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RESEARCH ARTICLES

A maternal high n-6 fat diet with fish oil supplementation during pregnancy and lactation in rats decreases breast cancer risk in the female offspring☆

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Abstract

The timing of dietary fat intake may modify breast cancer risk. In addition, n-3 fatty acids reduce, and n-6 fatty acids increase, the risk of breast cancer and a maternal high n-6 fat diet results in a greater risk of breast cancer in the female offspring. We hypothesized that the timing of n-3 fatty acid-enriched fish oil supplementation would be important for reducing the risk of breast cancer. Female rats were fed to a high n-6 fat diet containing 20% of the sunflower oil by weight during pregnancy and lactation, and the female offspring were exposed to fish oil by oral gavage either during the perinatal period via maternal intake or during puberty or adulthood. Exposure during the perinatal period to a maternal high n-6 fat diet with fish oil supplementation significantly reduced the incidence of carcinogen-induced mammary tumors in the female offspring compared to a maternal high n-6 fat diet with no fish oil supplementation or fish oil supplementation later in life (P=.0228 by Cox proportional hazards model). We found that a maternal high n-6 fat diet during pregnancy is more important in increasing the risk of mammary tumors in the female offspring than a maternal high n-6 fat diet during lactation. This study suggests that fish oil supplementation during the perinatal period decreases the effect of a maternal high n-6 fat diet on subsequent carcinogen-induced mammary tumor risk, whereas fish oil supplementation during puberty or adulthood does not.

Keywords: Breast cancer; High-fat diet; n-6 fatty acids; Fish oil; Life stage; Estradiol

1. Introduction

Most animal or cell culture studies have shown that n-3 fatty acids reduce, and n-6 fatty acids increase, the risk of breast cancer [1–3]. However, epidemiologic studies, in which data on dietary intake were collected using a dietary recall based on a food frequency questionnaire or dietary history, have provided conflicting results about a potential effect of dietary n-3 fatty acids in reducing breast cancer risk [4–8]. It has been proposed that the timing of dietary fat intake may modify breast cancer risk [9], that breast cancer may have a fetal origin [10–12] and that a maternal high n-6 fat diet results in a greater risk of breast cancer in the female offspring [13,14]. Here, we examined whether the timing of n-3 fatty acids supplementation was critical in reducing the risk of breast cancer and whether rats exposed to a high n-6 fat diet with n-3 fatty acids supplementation during the perinatal period via the maternal intake showed a lower risk of breast cancer.

This study was designed to evaluate at which stage of life n-3 fatty acids supplementation is important in reducing the risk of breast cancer in rats exposed to a high n-6 fat diet via the maternal intake. Female rats were exposed to a high n-6 fat diet containing 20% of sunflower oil by weight with or without n-3 fatty acid-enriched fish oil supplementation either during the perinatal period via maternal intake or during puberty or adulthood. We also examined whether the *in utero* period or the perinatal period (*in utero* plus lactation period) was the critical stage for the effect of a maternal sunflower oil diet on subsequent carcinogen-induced mammary tumor risk. In addition, we examined whether maternal sunflower or safflower oil diets containing different amounts of n-6 fatty acids had different effects in modifying the risk of subsequent carcinogen-induced mammary tumorigenesis in the female offspring. We also examined the effect of n-3 fatty acids supplementation on serum estradiol levels in the pregnant dams fed the sunflower oil diet.

2. Materials and methods

2.1. Animals and study design

Pregnant Sprague-Dawley rats (8 weeks old) at Day 2 of gestation were obtained from BioLasco Taiwan, a technology licensee of Charles River Laboratories in Taiwan,

Abbreviations: DMBA, 7,12-dimethylbenzanthracene; 18:2n-6, linoleic acid.

