Breast Cancer Risk in Rats Fed a Diet High in n-6 Polyunsaturated Fatty Acids During Pregnancy

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Background: **Women who took the synthetic estrogen diethylstilbestrol during pregnancy exhibit an elevated risk of breast cancer, whereas those who suffered from preeclampsia, which is associated with low circulating pregnancy estrogens, exhibit a reduced risk. Since a high-fat diet may increase circulating estrogen levels and possibly breast cancer risk, dietary factors during pregnancy could influence the risk of developing this disease.** ***Purpose:*** **We tested the hypothesis that consumption of a high-fat diet during pregnancy increases carcinogen-induced mammary tumor incidence in rats.** ***Methods:*** **Pregnant or virgin female Sprague-Dawley rats that had been previously treated with 10 mg 7,12-dimethylbenz[*a*]anthracene (DMBA) by oral gavage when 55 days old were assigned to one of two isocaloric diets containing either 16% calories from fat (low-fat) or 43% calories from fat (high-fat) for the length of pregnancy or for the equivalent time of approximately 21 days. There were 20 pregnant and 10 nonpregnant DMBA-treated rats per group. Ten additional pregnant animals (not previously treated with DMBA) per group were used for hormone analysis. The fat source used was corn oil, which is high in n-6 polyunsaturated fatty acids, primarily linoleic acid. The animals were checked for tumors at least once per week by palpation. The tumor size, number, and latency to appearance after carcinogen exposure were recorded. The statistical significance of observed differences was tested by use of appropriate two-sided tests.** ***Results:*** **Female rats on different diets had virtually identical food intakes and weight gains during pregnancy. On gestation day 19, serum estradiol levels were approximately twofold higher in rats fed a high-fat diet than in rats fed a low-fat diet ($P<.02$). The serum insulin levels and insulin/glucose ratios (an index of insulin resistance) in rats fed the high-fat diet were approximately twofold lower than in rats fed the low-fat diet, but the differences did not reach statistical significance ($P<.09$ and $P<.09$, respectively). On week 18 following DMBA administration, the number of rats developing mammary tumors was significantly higher in the group exposed to a high-fat diet (40% of animals) than in the group exposed to a low-fat diet (10% of animals) during pregnancy ($P<.05$). Tumor multiplicity, latency to tumor appearance, and size of tumors upon first detection were similar among the dietary groups. No intergroup differences in the mammary tumor incidence were noted in virgin animals that were exposed to the high- or low-fat diets for an equivalent period of time.** ***Conclusions:*** **Our findings indicate that consumption of a diet high in fat (primarily in the form of n-6 polyunsaturated fatty acids) during pregnancy increases the risk of developing carcinogen-induced mammary tumors, possibly by increasing the pregnancy levels of circulating estrogens.** ***Implications:*** **If further studies find that the results from animal model studies are applicable to humans, some human breast cancers may be preventable by dietary manipulations during pregnancy. [J Natl Cancer Inst 1996; 88:1821-7]**

Pregnancy influences breast cancer risk (*1*). If a mother is younger than 20 years at the time of her first full-term pregnancy, pregnancy is protective. However, a first pregnancy after the age of 30 increases breast cancer risk. Hormonal treatments during pregnancy or pregnancy-related conditions also affect breast cancer risk. Mothers who took diethylstilbestrol (DES) to prevent miscarriages during pregnancy exhibit a significantly increased breast cancer risk (*2*). Breast cancer risk is essentially reduced in women who suffered from pregnancy-induced preeclampsia and/or eclampsia (*3*). The possible mechanisms by which DES exposure increases and preeclampsia reduces breast cancer risk may be estrogenic. DES is a synthetic estrogen. In contrast, the circulating levels of estrogens in preeclamptic women are much lower than those in healthy pregnant women (*4*).

