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Review

Breast Cancer and the Western Diet: Role of Fatty Acids and Antioxidant Vitamins

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Epidemiological reports are inconsistent on the association between breast cancer risk and the dietary intake of either individual fatty acids or of antioxidant vitamins. It is postulated here that the inconsistencies are in part due to interactions between the two classes of nutrients at the level of the cell membrane, affecting their potential role in mammary carcinogenesis. In this review, the effects of specific dietary fatty acids and antioxidant vitamins on experimental mammary cancer systems are compared with reported epidemiological associations of the same agents with breast cancer risk in humans. An increased ratio of n-3 to n-6 polyunsaturated fatty acids (PUFAs) in the diet inhibits the growth of the rat mammary cancer model. There is also evidence that members of the n-3 PUFA series can inhibit the growth of human breast cancer cells both *in vitro* and in explants. Clinical trials of supplementary n-3 PUFAs in conjunction with a reduced fat intake have been proposed for breast cancer prevention. It is postulated that further dietary supplementation with vitamin E and a retinoid is likely to increase the effectiveness of such a diet. A study of this type allows better control of specific dietary components than prospective trials of dietary fat reduction which are presently under evaluation. In particular, it is suggested that studies focusing on a single nutrient often fail to recognise interactions with other nutrients.

Key words: antioxidants, breast cancer, fatty acids, hyperinsulinaemia, insulin-like growth factor, vitamin A, vitamin E, Western diet
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INTRODUCTION

CORRELATIONS OBSERVED between breast cancer rates and per capita animal fat consumption in different countries [1] have been followed by studies on the association between individual fat consumption and breast cancer risk in women. However, 10 prospective studies in developed countries have failed to show a clear association between a woman's daily total fat intake and her breast cancer risk [2]. Recent investigations have focused on the intake of individual fatty acids after a meta-analysis of case–control studies reported a greater risk for saturated and monounsaturated fats than for polyunsaturated fats [3]. Some reports, including a prospective study [4]. have suggested a protective effect from monounsaturated fatty acids, and possibly a deleterious effect from *trans* fatty acids [5].

In the rat mammary tumour model, polyunsaturated fatty acids (PUFAs) were originally identified as tumour-promoting agents, but subsequent studies showed that a high intake of the n-3 group of PUFAs (from fish oil supplements) was able to counteract the tumour-promoting effect of n-6 PUFAs (from corn oil supplements) [6]. There is evidence that PUFAs generate free oxygen radicals and lipid peroxides, and these may stimulate cell proliferation by an effect on cell membrane fluidity, enzymes or receptors [7]. Dietary antioxidants, especially vitamin E, may counteract these effects and protect against mammary tumour development in the animal model [8].

Based on the experimental evidence, some have proposed dietary supplements rich in n-3 PUFAs aiming at breast cancer protection in the human [9, 10]. This proposal is examined in the following review, in relation to four main lines of evidence: (a) ecological evidence that a higher n-3/n-6 PUFA ratio in the diet may be protective, and studies on human mammary cancer cells *in vitro* and in explants showing growth inhibition by n-3 PUFAs; (b) evidence that a higher PUFA intake requires increased intake of antioxidants to prevent peroxidation and that antioxidant vitamins such as

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vitamins A and E may protect against mammary carcinogenesis even when given alone; (c) evidence that a higher n-3/n-6 PUFA ratio in the human diet can lessen the risk of hyperinsulinaemia whose concomitants are thought to favour mammary carcinogenesis; and (d) evidence that in breast cancer patients, breast adipose tissue contains a higher n-6/n-3 PUFA ratio and a lower vitamin E level than in controls.

ROLE OF PUFAS IN MAMMARY CARCINOGENESIS

Populations of industrialised Western countries tend to have a high fat diet with a high n-6 PUFA content, particularly linoleic acid which is found in cheap vegetable oils such as corn and safflower oils. Higher consumption of n-6 linoleic acid is not only a substrate for lipid peroxidation and free radical formation, but in addition, can aggravate insulin resistance and hyperinsulinaemia [11]. It has been postulated that high n-6 PUFA in the diet may have a role in the high breast cancer incidence in Western women [9, 10].

In contrast, the n-3 class of PUFAs may be protective, and an inverse correlation has been reported in ecological studies between per capita consumption of fish oils, with a high n-3 PUFA content, and breast cancer rates [12–14]. The latest of these studies [14] reported a significant inverse correlation only in those countries with a high animal fat intake. Marine oils, such as fish oil, and vegetable oils, such as flaxseed oil, are particularly rich sources of n-3 PUFAs.

