Effects of dietary fatty acids on breast and prostate cancers: evidence from in vitro experiments and animal studies\textsuperscript{1–3}

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**ABSTRACT** Linoleic acid, an \(n-6\) polyunsaturated fatty acid, is essential for normal mammary tissue development, at least in part because it provides the metabolic precursor required for the biosynthesis of key eicosanoids. A similar requirement applies to the growth of estrogen-independent but apparently not to estrogen-dependent rodent mammary and human breast carcinoma cells in vitro. By way of lipoygenase products, \(n-6\) fatty acids also regulate expression of the invasive phenotype. High-fat, linoleic acid–rich diets promote chemically induced rat mammary carcinogenesis, virally induced mouse mammary tumor development, and the growth and metastasis of estrogen-independent human breast cancer cells in athymic nude mice. In contrast, saturated fatty acids have no discernible effects on mammary carcinogenesis or progression. Most mechanistic studies have focused on the cyclooxygenase and lipoygenase products of \(n-6\) fatty acid metabolism, and support is accumulating for interactions between these eicosanoids and growth factors and oncogenes. The investigation of dietary fatty acids in prostate cancer is at an early stage and has been handicapped by a lack of satisfactory animal models. However, there are indications that the \(n-6\) fatty acids perform functions in experimental prostate cancer progression similar to those described for breast cancer. *Am J Clin Nutr* 1997; 66(suppl):1513S–22S.

**KEY WORDS** Breast cancer, prostate cancer, fatty acids, linoleic acid, eicosanoids, animal models, \(n-6\) fatty acids

**INTRODUCTION**

There is evidence from both epidemiologic and experimental studies that dietary fatty acids influence the development and subsequent progression of both breast and prostate cancer. Moreover, specific fatty acids may exert opposing effects so that the net result is dependent on their relative concentration in the diet. This chapter will focus particularly on the \(n-6\) polyunsaturated fatty acids but, as will emerge in the companion review by Ip (1), the ratio of dietary \(n-6\) to \(n-3\) fatty acids may prove to be of critical importance in determining breast and perhaps prostate cancer progression.

Dietary polyunsaturated fatty acids are the sole source of fatty acids that cannot be synthesized by many animal species, including humans. Linoleic acid (18:2\(n-6\)), an \(n-6\) fatty acid, and \(\alpha\)-linolenic acid (18:3\(n-3\)), a short-chain \(n-3\) fatty acid, undergo desaturation and chain elongation to yield arachidonic acid (20:4\(n-6\)) and eicosapentaenoic acid (20:5\(n-3\)), respectively; 20:4\(n-6\) and 20:5\(n-3\) are the substrates for the biosynthesis of dienoic and trienoic eicosanoids and tetroaene and pentaene leukotrienes.

In general, the \(n-6\) fatty acids have been associated with enhancement of the promotional phase of experimental mammary carcinogenesis and with tumor cell invasion and expression of the metastatic phenotype; the long-chain \(n-3\) fatty acids present in fish oils inhibit this spectrum of the cancer process. However, despite the supportive findings from experimental studies in animal models, the involvement of dietary fatty acids in the etiology of human breast and prostate cancers remains controversial. As we examine the evidence, it will become apparent that a distinction must be made between the process of neoplastic transformation and the subsequent behavior of the cancer cell. For example, a particular fatty acid or its metabolite products may have no effect on the conversion of a normal cell to a cancer cell but will enhance the capacity of that cell to invade and establish metastatic colonies.

**EXPERIMENTAL MAMMARY CARCINOGENESIS AND TUMOR PROGRESSION**

\(n-6\) Fatty acids and normal and preneoplastic mammary epithelium

The presence of white adipose tissue rich in 18:2\(n-6\) (2, 3) and an adequate intake of this \(n-6\) fatty acid (4, 5) are essential for normal development of the mouse mammary gland. Bandyopadhyay et al (6, 7) showed that 18:2\(n-6\) stimulated the proliferation of mouse mammary epithelial cells in vitro, a response requiring the presence of epidermal growth factor (EGF). This growth-enhancing effect of 18:2\(n-6\) was inhibited by indomethacin, a cyclooxygenase inhibitor, and partially reproduced by the cyclooxygenase product prostaglandin E\textsubscript{2} (PGE\textsubscript{2}), which bypasses the metabolic block (5). However, the

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growth response to PGE₂ was less than that to 18:2n–6; for full stimulation both PGE₂ and one of the three hydroxyeicosatetraenoic acids formed from arachidonic acid under the influence of lipooxygenase activity (Figure 1) were required. Thus, an optimal growth response of mouse mammary epithelial cells to 18:2n–6 appears to represent a synergism between hydroxyeicosatetraenoic acids and PGE₂, which occurs under the added influence of EGF (7).