All pregnant women are exposed to a relatively high estrogenic environment. The fetoplacental unit is a major source of estradiol (E_2), estrone, and estriol, with concentrations increasing throughout pregnancy to reach their highest levels at birth (*5*). However, in women experiencing a normal pregnancy, plasma estrogen levels show a marked interindividual variability (*6*). Dietary factors may play an important role in causing these variations. For example, several clinical studies (*7-9*) have

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See "Notes" section following "References."

shown that a high-fat diet increases and a low-fat diet reduces the levels of circulating estrogens.

The role of dietary fat intake in breast cancer is controversial. The n-6 polyunsaturated fatty acid (PUFA) linoleic acid has been strongly implicated as a promotional agent in both carcinogen-induced and spontaneous rodent mammary tumor models (*10,11*). In humans, international comparisons and case–control studies (*12*) have suggested a relationship between total fat consumption and elevated risk for breast cancer. However, most cohort studies on breast cancer have found either a borderline or no relationship (*13*). None of the previous human or animal studies have investigated the role of dietary fat exposure during pregnancy. Thus, an important period in life, when the mammary gland may be sensitive to the effects of a high-fat diet, could have been overlooked.

Until recently, relatively low weight gain and restricted consumption of meat and fat were recommended for pregnant mothers (*14-16*). This recommendation was made primarily to facilitate delivery by producing healthy but relatively low-birthweight babies. The emphasis of the prenatal diet was changed in the 1950s, when children born to mothers exhibiting low gestational weight gain were noted to be at an increased risk of infant mortality and many developmental delays and abnormalities (*17*). The dramatic increase in breast cancer incidence after the 1960s (*18*) closely followed the change in dietary fat intake in pregnant women (*19-21*).

In this study, we tested the hypothesis that exposure to a high-fat diet during pregnancy may influence carcinogen-induced mammary tumors in a rat model of breast cancer.

Materials and Methods

Carcinogen Exposure

Seven-week-old female Sprague-Dawley rats were purchased from Charles River (Wilmington, MA). The rats were administered 7,12-dimethylbenz[*a*]anthracene (DMBA) to induce mammary tumors. While DMBA is an experimental carcinogen rather than a causative factor for human breast cancer, it produces mammary tumors that are comparable to those in humans in terms of their long relative latency, histotypes, and endocrine responsiveness (*22*). At the age of 55 days, a total of 68 female rats were treated with 10 mg of DMBA (Sigma Chemical Co., St. Louis, MO) by oral gavage. The carcinogen was dissolved in peanut oil and given in a volume of 1 mL. The animals were housed in a temperature- and humidity-controlled room at the Georgetown University Research Resource Animal Facility under a 12-hour light–dark cycle. All animal procedures were approved by the Animal Care and Use Committee of Georgetown University, and the experiments were performed following the National Institutes of Health guidelines for the proper and humane use of animals in biomedical research.

Pregnancy

Terminal end buds in the mammary gland differentiate to alveolar buds and lobules during pregnancy, and these differentiated epithelial structures do not give rise to DMBA-induced cancers (*22*). Thus, a parous animal treated with DMBA does not develop mammary tumors. A female rat that is first treated with DMBA and then undergoes pregnancy, however, will develop mammary tumors. In the present study, the animals were first treated with DMBA and then made pregnant.

When the female rats were 75 days old, 48 of them were bred with male rats (group 1—pregnant), and 20 were housed with females only (group 2—virgin). Those bred with males were housed as two females together with one male. The males were kept with the female rats until a few days before the litters were delivered. Thereafter, the females were housed individually with their offspring. The offspring were weaned 3 weeks after birth, and the mothers were rehoused in groups of three or four. The virgin females also were housed in groups of three or four.