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Fig. 1. Study design for female rats exposed to a high n-6 fat diet during the perinatal period via maternal intake with or without n-3 fatty acid-enriched fish oil supplementation at different life stages. Group S: Sunflower oil diet for the dam during pregnancy and lactation; pups fed chow diet. Group $SF_{perinatal}$: Sunflower oil diet with fish oil supplementation for the dams during pregnancy and lactation; pups fed chow diet. Group $SF_{puberty}$: Sunflower oil diet for the dams during pregnancy and lactation; pups fed chow diet with fish oil supplementation during Postnatal Days 30–71. Group SF_{adult}: Same as group $SF_{puberty}$, but with fish oil supplementation during Postnatal Days 110–151 instead of 30–71. Group $S_{in\ utero}$: Sunflower oil diet for the dams during pregnancy and chow diet during lactation; pups fed chow diet. Group safflower: Safflower oil diet for the dams during pregnancy and lactation; pups fed chow diet.

and were immediately assigned to the experimental high n-6 fat diets (the study design is shown in Fig. 1). All rats were housed in a humidity-controlled room at 24±1°C on a 12-h light–dark cycle with free access to tap water and the diet. The protocols and animal treatments used in this study were approved by the Animal Care and Use Committee of the National Taiwan University College of Medicine.

In the S, $SF_{puberty}$ and SF_{adult} groups, the dams (n=17) were fed a sunflower oil diet (Table 1), prepared in our laboratory, from Day 2 of gestation throughout pregnancy and lactation. After weaning at postnatal day 22, the pups were fed chow diet (5001, LabDiet) throughout their lifetime. On Postnatal Day 28, the female offspring were randomly assigned to group S (n=29), $SF_{puberty}$ (n=31) or SF_{adult} (n=33), which were given no fish oil supplementation (group S) or were supplemented by oral gavage with fish oil [18 mg of eicosapentaenoic acid (20:5n-3) and 12 mg of docosahexaenoic acid (22:6n-3), Leiner Health Products, California, USA] per day for 42 days on Postnatal Days 30–71 (puberty) (group $SF_{puberty}$) or Postnatal Days 110–151 (adulthood) (group SF_{adult}).

In group $SF_{perinatal}$, the dams (n=5) were fed sunflower oil diet supplemented by oral gavage with fish oil containing 36 mg of 20:5n-3+24 mg of 22:6n-3 during pregnancy and 72 mg of 20:5n-3+48 mg of 22:6n-3 during lactation. The amounts of n-3 fatty acids used were based on how much diet was eaten in order to meet the n-3 fatty acid dietary recommendation of about 0.4% of the energy source [16]. The female offspring (n=33) were fed chow diet till Postnatal Day 230.

Table 1
Components of the high n-6 fat diets

Ingredient (g/kg diet)	
Sunflower oil/safflower oil	200
Casein	238
DL-Methionine	3.5
Corn starch	150
Sucrose	294.3
Alphacel	58.8
AIN-76 vitamin mix	11.8
AIN-76 mineral mix	41.2
Choline chloride	2.4
Fat (% of energy)	40
Protein (% of energy)	21
Carbohydrate (% of energy)	39

The compositions of the high n-6 fat diets (sunflower oil or safflower oil diet) were modified according to those of the AIN-76 purified diet [15]. The amounts of casein, methionine, fiber, vitamin mixtures, mineral mixtures and choline in the high n-6 fat diet were adjusted to have the same nutrient/energy ratio as the AIN-76 purified diet.

In group $S_{in\ utero}$, the dams (n=5) were fed sunflower oil diet throughout pregnancy, then were changed to chow diet during lactation, and the female offspring (n=28) were maintained on chow diet till Postnatal Day 230.

In group safflower, the dams (n=6) were fed safflower oil diet throughout pregnancy and lactation and the female offspring (n=32) were fed chow diet till Postnatal Day 230.

The age of puberty onset was determined by examining the vaginal opening daily, starting on Postnatal Day 28.

2.2. Diet composition

The sunflower and safflower oil diets (Table 1) containing 20% of oil by weight were modified according to those of the AIN-76 purified diet to maintain the same nutrition density [15]. The sunflower or safflower oil diet contained, respectively, 60% or 23% of the total fatty acids as linoleic acid (18:2n-6), representing 12% or 4.6% by weight as n-6 fatty acids (Table 2). All diet ingredients were obtained from MP Biomedicals (Ohio, USA), except the methionine and choline, which were from Sigma-Aldrich Chemical (St Louis, MO, USA), and the sunflower oil, safflower oil, corn starch and sucrose, which were purchased from a local supermarket.