In carcinogen-induced mammary tumours in rats, feeding with saturated fats has a weak promoting effect, n-6 PUFAs have a strong promoting effect, while n-3 PUFAs have an inhibiting effect [15]. Similarly, n-6 PUFAs stimulate the growth of human mammary cancer cells *in vitro*, and supplementary corn oil (rich in n-6 PUFAs) can stimulate growth and metastasis in human mammary cancer cells growing in explants [16]. In contrast, long chain PUFAs of the n-3 series inhibit the growth of human mammary cancer cells *in vitro*, and supplementary fish oil (rich in n-3 PUFAs) inhibits growth and metastasis in human mammary cells in explants [17]. There is also evidence that flaxseed oil fractions can protect against chemically induced mammary carcinogenesis in the rat, both in the promotion and progression stages [18]. Flaxseed oil contains lignan precursors, but its effect on carcinogenesis is attributed to its n-3 PUFA content and is not related to urinary lignan levels [18].

A recent dietary intervention study in breast cancer patients showed a significantly increased n-3/n-6 PUFA ratio in both plasma and breast adipose tissue following a change to a low fat diet with daily fish oil supplements for 3 months [10]. The relative roles of n-3 PUFAs derived from fish oil and those derived from vegetable oil are still uncertain [19], but their use as dietary supplements could be a suitable alternative to a controlled feeding study [20].

The opposing effects of n-3 and n-6 PUFAs on the growth of mammary cancer are generally explained on the basis of different effects on eicosanoid production [21]. Experimental observations suggest that eicosanoids can affect cell proliferation, immune responsiveness and tumour invasiveness and metastasis [22]. Prostaglandin E2 has been shown to inhibit the growth of human breast cancer cell lines [23], but there is no direct evidence that the effect of PUFAs on experimental tumours or on breast cancer cell lines is mediated by either prostaglandins or leukotrienes. Indomethacin, a prostaglandin inhibitor, can block stimulation of rat mammary tumour growth by an n-6 PUFA-rich diet [21], but there is no evidence that non-steroidal anti-inflammatory agents can reduce breast cancer risk in women [24].

Multiple studies confirm that human mammary cancers contain high levels of prostaglandin E2, but its function is not clear [25]. In humans, diets rich in n-6 PUFAs have been reported to cause no change in the urinary excretion or plasma levels of prostaglandin E2 [26]. Because of these observations, it has been proposed that eicosanoids may increase tumour invasiveness or metastasis, but do not necessarily increase carcinogenesis and the risk of developing breast cancer [27].

ROLE OF ANTIOXIDANT VITAMINS

The concept that diets rich in PUFAs promote tumour growth by the generation of lipid peroxides or free oxygen radicals is supported by evidence from carcinogen-induced mammary tumours in rats [7]. Free radicals can cause lipid peroxidation in cell membranes and damage its function, and antioxidant vitamins have been found to be effective scavengers of these radicals. Such an effect may protect against carcinogenesis. Thus, vitamin A administration can suppress mammary tumorigenesis in rats fed high dietary levels of PUFAs [28], whilst vitamin E deficiency increases tumorigenesis in rats fed similarly [29].

Numerous epidemiological studies have investigated antioxidant vitamins such as beta-carotene (provitamin A), retinol (vitamin A), alpha-tocopherol (vitamin E) and ascorbic acid (vitamin C) for a protective effect against breast cancer. In general, the results of case–control and prospective studies are unconvincing in the case of beta-carotene, vitamin E and vitamin C. A large case–control study in five European countries (EURAMIC study) found no association for beta-carotene, vitamin E or selenium [30], and a recent meta-analysis of 14 prospective and 33 case–control studies found conflicting associations for beta-carotene, vitamin E and vitamin C [31]. Studies to establish associations with blood levels of beta-carotene, vitamin E and vitamin C have also yielded conflicting results [32].

In the case of vitamin A, experimental evidence confirms its ability to block mammary carcinogenesis, but the epidemiological evidence is somewhat less convincing [33]. Older clinical trials of vitamin A supplements were limited by toxicity, but more potent and less toxic synthetic vitamin A analogues have been synthesised. Trials of *N*-(4-hydroxyphenyl) retinamide (fenretinide) are currently underway in Italy, aiming to reduce the growth of second breast cancer after primary treatment [34].