Dietary Manipulations

Upon arrival at our laboratory, the animals were fed Purina Rodent Laboratory Chow 5001, which contains 12% calories from fat (saturated and unsaturated). The physiologic caloric value of this diet is 3.3 kcal/g. When the female rats were housed together with the males (group 1—pregnant) or left in cages containing females only (group 2—virgin), they were introduced to diets that had a high (43% calories from fat) or a low (16% calories from fat) fat content. The fat was derived from corn oil, which contains 59% of n-6 PUFA linoleic acid. Twenty females in the high-fat group and 20 females in the low-fat group became pregnant, and there were 10 females in each of the unmated groups. Animals were fed food and water ad libitum. The diets were within the range of fat consumed by North Americans; an average North American woman consumes 37% of calories from fat (*23*). By comparison, North American vegetarians consume significantly fewer calories from fat (*24*), with levels as low as close to 20% achieved in low-fat dietary intervention studies (*25*).

The semipurified animal diets were prepared commercially by Bioserv Inc. (Frenchtown, NJ) in accordance with the guidelines of the American Institute of Nutrition (AIN) (*26*) (Table 1). We made the diets isocaloric by adjusting their fiber content. We adjusted the proportion of dietary components other than fat to ensure an adequate intake of protein (casein), vitamins, and trace elements, and the amounts of these components per diet were approximately constant with regard to energy. On the day the offspring were born, the special diets were switched back to the standard laboratory diet. The virgin female rats (group 2) were removed from the special diets at the time that the pregnant females (group 1) began to give birth. Therefore, the animals in both groups were fed the special (i.e., high- or low-fat) diets for 3 weeks (approximately 21 days).

Hormonal Assays

The effect of diet on serum hormone levels was studied by use of a separate group of 20 female Sprague-Dawley rats that arrived in our laboratory on day 7 of gestation. Immediately upon arrival, 10 of these rats were placed on the high-fat diet (i.e., containing 43% of the total calories from corn oil), and 10 were placed on the low-fat diet (i.e., containing 16% of the total calories from corn oil). The animals in these two experimental groups were not exposed to DMBA. Both the various estrogens and total estrogen levels are closely similar in pregnant rodents and pregnant humans (*27,28*). Thus, the total E_2 levels have been found to increase throughout pregnancy in rats and to reach their highest levels before parturition. To determine the levels of total circulating E_2, we collected blood from five animals per group on gestation days 12 and 19.

Table 1. Dietary formulations

Ingredients*	Diet	
	Low-fat	High-fat
Fat—total (corn oil), g	70	194
Protein (casein, L-cystine), g	203	203
Carbohydrates, g	629.5	340
Cornstarch	397.5	140
Maltose	132	100
Sucrose	100	100
Fiber (alphacel), g	50	215.5
AIN mineral mix, g	35	35
AIN vitamin mix, g	10	10
Choline chloride, g	2.5	2.5
TBHQ, g	0.014	0.014
Total, g	1000	1000
kcal density/g	3.72	3.75
% kcal from fat	15.7	43.2
% kcal from protein	20.5	19.2
% kcal from carbohydrates	63.8	37.6

*AIN = American Institute of Nutrition; TBHQ = tertiary butyl hydroquinone; kcal = kilocalories.

In addition to E_2 levels, serum insulin and glucose levels were determined from the same female rats. The animals were not fasted, which induced variability into the insulin and glucose levels. The diets, however, were isocaloric, and the animals in the different dietary groups had similar food intakes (*see* "Results" section). Our rationale for measuring serum insulin levels was fourfold. First, dietary fat stimulates insulin release from the pancreas. Second, insulin is among the most critical biologic factors associated with normal and malignant growth of mammary tissue (*29,30*). For example, deprivation of insulin by destroying pancreatic insulin-producing beta cells reduces the growth of DMBA-induced mammary tumors in female rats (*31*). Third, high levels of insulin are present in colostrum and milk (*32*). Finally, it is known that high insulin levels during pregnancy are associated with subsequent cardiovascular diseases, hypertension, and diabetes in the mother and her offspring (*33-35*), suggesting that circulating insulin levels during pregnancy may play a role in altering the risk of developing some diseases.

