Consumption of a High-Fat Diet Alters Estrogen Receptor Content, Protein Kinase C Activity, and Mammary Gland Morphology in Virgin and Pregnant Mice and Female Offspring

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ABSTRACT

Previous studies have shown that a diet high in polyunsaturated fatty acids increases mammary tumor incidence in adult and pregnant mice and rats and in the female offspring. The present study investigated whether a high-fat diet alters the number of estrogen receptor (ER) binding sites and protein kinase C (PKC) activity in the mammary gland of these animals. In the female offspring, the effects of maternal exposure to a high-fat diet during pregnancy on development of the mammary epithelial tree were studied also. BALB/c mice were kept on a diet containing either 43% (high-fat) or 16% (low-fat) calories from corn oil, which consists mostly of n-6 polyunsaturated fatty acids, for 1 month. In adult female mice, a 6-fold increase in the number of ER binding sites and 2-fold increase in PKC activity were found in the mammary glands of the high-fat mice when compared with the low-fat mice. In pregnant mice, a high-fat diet increased ER binding sites by 61% and PKC activity by 51%. In contrast to adult and pregnant mice, females exposed to a high-fat diet only in utero through their pregnant mother exhibited a significantly reduced number of mammary ER binding sites by age 45 days (78% decrease) and a reduction in PKC activity by ages 30 and 100 days (44 and 20% decrease, respectively). The mammary epithelial tree of the high-fat offspring contained more terminal end buds and was less differentiated than that of the low-fat offspring. These findings show that consumption of a high-fat diet increases ER and PKC in the adult and pregnant mouse mammary gland, perhaps contributing to the fat-induced promotion of mammary tumorigenesis. In contrast, reduced ER and PKC following a high-fat exposure in utero may be associated with increased susceptibility to carcinogenesis, possibly due to an increased number of terminal end buds that are the sites of neoplastic transformation in the mammary gland.

INTRODUCTION

The causative relationship between a high dietary fat intake in women and increased breast cancer risk remains controversial (1, 2). This may partly be explained by the lack of consensus of the mechanism of action of dietary fat on mammary cells. The pathways proposed include changes in lipid peroxidation (3), prostaglandin E2 content (4), and/or 12-hydroxyeicosatetraenoic content (5) in the mammary gland and tumors, and increased circulating estrogen levels (6-9). The timing of dietary fat exposure also may be important. We have shown that a high-fat diet increases breast cancer risk if the exposure occurs during critical developmental periods, when rapid, hormone-induced proliferation of mammary epithelial cells takes place. Rats kept on a diet high in polyunsaturated fatty acids during pregnancy and their female offspring exhibit a significant increase in carcinogen-induced mammary tumor incidence (10, 11). Furthermore, serum estradiol levels are elevated by a high-fat diet during pregnancy (10, 11). However, among the offspring, the estradiol levels are normal.

In our studies, we have focused on the possibility that maternal fat intake during pregnancy may alter mammary tumor development in the mother and her offspring by increasing pregnancy estrogen levels. The effects of estrogens on mammary glands and tumors are likely to be mediated through the ER.3 Studies done in mice (12) and rabbits (13) indicate that the ER is present in utero, and the ER concentrations in the mammary gland increase dramatically during the first month of life (14). Thereafter, the ER content remains at a relatively constant level. In the fetal mammary gland, ER is located in the stroma and appears on day 14 of gestation (12), whereas after birth, at least from postnatal week 2 onward, ER is located both in the stromal and epithelial mammary cells (15). Results obtained in rabbits and rats show that during pregnancy, the ER concentrations are low but detectable (13, 16). A marked increase in mammary ER content occurs during lactation (16). The changes in ER levels during pregnancy and lactation reflect the down-regulation of ER by estrogens; estrogen levels are high during pregnancy and low during lactation. The effect of dietary fat on ER content/expression has not been studied.

Estrogens may regulate cellular functions by affecting the expression/activity of genes in the signal transduction pathways, including the PKC family (17, 18). PKC also is dependent on diacylglycerol (a fatty acid metabolite) and calcium for activation. Estrogens increase PKC8 expression in the uterus (19), and activation of PKC isoenzymes decreases ER expression in human breast cancer cell lines (20-22). These studies suggest a cross-regulation between the estrogen receptor and PKC pathways. Diet also influences PKC activity. A high-fat diet increases PKC activity (23), whereas caloric restriction inhibits PKC activity (24) 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 (25).