Several groups of investigators have examined the effect of 18:2n–6 on the growth of nontransformed human breast epithelial cells, either when freshly isolated from tissue obtained during reduction mammoplasty (9) or after the establishment of cell lines (10, 11). As for mouse mammary epithelial cells, the human counterparts exhibited a growth response to 18:2n–6 or PGE₂ in the presence of EGF and insulin (9). Grammatikos et al (10) used the nonneoplastic, spontaneously immortalized, MCF-10A human mammary epithelial cell line and this same line expressing the c-Ha-ras protooncogene (10) in a series of experiments that again showed a growth response to 18:2n–6 at the lower doses tested; in addition, inhibition occurred with the long-chain n–3 fatty acids.

In contrast with their normal progenitors, human epithelial cells isolated from noncancerous benign tumors, fibroadenomas (9), and MCF-10A cells expressing an activated c-Ha-ras oncogene (11) failed to show growth stimulation in vitro when cultured in the presence of 18:2n–6. The fibroadenoma-derived epithelial cells underwent no further growth acceleration when 18:2n–6 was added to the medium containing EGF and insulin, a feature that was accompanied by an impairment in the capacity of these cells to form 20:4n–6 from 18:2n–6. Similarly, the MCF-10A cells with the activated c-Ha-ras had lost their ability to perform Δ6 desaturation, which is the rate-limiting step in 20:4n–6 biosynthesis. We shall return to this issue of desaturase enzyme loss; however, note that in the study of fibroadenoma epithelial cells, the growth response to PGE₂ was lost as well as that to 18:2n–6 (9).

As in the human disease, mouse mammary tumors develop through a multistep process (12). An intermediate, pathologically defined stage is the formation of hyperplastic alveolar nodules, which appear with increasing frequency over time in mice expressing the mouse mammary tumor virus (MMTV). Telang et al (13, 14) investigated the effects of fatty acids on the hyperplastic alveolar nodule–like mammary alveolar lesions that develop in explants cultured from the mammary tissue of mice expressing MMTV. They found that both 18:2n–6 and 20:4n–6 enhanced replicative DNA synthesis in these lesions, whereas both stearic acid (18:0) and the long-chain 20:5n–3 were inhibitory. These alterations in proliferation were associated with changes in reverse transcriptase activity and c-rasH p21 expression. The demonstration that fatty acids affect events associated with cancer initiation, such as expression of MMTV and c-rasH p21, in nontransformed tissue has important implications for breast cancer prevention.

**Fatty acids and mammary cancer cell growth in vitro**

Wicha et al (15) studied the effects of several classes of fatty acids on the growth of a cell line derived from a dimethylbenz[a]anthracene (DMBA)-induced rat mammary carcinoma. The addition of 18:2n–6 to the culture medium resulted in a stimulation of proliferation; oleic acid (18:1), an n–9 monounsaturated fatty acid, had an even greater stimulatory effect whereas 18:0 produced a dose-related inhibition. Similar results were obtained by Rose and Connolly (16, 17) with the estrogen-independent MDA-MB-231 human breast cancer cell line; in these experiments, 18:2n–6 was stimulatory with an optimal effect at a concentration of 0.75 mg/L but was inhibitory at higher concentrations.

Care is needed in designing and interpreting cell culture experiments with added fatty acids because fatty acids normally exist in the bound form. There is a requirement for binding to albumin, which avoids exposure to high concentra-

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**FIGURE 1.** The biosynthesis of eicosanoids from linoleic acid. HPETE, hydroperoxy-5,8,10,14-eicosatetraenoic acid; HETE, hydroxy-5,8,10,14-eicosatetraenoic acid. Adapted from a figure published previously (8).
tions of free fatty acids and consequent artifactual cytotoxic effects (17).