On the day that the serum samples were collected, the pregnant rats were anesthetized by use of methoxyflurane inhalant, and blood was collected by cardiac puncture between 10 AM and 11 AM. The rats were killed immediately afterward by cervical dislocation. Their blood was placed in tubes, centrifuged at 1000 rpm for 10 minutes at room temperature, and stored at −70 °C. Serum E_2 and insulin concentrations were determined from the samples by use of specific double-antibody kits from ICN Biomedicals, Inc. (Irvine, CA), according to the manufacturer's instructions. Glucose levels were determined by a glucometer (Beckman Instruments, Inc., Fullerton, CA).

Determining Mammary Tumor Incidence

The rats were checked regularly for mammary tumors by palpation at least once per week. The end points for data analysis were latency to tumor appearance, the size of the tumor upon detection, and the number of tumors. To determine the tumor size, we immobilized the animal by holding it in a firm grip with one hand and with the other hand measuring the length, width, and height of the tumor with the use of a caliper. The rats were killed when the detectable tumor burden was equivalent to approximately 10% of total body weight. The surviving animals and the animals that did not appear to develop mammary tumors were killed 18 weeks after the administration of DMBA.

Statistical Analyses

Statistical tests were performed by use of the SOLO software (BMDP Statistical Software, Los Angeles, CA). One- or two-way analysis of variance (ANOVA) (*36*) was used to analyze results for the body weight, food intake, and other parameters associated with pregnancy, hormonal data, and data for tumor size and latency. Where appropriate, between-group comparisons were performed using Fisher's least significant difference test. The logrank test was used to analyze tumor incidence for the two groups. In addition, the chi-squared test was used to analyze the difference in tumor incidence at the last week of tumor measurements (week 18). All statistical tests were two-sided.

Results

Pregnancy

The food intake and body weight gain of the pregnant rats were equivalent in each group. Thus, the pregnant animals kept on a high-fat diet consumed 20.9 g ± 0.3 g of food per day and 77.6 kcal ± 1.0 kcal per day (mean ± standard deviation of the mean [SEM]), and the animals kept on a low-fat diet consumed 20.6 g ± 0.2 g of food daily and 77.2 kcal ± 0.6 kcal per day. The body weights increased from 245.5 g ± 3.6 g (mean ± SEM) at the beginning of pregnancy to 335.8 g ± 9.2 g on day 20 of pregnancy in the high-fat group and from 251.6 g ± 4.1 g at the beginning of pregnancy to 344.2 g ± 8.5 g on day 20 of pregnancy in the low-fat group. Furthermore, as we will report,[1] the duration of pregnancy and the number of pups born and their body weights did not differ among the groups (data not shown).

Effects of Diet on E_2, Glucose, and Insulin Levels During Pregnancy

The serum levels of E_2, glucose, and insulin were measured in dietary manipulated female rats on days 12 and 19 of gestation. The E_2 levels were higher on gestation day 19 than on day 12 (two-way ANOVA: $F = 4.53$; $df = 1, 14$; $P<.05$). Furthermore, on gestation day 19, the plasma levels of total E_2 were significantly (approximately twofold) higher in the pregnant females fed a high-fat diet when compared with the pregnant females fed a low-fat diet (Student's t test: $t = 3.08$; $df = 8$; $P<.02$) (Fig. 1). On day 12, when the females had been on the special diets for only 5 days, no differences in the E_2 levels were seen (Fig. 1).

Serum glucose levels were significantly higher on gestation day 12 than on gestation day 19 (two-way ANOVA: $F = 6.67$; $df = 1$ and 14; $P<.02$) (Fig. 2). There was a nonsignificant tendency for the insulin levels (Student's t test: $t = 1.88$; $df = 8$; $P<.09$) and the insulin/glucose ratio (an index of insulin resistance) (Student's t test: $t = 1.91$; $df = 8$; $P<.09$) to be reduced on gestation day 19 in the pregnant rats that were fed a high-fat diet when compared with levels obtained in 19-day-pregnant rats kept on a low-fat diet. Both these measures were approximately twofold lower in the rats fed the high-fat diet than in the rats fed the low-fat diet (Fig. 2). We have subsequently repeated these experiments and found that, in BALB/c mice that consumed a 43% high-fat diet throughout pregnancy, the serum insulin levels and insulin/glucose ratio were significantly reduced on the 3rd (last) gestation week (data not shown).

