Molecular and Cellular Biochemistry **188:** 5–12, 1998.

Timing of dietary fat exposure and mammary tumorigenesis: Role of estrogen receptor and protein kinase C activity

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Abstract

The possible association between a high fat diet and increased breast cancer risk has remained controversial. This largely reflects the conflicting data obtained from migrant, case control and animal studies, which generally support this association, and cohort studies which often fail to show a link between fat and breast cancer. The mammary gland is particularly sensitive to estrogens during fetal development, leading us to hypothesize that dietary fat levels during this period may significantly influence breast cancer risk. Using chemically-induced mammary tumors in rats as our experimental model, we have demonstrated the ability of a maternal diet, high in the polyunsaturated fatty acid (PUFA) linoleic acid, to alter mammary gland differentiation, accelerate the onset of sexual maturation, and increase breast cancer risk. The mammary glands of female rats exposed to a high-fat diet *in utero* have more of the undifferentiated structures (terminal end buds) and fewer of the differentiated structures (alveolar buds) than the glands of rats exposed to a low-fat diet *in utero*. Furthermore, these mammary glands contain lower levels of total estrogen receptors and have reduced total protein kinase C activity. These effects appear to be mediated by an increase in the serum estradiol levels of pregnancy, which are elevated at least 30% in pregnant dams fed a high-fat diet. Furthermore, the administration of estradiol to pregnant dams produces effects on mammary gland development, onset of puberty and sensitivity to chemical carcinogenesis comparable to those seen in the offspring of rats fed a high fat diet during pregnancy. Our results, thus, support the hypothesis based on epidemiological data that high maternal estrogen levels increase daughters' breast cancer risk. The results also suggest that a high-fat diet may be an important factor in increasing pregnancy estrogenic activity. (Mol Cell Biochem **188**: 5–12, 1998)

Key words: breast cancer, estrogen, dietary fat, polyunsaturated fatty acid, estradiol, pregnancy

Dietary fat and breast cancer

The greatest potential for future success in reducing breast cancer mortality is to identify new prevention strategies for this disease. Diet is suggested to be involved in the multistep process that leads to clinical manifestation of breast cancer. It has been estimated that 35% of breast cancers could be prevented by appropriate dietary modifications [1]. However, despite extensive human and animal research, controversies exist concerning the possible link between nutritional components, including fat, fiber and vitamins, and breast cancer risk.

The initial observation linking dietary fat to breast cancer was the high correlation noted between national fat intake and breast cancer risk across different countries [2]. This observation led to a series of case control studies, the majority of which implicated that cases consumed more fat than controls [3, 4]. Numerous animal studies also showed that feeding a diet high in polyunsaturated fatty acids (PUFAs) promoted carcinogen-induced mammary tumorigenesis [5, 6]. Other types of fats, including saturated fats, were less effective in animals [5]. In humans, total or saturated fat intake appeared to be most closely linked to breast cancer [4]. Additional controversy arose when it became apparent that

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the majority, but not all, cohort studies did not establish an increased breast cancer risk associated with a high-fat diet [7].

Sensitive periods and a high-fat exposure

We have proposed that a high-fat diet increases breast cancer risk if consumed during periods when the mammary gland is sensitive to endogenous estrogens, such as fetal life [8]. Evidence in support of the hypothesis includes the observation that Asian women, who consume a low-fat diet in their home country and have a low breast cancer incidence [9], when immigrating to the United States reach the high breast cancer incidence of Western countries within a few succeeding generations [10, 11]. Breast cancer risk among Asian immigrants is already 80% higher after a decade of living in the US, when compared with the risk of Asian women living in the East [11]. The most dramatic increase in risk occurs between Asian-Americans born in the West and those born in the East [11]. A higher dietary fat intake during pregnancy in the West than in the East may be responsible for the transition towards higher breast cancer risk between Asian generations living in the West.

Animal studies provide more direct evidence that a maternal intake of a high-fat diet during pregnancy increases spontaneous or carcinogen-induced mammary tumor incidence in the female offspring [12, 13]. The dietary fat source in these studies was corn oil that is high in PUFAs, particularly linoleic acid. The earlier studies have generally explored the effect of fat on promotion or progression of breast cancer. None of these studies were constructed/performed in a manner that would enable them to address our hypothesis, that dietary exposure during early life can significantly increase breast cancer risk in later life. Thus, an important period during which the mammary gland may be sensitive to dietary fat, might have been overlooked.