As for normal mammary epithelium, the stimulation of breast cancer growth in vitro by 18:2n-6 involves the further metabolism of 20:4n-6 to eicosanoids. The demonstration that pharmacologic inhibition of lipoygenase, but not cyclooxygenase, activity could block the MDA-MB-231 cell growth response to 18:2n-6 (17) was extended by Buckman et al (18) to include a mouse mammary tumor cell line, the metastasis of which is enhanced by a diet rich in 18:2n-6. Somewhat similar results were also reported by Earashi et al (19), although in their study very high concentrations of piroxicam, primarily a cyclooxygenase inhibitor, also partially suppressed MDA-MB-231 cell growth. Lipoygenase products are believed to exert their biological actions partly through cyclic GMP, and cyclic GMP was as effective as 18:2n-6 in stimulating the growth of 4526 mouse mammary tumor cells [a cell line derived from a DMBA-induced mammary carcinoma (15)] (18).

The 4526 mouse mammary tumor cell line (18), MDA-MB-231 cells (17), and the MDA-MB-435 human breast cancer cell line (the growth of which is also stimulated by 18:2n-6) (20) are all estrogen independent. Although these cell lines all exhibit a clear-cut growth response when cultured in vitro with 18:2n-6, the situation with respect to estrogen-dependent breast cancer cells is uncertain. Originally, we reported that growth of the estrogen receptor–positive MCF-7 human breast cancer cell line was stimulated, albeit modestly, when 18:2n-6 was added to a culture medium supplemented with 1% fetal bovine serum (16). Later, it emerged that these particular cells were of questionable origin and hormone dependence (21). With authentic MCF-7 cells from another source, we showed estrogen dependence while establishing that this cell line shows no mitogenic response to 18:2n-6. In an unpublished experiment, we (JM Connolly and DP Rose, 1994) found that the growth of another estrogen-dependent human breast cancer cell line, ZR-75-1, was also unaffected by 18:2n-6 in vitro.

Grammatikos et al (10) also found that 18:2n-6 had no effect on MCF-7 cell growth, apparently because the cell line had a defect in desaturase activity similar to that observed by these same investigators in human breast epithelial cells expressing an activated c-Ha-ras oncogene (11). A similar study showed a deficiency in Δ6 and Δ4 desaturases in the T47D estrogen-responsive human breast cancer cell line (22).

Taken together, the observation that the three estrogen-independent cell lines (MDA-MB-231, MDA-MB-435, and 4526) studied so far all exhibit a growth response when cultured in the presence of 18:2n-6 whereas the three estrogen-dependent lines (MCF-7, ZR-75-1, and T47D) do not is of considerable interest. It suggests that the n-6 fatty acids, most likely by way of lipoygenase metabolic products, are involved in the regulation of expression of the metastatic phenotype. In an ongoing study, we have approached this important issue by first showing that MCF-7 cells possess a relatively low amount of 12-lipoygenase activity and then transfecting the cells with the complementary DNA for the enzyme. The MCF-7 cell transfectants, now overexpressing 12-lipoygenase, had an accelerated growth rate, which was stimulated further by 18:2n-6 even in the absence of estrogen. Moreover, in contrast with MCF-7 parental cells, the transfectants were highly invasive in an in vitro assay (X-H Liu, JM Connolly, and DP Rose, unpublished observations, 1996). Because the 12-lipoygenase transfectant cells had become responsive to 18:2n-6, with respect to both their growth and invasive capacity, characteristics in estrogen-independent breast cancer cell lines are associated with products of 20:4n-6 metabolism, it appears that they had reestablished expression of desaturase activity.

**Dietary fatty acids and experimental mammary carcinogenesis**

In this section, I discuss the n-6 polyunsaturated fatty acids and the saturated fatty acids; the influence of the n-3 and n-9 fatty acids on mammary carcinogenesis is discussed by Ip (1). Since the original descriptions by Tannenbaum (23) and Silverstone and Tannenbaum (24), many investigators have confirmed the enhancing effects of high-fat diets, specifically diets rich in n-6 fatty acids, on mammary tumor development in rodents. Reviews of this research effort have been published by several authors, including Rose (8), Welsch and Aylsworth (25), Cohen (26), and Rogers and Lee (27).

One of the earlier reports, by Davis et al (28), described an enhancing effect of a high-fat diet on the lifetime development of spontaneous tumors in female Sprague-Dawley rats, but most experimental studies in this species used either DMBA or N-nitrosomethylurea (NMU) to induce carcinogenesis. Of the two, NMU has the advantage that it is a direct-acting, water-soluble carcinogen and that the tumors induced are principally estrogen rather than prolactin dependent, thus approximating more closely human breast cancer (29, 30).

