The Influence of Maternal Diet on Breast Cancer Risk Among Female Offspring

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ABSTRACT

The induction of breast cancer is a long process, containing a series of biological events that drive a normal mammary cell towards malignant growth. However, it is not known when the initiation of breast cancer occurs. One hypothesis is that a high estrogenic environment during the perinatal period increases subsequent breast cancer risk. There are many sources of extragonadal estrogens, particularly in the diet. The purpose of this paper is to review the evidence that a high maternal intake of dietary fats increases serum estrogens during pregnancy and increases breast cancer risk in daughters. Our animal studies show that a high maternal consumption of corn oil consisting mainly of linoleic acid (ω-6 polyunsaturated fatty acid, PUFA), increases both circulating estradiol (E2) levels during pregnancy and the risk of developing carcinogen-induced mammary tumors among the female rat offspring. A similar increase in breast cancer risk occurs in female offspring exposed to injections of E2 through their pregnant mother. Our data suggest that the mechanisms by which an early exposure to dietary fat and/or estrogens increases breast cancer risk is related to reduced differentiation of the mammary epithelial tree and increased number of mammary epithelial cell structures that are known to the sites of neoplastic transformation. These findings may reflect our data of the reduced estrogen receptor protein levels and protein kinase C activity in the developing mammary glands of female rats exposed to a high-fat diet in utero. In summary, a high dietary linoleic acid intake can elevate pregnancy estrogen levels and this, possibly by altering mammary gland morphology and expression of fat- and/or estrogen-regulated genes, can increase breast cancer risk in the offspring. If true for women, breast cancer prevention in daughters may include modulating the mother's pregnancy intake of some dietary fats. *Nutrition* 1999;15:392–401. ©Elsevier Science Inc. 1999

Key words: dietary fat, developmental periods, breast cancer

The sources of the high incidence of breast cancer, a disease that one out of every nine women in the US will face during their lifetime, continue to be unknown.[1,2] Advancing molecular techniques have identified multiple factors that are associated with the neoplastic transformation of the breast. For example, estrogens are involved in breast cancer together with other hormones, growth factors, oncogenes, and tumor suppressor genes.[3,4] However, the precise role of these factors in the events that lead to breast cancer remains unclear.

Prevention is the most powerful means to reduce breast cancer incidence. Many environmental factors have been linked to breast cancer. The etiology of breast cancer may be associated with a high or low consumption of fat, fiber, fruits, vegetables, beta carotene, vitamin C, zinc, phytoestrogens, and alcohol.[5–11] The focus of our studies has been dietary fat and phytoestrogens. Studies comparing the incidence of breast cancer and high-fat diet have shown that in countries where fat consumption is high, such as the US, breast cancer incidence is high. In East Asian countries, where the levels of fat intake are low, breast cancer incidence also is low.[2,12,13] Because some phytoestrogens, such as genistein, are consumed at significantly higher levels in the East Asian diet than in the Caucasian diet,[14] the potentially protective plant-derived phytoestrogens may explain some of the differences in breast cancer incidence between East Asian and Caucasian women. For granddaughters of East Asian women who move to the US, breast cancer risk increases to the level of Americans.[2,15] Therefore, altered dietary habits, rather than a genetic difference between populations, may be responsible for the increased risk.

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TABLE I.

DIET FORMULATIONS

	Isocaloric				Non-isocaloric	
	Low fat		High fat			
Ingredients (g)	12% fat	16% fat	43% fat	46% fat	Low fat 11% fat	High fat 46% fat
Fat—total (corn oil)	5.1	7.0	19.4	20.1	5.0	24.6
Protein (casein)	16.6	20.3	20.3	13.3	20.3	25.1
Carbohydrates (cerelose, corn starch)	68.5	62.9	34.0	39.1	65.0	38.3
Fiber	2.5	5.0	21.5	20.7	5.0	6.2
AIN mineral mix	1.3	3.5	3.5	1.1	1.0	1.2
AIN vitamin mix	6.0	1.0	1.0	5.7	3.5	4.3
Choline chloride		0.3	0.3		0.2	0.3
Total grams	100	100	100	100	100	100
kcal density/g	3.8	3.7	3.8	3.8	3.8	4.8
% kcal from fat	12	16	43	46	11	46
% kcal from protein	17	20	19	14	21	22
% kcal from carbohydrates	71	64	38	40	68	32

Dietary fat consumption has been a popular target of investigations that seek to relate it to breast cancer risk, partly because it may lead to an increase in circulating estrogen levels. Consumption of a high-fat diet increases the circulating estrogens,[16,17] while estrogens are reduced by a low-fat diet.[18–20] Thus, estrogens, which are among the most critical biological factors driving breast cancer,[3] may connect dietary fat to this disease. Further, it is relatively easy to control for fat intake, and perhaps reduce the risk of breast cancer in some women. In support of the role of dietary fat in breast cancer, most case-control studies[10,21] and studies performed using animal models[22,23] suggest that a high-fat diet is involved in promotion of breast cancer. The majority of prospective cohort studies have failed to find an association between dietary fat intake and breast cancer risk,[24] with few exceptions.[10,25] These exceptions include a recent prospective study in which a high intake of ω-6 polyunsaturated fatty acids (PUFA) significantly increased, and a high intake of monounsaturated fatty acids reduced, breast cancer risk in a cohort of 61 471 Swedish women.[25] Thus, not all fats are equal in terms of breast cancer risk, and a high ω-6 PUFA intake in particular may be associated with elevated breast cancer risk. This conclusion is strongly supported by animal data.[22,23] Survival from breast cancer also appears to be shortened, and breast cancer mortality to be increased, in women consuming a high-fat diet.[6,26,27] Again, these effects differ according to the type of fat consumed, with dietary fish fat (ω-3 PUFA) reducing, and ω-6 PUFA vegetable fat and saturated fat increasing mortality.[6,26]