2.3. Serum estradiol levels in the pregnant dams

The effect of n-3 fatty acids supplementation on serum estradiol levels in pregnant dams fed the high n-6 fat diet was examined using a separate group of Sprague-Dawley rats. Chow diet-fed 8-week-old female rats were mated and conception confirmed by the presence of vaginal plugs. The dams were fed the sunflower oil diet with or without fish oil supplementation, then blood was collected by cardiac puncture on Gestation Day 19 and serum immediately prepared by centrifugation and stored at −80°C until analysis. Serum estradiol levels were measured using an EIA kit (Cayman Chemical, Michigan, USA) according to the manufacturer's instructions.

2.4. Tumor induction and mammary tumorigenesis

Mammary tumors were induced by a single intragastric administration of 10 mg of 7,12-dimethylbenzanthracene (DMBA) (Sigma) (10 mg/ml in peanut oil) to the female offspring on Postnatal Day 55. The DMBA-induced mammary tumor is an adenocarcinoma resembling human hormone-dependent breast cancer [18–20]. The rats were checked once a week for mammary tumors by palpation, and the appearance of palpable mammary tumors and the number of tumors were recorded. The latency was calculated as the average week of appearance of the first tumor. Rats were anesthetized with CO_2 and killed on Postnatal Day 230 (25 weeks after DMBA administration). The mammary tumors were dissected out and weighed.

Table 2
Fatty acid composition of the diets and fish oil

Weight %	Sunflower oil diet	Safflower oil diet	Fish oil
14:0	0.1±0.0	0.2±0.0	15.6±0.6
16:0	7.9±0.3	8.1±0.3	25.1±0.6
18:0	3.6±0.2	2.8±0.1	3.4±0.1
20:0	0.1±0.0	0.1±0.0	0.1±0.0
Sum	11.6±0.3	11.4±0.4	43.8±0.8
16:1(n-7)	0.0±0.0	0.0±0.0	15.0±0.3
18:1(n-9)	28.3±0.5	63.6±1.1	9.3±0.2
18:1(n-7)	0.4±0.2	0.7±0.2	3.3±0.2
20:1(n-9)	–	0.1±0.0	0.5±0.1
22:1(n-9)	–	–	–
Sum	28.7±0.6	64.7±1.3	28.2±0.5
18:2n-6	59.6±0.6	23.3±1.0	1.5±0.3
20:2n-6	–	–	0.4±0.2
20:3n-6	–	–	0.4±0.1
20:4n-6	–	–	0.2±0.2
22:4n-6	–	–	0.1±0.0
22:5n-6	–	–	–
Sum	59.6±0.6	23.3±1.0	2.7±0.3
18:3n-3	–	0.5±0.1	0.5±0.1
20:5n-3	–	–	15.9±0.8
22:5n-3	–	–	1.2±0.1
22:6n-3	–	–	8.0±0.1
Sum	–	0.5±0.1	25.4±0.3

The data are presented as the mean±S.E.M. (n=6). The fatty acid composition was analyzed by gas chromatography [17]. Fatty acids that account for less than 0.1% of total fatty acids are not shown. The amount of each fatty acid is expressed as the weight % of total fatty acids.

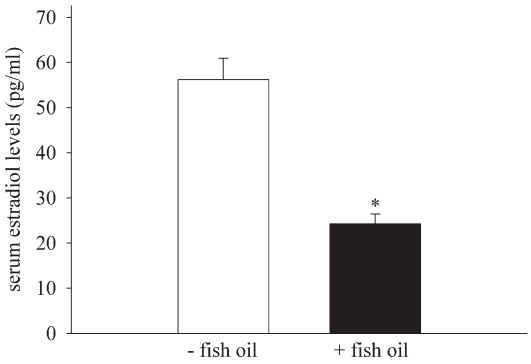

Fig. 2. Serum estradiol levels on Gestation Day 19 in pregnant rats fed the sunflower oil diet without or with fish oil supplementation. The data are presented as the mean±S.E.M. (n=7–8 rats/group). * indicates a significant difference between groups (P<.0001).