One issue that is frequently raised when the role of dietary fat in experimental mammary carcinogenesis is discussed is whether any observed relations are due to fat per se or, given that fat is the most energy-dense nutrient, to associated differences in energy intake. Although Boissonneault et al (31), Pariza et al (32), and Kritchevsky and Klurfeld (33) championed the view that the promotional effects of a high-fat diet on chemically induced rat mammary carcinogenesis are ascribable primarily to high energy intake and use, others have taken an intermediate position and proposed that there are dual and distinct effects of fat or specific fatty acids and energy intake (34, 35). One convincing argument for a direct influence of dietary fat is that the effects observed vary with the specific fatty acids that contribute to the total fat intake.

Carroll and Hopkins (36) showed that when a high-fat (20% by wt) diet was provided in the form of corn oil or sunflower oil rich in 18:2n-6, there was a pronounced enhancement of mammary tumor development in rats exposed to DMBA. In contrast, equivalent total fat intake in the form of saturated fatty acid–containing coconut oil, butter, or tallow produced no significant increase in tumor formation compared with that seen in rats fed an 0.5%–corn oil diet. These observations have been confirmed and extended by many investigators.

Cohen et al (37) compared NMU-induced mammary carcinogenesis in rats fed different amounts of corn oil as the sole source of dietary fat. They found a tumor-promoting effect that appeared to have a threshold rather than a linear dose response. Thus, tumor incidence was no different in animals fed 16% or 23% (by wt) corn oil but was two- to threefold more than that in the animals fed diets containing 5% or 10% corn oil. Body weight gains throughout the course of this experiment were not different among the four dietary groups, which was interpreted as indicating that the observed effects on tumorigenesis could not be ascribed to variations in energy intake.
Both Hopkins and Carroll (38) and Ip et al. (39) performed experiments to determine what proportion of the total lipid consumed was required to be in the form of 18:2n-6 to achieve an optimal enhancement of mammary tumorigenesis in the DMBA-induced rat mammary carcinoma model. Although there were differences in the experimental designs and in the statistical evaluation of the results obtained, the conclusions were similar: in both cases when the total dietary fat was 20% (by wt.), ≈3-4% 18:2n-6 was required for maximal tumor development, the rest being provided as saturated fatty acids. Note that dietary 18:2n-6 intakes at the 3% level (eg, a 5%-corn oil diet) do not produce these high tumor yields; the high total fat consumption is necessary, perhaps to provide the required energy intake.

In contrast with the polyunsaturated fatty acids discussed here and in the following review by Ip (1), saturated fatty acids do not appear to have a direct promotional effect on experimental mammary carcinogenesis. Cohen et al. (40) found no enhancing activity of the saturated fatty acids present in coconut oil on NMU-induced rat mammary carcinogenesis, and similar negative results were obtained when 18:0 was assessed in strains of mice with a high incidence of spontaneous mammary tumors (41, 42). Indeed, in C3H mice there was both an increase in latency and a reduction in tumor incidence (41). As already noted, in their study using the DMBA tumor model, Carroll and Hopkins (36) also found no promotional effect of saturated fatty acids.

**Models of breast cancer progression: transplantable rodent carcinomas and human breast cancer xenografts in nude mice**

In addition to the influence of fatty acids on the process of neoplastic transformation, more recent studies emphasized their involvement in subsequent expression of the metastatic phenotype. Gabor and Abraham (43) first observed the stimulatory effect of an 18:2n-6-rich diet on the growth of a transplantable mouse mammary adenocarcinoma, and in a series of studies we showed that this same diet both stimulated growth and enhanced the metastasis of human breast cancer cells after their injection into the thoracic mammary fat pads of athymic nude mice (44-47).

The first step in the metastatic process involves local invasion of tumor cells through the extracellular matrix, and at least one biological mechanism for the enhancement of metastasis in mice fed diets rich in n-6 fatty acids appears to operate at this level. Thus, in an in vitro assay the invasive capacity of the MDA-MB-435 human breast cancer cell line was stimulated by 18:2n-6 (48), an effect that was associated with 12-hydroxyicosatetraenoic acid–mediated induction of the 92-kDa isoform of type IV collagenase (matrix metalloproteinase-9) but not the 72-kDa isoform (metalloproteinase-2) (49). In contrast with dietary 18:2n-6, feeding long-chain n-3 fatty acids suppressed expression of the metastatic phenotype by human breast cancer cell xenografts in nude mice (50, 51), and in preclinical experiments n-3 fatty acids showed promise as a novel approach to nutritional adjuvant therapy (52).