Mechanisms mediating the effects of dietary fat

Diet and estrogens

The mechanisms through which dietary fat alters mammary tumorigenesis, are unclear. Several possible mechanisms exist, including direct effects on lipid signaling pathways, and indirect effects mediated through perturbations in sex steroid levels (Table 1). Since estrogens have been extensively implicated in affecting breast cancer risk [14], one likely factor is the apparent ability of fat to alter serum estrogen levels (Table 2). Several clinical studies show that both a high fat and/or total caloric intake increase the levels of circulating free estrogens, whereas a low-fat diet is associated with low plasma estrogen levels [15–18]. Results obtained in animal studies are less clear, but support the link between an isocaloric high-fat diet and high serum estrogen levels [19, 20]. Our findings clearly indicate that a diet high in corn oil increases serum estradiol (E2) levels in pregnant rats [13, 21]. This increase does not persist in the offspring after birth, which is consistent with the rapid clearance of maternal estrogens from neonates.

At least three possible mechanisms exist for a high-fat diet to increase circulating estrogens. Firstly, a high fat intake tends to lead to accumulation of adipose tissue, which is an important site for the conversation of androstenedione to estrone [25]. Secondly, arachidonic acid, a metabolite of PUFAs, activates P450 aromatase that then increases conversion of androstenedione to estrone [26]. Finally, PUFAs can reduce the binding of estrogens to serum binding proteins, including both sex-hormone binding globulin (SHBG) and albumin, thereby increasing the circulating levels of biologically potent estrogens [27].

Estrogen receptor and breast cancer

The fat-induced elevation in circulating estrogens is likely to indirectly affect mammary glands and tumors by influencing

Table 1. Putative mechanisms of action of dietary fat.

Generation of active lipid peroxides [22]
Indirect effects on signal transduction and gene expression through alterations in cellular membrane structure and function [23]
Direct effects on lipid-mediated signal transduction pathways, e.g., by altering the levels of arachidonic acid and eicosanoids [24]
Alterations in the levels/bioavailability of sex steroid hormones and their receptors [15–21]

Table 2. Observations demonstrating potential associations between dietary fat consumption, perturbations in serum estrogen levels and breast cancer risk.

Lifetime exposure to estrogens is lower in Asian women, who also consume a low fat diet and have a lower breast cancer risk [9]
A low fat diet can reduce serum estrogen levels [18]
Elevated serum estrogen levels are associated with increased breast cancer risk in some women [73]
Obese women have elevated serum estrogen levels, and postmenopausal obesity is associated with increased breast cancer risk [74]

estrogen's interactions with its nuclear estrogen receptors (ER). The ER is present *in utero* [28], and its concentrations in the mammary gland increase between birth and the first week of life [29]. The ER content remains at a relatively constant level after the prepubertal period, but appears to vary in concert with the estrus cycle [30]. A change in mammary ER levels also occurs during other periods when the levels of estrogens vary. For example, during pregnancy when circulating estrogen levels are high, the ER concentrations are low, but detectable [31]. A marked increase in mammary ER content and a decrease in serum estrogens occurs during lactation [32]. Thus, the changes in ER levels during pregnancy and lactation reflect the downregulation of ER by estrogens [33].

ER-positive breast tumors, which account for about 60% of all breast cancers [34] probably arise from within the ER positive epithelial cell populations of normal breast tissue [14]. Some 90% of these are associated with adjacent, ER-positive, non-neoplastic tissue [35]. ER-positive tumors tend to be more differentiated [36–38], exhibit a slower growth pattern [39, 40] and a better overall prognosis [41, 42]. A lower proportion of ER-positive tumors are found in premenopausal than postmenopausal breast cancer patients [43]. These findings could suggest that low mammary ER levels may be associated with an increased risk to develop premenopausal breast cancer. Since the data are based on ERα measurements, the role of ERβ in affecting breast cancer risk remains to be determined.

Estrogen receptor and dietary fat intake

Since a high-fat intake elevates serum estrogens, a logical consequence of a high dietary fat consumption is an alteration in mammary ER status. Therefore, we have explored whether a diet high in n-6 PUFA affects ER protein levels in the mammary gland. Surprisingly, female mice consuming a high-fat diet exhibit a 6-fold increase in the mammary ER content [44]. A similar increase has been seen in DMBA-induced mammary tumors in female rats fed with a high corn oil diet [20]. These results would suggest that PUFAs may up-regulate ER, and this increase cannot be reversed with a simultaneous increase in circulating estrogens.