One possible explanation for the discrepancy between different studies is that the timing of fat exposure may be critical. An exposure around certain periods, when the mammary gland is particularly sensitive to endogenous estrogens, such as in utero, puberty, and pregnancy, may be required for dietary fat to influence breast cancer risk. In this paper, we will review evidence that maternal exposure to a high-fat diet increases circulating estrogen levels during pregnancy and breast cancer risk among female offspring. In addition, we suggest that maternal dietary fat intake alters offspring's breast cancer risk by increasing the number of epithelial structures that are sites for neoplastic transformation, and by altering the pattern of expression of fat- or estrogen-regulated genes.

HYPOTHESIS OF THE ORIGIN OF BREAST CANCER

Recently, it has been proposed that breast cancer originates in utero.[28–30] According to this hypothesis, the increasing concentrations of estrogens during pregnancy may increase the probability of daughters getting breast cancer by creating a "fertile soil" for subsequent cancer initiation. Epidemiologic and experimental evidence to support the hypothesis has begun to emerge. Increased breast cancer risk is reported in dizygotic twins,[31] who have a higher in utero estrogenic environment than single fetuses, and in women exhibiting high birth weight.[32,33] Birth weight is suggested to reflect high fetal estrogenic environment.[34] There is a significant reduction in breast cancer risk among daughters of mothers who suffered from pre-eclampsia/eclampsia during pregnancy,[35,36] eclampsia being associated with reduced circulating estrogen concentrations. According to animal data, early postnatal exposure to the synthetic estrogen diethylstilbestrol (DES) induces an increased incidence of mammary tumors.[37–39] These data would predict that the daughters of those women who took DES also will exhibit an increased incidence of breast cancer.

Clearly, it is critical to determine what the factors are that alter normal maternal estrogenic activity and affect breast cancer risk among daughters. Although pregnancy is characterized by high circulating estrogen levels,[40] these levels may vary up to six-fold between any two women undergoing normal pregnancy.[41] One of the sources of the interindividual variations in maternal estrogens may be diet. For example, maternal weight gain[42] and high intake of PUFAs[43,44] correlate with birth weight, which in turn increases breast cancer risk.[32,33] In addition, preliminary evidence indicates that quantitative aspects of diet during pregnancy, as reflected in pregnancy weight gain, may influence estrogens.[45] In the following, we will summarize our animal data, which indicate that a maternal high-fat diet during pregnancy increases the incidence of breast cancer among female offspring.

IN UTERO DIETARY FAT EXPOSURE AND BREAST CANCER RISK

Dietary Manipulations

Transgenerational studies to investigate the role of in utero dietary fat exposure in breast cancer cannot easily be performed in humans, as they are complex and time consuming. We chose the

rat as our experimental model. The development and endocrine responsiveness of the mammary gland, and the general structure of the critical epithelial components of the gland that are associated with tumorigenesis, are believed to be broadly comparable in rats and humans.[46] In our study,[47] female rats were fed with either a diet high in corn oil, containing 43–46% calories from fat, or a diet lower in corn oil, containing 12–16% calories from fat (Table I). Corn oil contains 59% of the ω-6 polyunsaturated fatty acid (PUFAs) linoleic acid. These percentages of dietary fat (although not the high proportion of dietary linoleic acid in corn oil) are broadly comparable with the high- and low-fat consumption among various human populations, for example Caucasian and East Asian women.[48–50]

The ω-6 PUFA linoleic acid has been strongly implicated as a promotional agent in the carcinogen-induced rat and spontaneous or transplanted mouse mammary tumor models, and in tumor metastasis.[51–54] It is also possible that ω-6 PUFA accelerates mammary tumor growth and metastasis in humans.[6,25,26] Linoleic acid is a nutritionally essential fatty acid, and its only source for the human and rodent adult and fetus is the (maternal) diet. The other essential fatty acid that can only be obtained from the diet, is α-linolenic acid (ω-3 PUFA). Together with ω-6 PUFA, ω-3 PUFA is particularly important for the normal development of the fetal central nervous system.[55,56] In contrast to linoleic acid, a diet high in ω-3 PUFA possibly inhibits mammary tumor growth and mammary metastasis.[57–60] The maternal diet also provides approximately 50% of the total non-essential fatty acid requirement of the fetus, although the fetus is capable of metabolizing these acids from the nutritionally essential fatty acids.[61,62]

We used two different dietary formulations based on AIN-76 (American Institute of Nutrition) recommendations[63] (Table I). One group of pregnant rats was fed with non-isocaloric high- and low-fat corn oil diets used in most dietary fat experiments performed in adult animals.[64] A second group of pregnant rats received isocaloric high- and low-fat diets. The caloric contents of the isocaloric diets were adjusted with with alphacel. Thus, the high-fat diet contained more fiber than the low-fat diet.