### Mechanisms

**Hormones**

The primary action of fatty acids in mammary carcinogenesis is generally regarded as involving promotion rather than initiation. In one study, the effect of a high-fat diet was evident even when it was commenced 20 wk after exposure to a chemical carcinogen (53). The dependence of a promotional effect of fatty acids on normal endocrine function has been shown by numerous investigators, but the nature of any interaction remains in question. Both bilateral ovariectomy (54) and the inhibition of prolactin release by bromocriptine (55) inhibit rat mammary tumor development after exposure to a chemical carcinogen, and early reports suggested that high-fat diets might exert their effects on mammary tumorigenesis by altering blood hormone concentrations (55, 56). However, these observations were questioned because anesthesia, which may stimulate prolactin release, was used to facilitate blood collection. Later studies of unanesthetized animals with indwelling cardiac catheters (to provide for multiple sampling throughout the estrous cycle) showed no changes in plasma estradiol, progesterone, or prolactin concentrations after a high-fat diet rich in 18:2n-6 was fed (57).

Even in the absence of increased hormone secretion or altered metabolism, enhanced hormonal activity could still result from changes in receptor binding capacity or accelerated postreceptor molecular signaling. In common with other polypeptide hormones and growth factors, prolactin binds to receptor sites located in the cell membrane, which therefore may be influenced by changes in the membrane phospholipid fatty acid composition. Cave and others (58, 59) found that severe reductions in polyunsaturated fatty acid intake produced a decrease in prolactin binding to rat mammary carcinoma cell membranes as well as reduced tumor growth; increasing the 18:2n-6 intake reversed this effect but only to a concentration of 3% corn oil, after which no further change in prolactin binding capacity occurred.

The interactions between dietary fatty acids and growth factors constitute a priority area for further research. EGF, transforming growth factors α and β, insulin-like growth factors I and II, platelet-derived growth factors, and the family of fibroblast growth factors are all associated with various aspects of breast cancer progression and the related process of angiogenesis (60-62). We have already noted that there is a requirement for EGF in 18:2n-6-stimulated mammary epithelial cell proliferation (6, 7), and in another study EGF receptor signal transduction was observed to be down-regulated by 18:1 (63). Whether this effect is specific for n-9 fatty acids is unknown, as is its possible role in the reported inhibitory effect of 18:1-rich olive oil on NMU-induced rat mammary carcinogenesis (40).

**Eicosanoids**

I have already reviewed the evidence that 20:4n-6-derived eicosanoids are involved in growth of mouse mammary carcinoma and human breast cancer cell lines that is stimulated by n-6 fatty acids. This evidence is largely indirect and stems from the use of semiselective pharmacologic inhibitors. Other investigators have taken a similar experimental approach to assess the roles of prostaglandins and leukotrienes in the promotion of mammary carcinogenesis by dietary n-6 fatty acids.
(64–68). Hillyard and Abraham (64) showed that the stimulation of growth of transplantable rodent mammary adenocarcinomas by a diet high in corn oil could be blocked by indomethacin, which is primarily a cyclooxygenase inhibitor. Carter et al (65) obtained similar results with the DMBA-induced rat mammary carcinogenesis model. However, in the latter experiments, another cyclooxygenase inhibitor, carprofen, had no such effect, even though it was at least as effective in reducing mammary epithelial cell PGE2 concentrations.

At relatively high concentrations, indomethacin also inhibits lipooxygenase activity (69) and several studies (66–68), including our own (17, 51), have shown that the relations among mammary carcinogenesis, tumor progression, and the eicosanoids involve lipooxygenase products as well as the prostaglandins formed by the cyclooxygenase-mediated metabolic pathways. For example, Abou-El-Ela et al (66) found that high concentrations of both leukotriene B4 and PGE2 were present in DMBA-induced tumors, the development of which had been stimulated by a 20%-corn oil diet; in contrast, concentrations of these eicosanoids were relatively low in the smaller tumors that did develop in animals fed a 20%-menhaden oil diet. In another study performed by this group of investigators, a diet containing 15% menhaden oil and 5% corn oil reduced mammary carcinogenesis, PGE2 synthesis, and leukotriene B4 synthesis by the tumor cells compared with a diet containing 20% corn oil (67). This involvement of lipooxygenase products was also shown directly by experiments that used inhibitors of this family of enzymes possessing greater specificity than that obtainable during indomethacin treatment (17, 18, 49, 68).