The effect of fat intake on ER in the breast has not been directly explored in human populations. Indirect evidence to suggest that a high-fat diet may induce ER is available from studies showing that either obese women, or women consuming a high-fat diet, are more likely to develop ER-positive mammary tumors than women consuming a low-fat diet [45]. Thus, a dietary fat appears to increase mammary ER content both in animals and humans. This increase is not likely to be caused by a fat-induced elevation in serum estrogens. However, it appears that the presence ER may be required for fat to promote tumor growth and, perhaps, elevate the levels of this receptor. There is no correlation between the recurrence of ER negative breast tumors and dietary fat intake, while ER positive tumors are more likely to metastasize if a woman is consuming a high-fat diet [46].

Where associations between dietary fat and increased breast cancer risk have been reported, these are almost exclusively found in postmenopausal patients [47, 48]. A higher proportion of ER-positive tumors arise in post-menopausal women [43]. However, a significant proportion of human breast tumors are ER-negative [34], whereas the carcinogen-induced rodent mammary tumor models are ER-positive and strongly estrogen-dependent [49]. An exposure to a high-fat diet promotes the growth of mammary tumors in animal models [5]. These observations are consistent with the hypothesis that a high-fat diet preferentially affects breast cancer in ER-positive mammary glands. Thus, the effect of dietary fat on mammary gland ER content may explain some of the difference between animal studies and human cohort studies concerning a high-fat diet and breast cancer. In humans, a high-fat diet would preferentially increase breast cancer growth among the approximately 60% of the study population with ER-positive tumors. This could impact the power of some cohort studies to detect a significant overall trend for increased breast cancer risk in women consuming a high fat diet [7].

Estrogen receptor and dietary fat exposure during sensitive periods in mammary gland development

The results in humans and animals suggest that high ER levels in the mammary gland are associated with fat-induced promotion and progression of breast cancer. However, high ER levels prior to the carcinogen exposure may not affect the susceptibility to develop carcinogen-induced mammary tumors. An exposure to a high-fat diet prior to breast cancer initiation in adult animals does not appear to have significant effects on mammary tumorigenesis [5]. However, fat is likely to increase mammary ER content both before and after carcinogen exposure. Thus, high mammary ER levels in normal adult mammary glands do not increase the risk to develop carcinogen-induced tumors.

It was of interest to determine whether *in utero* exposure to a high-fat diet, that affects preinitiation events that increase susceptibility to develop mammary tumors, also affects mammary ER levels. Our data show that a maternal exposure to a high-fat diet induces a 4-fold reduction in the ER content in the offspring's mammary gland, when compared with ER content in the low-fat offspring [44]. This finding is similar to that showing that early postnatal treatment with DES causes a permanent reduction in the concentrations of ER

in the mouse mammary gland and DMBA induced tumors in rats [50, 51]. The decrease in the mammary ER content both in the high-fat and DES-exposed offspring suggest that the factor(s) responsible may be a high maternal estrogenic environment. There are several clinical observations that support the link between high maternal estrogen levels during pregnancy and increased breast cancer risk among daughters (Table 3). Thus, elevated circulating E2 levels during pregnancy in the mothers consuming a high-fat diet as well as maternal DES exposure could have resulted in a permanent down-regulation of ER in the offspring's mammary gland.

We do not have a clear explanation for why a high-fat diet increases ER levels when consumed by adult animals (and in breast tumors in humans), and why it reduces ER levels if the exposure occurs through a pregnant mother. In both cases, a high-fat diet increases serum E2 levels. However, the developmental state of the mammary gland, and the respective endocrinologic environments, are quite different in fetal and adult life. This suggests that the response of the gland to fat/E2 may vary during lifetime.

Another explanation for differences between adult and fetal exposure may be that *in utero* dietary fat may predominantly affect ERβ receptors. These may be the predominant ER form during fetal life. We have used a ligand binding assay, which does not distinguish between ERα and ERβ. The ERβ cDNA was recently cloned from the rat prostate [52]. The DNA-binding domain of this ERβ is highly homologous to that in the 'classical' ERα protein, while the C-terminal ligand binding domain bears only 55% homology. Most estrogenic substances or estrogenic antagonists compete with E2 for binding to both ER subtypes with identical preference and potency, but transcript tissue distribution is quite different for the ERα and ERβ mRNA [53]. ERβ mRNA expression is high in the ovary and prostate, moderate in uterus and testis, and low in the brain [53]. The distribution of ER subtypes in the breast is still unclear. However, human breast cancer cell lines appear to express less ERβ mRNA than normal breast tissues [53, 54].