In the present study, we investigated the effects of the consumption of a high-fat diet during pregnancy on the number of ER binding sites and PKC activity in the mammary gland of mothers and their female offspring. We compared these findings with the changes in mammary gland morphology in the offspring. The data indicate that a high-fat diet increases the number of ER binding sites and PKC activity in the virgin mammary gland and tends to have a similar effect in pregnant animals. However, in the female offspring exposed to a high-fat diet through the pregnant mother, the amount of ER and PKC activity in the mammary gland is significantly reduced. The reduced levels of estradiol binding and PKC activity is accompanied by a decrease in epithelial differentiation and a persistence of structures that are known to be the targets of malignant transformation.

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3 The abbreviations used are: ER, estrogen receptor; PKC, protein kinase C; PUFA, polyunsaturated fatty acid; TEB, terminal end bud; DES, diethylstilbestrol.
MATERIALS AND METHODS

Animals and Diets. BALB/c mice, purchased from Charles River (Wilmington, MA), were used. These animals were kept either on a regular mouse diet (Purina laboratory rodent chow 5001) or on AIN-93 (American Institute of Nutrition)-based (26) high- or low-fat diets that were prepared by Bioserv, Inc. (Frenchtown, NJ). Description of the diets is provided in Table 1. Purina chow contained 12% calories from fat. The physiological caloric value of this diet was 3.3 kcal/g. The AIN-93-based high-fat diet contained 43% calories from fat, and the low-fat diet contained 16% calories from fat. The fat source was corn oil that is high in n-6 PUFAs, particularly linoleic acid (59% of total fatty acids). Although the diets had significantly different amounts of fat, they were isocaloric (caloric density ~3.7 kcal/g) due to adjustments made by the addition of fiber (Alphacel). Thus, the high-fat diet contained high levels of nondigestible dietary fiber. The proportion of dietary components other than fat were adjusted to ensure an adequate intake of protein (casein and L-cystine), carbohydrates (corn starch, maltose, and sucrose), vitamins (AIN vitamin mix), and trace elements (AIN mineral mix), and the amounts of these components per diet were approximately constant with regard to energy.

Three different feeding regimens were used. (a) Adult exposure: Two-month-old virgin female mice were kept on the high- or low-fat diet for 4 weeks, after which the mice were sacrificed and their mammary glands were removed. (b) Exposure during pregnancy: Two-month-old female mice were kept on a high- or low-fat diet. On week 2 of this feeding regimen, the females were mated by housing them with male mice. The pregnant female mice were sacrificed on day 14 or 19 of gestation, and their mammary glands were removed. (c) In utero exposure: Female mice were kept on a high- or low-fat diet. On week 2 of this feeding regimen, the females were mated by housing them with male mice. High- or low-fat feeding continued throughout pregnancy, and the animals were switched to Purina laboratory chow immediately after the offspring were born. Mammary glands of the offspring, exposed to a high- or low-fat diet only through their pregnant mother, were removed on postnatal days 30, 40, 45, 50, and 100.

ER. The number of ER binding sites in mammary glands were determined from adult mice kept on the high- or low-fat diets for 4 weeks (adult exposure), pregnant mice on gestation day 14 kept on the high- or low-fat diet (exposure during pregnancy), and 45-day-old female offspring of mothers kept on the high- or low-fat diets during pregnancy (in utero exposure). In the adult and pregnant mice, mammary glands from five female mice in the high-fat and five female mice in the low-fat group were assayed for ER content. In the offspring, glands from 12 high-fat and 10 low-fat animals were assayed. The number of ER binding sites were determined from the fourth abdominal mammary glands using a ligand binding assay as described by Nelson et al. (27).