2.5. Statistical analysis

All statistical analyses were performed using SAS statistical software (version 9.1.3, SAS Institute, Cary, NC, USA). A two-sided P≤.05 was considered statistically significant. Continuous data are presented as the mean±S.E.M., whereas sample proportions were computed for categorical data. Mean differences in the characteristics of the mammary tumors with continuous measurements (e.g., tumor weight, tumor number, etc.) were analyzed by one-way ANOVA, followed by post hoc Duncan's multiple range tests for multiple comparisons. The time to occurrence of mammary tumor was analyzed using the log rank test and further analyzed using Cox proportional hazards model. The goal of regression analysis is to find parsimonious regression models that fit the observed data well for answering the scientific questions under study. In particular, we wish to validly estimate the effects of important risk factors or predictors on the occurrence of mammary tumor. To ensure the quality of analysis results, basic model-fitting techniques for variable selection, goodness-of-fit (GOF) assessment and regression diagnostics were used in our regression analysis. Specifically, in the stepwise variable selection procedure, all the univariate significant and nonsignificant covariates, including experimenter and fish oil group, were considered and the significance levels for entry and for stay were set as .15 or larger. Any discrepancy between the results of univariate analysis and multivariate analysis was likely due to the confounding effects of the uncontrolled covariates in the univariate analysis. Both the adjusted generalized R^2 and the Grønnesby–Borgan GOF test were used to assess the GOF of the fitted Cox proportional hazards model. Statistical tools for regression diagnostics, such as verification of the proportional hazards assumption, residual analysis, detection of influential cases, and check for multicolinearity, were applied to discover model or data problems.

3. Results

3.1. Serum estradiol levels in the pregnant dams

Serum estradiol levels on Gestation Day 19 were significantly lower (P<.0001) in pregnant rats fed the sunflower oil diet with fish oil supplementation than in those without fish oil supplementation (Fig. 2).

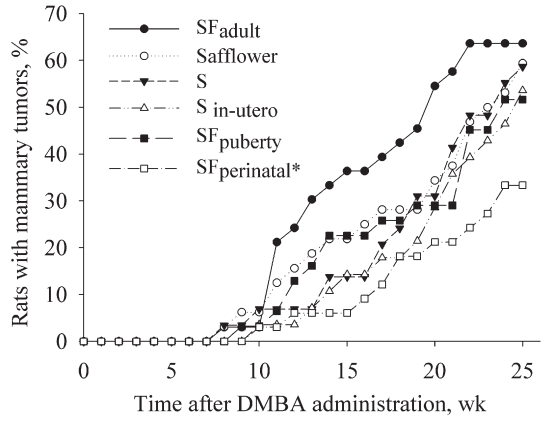

Fig. 3. Percentage cumulative mammary tumor incidence in rats exposed to a high n-6 fat diet during the perinatal period with or without fish oil supplementation. Group S, n=29; group $SF_{perinatal}$, n=33; group $SF_{puberty}$, n=31; group SF_{adult}, n=33; group $S_{in\ utero}$, n=28; group safflower, n=32. The appearance of mammary tumors was examined by palpation once a week after DMBA administration. * indicates a significant difference compared to group S.

3.2. Onset of puberty and body weight

Onset of puberty, determined by examining vaginal opening, occurred significantly earlier in the safflower group than in all other groups except the $SF_{perinatal}$ group (Table 3).

There was no significant difference between the groups in mean body weight throughout puberty (data not shown) or at age 230 days when the rats were killed (Table 3).

3.3. Mammary tumorigenesis

3.3.1. Tumor latency

The appearance of mammary tumors was examined by palpation once a week after DMBA administration. The first tumor appeared on Week 8 in groups S, SF_{adult} and safflower; Week 9 in group $SF_{puberty}$; and Week 10 in groups $SF_{perinatal}$ and $S_{in\ utero}$ (Fig. 3). The mean latency to the appearance of the first tumor was shorter in group SF_{adult}, but was not significantly different from the latencies in the other groups except group $S_{in\ utero}$ (Table 3).