Taken together, the observations described above strongly suggest that both the timing and duration of exposure to a high-fat diet may induce different responses in the mammary gland. Whether this is due to differential expression of ERβ vs. ERα remains to be established. Nevertheless, the potential for the mammary gland to exhibit different responses to estrogens at different times could have considerable impact on how we think about estrogenic exposure and breast cancer risk, and how to modulate risk through chemoprevention or other dietary means.

Table 3. Observations associating breast cancer risk with *in utero* estrogenic exposure.

Dizygotic twins have a high estrogenic pregnancy environment. The daughters of these pregnancies have an increased breast cancer risk [75–77].
High birth weigh is associated with high levels of estrogens during pregnancy. The daughters of these pregnancies have an increased breast cancer risk [78, 79].
Preeclamptic and eclamptic pregnancies are accompanied by low serum estrogen levels. The daughters of these pregnancies have a lower breast cancer risk [80, 81].

Protein kinase C and breast cancer

Hormones can regulate cellular functions by activating some of the several isoforms of protein kinase C (PKC) [55, 56]. For example, E2 increases PKCδ expression in the uterus [57]. In human breast cancer cell lines PKC isoenzymes down-regulate ER mRNA expression [58–60]. Protein kinase C also is dependent on diacylglycerol (a fatty acid metabolite) and calcium for activation.

While PKC is linked to breast cancer [55, 61], its role is not fully understood. PKC activity is higher in the malignant than benign breast tissues [61], and higher in the more aggressive than in the less aggressive phenotype of human breast cancer cell lines [62]. However, reduced expression of some PKC isoforms, such as PKCη, is associated with increased neoplastic transformation in the mammary gland, while expression of other isoforms, such as PKCΘ, is linked to a more aggressive neoplastic process [62, 63].

Protein kinase C and dietary fat intake

Diet influences PKC activity. A high-fat diet increases PKC activity [64], whereas caloric restriction inhibits PKC activity [65] in epidermal cells in Sencar mice. A high corn oil diet also enhances PKC activity in the colon and carcinogen-induced colon tumors in male rats [66]. Further, a diet high in corn oil can block the inhibition of skin carcinogenesis, and reverse the reduction in PKC activity induced by moderate energy restriction [67]. Our data indicate that similarly to skin and colon, a diet high in n-6 PUFA increases PKC activity in the mouse mammary gland [44]. Since a high-fat intake increases mammary tumor incidence in animal models [5], the higher PKC activity in the mammary glands of the high-fat fed mice parallels the observed association between malignant progression and high PKC activity.

Protein kinase C and dietary fat exposure during sensitive periods

In contrast to the results obtained in adult mice fed with a high n-6 PUFA diet, PKC activity appears to be reduced in the

mammary gland in the offspring of mothers that were kept on a high-fat diet during pregnancy [44]. It is possible that the reduced PKC activity reflects specific morphological changes in the mammary gland that increase susceptibility to neoplastic transformation. *In utero* exposure to a high-fat diet alters the normal development of a mammary gland [13, 44]. The number of terminal end buds, structures that are the targets of malignant transformation in the rodent mammary gland and possibly in the human breast [68, 69], is higher in the high-fat offspring than in a low-fat offspring. Thus, these structures that are sensitive to neoplastic changes, persist in the mammary glands of female mice exposed to a high-fat diet *in utero*. Persistent TEBs also have been reported in transgenic mice [70] and in rats exposed to either a high-fat diet or estradiol (E2) *in utero*, or during early postnatal period [13, 71]. All these groups exhibit an increased incidence of malignant growth in the mammary glands. These findings suggest that low PKC activity (as well as low ER content) in the mammary gland may increase the subsequent susceptibility to develop mammary tumors.