PKC Activity in the Mammary Gland. The activity of PKC was determined in the seventh mammary gland from the adult and pregnant mice (on gestation day 19) and from 30- and 100-day-old female offspring exposed to the high- or low-fat diet through a pregnant mother. The number of mice per group in each assay was four to five. PKC activity was determined using the Biotrak PKC enzyme assay system obtained from Amersham Life Sciences (Arlington Heights, IL), following the manufacturer’s instructions. In this assay, the level of PKC activity was determined using a specific peptide derived from the epidermal growth factor receptor. Samples were frozen at −70°C until assayed. All reactions were set up on ice. Samples were homogenized in a buffer containing 50 mM Tris/HCl (pH 7.5), 0.3% (w/v) β-mercaptoethanol, 5 mM EDTA, 10 mM EGTA, and 50 μg/ml phenylmethylsulfonyl fluoride. Blanks were used to correct for nonspecific effects of [32P]ATP or the binding of its radiolytic decomposition products. Extracts from the MDAMB231 human breast cancer cell line, which contain high PKC activity, were used as a positive control.

Mammary Gland Morphology. Whole mounts of the fourth abdominal glands were obtained from five 30-, 40-, 50-, and 100-day-old female offspring of high- and low-fat mothers per treatment group. The harvested glands were stained with carmine aluminum, as described previously (28), and examined under an Olympus dissecting scope. The presence of the three characteristic morphologies of the mammary glands were evaluated in a double-blind manner using a five-point scale (0, absent; 1, few; 2, low–moderate; 3, high–moderate; and 4, numerous). The scored characteristics were the relative density of (a) epithelial ducts; (b) terminal ducts and lobulo-alveolar units; and (c) crossing epithelial ducts (pattern of branching). We have validated and successfully used this scale in our earlier studies (10, 29). The number of TEBs per total mammary gland also was determined.

Statistical Analysis. Statistical tests were performed using the SOLO statistical system (BMDP Statistical Software, Los Angeles, CA). Body weights and other general effects in the mother and offspring were analyzed using Student’s t test. Data obtained on physical maturation were analyzed using the t test (day of eye-lid and vaginal openings) and Log-Rank test (proportion of animals with eye-lid and vaginal openings on each day). Estrogen receptor binding sites and PKC activity data obtained from virgin and pregnant mice were analyzed using two-way ANOVA, with age and treatment being the variables. In the offspring, ER content on day 45 was analyzed using Student’s t test. PKC activity on days 30 and 100 was analyzed using two-way ANOVA. Results for the relative density of different parenchymal structures and number of TEBs were determined using two-way ANOVA. Posthoc comparisons between the groups after ANOVA were made using Fisher’s least significant difference test. All probabilities are two-tailed.

RESULTS

Effects of Dietary Manipulations on Body Weights and Pregnancy Outcomes. At the beginning of the study, adult female mice to be fed with the low-fat diet [mean body weights ± SE: 18.5 ± 0.1 g (n = 30)] weighed the same as the female mice to be fed with the high-fat diet [18.5 ± 0.1 g (n = 30)]. However, when kept on the isocaloric diets for 10 days, the low-fat consuming adult mice [18.3 ± 0.1 g (n = 30)] weighed significantly less than the high-fat mice [19.4 ± 0.1 g (n = 30); t = 6.37, df = 58, P < 0.0001]. During the course of pregnancy, the difference in weight gain disappeared (Table 2), indicating that pregnant mice in the low-fat diet consumed at least as much energy as the pregnant mice in the high-fat diet. These results are essentially similar to those obtained previously in pregnant rats consuming either a high- or low-fat diet (10). The duration of pregnancy, the percentage of successful deliveries, the number of pups per mother, birth weight, and body weight gain were equivalent in the low- and high-fat groups (Table 2).

Effects of Maternal Dietary Manipulation on the Physical Maturation in Female Offspring. As indexes of early physical maturation, we determined the age when the eye-lid opening and vaginal openings occurred. Beginning on postnatal day 12, the litters were examined daily for eye-lid opening. There was no difference in the onset of eye-lid opening in the high-fat (postnatal day 15.7 ± 0.2, mean ± SE, n = 56) and low-fat offspring (postnatal day 16.0 ± 0.1, mean ± SE, n = 56).

