to cause kidney or liver tumours in certain animal models indicates that 4-hydroxylated oestrogens are carcinogenic metabolites.

High intake of the ω-6 PUFA linoleic acid and arachidonic acid inhibits the detoxification of oestrogens by 2-hydroxylation (70) and increases 16α-hydroxylation, resulting in metabolites that can undergo redox cycling and generate hydroxyl radicals. Whether ω-6 PUFA s also enhance the formation of 4-hydroxylated oestrogens is not known.

Increased activity of oestradiol 4-hydroxylase in target tissues of oestrogens may play an important role in the development of oestriadiol-induced tumourigenesis (69). A high degree of activity is expressed in the kidneys of male Syrian hamsters, the uterus of CD-1 mice and the pituitary gland of rats, which are susceptible to oestrogen-induced cancer. Each of these target organs contains a very high concentration of endogenous catecholamines, which may significantly inhibit catechol-O-methyltransferase-catalysed O-methylation of 4- and 2-hydroxyoestradiol in vivo. Moreover, catechol-O-methyltransferase-catalysed O-methylation of 4-hydroxyoestradiol is inhibited by 2-hydroxy-17α-oestradiol, whereas the O-methylation of 2-hydroxy-17β-oestradiol is not inhibited by 4-hydroxy-17α-oestradiol. Therefore, it is likely that 4-hydroxy-17β-oestradiol accumulates in these target organs because of inhibition of its O-methylation and also because of its rapid formation. Furthermore, greater oestradiol 4-hydroxylase activity has been observed in human breast cancer tissue than in normal breast tissue (70) and 4-hydroxyoestradiol appears to be the most abundant oestrogen metabolite in human breast cancer tissue. Studies are needed to confirm these findings.

Several attempts have been made to characterize CYP isoforms with high oestradiol 4-hydroxylase activity in oestrogen target tissues (69). In human breast cancer cells and uterine myoma, oestradiol 4-hydroxylation is catalysed predominantly by CYP1B1. The expression of its mRNA is regulated by multiple endogenous factors, including cAMP, and by lipid-soluble xenobiotics. It will be important to characterize the selective expression and differential regulation of CYP1B1 and other CYP isoforms with oestradiol 2- and 4-hydroxylase activity in different cell types in human breast and the effects of various fatty acids. Human biomarkers should now be
Fig. 4. Hypothetical scheme linking arachidonic acid (AA) metabolism, persistent oxidative stress and DNA damage to multistage carcinogenesis. In initiated or preneoplastic cells (e.g. in the colon), PLA2, COX-2 and LOX are often constitutionally overexpressed. This leads to increased release of AA and faster AA oxygenation, resulting in higher levels of ω-6 eicosanoids, accompanied by generation of reactive oxygen species (ROS). These can cause DNA damage and trigger lipid peroxidation (LPO) of polyunsaturated fatty acids (PUFAs) in a self-perpetuating process, leading to various forms of exocyclic DNA base modifications (e.g. via 4-HNE and MDA). A tight link between the formation of HETE (via LOX) and exocyclic DNA adducts during two-stage skin carcinogenesis in mice was recently demonstrated (Nair et al., submitted for publication). In rapidly dividing cells, the resulting genetic changes and disrupted signalling pathways may drive premalignant cells to genetic instability and malignancy. ω-3 PUFAs inhibit AA metabolism and COX activity, thus blocking the formation of ω-6 eicosanoids from diet-derived linoleic acid (LA), which have been linked to tumour growth and metastasis (see also Effects on cell proliferation and signal transduction). 4-HNE, trans-4-hydroxy-2-nonenal; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; LOX, lipoxygenase; MDA, malondialdehyde; PLA2, phospholipase A2.

Effects on cell proliferation and signal transduction

Fat may regulate cellular functions by affecting the expression or activity of genes in the signal transduction pathway related to the control of cell growth and apoptosis. High intake of ω-6 PUFAs induces various physiological and metabolic effects (5,72): (i) increased ornithine decarboxylase activity in colonic mucosa, resulting in enhanced epithelial polyamine levels and increased colonic crypt cell proliferation; (ii) enhanced activities of protein kinases like protein kinase C in rodent mammary gland and an increased number of oestrogen receptor binding sites (73); (iii) increased prostaglandin concentrations. Prostaglandins, thromboxanes, leukotrienes and hydroxy and hydroxyperoxy fatty acids (collectively referred to as eicosanoids) are involved in tumour initiation and promotion, cell proliferation, tissue invasion and metastatic spread (Figure 4). Tumour cells produce larger amounts of eicosanoids than their normal cell counterparts and eicosanoids ultimately derived from linoleic acid (ω-6 eicosanoids) have been linked to increased growth and metastasis. The finding that oleic acid and ω-3 PUFAs, specifically eicosapentaenoic acid, block the desaturase reaction, the first step from linoleic acid to eicosanoids, may partially explain their inhibitory effects on tumourigenesis. Indeed, a stepwise reduction in eicosapentaenoic acid concentration was seen in diseased mucosa from benign adenoma to the most advanced colon cancer, indicating that changes in the ω-3:ω-6 ratio may participate in the early phases of human carcinogenesis (74).