Among the pregnant rats exposed to isocaloric diets, the food consumption and body weight gain were equivalent in the high- and low-fat groups. Furthermore, both the number of pups born and their body weights did not differ between the groups (Fig. 1), indicating that the low-fat diet contained a sufficient amount of essential fatty acids to allow normal fetal development. Among the pregnant rats consuming non-isocaloric diets, the feed intake and total caloric consumption were significantly *lower* in the *high-fat group* (Fig. 1). The offspring of mothers fed a non-isocaloric high-fat diet during pregnancy also were significantly *lighter* than the offspring of low-fat mothers. Similar observations concerning the offspring's body weight have been reported in an earlier study in which pregnant rats consumed a diet high in corn oil with a caloric density of 5.6 kcal/g, or a low-fat diet with a caloric density of 3.6 kcal/g.[65] Thus, during a rodent's pregnancy, the high caloric density of a diet is compensated with reduced food consumption, which leads to reduced body weight among the offspring.

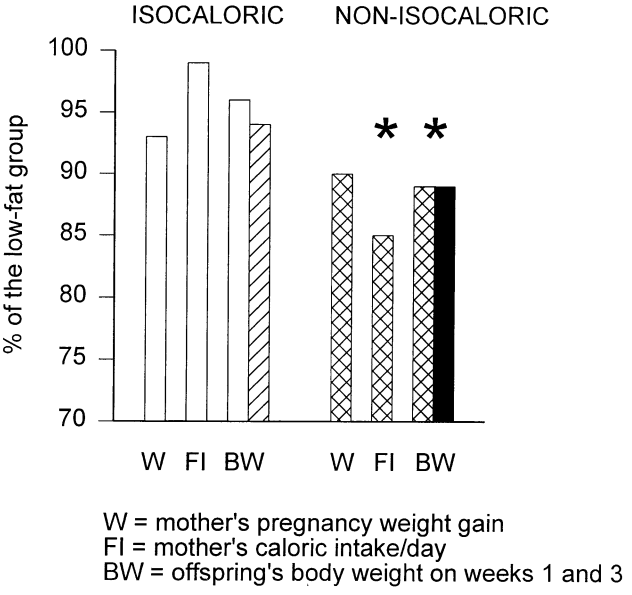

FIG. 1. Maternal weight gain, food intake, and offspring's body weight in the groups fed either with an isocaloric or non-isocaloric high- or low-fat ω-6 PUFA diet during pregnancy. The data for high-fat groups are presented as a percentage of an appropriate low-fat group. Statistically significant difference: $*P < 0.05$. Partially adapted from the data presented in our previous paper.[47]

Mammary Tumorigenesis

Rats require exposure to chemical carcinogens to induce a high and reproducible incidence of mammary tumors. We have used administration of 7,12-dimethylbenz(a)anthracene (DMBA) as our experimental model, since it produces mammary tumors that are comparable with human breast tumors in terms of their long relative latency, histotypes, and endocrine responsiveness.[46] It has been known for several years that the sensitivity of the mammary gland to DMBA is age dependent. Highest tumor incidence is obtained when DMBA is administered on week 6 or 7. If DMBA is given after week 8, a significantly lower tumor incidence is obtained. However, it also is known that the DMBA-induced tumor incidence varies across different laboratories.[66] The factors contributing to this variability may include diet and animal maintenance practices.

We found that a maternal exposure to an isocaloric corn oil diet, high or low, in fat significantly affects tumorigenesis in female offspring. The offspring were maintained on a Purina (PMI Feeds Inc., Richmond, IN, USA) rodent laboratory chow from birth onwards. Mammary tumor incidence was higher and latency for tumor appearance shorter among the rats that were exposed in utero via a maternal feeding to an isocaloric diet high in linoleic acid, when compared with the offspring of mothers fed a low-fat diet (Fig. 2).[47]

Among the female rats exposed to non-isocaloric dietary fat manipulations in utero, mammary tumor incidence was slightly higher in the high-fat group, but the difference did not reach statistical significance (Hilakivi-Clarke, unpublished data). Thus, on week 13 after DMBA administration, 23% of the offspring of high-fat mothers and 5% of the offspring of low-fat mothers had developed mammary tumors, but on week 18 the incidences were 43% and 33%, respectively. Thus, the tumor latency was shortened, but the total number of animals developing tumors was similar in the offspring of mothers consuming non-isocaloric high- or low-fat diets. These results suggest that, although maternal consumption of a diet containing high levels of ω-6 PUFA increases mammary tumor incidence among female offspring, this effect can be partly reversed, possibly due to reduced maternal caloric intake. In adult animals, the effects of fat on mammary tumorigenesis are often inseparable from the effects of food's caloric content,[22] and caloric restriction reduces mammary tumorigenesis in mice and rats.[67,68]