Table 1 Dietary formulations

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>Low-fat</th>
<th>High-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat, total (corn oil)</td>
<td>50</td>
<td>300</td>
</tr>
<tr>
<td>Protein (casein, L-cystine)</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Carbohydrates (corn starch, maltose, sucrose)</td>
<td>629.5</td>
<td>340</td>
</tr>
<tr>
<td>Fiber</td>
<td>50</td>
<td>215.5</td>
</tr>
<tr>
<td>AIN mineral mix</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>AIN vitamin mix</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>TBHQ&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>Total grams</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>kcal density</td>
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<td>3.75</td>
</tr>
<tr>
<td>% kcal from fat</td>
<td>15.7</td>
<td>43.2</td>
</tr>
<tr>
<td>% kcal from protein</td>
<td>20.5</td>
<td>19.2</td>
</tr>
<tr>
<td>% kcal from carbohydrates</td>
<td>63.8</td>
<td>37.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> In mg/kg diet: calcium 5000, phosphorus 1992, potassium 3600, sulfur 300, sodium 1019, chloride 1571, magnesium 507, iron 35, zinc 30, manganese 19, copper 6, iodine 0.2, molybdenum 0.15, selenium 0.15, silicon 5, chromium 1, fluoride 1, nickel 0.5, boron 0.5, lithium 0.1, and vanadium 0.1.

<sup>b</sup> In units/kg diet: nicotinic acid 30 mg, pantotenate 15 mg, pyridoxine 6 mg, thiamin 5 mg, riboflavin 6 mg, folic acid 2 mg, vitamin K 750 μg, D-Biotin 200 μg, vitamin D<sub>12</sub> 25 μg, vitamin A 4000 IU, vitamin D<sub>3</sub> 1000 IU, and vitamin E 75 IU.

<sup>c</sup> TBHQ, tertbutylhydroperoxide.
Puberty onset

Fig. 1. The proportion of female mice exposed in utero via their mother to high (n = 32) or low n-6 PUFA diets (n = 31; fat source was corn oil) with vaginal opening between postnatal days 23 and 29 days. Vaginal opening occurred significantly earlier in the high-fat versus low-fat group (P < 0.004).

Table 2 Effects of a high (43%)- or low (16%)-fat diet on pregnancy and early developmental in Balb/c mice

<table>
<thead>
<tr>
<th>Dietary fat content</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>% successful pregnancies</td>
<td>83.3 (10/12)</td>
<td>91.7 (11/12)</td>
</tr>
<tr>
<td>Pregnancy weight gain (g)</td>
<td>8.7 ± 1.0</td>
<td>8.3 ± 0.7</td>
</tr>
<tr>
<td>Time from housing with males to giving birth (days)</td>
<td>26.3 ± 1.6</td>
<td>25.3 ± 1.0</td>
</tr>
<tr>
<td>Number of pups/litter</td>
<td>6.3 ± 0.5</td>
<td>5.5 ± 0.6</td>
</tr>
<tr>
<td>Pup weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>2.2 ± 0.2</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.7 ± 0.4</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>Day 21</td>
<td>6.8 ± 0.2</td>
<td>7.0 ± 0.4</td>
</tr>
</tbody>
</table>

*The values are means ± SE.

Fig. 2. ER protein levels, obtained using a ligand binding assay, in the fourth mammary gland in female mice that were exposed to a high (43% calories from corn oil)- or low (16% calories from corn oil)-fat diet. The values were obtained from virgin mice that were kept on the high- or low-fat diets for 4 weeks, from pregnant mice that were kept on the diets for 2 weeks prior to pregnancy until sacrificed on day 14 of gestation, and from 45-day-old female offspring of mothers that were kept on the diets during pregnancy. Means obtained from 5 mammary glands per group in adult and pregnant mice and 10 from low-fat and 12 from high-fat mammary glands are shown; bars, SE. Significantly different from the low-fat group: * P < 0.05.

Discussion

The number of ER binding sites was significantly reduced in the female offspring of mothers fed a high-fat diet during pregnancy when compared with the female offspring of mothers fed a low-fat diet (t = 2.1, df = 16, P < 0.05; Fig. 2). This result is opposite to that seen in adult female mice kept on the high- or low-fat diets at the time of determining the mammary content of ER. However, a decrease in the mammary ER amounts has been reported in offspring of mothers exposed to the synthetic estrogen DES (31, 32), suggesting that maternal intake of a high-fat diet during pregnancy may reduce mammary ER content in the female offspring possibly by increasing pregnancy estrogenic activity (10, 11).