Conclusions

In conclusion, a diet high in PUFAs, when exposure occurs during adult life (mammary gland is well developed) increases the amounts of ER and PKC in the mammary gland. These events may be linked to the fat-induced increase in mammary tumorigenesis in animal models [5] and the effects (or lack of them) on breast cancer risk in humans [3, 7, 72]. In marked contrast, maternal intake of a high-fat diet during pregnancy reduces the ER content and PKC activity in the offspring's mammary gland and alters the gland's state of differentiation (Fig. 1). Since these offspring are at an increased risk to develop spontaneous and carcinogen-induced mammary tumors [12, 13], low amounts of ER and low PKC activity in the mammary gland may predict an increased breast cancer risk. In addition, the expression of ERβ may be higher in the fetal than adult mammary gland [82], and therefore a maternal high-fat diet may affect offsprings' breast cancer risk by affecting this novel ER subtype. Our future studies will determine whether a maternal high-fat intake specifically affects the ERα or ERβ in the offspring. We also plan to investigate whether the low PKC activity reflects a reduction in activity of a specific isoform of PKC family of genes.

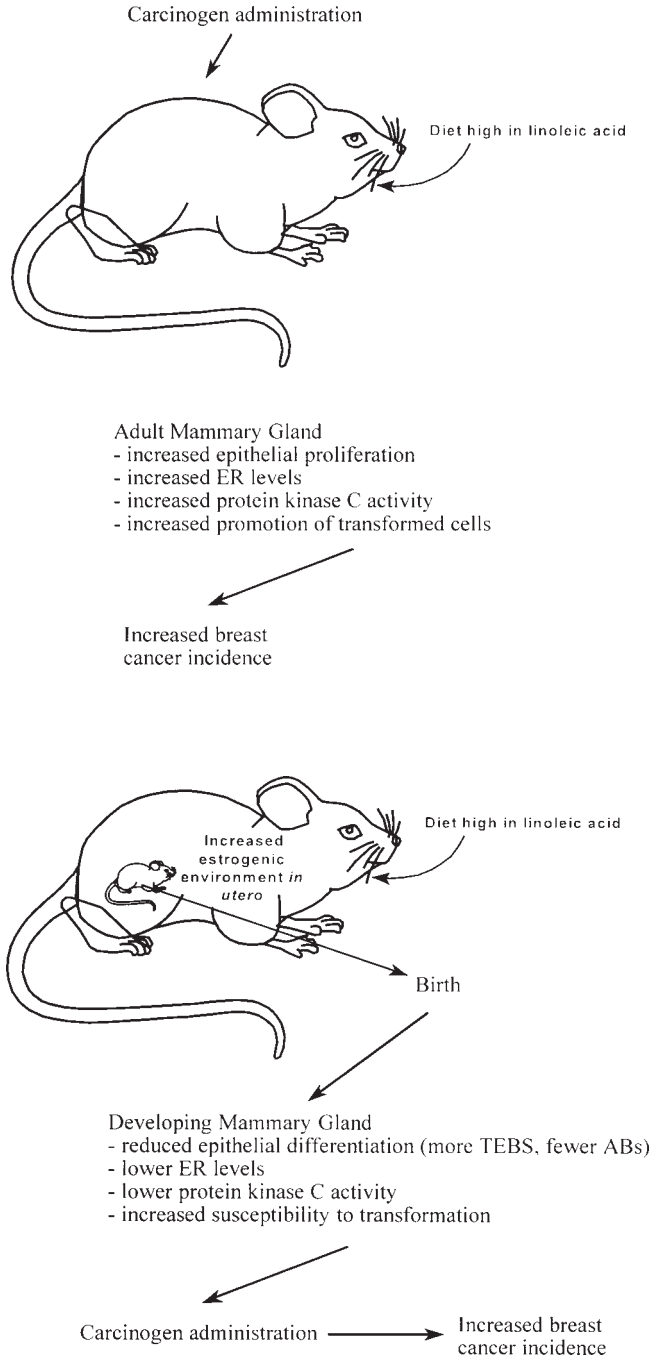

Fig. 1. Plausible mechanisms mediating the effects of an exposure to a diet high in polyunsaturated fatty acids (a) after a carcinogen administration or (b) *in utero* on breast cancer risk. TEB – terminal end bud; AB – alveolar bud; ER – estrogen receptor (ERα and ERβ); PKC – Protein kinase C.

Acknowledgments

This work was supported by grants from the American Cancer Society (CN80420 to L.H.-C.), and the Public Health Service (P50-CA58185 to R.C.) and the Lombardi Cancer Center Shared Animal Resource Facility (P30-CA51008).

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