In utero exposure to a high-fat diet in mice[39] or high body weight gain during the juvenile period in pet dogs[69] has been

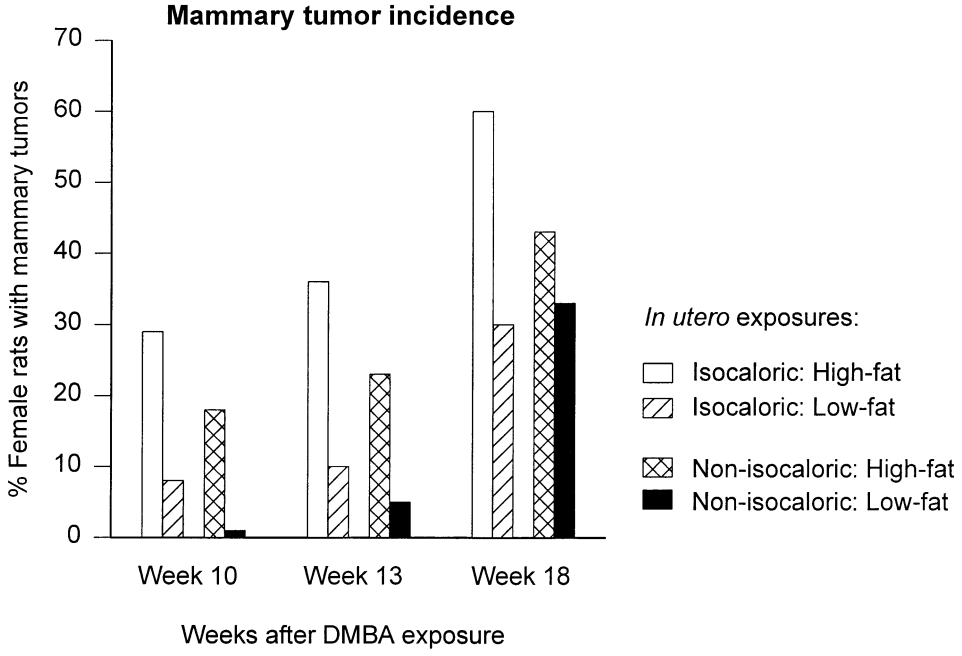

FIG. 2. The proportion of female rats exposed in utero to isocaloric or non-isocaloric high- and low-fat diets through their pregnant mother with DMBA-induced mammary tumors. The difference is significant between the offspring of mothers fed with isocaloric diets, but not between offspring of mothers fed non-isocaloric diets. Log-rank test was used to analyze the statistical significance between the groups. Partially adapted from the data presented in our previous paper.[47] DMBA, 7,12-dimethylbenz(a)anthracene.

shown to increase spontaneous mammary tumor incidence. Although there are no human studies that have looked at the association between mothers' dietary habits during pregnancy and breast cancer risk in their daughters, the animal data strongly suggest that such a link may exist.

Mechanisms of Action of ω-6 and ω-3 Fatty Acids on Mammary Tumorigenesis

Several mechanistic pathways for dietary fat to alter neoplastic growth have been proposed, including changes in lipid peroxidation.[70] Malignant cells show decreased peroxidation of essential fatty acids, when compared with normal cells.[71] Since ω-3 PUFA both more readily undergoes peroxidation and arrests malignant cell growth,[72] the mechanism of this fatty acid in inhibiting growth, or killing breast cancer cells, is thought to be linked to increased susceptibility to peroxidative damage, which ultimately leads to cell death.[73] Some recent studies, however, question the role of lipid peroxidation as a mechanism of fatty acid action on tumor growth.[74]

Consumption of a diet high in ω-6 PUFA alters the composition of adipose tissue that surrounds mammary epithelial cells, thereby possibly altering membrane structure and function in these cells.[60,75,76] In the cell membranes, linoleic acid is metabolized to arachidonic acid (AA) and, thus, a diet high in linoleic acid will increase membrane AA content.[77,78] High levels of ω-3 PUFA, in contrast, will reduce AA component of the cell membranes.[60] While Rao et al.[78] have suggested that arachidonate is the major fatty acid regulating mammary tumor growth, some other investigators have failed to find a link between high AA levels and mammary tumor size.[53,79]

Prostaglandins, synthesized from AA, may play a role in mediating the effects of ω-6 and ω-3 fatty acids.[54,80] Prostaglandin synthesis inhibitors can counteract the effects of ω-6 fatty acids on transplantable mouse mammary tumors and human breast cancer cell lines and, therefore, this fatty acid might affect tumor growth through elevating tissue prostaglandin levels.[81,82] In support of this view, a diet high in ω-3 PUFA reduces mammary tumor concentrations of prostaglandin E_2.[60] However, no clear relationship exists between mammary tumor prostaglandin content and the growth and metastatic potential of the tumor.[60,81]