Effects of Dietary Manipulations on PKC. In adult and pregnant female mice, PKC activity was significantly elevated in the mammary glands of female mice consuming a high-fat diet when compared with activity detected in the glands of mice fed with a low-fat diet [F(1,14) = 4.57, P < 0.05; Fig. 3]. In the adult mice, a high-fat diet increased PKC activity by 81%, and in the pregnant mice, a high-fat diet increased activity by 51%. There also was a difference in the amount of PKC activity between adult and pregnant mice independent of their diet. The activity in the pregnant animals was significantly higher than in the virgin animals [F(1,14) = 6.30, P < 0.025]. Earlier studies in rats have reported an induction in PKC activity in the ovarian cells during pregnancy (19).

The PKC activity was significantly lower (44 and 20%) in the mammary glands of 30- and 100-day old offspring of mothers fed a high-fat diet during pregnancy than in the low-fat offspring [two-way analysis of variance (ANOVA) of variance; P < 0.05].

Effect of Dietary Manipulations on Estrogen Receptor. Adult and pregnant female mice consuming a high-fat diet exhibited a significantly higher number of estradiol binding sites in the mammary gland when compared to animals exposed to a low-fat diet [F(1,16) = 13.66, P < 0.002; Fig. 2]. The difference was particularly significant in adult mice; the high-fat animals had a 6-fold (574%) higher amount of ER binding sites than the low-fat animals. During pregnancy, 61% higher ER binding sites were seen in the high-fat than low-fat-fed mice. In addition to the dietary difference, there was a difference between adult versus pregnant mice in the number of estradiol binding sites of the mammary gland. The adult animals had a higher number of ER protein than the pregnant animals [F(1,16) = 11.87, P < 0.003]. This finding is in accordance with previous data, indicating that pregnancy reduces ER content in the mammary gland (13). We also studied the possible effect of dietary fat on progesterone receptor. The high-fat diet did not have an effect on mammary gland progesterone receptor content (data not shown).

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associated with a subsequent increase in susceptibility to develop pregnancy. Data are means obtained from four to five glands; bars, SE. * P < 0.05.

on the diets from 2 weeks prior to pregnancy until sacrificed on day 19 of gestation, and or low (16% calories from corn oil)-fat diet. The values were obtained from virgin mice on days 40 (P < 0.05) and 50 (P < 0.05) but not on days 30 and 100.

pregnancy when compared with the offspring of low-fat mothers reduced in the offspring of mothers kept on a high-fat diet during Morphology. The density of epithelial structures was significantly increased with age [F(1,13) = 13.13, P < 0.003; Fig. 3]. These findings indicate that low PKC activity in the mammary gland may be involved in this process. Our earlier studies clearly indicate that a high-fat diet increases circulating estradiol levels in the pregnant and nonpregnant mice and rats (10, 11, 34) but not in the offspring. Because estrogens regulate ER and PKC, these parameters were determined in the adult and pregnant mammary glands of mice consuming either a high- or low-fat diet. Additionally, the number of ER binding sites and PKC activity were measured in the mammary glands of female offspring exposed to a high- or low-fat diet through a pregnant mother.

Adult mice consuming a high-fat diet exhibited a 6-fold increase in ER binding sites in the mammary gland. To our knowledge, this is the first time that a direct effect of dietary fat on ER in the breast has been shown. Indirect evidence, to suggest that a high-fat diet may induce ER, is available from studies that show that 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 (35). The effect of dietary fat on the mammary gland ER content may explain the difference between animal studies and human cohort studies concerning a high-fat diet and breast cancer. In animal models, an exposure to a high-fat diet promotes mammary tumor growth (36). Because the carcinogen-induced rodent mammary tumor models are ER positive and strongly estrogen dependent (37), a fat-induced increase in circulating estrogens and mammary ER content may stimulate tumor growth. In humans, the link between a high-fat diet and breast cancer is not as clear. Although most case-control studies suggest a link between a high-fat diet and breast cancer (1, 38), large

ANOV: $F(1,13) = 5.86$, $P < 0.04$; Fig. 3]. PKC activity also increased with age [$F(1,13) = 13.13$, $P < 0.003$; Fig. 3]. These findings indicate that low PKC activity in the mammary gland may be associated with a subsequent increase in susceptibility to develop mammary tumors.