Intervention with pharmacological agents that inhibit eicosanoid synthesis, such as non-steroidal anti-inflammatory agents (NSAIDs) results in inhibition of tumourigenesis. Long-term ingestion of NSAIDs is associated with a reduced risk of colon cancer, a reduction in the number and size of colonic polyps and adenomas in patients with familial adenomatous polyposis and protection against chemically induced colon cancer in animal models (75). Cyclooxygenase (COX)-1 and COX-2 are among the targets of NSAIDs and treatment with NSAIDs is associated with a decrease in COX-2 activity in colon tumours. The ω-3 PUFAs eicosapentaenoic acid and docosahexaenoic acid inhibit COX activity and arachidonic acid metabolism. Rats fed ω-3 PUFAs showed selective incorporation of ω-3 PUFAs with a concomitant reduction in ω-6 PUFAs in the membrane phospholipid pool of cells in various tissues. Recent results suggest that ω-6 PUFAs promote colon and mammary tumourigenesis by up-regulating the expression of COX-2 and p21ras, whereas ω-3 PUFAs may exert their antitumour effect by inhibiting COX-2 expression (76–78). Docosahexaenoic acid was also shown to suppress intestinal polyp development in Apc<sup>−/−</sup> knockout mice (a model for human familial adenomatous polyposis), possibly by interfering with the metabolic pathways of arachidonic acid (79), i.e. by inhibiting COX-2 (80).

Nitric oxide is an important physiological mediator, but its excessive production during inflammation is thought to cause cellular injury and, in the long term, cancer (81). Stimulation of nitric oxide production in a murine macrophage cell line with lipopolysaccharide was suppressed by docosahexaenoic acid, eicosapentaenoic acid and α-linolenic acid in a dose-dependent fashion (82). No inhibition was observed with ω-6 PUFAs (linoleic acid), ω-9 PUFAs (oleic acid) or a saturated
fatty acid (stearic acid). Inhibition of inducible nitric oxide synthase gene expression could further contribute to the cancer-preventive activity of ω-3 PUFAs.

Consumption of a fish oil diet was associated with increased apoptotic cell death and suppression of proliferation in colonic crypt cells of rats 24 and 48 h after administration of 1,2-dimethylhydrazine. The reduced incidence of aberrant crypt foci suggests that ω-3 PUFAs can protect against colon carcinogenesis by mediating changes in the balance of proliferation and cell death (83).

Conclusions and prospects for prevention

The hypothesis that a high fat diet promotes the development of breast cancer in post-menopausal women is supported by the results of international studies that show strong correlations between fat intake and breast cancer rates and modest positive associations with high fat diets in case control studies. Dietary fat intake was found to be unrelated to the risk of breast cancer in cohort studies. In view of these conflicting findings, it has been difficult to make nutritional recommendations for the prevention of breast cancer (28,29). Studies in animal models and recent observations in humans have, however, provided evidence that a high intake of ω-6 PUFAs stimulates several stages in the development of mammary and colon cancer, from an increase in oxidative DNA damage to effects on cell proliferation, free oestrogen levels and hormonal carcinobism. In contrast, fish oil-derived ω-3 fatty acids seem to prevent cancer by influencing the activity of enzymes and proteins related to intracellular signalling and, ultimately, cell proliferation. Directed studies are now needed, which include relevant biomarkers, to unravel the contributions of different types of fat, their interactions with hormonal carcinobism, protective nutritional factors and human cancer risk.

Although a reduction in total fat consumption has been shown to reduce circulating oestriadiol levels by 7% in pre-menopausal women and 23% in post-menopausal women in western populations (84), consideration should be given to the dietary balance between ω-3 and ω-6 fatty acids, especially in women. Diets rich in linoleic acid have not resulted in lowered mortality from cancer and heart disease in Israel, however, which has the highest intake, which suggests that a diet rich in ω-6 PUFAs (in particular linoleic acid) is not beneficial for the population at large. Even if the optimal ratio of ω-6:ω-3 fatty acids has yet to be determined, there are reasons to counter its increase. A ratio of 8–5:1 might be recommended, as active people who are in energy balance consume up to 30% of their total energy intake from dietary fat, with 7% from saturated fats, 10–15% from monounsaturated fatty acids and up to 10% from PUFAs.

Should the inverse association between monounsaturated fatty acid intake and the positive association between ω-6 PUFAs and the risk for breast cancer be confirmed by additional studies, dietary recommendations should focus on substitution of margarine and various vegetable oils (in which the monounsaturated fatty acid:PUFA ratio is most often 1:2) by olive oil (5:1). An adequate intake of antioxidants like vitamin E and phenolics and other chemopreventive agents (squalene) found in olive oil may be a further basis for the cancer-protective effect of the Mediterranean diet, and dietary advice should be based on this consistent observation.

References


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