Kitagawa and Noguchi (68) compared the effects of the cyclooxygenase inhibitor piroxicam and esculetin, an inhibitor of 5- and 12-lipoxygenase, on DMBA-induced rat mammary carcinoma cell proliferation in vivo. Although esculetin significantly suppressed both mammary tumorigenesis and cell proliferation rates, piroxicam had no effect.

**Gene expression**

**MMTV-specific transcripts.** The C3H Heston foster-nursed mouse (C3Hf mouse) is unique in that the initial events of mammary carcinogenesis have been determined at the molecular genetic level. These involve hormonally controlled expression of a single MMTV endogenous proviral DNA that is present at the Mtv-1 locus (70, 71) and activation of the Wnt-1 and Int-2 protooncogenes (72).

Etkind (73) used this model to study the effect of dietary fat on the initiation stage of mammary carcinogenesis; C3Hf mice were fed either a high-fat (23.5% corn oil) or a low-fat (5% corn oil) diet. The high fat intake resulted in an acceleration of the expression of the MMTV proviral DNA sequence present at the Mtv-1 locus and earlier development of mammary tumors. This work connects nicely to studies showing effects of dietary fat intake on mammary tumorigenesis in MMTV/\(v\)-Ha-ras transgenic mice in which the activated ras oncogene is integrated into the genome (74, 75). DeWille et al (75) used this transgenic model to show that a high-fat, energy-rich diet enhanced the incidence of these ductal adenocarcinomas and that ras transgene messenger RNA levels were higher.

**Ha-ras.** Although mutation of the ras protooncogene is rare in human breast cancer, a point mutation in the Ha-ras gene occurs in ~80% of rat mammary tumors induced by NMU (76). Ronai et al (77) reported that point mutations in codon 12 of the Ha-ras oncogene were present in the mammary gland as early as 3 wk after exposure to NMU, but at no time in the carcinogenic process were differences seen between animals fed diets containing either 23% corn oil or equal amounts of menhaden oil (rich in n-3 fatty acids) and corn oil. Hu et al (78) did find that 30 and 75 d after NMU administration the mammary glands from rats fed a diet containing 20% lard had a higher proportion of cells with a mutated Ha-ras than did those from rats fed a 4% lard-containing diet, but it should be noted that the diets had different energy densities: 19.0 and 15.6 kJ/kg (4.54 and 3.73 kcal/g), respectively. As in the study by Ronai et al (77), it was also observed that the frequency of Ha-ras mutations in the mammary glands was greater than that of mammary tumors, indicating that a ras mutation alone does not result in tumor production and that an additional later event is necessary to stimulate the expansion of preneoplastic cells.

In other studies by Telang et al (14, 79), n-6 fatty acids increased and n-3 fatty acids decreased the expression of C-rasH p21 expression in mammary explant cultures prepared from mice harboring MMTV or explants exposed to DMBA in vitro.

**p53 Protein expression.** Increases in the concentrations of wild-type p53 tumor suppressor protein were correlated with the induction of a block at the restriction point in the G1 phase of the cell cycle (80, 81). In normal human breast epithelial cells, transfection with a mutated p53 gene increased proliferation and immortalization may have occurred (82). Tillotson et al (83) suggested that fatty acids may influence mammary tumor cell proliferation through effects on cell cycle progression. When they cultured an NMU-induced tumor cell line in a medium containing a low serum concentration, the addition of 18:2n-6 enhanced DNA synthesis and shortened the duration of the G phase, thus increasing the number of cells in the S phase of the cycle; this was associated with a decrease in wild-type p53 protein. Conversely, docosahexaenoic acid (22:6n-3), inhibited mammary tumor cell growth and upregulated the expression of p53.

**PROSTATE CANCER**

**Experimental carcinogenesis**

In contrast with breast cancer, there is only a sparse and somewhat inconsistent literature concerned with the influence of dietary fat and individual fatty acids on prostatic carcinogenesis and prostate cancer progression (8). Indeed, there continues to be a lack of a universally accepted animal model for the human disease. Wynder et al (84) found only five publications before 1994 that examined the influence of a high-fat diet on experimental prostatic carcinogenesis (85–89). In only one case was it reported that an effect occurred, and in this case the tumor incidence was increased only from 5% to 17% in animals fed a high-fat, 18:2n-6-rich diet compared with a low-fat diet (87).