Effects of in Utero Dietary Manipulations on Mammary Gland Morphology. The density of epithelial structures was significantly reduced in the offspring of mothers kept on a high-fat diet during pregnancy when compared with the offspring of low-fat mothers [$F(1,30) = 8.98$, $P < 0.0054$; Figs. 4 and 5]. The difference was seen on days 40 ($P < 0.05$) and 50 ($P < 0.05$) but not on days 30 and 100. Older animals also had a more dense epithelial tree than the younger animals [$F(3,30) = 5.65$, $P < 0.0034$], except a drop in density was observed in the high-fat offspring between days 30 and 40.

In utero exposure to a high-fat diet significantly altered the number of TEBs present in the mammary gland. This effect was age specific [$F$ for interaction between fat and age ($3,30) = 6.87$, $P < 0.0012$]. On day 30, the high-fat offspring had significantly more TEBs than the low-fat offspring ($P < 0.05$). However, on days 40 and 50, no significant differences were seen. On day 100, when all TEBs in the low-fat offspring had differentiated to terminal ducts and lobulo-alveolar units, the high-fat offspring still exhibited TEBs. Thus, TEBs persisted for a longer time period following an in utero high-fat exposure than a low-fat exposure. Significantly fewer differentiated terminal ducts and lobulo-alveolar units were present in the mammary glands of female mice exposed to a high-fat diet in utero than in the glands of low-fat animals by days 40, 50, and 100 [$F(1,23) = 19.77$, $P < 0.0002$]. Few of these structures were seen on day 30 and consequently were not scored at this age. A significant increase by age in the density of terminal ducts and lobulo-alveolar units was observed [$F(2,23) = 10.65$, $P < 0.0005$].

Significantly more epithelial criss-crossing was scored in the mammary glands of the high-fat offspring than of the low-fat offspring by day 30 [$F(1,30) = 8.31$, $P < 0.0072$]. After that age, the difference between the two groups was not significantly different.

**DISCUSSION**

We (10, 11) and others (33) have shown that consumption of a high-fat diet, during periods when the mammary epithelial cells are growing rapidly (pregnancy and fetal life), increases subsequent breast cancer risk in the mothers and their female offspring. The present study investigated the potential mechanisms of action involved in this process. Our earlier studies clearly indicate that a high-fat diet increases circulating estradiol levels in the pregnant and nonpregnant mice and rats (10, 11, 34) but not in the offspring. Because estrogens regulate ER and PKC, these parameters were determined in the adult and pregnant mammary glands of mice consuming either a high- or low-fat diet. Additionally, the number of ER binding sites and PKC activity were measured in the mammary glands of female offspring exposed to a high- or low-fat diet through a pregnant mother.

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cohort studies have failed to confirm this link (2). A significant proportion (from 20% in postmenopausal to 60% in premenopausal) of breast tumors are ER negative (39) and do not respond to estrogens and perhaps not to dietary fat. The lack of effect of a high-fat diet on ER-negative breast tumors in women is supported by the observation that there is no correlation between the recurrence of ER-negative breast tumors and dietary fat intake, whereas ER-positive tumors are more likely to metastasize if a woman is consuming a high-fat diet (40).

In pregnant mice, a high-fat diet failed to significantly increase the amount of mammary ER content. This may be due to the fact that high pregnancy estrogen levels are linked to low ER content in the mammary gland (13). Thus, high serum levels of estrogens during pregnancy may prohibit the effects of a high-fat diet on ER. It is presently unclear how dietary fat increases ER in the mammary gland. The elevated serum estradiol levels in the high-fat-fed animals (adult and pregnant) are not likely to cause an induction of ER protein, because estradiol down-regulates this receptor (41).