In addition to the cyclooxygenase pathway of AA metabolism that results in prostaglandins, AA is metabolized through lipoxygenase pathway. Some studies suggest that a high dietary intake of ω-6 fatty acid correlates with elevated levels of 12-hydroxyeicosatetraenoic (12-HETE) and 15-hydroxyeicosatetraenoic (15-HETE) eicosanoids of lipogenase pathway in the human mammary tumors growing in nude mice.[60] In contrast, dietary intake of eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) reduces the mammary tumor levels of 12-HETE and 15-HETE. Thus, 12-HETE may be an important factor in mediating at least some of the biological actions of ω-6 and ω-3 PUFAs on mammary tumorigenesis.[60]

Additional mechanisms of action of linoleic acid include effects on prostaglandin-induced metabolic events that lead to either an increased formation of diacylglycerol, which in turn activates protein kinase C,[83,84] or altered phospholipase C activity that increases synthesis of AA from diacylglycerol. Emerging evidence links protein kinase C (PKC), diet, and tumor growth. A diet high in linoleic acid increases,[85] and dietary energy restriction inhibits,[86] PKC activity in epidermal skin cells in Sencar mice. A high-fat (corn oil) diet can block the inhibition of skin carcinogenesis and reduce PKC activity induced by moderate energy restriction.[87] These observations suggest that PKC may be an important mediator of the effects of fat, at least ω-6 fatty acids, on tumorigenesis.

Diet and Serum Estrogens

Dietary fat may alter tumor growth by affecting circulating estrogen levels. An increase in the serum estrogen levels by a high-fat diet, on the other hand, may be due to an increase in the size of the adipose tissue. Aromatization of androstenedione to estrone occurring in adipose tissue contributes one-third of the circulating estrogens of premenopausal women, and is the main source of estrogens in postmenopausal women.[88,89] Further, AA activates P450 aromatase that then increases conversion of androstenedione to estrone.[90]

We have measured the plasma or serum levels of 17β-estradiol (E2) in the dietary manipulated pregnant mothers and their offspring. The results indicate that during pregnancy the circulating levels of total E2 are significantly higher (by approximately 30–100%) in the pregnant females fed an isocaloric high-fat diet, when compared with the animals fed a low-fat diet.[47,91] Although the difference may not seem large, it may be biologically critical, e.g., in terms of receptor occupancy. Significantly, there is a 30% difference in the circulating E2 levels between East Asian and Caucasian women,[92] and this may be sufficient to explain the differences in breast cancer risk between these populations. One factor that could have contributed to the difference in estrogen levels between pregnant rats kept on high- and low-fat diets may be the high levels of fiber in the high-fat diet. Fiber reduces serum estrogens by increasing fecal excretion of this hormone.[16]

In pregnant rats consuming non-isocaloric high- and low-fat diets, no differences in serum E2 levels between the high- and low-corn oil groups have been noted (Hilakivi-Clarke et al., unpublished data). This lack of difference could result from the fact that both food and caloric intakes were significantly higher in pregnant rats kept on a non-isocaloric low-fat diet, than in pregnant rats kept on a high-fat diet. High caloric intake and obesity are associated with elevated circulating E2 levels.[93] Our data also show that a maternal diet high in linoleic acid does not significantly increase mammary tumor incidence in female offspring if the mother's serum E2 levels are not elevated during pregnancy. In contrast, an exposure of pregnant rats to a physiologic dose of E2 between gestation days 14 and 20 results in a significantly higher incidence of DMBA-induced mammary tumors in the female offspring.[47] These findings in rats could help to explain why, in women, a high dietary fat intake is not consistently associated with elevated breast cancer risk.

Since after birth and throughout adulthood the serum E2 levels are similar in the offspring of high- and low-fat offspring,[47] maternal diet does not cause a permanent alteration in ovarian steroid production. The offspring of high- and low-fat exposed groups also have normal estrus cycle length and uterine wet weight.[47] It is, therefore, unlikely that the elevated tumor incidence among the female rats exposed to a high linoleic acid diet in utero results from changes in the overall hormonal environment. Rather, in utero high-fat exposure may have caused alterations in the mammary gland itself, which then predisposes it to neoplastic transformation.

Development of Breast

According to our data, fetal exposure to high dietary ω-6 PUFA through the pregnant mother increases sensitivity to carcinogen-induced mammary tumorigenesis, possibly by elevating circulating estrogen levels during pregnancy. Estrogens have multiple effects on both the developing mammary gland morphology and gene expression in various target organs. Results from studies with mice and rats show altered patterns of mammary epithelial morphology after neonatal treatment with DES.[94–97] In humans, precocious breast growth (thelarche) has been reported in infants whose nursing mothers took estrogenic birth-control pills or in children who were exposed to other environmental factors, which either act as estrogens and/or increase serum/urinary levels of endogenous estrogens.[98]