Effects of Diet on Mammary Tumorigenesis

The mammary tumor incidence was significantly higher among the rats fed the high-fat diet during pregnancy than among the rats fed the low-fat diet during pregnancy (logrank test = 4.64; $df = 1$; $P<.031$) (Fig. 2). Thus, at week 18 after DMBA administration, 40% of the animals fed the high-fat diet had developed mammary tumors, whereas 10% of the animals

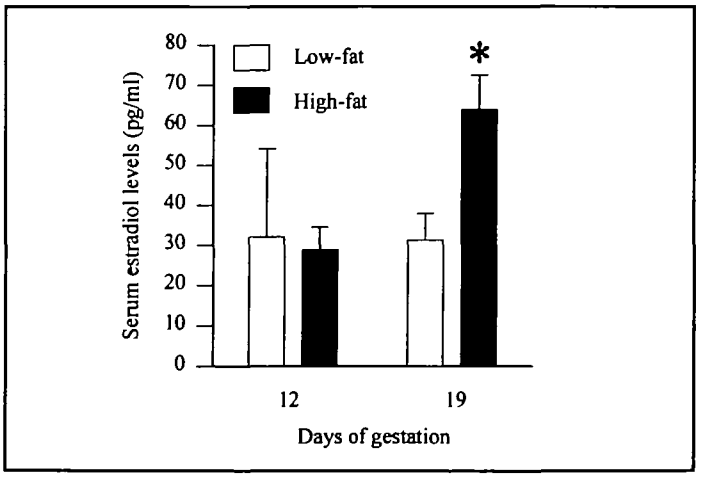

Fig. 1. Serum levels (mean value ± standard error of the mean) of total estradiol in pregnant female Sprague-Dawley rats that were fed isocaloric high-fat (43% calories from fat) or low-fat (16% calories from fat) diets from gestation day 7 onward. Each group contained four or five animals. Asterisk indicates statistical significance at the $P<.05$ level.