In contrast to the effects in adult female mice, the mammary gland ER was reduced in the offspring of mothers who consumed a high-fat diet during pregnancy. Because female mice and rats exposed to a high-fat diet through their pregnant mothers exhibit an increase in breast cancer risk (spontaneous or carcinogen induced; Refs. 10 and 33), low mammary ER content in the offspring may be predictive of an elevated breast cancer risk. Previous studies also show that high in utero estrogenic activity, induced by a maternal exposure to DES, causes a reduction in mammary gland and tumor ER content and an increase in mammary tumor risk (31, 32). 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. For example, elevated circulating estradiol levels during pregnancy in the mothers consuming a high-fat diet as well as maternal DES exposure could have resulted in a decrease in ER in the mammary glands of the offspring.

Several studies suggest an association between PKC activity and breast cancer risk. PKC activity is higher in the malignant than benign breast tissues (42) and higher in the more aggressive than in the less aggressive phenotype of human breast cancer cell lines (43). Because a high-fat intake promotes mammary tumorigenesis in animal models (36), 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. The increased PKC activity in the mammary glands of adult female mice also is in accordance with data showing an induction in PKC activity by dietary fat in the normal and/or transformed colon and skin epidermal cells (23, 25). Furthermore, a diet high in corn oil can block the inhibition of skin carcinogenesis and reverse the reduction in PKC activity induced by energy restriction (44).

During pregnancy, a moderate elevation in the PKC activity by a high-fat diet was seen. Previous studies have demonstrated that pregnancy itself increases the activity/expression of some PKC isoforms in the ovary (19) and mammary gland (45), and a similar increase in PKC activity was found in this study. Pregnancy has been shown to increase breast cancer risk within 3–9 years following the birth of a child (46, 47), after which the risk decreases below that detected in nulliparous women (48). Our previous animal study also shows that an exposure to a high-fat diet during pregnancy increases breast cancer risk (11). Thus, the high-fat (and pregnancy)-induced increase in PKC activity is not likely to cause a decrease in ER content in the mammary glands of the offspring.

Rig. 5. Representative mammary whole mounts of the fourth abdominal gland obtained from 30-, 40-, and 100-day-old female mice exposed to a high- or low-fat diet in utero through their pregnant mother. Tissues were prepared as described by Haslam (28) using carmine staining. By day 30, more TEBs are present in the high-fat group. By day 40, the epithelial density is reduced in the high-fat offspring. By day 100, the glands of the high-fat offspring still contain TEBs, although the glands of the low-fat offspring are differentiated to terminal ducts and lobulo-alveolar units. X6.3.

Fig. 5. Representative mammary whole mounts of the fourth abdominal gland obtained from 30-, 40-, and 100-day-old female mice exposed to a high-or low-fat diet in utero through their pregnant mother. Tissues were prepared as described by Haslam (28) using carmine staining. By day 30, more TEBs are present in the high-fat group. By day 40, the epithelial density is reduced in the high-fat offspring. By day 100, the glands of the high-fat offspring still contain TEBs, although the glands of the low-fat offspring are differentiated to terminal ducts and lobulo-alveolar units. X6.3.
activity may be associated with an increased incidence of carcinogen-induced mammary tumors in rats fed with a high-fat diet during pregnancy (a temporal increase in breast cancer risk in women after pregnancy).

In contrast to the results obtained in adult and pregnant mice, PKC activity was reduced in the mammary gland in the offspring of mothers that were kept on a high-fat diet during pregnancy. This appears paradoxical, because mammary tumor incidence increased in the offspring (10, 33). However, reduced expression of some PKC isoforms, such as PKCα, is associated with increased neoplastic transformation in the mammary gland, whereas expression of other isoforms, such as PKCβ, is linked to a more aggressive neoplastic process (43, 49). Because we only measured total PKC activity, it is not known whether the reduced PKC activity in the offspring results from a reduction of activity of one particular isoform.