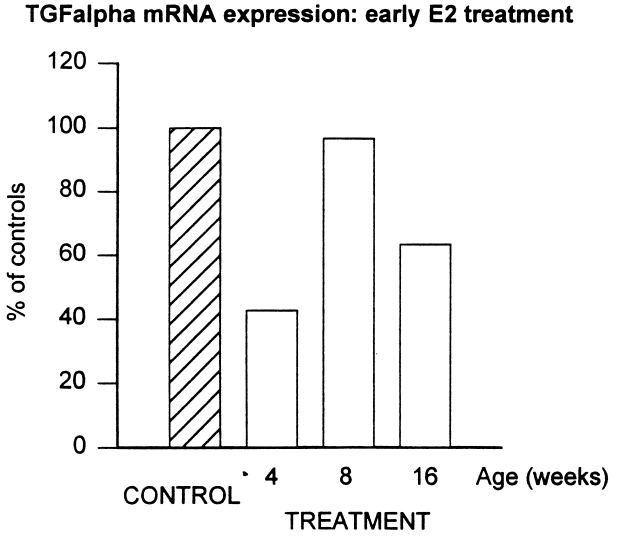

FIG. 3. Effect of early postnatal treatment of 2–4 μg E2 on TGFα mRNA expression in the fourth abdominal mammary glands obtained from 4- to 16-wk-old female mice. Relative densitometric units of TGFα mRNA after correction for GAPDH. Densitometric analysis of the autoradiogram was done, and the amount of TGFα mRNA was calculated as the proportion of control in relative densitometric units after correcting for the intensity of the band for GAPDH. Each bar represents data obtained from three glands.

Among human infants and adults, mammary parenchymal patterns vary substantially between individuals.[99,100] Variations in parenchymal patterns in newborns may be indicators of a high fetal estrogenic environment, such as high placental or birth weight.[101] Our animal studies directly support the role of estrogenic environment in utero in causing significant variations in mammary parenchyma. We have found that a perinatal endogenous exposure to physiologic concentrations of E2 causes permanent changes in the mammary gland morphology. In mice and rats, either an in utero or early postnatal exposure to E2 increases the number of epithelial terminal end buds (TEBs), and temporarily inhibits their differentiation to lobulo-alveolar units.[47,102] TEBs are considered the primary targets for neoplastic transformation in the rodent mammary gland, although some tumors also may arise in the terminal ducts.[103] In humans, most mammary carcinomas originate from lobules type 1 (terminal ductal-lobular units [TDLU]), which arise directly from the TEBs and are composed of terminal ducts and ductules.[46] We also have found that in utero exposure to a high-fat diet alters mammary gland morphology in a manner similar to early E2 exposure.[47] These data suggest that an exposure to a diet high in ω-6 fatty acids during fetal life increases the number of structures that give rise to mammary tumors.

Alterations in Gene Expression

Altered morphology of the mammary gland in individuals exposed to in utero estrogenic manipulations may be associated with changes in the expression of specific genes. Early postnatal exposure to DES produces an ovary-independent induction of the mRNAs and protein encoded by the epidermal growth factor (EGF) and lactoferrin genes in the mouse uterus and vagina.[104] We found a reduction in expression of the estrogen-regulated transforming growth factor α (TGFα) in the mammary glands of female mice exposed to estrogenic manipulations during the early

postnatal period (Fig. 3). Since overexpression of TGFα is generally associated with malignant transformation in the breast, our finding that the expression of this growth factor is reduced in animals exhibiting an elevated breast cancer risk appears controversial. However, in previous studies TGFα overexpression has been detected in vitro in the human breast cancer cell lines and in vivo in breast tumors,[105–107] while in our study the lowered TGFα expression was seen in otherwise normal mammary glands that are prone, after an insult to a carcinogen, to develop a high incidence of mammary tumors. This would indicate that the pattern of TGFα expression may change in parallel with the transformation from normal to abnormal malignant growth.

Insulin may be associated with in utero exposure to fatty acids and increased mammary tumor incidence. Fat stimulates pancreatic insulin production, and the hyperinsulinemia resulting from visceral obesity is closely linked to increased breast cancer risk.[108] Animal and cell culture data generally support the role of insulin being associated with increased tumor growth. Insulin is a major mitogen for human breast cancer cells and is able to stimulate their proliferation even in the absence of other mitogens.[109–111] A series of experiments with diabetic animals have shown that deprivation of insulin causes a rapid regression of most of the carcinogen-induced rat mammary tumors, and administration of insulin stimulates growth of these tumors.[112–115] In addition, insulin receptors (IRs) have been identified in human breast cancer cell lines, tumor biopsies, rodent mammary tumors, and normal human and animal mammary tissue.[116,117] Recently, we have found insulin to be synthesized by several extrapancreatic tissues,[118] including mammary gland (Raygada and Hilakivi-Clarke, unpublished data). Our data also indicate that insulin mRNA expression is higher in the malignant than in the normal human breast tissues, and is particularly high in large DMBA-induced mammary tumors in rats, when compared with normal mammary gland or small DMBA tumors (Raygada and Hilakivi-Clarke, unpublished data). However, in the offspring of high-fat mothers, the insulin mRNA levels in the normal mammary gland are lower than in the offspring of low-fat mothers. This unexpected finding is in parallel with that of TGFα, which expression also is reduced in the animals who are at an increased risk to develop mammary tumors, if exposed to a carcinogen.