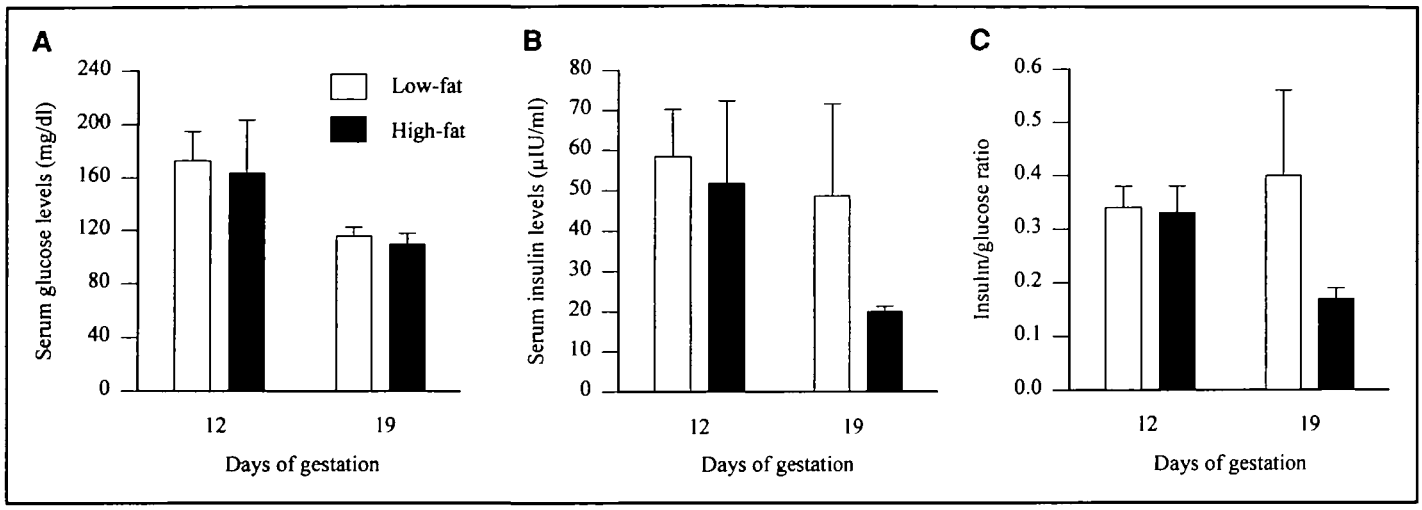

Fig. 2. Serum levels (mean value ± standard error of the mean) of glucose (**A**), insulin (**B**), and insulin/glucose ratio (**C**) in pregnant female Sprague-Dawley rats that were fed isocaloric high-fat (43% calories from fat) or low-fat (16% calories from fat) diet from gestation day 7 onward. Each group contained four or five animals.

fed the low-fat diet had tumors (chi-squared = 4.80; *df* = 1; *P*<.05). Other parameters of tumorigenesis were unaffected by dietary exposure during pregnancy. The latency to tumor appearance, the size of the tumors upon first detection, and tumor multiplicity (number of tumors per animal) were similar in rats exposed to a high- or a low-fat diet during pregnancy (Table 2).

The mammary tumor incidence was not altered among virgin female rats exposed to special diets for a 3-week period (Fig. 3). In the present study, the overall level of mammary tumor incidence in rats treated with DMBA was lower than in our other studies (*37*)[1]. In our experience, DMBA given to 55-day-old rats typically produces a 50% tumor incidence, and those animals that do develop mammary tumors have one to three tumors per animal. In the present study, the rats were handled; they were weighed frequently, and their food intake was measured. Handling rats after DMBA administration has been reported to reduce the incidence of DMBA-induced mammary tumors [*see* (*38*)].

Discussion

Our data show that the consumption of a diet high in fat, primarily in the form of n-6 PUFA, by female rats during pregnancy increases their susceptibility to mammary tumorigenesis. Such rats exhibited a significantly higher incidence of DMBA-induced mammary tumors if they were fed a diet containing 43% calories from fat (in the form of corn oil) than if they were fed a diet containing 16% calories from fat (corn oil) during pregnancy. We also generated data indicating that short-term exposure (3-week exposure occurring 21 days after DMBA administration) of virgin animals to a high-fat diet does not alter the incidence of carcinogen-induced mammary tumorigenesis. The lack of an effect by this short-term exposure is in line with the findings of several other investigators [reviewed in (*10*)]. Although a diet high in linoleic acid generally increases tumorigenesis in carcinogen-induced or spontaneous mammary tumor models, a long rather than a short exposure is required (*39*). In addition, our data indicate that a diet high in linoleic acid increases tumor incidence if this diet is consumed during pregnancy.

Our finding that pregnancy is a period during which a rat is vulnerable to the tumor-promoting effects of dietary fat may reflect the functional changes occurring in the mammary gland during pregnancy. Two major events occur in the mammary gland during pregnancy. First, the number of epithelial structures increases substantially as a result of rapid cell proliferation (*40*). Second, terminal end buds and terminal ducts differentiate into alveolar structures in rodents (*40*). In humans, type 1 lobules differentiate into type 3 lobules (*41*). The pregnancy-associated changes in the mammary gland structure and function result from the increase in circulating hormone levels (*40*). Estrogen is a promoter of breast cancer (*42*), although the exact molecular pathways for its action on mammary epithelial cells have remained unclear. In our present and in another study,[1] we found an increase in the serum levels of total E_2 in pregnant rats exposed to a high-fat diet. Since breast cancer risk is elevated in women who were exposed to DES during pregnancy (*2*) and reduced in women who had low estrogen levels during pregnancy (*3*), our results obtained in rats may be associated with the fat-induced increase in circulating E_2 levels. Estrogens might induce proliferation of the mammary glands during pregnancy, since the role of this hormone is closely linked to mammary