**Human prostate cancer cell lines**

Rose and Connolly (90, 91) found that 18:2n-6 exerted a stimulatory effect on the growth of the androgen-independent PC-3 human prostate cancer cell line in vitro. Another androgen-independent prostate cancer cell line, DU145, has been used in several studies in vitro and in vivo. Although the original
parent line was unresponsive when cultured with 18:2n-6, the growth of a high-passage variant was stimulated by the n-6 fatty acid (92), an effect that was shown to require an intact, functional, EGF-mediated autocrine loop.

The DU145 cell line readily formed solid tumors when injected subcutaneously into male athymic nude mice; its growth was inhibited by feeding a diet containing a high concentration of long-chain n-3 fatty acids (93, 94). In contrast, a diet supplemented with 18:3n-3 was ineffective in suppressing prostate cancer cell growth in nude mice (Figure 2), perhaps because of the relatively weak ability of the short-chain n-3 fatty acids to displace 20:4n-6 from the cell membrane phospholipids.

Only one published report described the effect of a high-fat, 18:2n-6-rich diet on the growth of an androgen-responsive human prostate cancer cell line in nude mice. Wang et al (95) used the LNCaP cell line, which secretes prostatic-specific antigen, a biomarker of prostate cancer activity and tumor burden, to assess the effects of diets containing 40.5%, 30.8%, 21.2%, 11.6%, or 2.3% of energy as fat on the growth of subcutaneously located tumors. There were no significant differences in total energy intake or in body weight gains among the five dietary groups. Tumor growth rates and the ratios of tumor weight to body weight were significantly greater in mice fed the 40.5%-fat diet compared with those of the other groups; in fact, the only difference in tumor size was in the mice consuming the highest fat intakes; the prostate-specific antigen concentrations in sera collected at the end of the experiment were also highest in the 40.5%-fat dietary group. Serum testosterone concentrations were unaffected by amount of fat consumed and were not correlated with tumor progression.

The androgen-responsive LNCaP prostate cancer cell line, like the estrogen-dependent MCF-7 breast cancer cell line, does not metastasize when growing in the orthotopic site in nude mice (96). However, the androgen-independent PC-3M human prostate cancer cell line, which was obtained from a relatively uncommon PC-3 cell metastasis, was reported to be highly metastatic in the nude mouse model (96). Like the parental cell line, PC-3M cells showed a growth response to 18:2n-6 in vitro along with an enhanced invasive capacity (JM Connolly and DP Rose, unpublished observations, 1995). No data have been published concerning the effects of dietary 18:2n-6 on the metastatic potential of tumors formed by PC-3M prostate cancer cells in nude mice.

Mechanisms

Little work has been done to determine the mechanisms by which dietary fat and specific fatty acids may influence prostate cancer development and progression, but this is true of the whole research area of diet and prostate cancer. However, a role for the eicosanoids formed from 20:4n-6 does seem likely. Human prostate epithelial cells synthesize prostaglandins, particularly PGE2, in culture (97), and growth of both the DU145 and PC-3 cancer cell lines is suppressed by inhibitors of eicosanoid biosynthesis and by long-chain n-3 fatty acids in vitro (90).

In contrast with the human breast cancer cell lines discussed earlier, both piroxicam (90) and lipoxigenase inhibitors (90, 91) suppress PC-3 and DU145 cell growth in vitro. Moreover, the inhibition of DU145 cell solid tumor growth by a fish oil preparation (MaxEPA; Seven Seas, Hull, United Kingdom) was associated with reduced tumor PGE2 concentrations compared with those in tumors from mice fed a high-fat, 18:2n-6-rich diet (93). The relation of the eicosanoids to prostate cancer growth and invasion is, however, likely to prove to be complex. This was brought out by a result obtained from the PC-3 cell culture experiment that used three different inhibitors (90). Piroxicam and indomethacin are primarily cyclooxygenase inhibitors; esculetin is an inhibitor of 5- and 12-lipoxigenase but not cyclooxygenase. All three compounds inhibited cell growth but whereas the first two produced the expected block in PGE2 production, the concentration of this cyclooxygenase product was actually elevated by esculetin, perhaps because of the diversion of 20:4n-6 away from lipoxigenase-mediated metabolism.