Further support for the reduced expression of genes that are generally linked to malignant growth in the breast tissue (and whose levels are elevated in vitro and in vivo in the transformed mammary cells), is obtained in our studies concerning a high-fat diet and estrogen receptor (ER). Maternal exposure to a high-fat diet induces a long-lasting reduction (four-fold lower) in the ER content in the offspring's mammary gland.[119] These findings are similar to those 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.[120,121]

Our findings suggest that low TGFα and insulin mRNA expression, and low ER protein levels in the non-malignant mammary gland, may be associated with increased breast cancer risk. The cause for these contrasting findings with all the three genes in the offspring of high-fat or E2-exposed mothers, when compared with those in already malignant tissues, remains to be established.

DIETARY FAT IN PREGNANT RATS AND BREAST CANCER RISK

Recently, we have obtained evidence to suggest that not only may the offspring be at increased risk for breast cancer by mother's high-fat consumption during pregnancy, but the mother's risk also may be increased. Pregnancy is an established risk factor for breast cancer. In a woman who was younger than 20 y at the time of her first full-term pregnancy, the risk is reduced. In contrast, a woman whose first pregnancy occurred after the age of 35 has an increased risk.[122,123] Further, a short-term increase in breast cancer risk after a full-term pregnancy is seen in women who have no previous history of breast cancer.[122,124–128]

The pregnancy-associated changes in breast cancer risk in women who have recently given birth, when compared with nulliparous women or women who gave birth over 10 y ago, are likely to be linked with the high estrogenic environment during pregnancy. There is evidence that high estrogen levels during pregnancy are associated with the higher breast cancer risk. Severe nausea and vomiting are associated with higher estrogen levels during pregnancy,[129] and significantly increased subsequent risk of breast cancer.[130] In addition, women who used the synthetic estrogen DES exhibit significantly increased breast cancer risk.[131] Conversely, maternal breast cancer risk is essentially reduced in women who suffered from pregnancy-induced hypertension,[126] the hypertension being closely associated with pre-eclampsia. The circulating levels of estrogens in pre-eclamptic women are lower than those in healthy pregnant women.[132] Thus, within the normal physiologic variability of estrogen levels during pregnancy, those women who exhibit low levels may be at reduced risk for breast cancer, while women with high estrogen levels may be at elevated risk.

We have studied the effect of a high-fat intake during pregnancy on breast cancer risk in female rats. In our experimental paradigm, the DMBA model of breast cancer was used to investigate the association between dietary fat during pregnancy and breast cancer.[91] Pregnant female rats, treated with DMBA while still virgin, were introduced to a high-fat (43% calories from fat) or low-fat (16% calories from fat) diet, the source of fat being corn oil high in the ω-6 PUFA linoleic acid. When the offspring were born, the female rats were switched back to the Purina rodent laboratory diet. Our results indicate that the mammary tumor incidence is significantly higher among the rats who were exposed to a high-fat diet during pregnancy than in the low-fat group.[91]

Since ω-6 fatty acids promote the growth of DMBA-induced mammary tumors,[24,133,134] a critical control in our study was virgin female rats exposed to high levels of corn oil for a period corresponding to the length of gestation (3 wk). Mammary tumor incidence was not altered in the virgin, dietary manipulated female rats.[91] Thus, our results are consistent with previous data suggesting that a long-lasting presence of high-fat diet is required to affect mammary tumor growth in adult animals.[24,135]

As is the case with the offspring of pregnant mothers who develop an elevated incidence of mammary tumors if fed with a diet high in ω-6 PUFA through their mother during the fetal life,[47] the increased risk among mothers is probably associated with an increase in circulating estrogen levels. During pregnancy, estrogen and other placental hormones cause a rapid proliferation of the mother's epithelial structures.[136] Further, in rodents, TEBs are differentiated to alveolar structures,[137] and in humans, type 1 lobules are differentiated to type 3 lobules during pregnancy.[138] The first event (proliferation) is probably responsible for the increase in breast cancer risk in women who undergo their first pregnancy after the age of 35, by causing a stimulation of the growth of those cells that have already undergone the first steps of neoplastic transformation.[139] Older women presumably have acquired a higher number of initiated epithelial cells than younger women. The second event (differentiation) is suggested to cause an overall reduction in breast cancer risk between parous and nulliparous women.[138]

In conclusion, our data show that consumption of a high linoleic acid diet during pregnancy, possibly through an increase in circulating estrogen levels and increased growth of transformed TEBs, increases the risk of developing DMBA-induced mammary tumors in female rats.

TABLE II.

SUMMARY OF FINDINGS IN OFFSPRING OF MOTHERS FED WITH AN ISOCALORIC DIET HIGH IN ω-6 PUFA (43% OR 46% CALORIES FROM FAT) IN CORN OIL DURING PREGNANCY, WHEN COMPARED WITH OFFSPRING OF LOW ω-6 PUFA MOTHERS

Mother
High maternal dietary intake of corn oil during pregnancy
Increased circulating E2 during pregnancy
Female offspring
Physical development
Normal physical development
Early puberty onset
Normal adult estrogen levels
Normal estrus cycle
Mammary gland: before DMBA administration
Increased number of structures (TEBs) that are targets of malignant transformation
Reduced expression of insulin mRNA
Reduced levels of ER protein
Mammary gland: after DMBA administration
Significantly increased incidence of mammary tumors
Shortened latency to tumor appearance
No changes in the tumor size or growth pattern

CONCLUDING COMMENTS

Our data discussed in this review are summarized in Table II and Figure 4. We propose that a high maternal intake of ω-6 fatty acids during pregnancy increases breast cancer risk among the female offspring. This increase may result from the elevated estrogen levels during pregnancy that, in turn, alter the normal mammary gland development. The changes in the mammary gland include an increased number of epithelial targets that may be sites for carcinogen-induced or spontaneous mutations, or altered expression of fat/estrogen-regulated genes. Thus, in utero exposure to a high-fat diet may serve as a preinitiator of breast cancer.