Table 2. Mean latency to the appearance of a tumor, area of tumors at first detection, and tumor multiplicity in 7,12-dimethylbenz[*a*]anthracene-treated rats exposed to a diet containing 16% (low-fat) or 43% (high-fat) calories from fat throughout pregnancy (n = 20 per group) or for 21 days (virgin controls, n = 10 per group)

	No. of tumors	Tumor latency, wk*	Tumor area, mm^2*	Tumor multiplicity*
Mated rats				
Low fat	2	9.5 ± 1.5	95.0 ± 5.0	1.0 ± 0
High fat	8	10.4 ± 0.9	79.5 ± 11.0	1.1 ± 0.3
Unmated rats				
Low fat	3	12.3 ± 2.3	42.7 ± 13.3	1.5 ± 0.5
High fat	3	12.0 ± 0.1	42.0 ± 24.0	1.5 ± 0.5

*Values = means ± standard error for proliferating tumors.

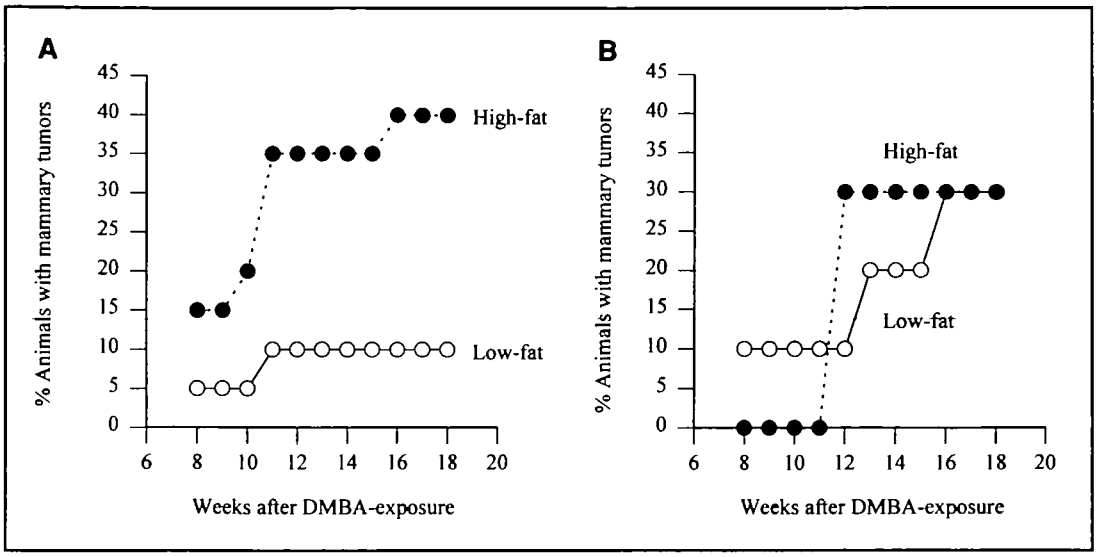

Fig. 3. Number of mammary tumors per group in 7,12-dimethylbenz[*a*]anthracene (DMBA)-treated female Sprague-Dawley rats. **A)** Rats that were exposed during subsequent pregnancy to a diet containing 43% (high-fat) or 16% (low-fat) calories from fat (n = 20 per group). **B)** Rats that were virgin and were fed a high- or low-fat diet during the same time period that the other females were pregnant. Tumor incidence was significantly higher among animals in the high-fat group that had undergone pregnancy than among animals in the low-fat group that had also undergone pregnancy (logrank = 4.64; $P<.031$).

epithelial cell growth (*43*). Thus, the mammary epithelial cells of pregnant rats that were pretreated with a carcinogen and exposed to elevated E_2 levels as a result of a high-fat diet may have been subjected to a higher degree of neoplastic transformation than the mammary epithelial cells of pregnant animals consuming a low-fat diet that results in normal pregnancy E_2 levels.