Many carotenoids are potent antioxidants, but the mechanism by which vitamin A may inhibit carcinogenesis is uncertain. It has both anti-initiating and antipromoting effects on mammary tumorigenesis and enhances cellular differentiation [7]. Not only does it inhibit tumour growth in rats on a standard diet, but it can also counteract the promoting effect of high PUFA levels [28, 35]. Retinoids are thought to reduce the concentration of free radicals, but they can also improve insulin sensitivity. They show significant effects on the insulin-like growth factor I (IGFI) system [34], as discussed more fully in a subsequent section.

In the case of vitamin E, the antioxidant properties are more clearly established, both in animals and in humans. Its administration reduces the incidence of carcinogen-induced rat mammary tumours [36]. The presence of vitamin E

deficiency increases tumour growth in animals fed high levels of PUFA, but not in those fed the standard diet [29].

Case–control studies show conflicting reports on the association of blood levels of tocopherol with breast cancer risk and prospective studies show no evidence of protection associated with higher blood levels [37, 38]. Tocopherol levels are reduced in the presence of diabetes or obesity [39] and increased consumption of PUFAs requires increased intake of vitamin E [40, 41]. Studies on the role of fish oil concentrates in protecting from coronary artery disease have added vitamin E as an antioxidant supplement [42].

In summary, it is postulated that interactions between PUFAs and antioxidants at the level of the cell membrane may explain the inconsistent epidemiological reports on the protective effects of individual fatty acids and individual antioxidant vitamins against breast cancer. It is possible that antioxidant vitamins protect against mammary carcinogenesis only in the presence of a high PUFA intake. A recent cohort study in The Netherlands, involving 62 573 women aged 55–69 years reported that beta-carotene and vitamin C intake showed an inverse association (non-significant) with breast cancer risk only among women with a high PUFA intake [43]. Similarly, on the basis of conflicting results in experimental studies, it has been postulated that the effect of vitamin E in inhibiting mammary carcinogenesis in animals must take into account the dietary intake of PUFAs. It is possible that the protective effects of antioxidant vitamins may be lost or even reversed by the presence of high levels of certain PUFAs [8].

POSTULATED ROLE FOR INSULIN ACTIVITY

Dietary fat intake influences the phospholipid composition of cell membranes leading to changes in cell surface permeability, receptor activity and cell-to-cell interaction [14]. Membrane changes also affect cell response to growth factors and hormones which stimulate activation of protein kinase C (PKC), a lipid-dependent enzyme which is widely distributed throughout the tissues [45]. It is a critical factor in the control of cell proliferation and differentiation.

The level of PKC is reported to be higher in breast cancer specimens than in normal breast tissue [45] and phospholipase levels are also higher [46]. PKC activity is increased by oestradiol, whilst the anti-oestrogen tamoxifen inhibits its activity in human breast cancer cell lines [47]. PKC overexpression in such cell lines has been associated with the expression of a more aggressive phenotype [48].

The PKC pathway is also involved in the pathogenesis of insulin resistance [49, 50] and variations in the fatty acid components of the phospholipids in human skeletal muscle are reported to influence insulin activity [44]. In obese pubertal children, insulin levels are reported to be low in those with high plasma levels of n-3 PUFAs [51], but high in those with high n-6 PUFA levels [52]. Case–control studies have shown hyperinsulinaemia to be a risk marker for breast cancer [53–56]. An increased risk of breast cancer has also been shown in patients with diabetes mellitus [57].

The major growth promoting effect of insulin *in vivo* is likely to be indirect through IGFI and IGFII. Many breast cancer cell lines produce IGFI and IGFII and also their binding proteins, and they can act either in an autocrine or a paracrine manner (reviewed in [58]). The cells express IGFI receptors and are very sensitive to stimulation by IGF. IGFI also circulates in an endocrine manner and six high-affinity binding proteins (IGFBP) control its bioavailability. Two case–control studies [59, 60] have reported an increased IGFI and decreased IGFBP3 level to be a risk marker for breast cancer in premenopausal women. Proteolysis of IGFBP3 is likely to be regulated by insulin [61].

It may be relevant that in breast cancer patients, administration of the synthetic retinoid fenretinide is shown to decrease circulating levels of IGFI and increase plasma levels of IGFBP3 [34]. Moreover, human breast cancer cell lines secrete IGFI and IGFBP3 and the addition of retinoic acid can inhibit cell growth and suppress IGFI activity [62, 63], whilst increasing IGFBP3 activity [64, 65]. It is likely that the growth inhibitory effect of retinoic acid on breast cancer cells, like those of anti-oestrogens and transforming growth factor beta, is mediated by IGFBP3 [66].