At present, we cannot rule out the possibility that a diet high in ω-6 PUFA during pregnancy alters breast cancer risk in the mothers and offspring through mechanisms other than an increase in serum estrogen levels. For example, diet high in ω-6 PUFA will reduce the metabolism of ω-3 PUFA,[140] because they compete for the same metabolic enzyme systems. Present in fish and other marine oils, ω-3 PUFA inhibits mammary tumor growth.[57–60] Thus, the increased breast cancer risk in mothers and their offspring fed with corn oil during pregnancy may result from the reduced metabolism of EPA or DHA. Alternatively, ω-6 PUFA linoleic acid, by causing an accumulation of arachidonic acid in the mammary gland, may alter estrogen's signal transduction pathways, those including protein kinase C, and other estrogen-regulated genes. The involvement of lipid peroxidation in explaining our results also remains to be determined.

It is to be emphasized that a balanced maternal diet is critical for the well-being of the fetus and its postnatal development. For example, a link between disproportionate fetal growth, fetal undernutrition, and increased risk for heart disease and diabetes has been proposed.[141] In addition, increasing evidence suggests that low birth weight and high placental weight in comparison to birth weight predisposes to hypertension.[141,142] The confirmed etiologies for intrauterine growth retardation are pregnancy-induced hypertension and smoking,[43,143] the role of nutrition and other factors being controversial. It appears that even a mild deficiency in essential fatty acids may reduce birth weights.[144]

Both ω-6 and ω-3 PUFAs are important for the fetal development. Dietary deficiency of ω-3 fatty acids during development leads to various impairments in the visual function and functions of the central nervous system.[55,56] Decreased maternal intake of ω-6 fatty acids may be linked to pregnancy-induced hypertension and intrauterine growth retardation.[145,146] However, high intake of

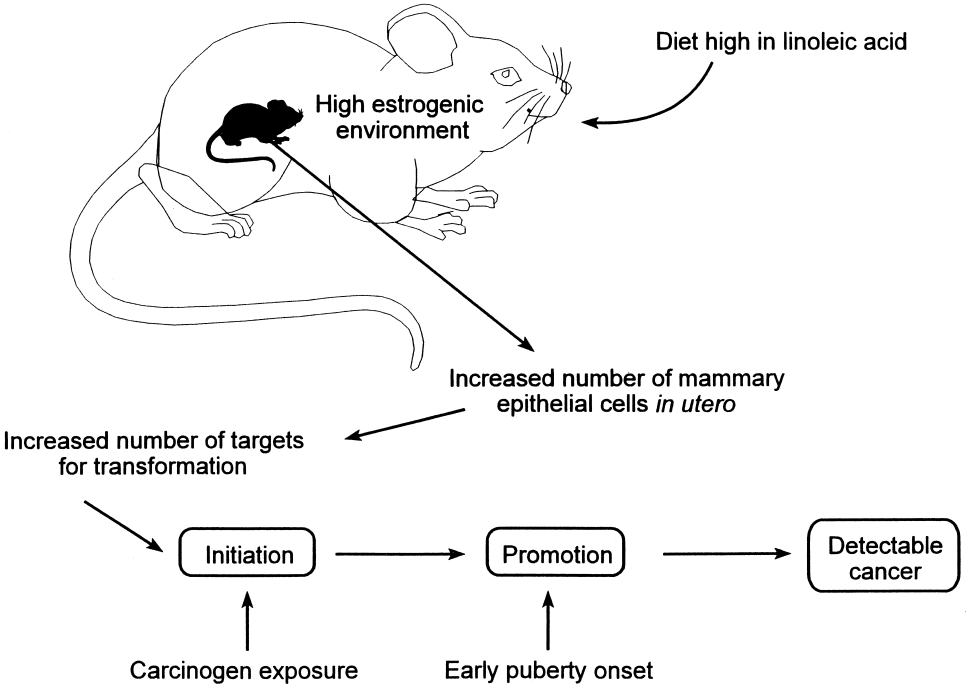

FIG. 4. The hypothetical pathway for breast cancer, preinitiated by maternal exposure to a diet high in ω-6 PUFA linoleic acid.

these PUFAs also may be detrimental, as indicated by impaired learning in rats fed a diet high in ω-6 PUFA,[147] and lower scores of psychomotor development in infants fed with formulas supplemented with marine oil.[148] Our animal data suggest that levels of ω-6 PUFA in excess of those required for a healthy pregnancy increase carcinogen-induced mammary tumor incidence in the mother and her female offspring. It remains a real possibility that the patterns of maternal dietary habits during pregnancy can permanently alter the risk of developing breast cancer in humans, both the mother and her daughter.

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