It also is possible that the reduced PKC activity reflects specific morphological changes in the mammary gland that increase the vulnerability to neoplastic growth. In utero exposure to a high-fat diet produced significant changes from the normal development of the mammary gland. The number of TEBs, structures that are the targets of malignant transformation in the rodent mammary gland and possibly in the human breast (50, 51), was significantly higher in the high-fat offspring than in a low-fat offspring at the age of 30 days. At the age of 100 days, when there were no visible TEBs in the glands of the offspring of the low-fat mothers, the high-fat offspring still had several TEBs. Thus, the structures that are sensitive to neoplastic changes persist in the mammary glands of female mice exposed to a high-fat diet in utero. Importantly, persistent TEBs also have been reported in transgenic mice (52, 53) and in rats exposed to a high-fat diet or estradiol in utero or during early postnatal period (10, 29). All of these groups exhibit an increased incidence of malignant growth in the mammary glands.

The relationship between various maternal manipulations that alter pregnancy estrogenic environment and the density of the mammary epithelial tree is not clear. In the present study, the epithelial density was significantly reduced in BALB/c mice, whereas in our previous study, the epithelial density is increased following in utero exposure to a high-fat diet in rats (10). We also have found that perinatal estrogen treatment significantly reduces mammary epithelial density in the 6-week-old female mice (29). In rats, prenatal estrogen administration does not significantly increase or decrease the density of mammary tree (10). Consistently in all of our animal studies (Refs. 10 and 29 and the present study), the differentiation of TEBs to terminal ducts and lobulo-alveolar units is reduced in mice and rats exposed to fetal or early postnatal dietary fat or estradiol treatment. Taken together, the increased number of TEBs at certain critical time points and their reduced differentiation to lobulo-alveolar units and terminal ducts in the mammary gland of the offspring of mothers exposed to a high pregnancy estrogenic environment may partly explain the increased risk for developing neoplasias.

The changes in the mammary gland morphology in the animals exposed to a high-fat diet in utero may be associated with the changes in PKC activity. High PKC activity is linked to increased cell proliferation and differentiation in the mammary gland (42). A high-fat diet (54) and pregnancy (37) both increase mammary epithelial proliferation and differentiation, and we found elevated PKC activity in adult female mice consuming a high-fat diet and in pregnant mice. In the offspring of mothers consuming a high-fat diet during pregnancy, mammary epithelial differentiation is reduced and PKC activity also is reduced. Thus, the reduced PKC activity in the mammary glands of female mice exposed to a high-fat diet in utero may be associated with the persistent TEBs and the reduced number of differentiated epithelial structures and possibly with the subsequent increase in the incidence of mammary tumors.

The increase in the number of ER binding sites and PKC activity in the mammary gland of female mice consuming a high-fat diet may indicate that this diet activates both ER and PKC, or it first activates ER, which then activates PKC, or vice versa. A cross-regulation between ER and PKC is suggested by findings that estrogens increase PKC expression in some target organs of estrogen (19). In vitro studies indicate that an elevation in PKC activity down-regulates ER expression (20–22). However, because both ER levels and PKC activity were increased in the mammary glands of female mice fed a high-fat diet and reduced in the high-fat offspring, the effects of fat on ER and PKC may have occurred independently from each other. The exact relationship among a high-fat diet, ER, and PKC remains to be studied.

In conclusion, a diet high in PUFA increases the estrogen receptor and PKC activity in the mammary gland. These events may be linked to the fat-induced increase in mammary tumorigenesis in animal models (36) and the effects or lack of them on breast cancer risk in humans (1, 2, 38). Maternal intake of a high-fat diet during pregnancy, in contrast, reduces the ER content and PKC activity in the offspring's mammary gland. Because these offspring are at an increased risk to develop spontaneous and carcinogen-induced mammary tumors (10, 33), low amounts of ER and low PKC activity in the mammary gland may predict an increased breast cancer risk. Recently, a novel rat ER cDNA was cloned from prostate, which was named rat ERβ (55). Transcript tissue distribution is quite different for the ERα and ERβ subtypes. ERβ mRNA expression is high in the ovary and prostate, moderate in uterus and testis, and low in the brain (56). Because expression of ERβ is probably higher in the developing than adult mammary gland or mammary tumors, a maternal high-fat diet may affect offspring's 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 the PKC family of genes.

REFERENCES


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Consumption of a High-Fat Diet Alters Estrogen Receptor Content, Protein Kinase C Activity, and Mammary Gland Morphology in Virgin and Pregnant Mice and Female Offspring

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