Although E_2 is a strong candidate to mediate the difference in mammary tumor incidence between female rats exposed to a high-fat diet during pregnancy and those exposed to a low-fat diet during pregnancy, it is possible that dietary fat was directly involved. For example, a high-fat diet may have induced changes in the structural composition of membranes (*44*) or metabolic pathways that lead to altered phospholipase C or protein kinase C activity (*45-47*). Since several of these events are also thought to be regulated by estrogens (*48-50*), the effects of high dietary fat intake during pregnancy on subsequent mammary tumor incidence could be due to both E_2 and fat.

Late pregnancy is characterized by hyperinsulinemia accompanied by insulin resistance (*51,52*). Since dietary fat stimulates insulin secretion from the pancreas (*28*), and insulin is linked to both the normal and malignant growth of mammary cells (*29,30*), we expected the serum levels of insulin to be elevated in pregnant rats consuming a high-fat diet. However, the opposite result was found. Insulin levels were lower by approximately twofold in the group fed the high-fat diet when compared with the group fed the low-fat diet during the last week of pregnancy. Serum glucose levels were not altered by dietary fat intake, but they declined with advancing gestation. This is also true for pregnant women (*53*), an exception being obese women who display progressive fasting hyperglycemia (*54*). In the present study, pregnant rats kept on a high-fat diet were not obese. They consumed the same amount of energy as the rats on the low-fat diet, and their body weights throughout the study were similar. On gestation day 19, the group given the high-fat diet had an insulin/glucose ratio that was twofold lower than that in the group given the low-fat diet. We have repeated these experiments in BALB/c mice and found that serum insulin levels and the insulin/glucose ratio were significantly reduced in pregnant female mice consuming a diet containing 43% calories from fat.[2]

The significance of these findings remains unclear. Women who suffer from pregnancy-induced hypertension and/or preeclampsia or eclampsia have a reduced subsequent breast cancer risk (*3*) and exhibit increased blood insulin levels and high insulin resistance (*33,55,56*). Incidentally, these women also have significantly lower serum levels of essential fatty acids, including linoleic acid (*57*). Our data show that high dietary intake of essential fatty acids by rats during pregnancy reduces their serum insulin levels. These rats, exposed to a high-fat diet during pregnancy, exhibit an increased incidence of mammary tumors. Thus, low levels of linoleic acid and high levels of insulin during pregnancy appear to protect against breast cancer (human data), while high levels of linoleic acid and reduced circulating levels of insulin during pregnancy increase mammary tumor incidence in rats.

Because of the high epithelial cell proliferation, pregnancy has been thought to be a risk factor for recurrence of breast cancer among breast cancer survivors. A significant proportion of treated breast tumors relapse within 5 years of original diagnosis. However, in the light of many studies (*58-60*), it is now evident that pregnancy does not pose an additional risk. The data obtained in the present study with an animal model are in support of these findings. The incidence of carcinogen-induced mammary tumors was 30% in the virgin animals and 10% (low fat) and 40% (high fat) in female rats who became pregnant after the carcinogen treatment. Thus, pregnancy that occurred 3 weeks after the carcinogen exposure did not increase breast cancer risk in the female rats.

In conclusion, we found that consumption of a high-fat diet during pregnancy increases the risk in female rats of developing DMBA-induced mammary tumors. Since a high dietary intake of fat was associated with elevated serum E_2 levels and previous data in humans suggest that high estrogen activity during pregnancy increases (*2*) and low estrogen levels reduce (*3*) breast cancer risk, E_2 may be an important mediator of the increased risk. Therefore, while our data may have important implications for the attempts to prevent some breast cancers by modulating

nutrition during pregnancy, we must caution that there are, to our knowledge, no published studies on women that have investigated the link between diet during pregnancy and subsequent breast cancer risk. Therefore, additional studies must be conducted to ascertain whether or not the results from animal model studies are applicable to humans.

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Notes

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