Hyperinsulinaemia may also increase breast cancer growth by an effect on tumour cell invasiveness. Urokinase plasminogen activator (UPA) is involved in the degradation of the basement membrane in malignant mammary cell invasion and metastasis, and a poor prognosis in breast cancer is related to overexpression of UPA [67]. Its activity is controlled by plasminogen activator inhibitors 1 and 2 (PAI 1, PAI 2) and the synthesis of PAI 1 is regulated by insulin and by IGFI. Markedly higher serum PAI 1 levels are associated with hyperinsulinaemia [68, 69]. Reports on the effect of n-3 PUFA and fish oil dietary supplements on plasma PAI 1 levels are conflicting [69, 70], but interventions which decrease insulin levels, such as a low calorie diet and physical training, are reported to decrease PAI 1 levels [71].

BIOLOGICAL MARKERS OF FATTY ACIDS

Examination of fatty acids in adipose tissue or blood can complement estimates of dietary intake and epidemiological studies, because fatty acid profiles may reflect long-term dietary intake. Studies of adipose aspirates are especially useful, because recall of past diet can be biased and also because marked changes have occurred in specific fatty acid composition of processed food over the past 10 years in the Western world [72]. Correlation between dietary intake and fatty acid profile of adipose tissue is generally much greater for PUFAs than it is for saturated and monounsaturated fatty acids [73].

Fatty acid profiles of adipose tissue have been compared in breast cancer patients and controls. Of six studies which examined the association with n-6 PUFAs, four showed a modest positive association with breast cancer [74]. In four studies which examined the association with n-3 PUFAs, no significant relationship was observed, whilst an association with *trans* fatty acids and monounsaturated fatty acids was inconsistent. A recent report of a five centre European study [75] showed a significant protective effect to be associated with a higher n-3/n-6 PUFA ratio in adipose tissue fatty acid content.

Comparisons have also been made between the fatty acid profile of breast cancer tissue and that of adjacent non-tumorous tissue. A French study reported higher levels of monounsaturated fatty acids and n-3 PUFAs, but lower levels of n-6 PUFAs in the cancer cell membrane phospholipids [76]. A Japanese study similarly showed higher n-3 PUFA levels and lower n-6 PUFA levels [77], whilst a Finnish study reported higher monounsaturated fatty acid and n-3 PUFA levels, as well as higher n-6 PUFA levels [78]. The differences may reflect dietary differences between the countries, but also suggest that the fatty acid profile of the cell membrane

which effects malignant change in mammary cells may be characteristic [76].

Studies have compared vitamin E levels in breast adipose tissue taken from breast cancer patients with that taken from controls [8, 79]. Both studies showed that breast cancer is associated with a lower vitamin E level in breast adipose tissue. In contrast, vitamin E levels in the blood have been shown to increase with advancing disease, both in breast cancer [80] and in other cancers [81]. The cause is unknown.

CONCLUSION

A major problem in linking dietary fat intake with breast cancer risk is that promotion of mammary carcinogenesis extends over many years. Marked changes have occurred in recent decades in the fatty acid and antioxidant vitamin profile of the diet in industrialised Western countries. Experimental studies on the effect of supplementary feeding with a variety of fatty acids and antioxidants are, therefore, relevant to the human disease, even if they involve animals with different genetic profiles, although consideration of specific genes in human mammary carcinogenesis is needed.

It is suggested that controlled dietary intervention trials in women at high risk of breast cancer may influence late promotion. Supplements of n-3 PUFAs in combination with vitamin E and a retinoid might be included as part of a low fat diet. Biological markers to act as intermediate endpoints in the assessment of cancer prevention need to be developed, but meanwhile, the effect on late stage breast cancer promotion could be tested in women at high risk of a second breast cancer after treatment of the primary breast cancer. The protocol should include measurement of the fatty acid profile of mammary adipose tissue as an intermediate marker of PUFA intake, and this could be correlated with assays of IGF system activity and sex hormone levels.

A study of this type permits better control of specific dietary components than do the prospective trials of dietary fat reduction which are currently under evaluation. In particular, the review highlights the problem that studies focusing on a single nutrient often fail to recognise possible interactions with other nutrients.

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