In addition to direct effects of fatty acids and their metabolic products on prostate cancer cells, a human study found that a low-fat dietary intervention caused a reduction in serum total testosterone and unbound, biologically available testosterone concentrations in adult male volunteers (98). Although this finding implies that so altered endocrine activity may also be involved in an effect of dietary fat on prostate cancer progression, essentially no data are available from experiments with animal models, other than the absence of a change in serum testosterone in the LNCaP cell experiment reported by Wang et al (95).

COMMENTARY

It is evident from this review that specific fatty acids differ in their effects on experimental mammary carcinogenesis and on the biological behavior of breast cancer cells both in vitro and in an appropriate host animal. Recent research efforts have gone some way in explaining these differences at both the cellular and molecular levels, although much remains to be done. In contrast with this progress, our knowledge of the effects of dietary fat and fatty acids on prostate cancer is rudimentary. Reported studies reflect the continued lack of...
appropriate animal models and published data tend to be fragmentary and inconsistent.

Justifiable concern has been expressed about the relevance to humans of dietary studies using animal models for breast cancer. One objection is that the published data rest heavily on experiments with chemically induced rat mammary carcinomas. Although this is true, similar conclusions have been reached from feeding experiments using spontaneously developing mouse mammary carcinomas (41, 42, 73, 75). Of more concern, perhaps, is that the tumors that develop after exposure to DMBA or NMU rarely metastasize; they do, however, exhibit a range of expression of the invasive phenotype (99).

The effects of fatty acids may be tissue specific and may also be influenced by the amounts present in the diet. Thus, although fish oil rich in n-3 fatty acids may inhibit NMU-induced rat mammary carcinogenesis (100), it has an enhancing effect in azaserine-induced pancreatic carcinogenesis (101). Moreover, in the mammary carcinogenesis model it was reported that there is a ratio of fish oil to corn oil in the diet that is inhibiting but that a greater proportion of the oil containing n-3 fatty acids actually stimulates tumor development (102).

Experiments performed in vitro with cancer cell lines need to be interpreted with particular caution because aside from the obvious problem of what is a biologically relevant concentration of the fatty acid under study, the absence of interaction between the cancer cells and the host tissue and microenvironment can lead to predictions of tumor response that are the opposite of in vivo responses (103). Nevertheless, in mechanistic studies such experiments are essential for separating direct effects on the cancer cell from the confounding influence of host cellular metabolism.

Another important distinction needs to be made between the influence that different concentrations of fatty acids may have as they occur in the human diet and the biological effects of these same fatty acids as constituents of the cellular microenvironment. For example, we have seen that the amount of dietary 18:2n-6 affects both the development of rodent mammary carcinomas and the progression of human breast cancer cell solid tumors in nude mice; in both situations the stimulatory effects of the n-6 fatty acid can be blocked by selective inhibitors of eicosanoid biosynthesis. However, it is difficult to translate these observations to the human situation. In one dietary study using adult male volunteers, feeding diets of equal total fat content (35% of total energy) but providing a daily 18:2n-6 intake of 30 g (8.4% of energy) or 10 g (2.8% of energy) produced no change in the plasma 20:4n-6 concentrations or in the urinary excretion of PGE metabolites (104). A similar result was obtained by James et al (105) when diets were fed that provided either 17.5% or 2.5% of total energy as 18:2n-6; there were positive correlations between dietary 18:2n-6 and the 18:2n-6 concentrations in neutrophil phospholipids, plasma triacylglycerols, and plasma cholesterol esters but no change in 20:4n-6 concentrations.

One interpretation of these findings is that changes in dietary 18:2n-6 alone are unlikely to affect cancer progression, although modification in the relative proportions of 18:2n-6 and other unsaturated fatty acids may do so. Alternatively, the recent demonstration that eicosanoids derived from n-6 fatty acids influence the invasive and metastatic capacity of human breast and prostate cancer cells suggests a chemosuppressive approach that utilizes pharmacologic inhibitors of lipoxygenase and cyclooxygenase activity (106).

Last, it may be necessary to distinguish between the process of neoplastic transformation and the clinical expression of disease progression once this has occurred. For example, dietary n-6 fatty acids may enhance tumor cell invasion and the eicosanoids formed from n-6 fatty acids are involved in angiogenesis, thus facilitating tumor growth and metastasis; these events do not necessarily imply any involvement of the fatty acids in carcinogenesis per se nor do they say anything about dietary fat and breast cancer risk.

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