3.3.2. Tumor incidence

The percentage of rats with mammary tumors at 25 weeks after DMBA administration was 63.6% (21/33) in group SF_{adult}, 59.4% (19/32) in group safflower, 58.6% (17/29) in group S, 53.6% (15/28) in group $S_{in\ utero}$, 51.6% (16/31) in group $SF_{puberty}$ and 33.3% (11/33) in

Table 3
Effect of the high n-6 fat diet with or without fish oil supplementation on puberty onset and DMBA-induced mammary tumorigenesis[1]

S	n	$SF_{perinatal}$	n	$SF_{puberty}$	n	SF_{adult}	n	$S_{in\ utero}$	n	Safflower	n
Age at puberty (days)											
42.0±0.7^{a}	29	40.3±0.8^{ab}	33	41.5±0.6^{a}	31	42.0±0.9^{a}	33	43.0±0.6^{a}	28	38.8±1.1^{b}	32
Mean latency to the first palpable tumor (weeks after DMBA administration)											
18.6±1.2^{ab}	17	18.6±1.4^{ab}	11	17.4±1.3^{ab}	16	15.2±1.0^{b}	21	19.3±1.2^{a}	15	17.6±1.3^{ab}	19
Tumor multiplicity (number of tumors/rat)											
0.93^{ab} (27/29)		0.45^{b} (15/33)		0.84^{ab} (26/31)		1.39^{a} (46/33)		0.68^{b} (19/28)		1.00^{ab} (32/32)	
Number of tumors per tumor-bearing rat											
1.6±0.2^{ab}	17	1.4±0.2^{b}	11	1.6±0.3^{ab}	16	2.2±0.3^{a}	21	1.3±0.2^{b}	15	1.8±0.2^{ab}	19
Tumor weight (g) per tumor-bearing rat											
2.2±0.8	17	2.0±0.9	11	6.7±3.4	15	3.9±1.1	20	4.7±3.2	13	2.5±1.2	16
Tumor weight/body weight (%)											
0.7±0.3	17	0.7±0.3	11	2.5±1.3	15	1.3±0.4	20	1.3±0.8	13	0.8±0.3	16
Body weight at death (g)											
282±6	27	288±6	31	285±7	26	301±5	30	295±9	26	304±6	30

[1] The data are presented as the mean±S.E.M. for the indicated number of rats. Different letters show significant differences between groups using post hoc Duncan's multiple range test.

group $SF_{perinatal}$ (Fig. 3). The log-rank test revealed that the incidence of mammary tumors over the whole 25-week period was significantly lower in the $SF_{perinatal}$ group than in the S group. There was no significant difference between the S, $SF_{puberty}$, SF_{adult}, $S_{in\ utero}$ and safflower groups.

The hazard ratio for the time to occurrence of DMBA-induced mammary tumors in the six groups was further analyzed using the Cox proportional hazards model. Since the hazard ratios in groups S, $SF_{puberty}$, SF_{adult}, $S_{in\ utero}$ and safflower were not significantly different from 1, they were pooled together as the reference group. The estimated hazard ratio in the $SF_{perinatal}$ group was 0.483 (95% confidence limits: 0.258 and 0.904, P=.0228).

3.3.3. Tumor multiplicity and tumor weight

At Week 25 after DMBA administration, mammary tumor multiplicity (number of tumors per rat) and the average number of tumors per tumor-bearing rat were significantly higher in the SF_{adult} group than in the $SF_{perinatal}$ and $S_{in\ utero}$ groups, with no significant difference between the S, $SF_{perinatal}$, $SF_{puberty}$, $S_{in\ utero}$ and safflower groups (Table 3). There was no statistical difference in the mean tumor weight per tumor-bearing rat and tumor weight as a percentage of body weight between the six groups.

4. Discussion

Whether dietary n-3 fatty acids have an effect in suppressing breast cancer risk is still controversial [4–8]. It has been proposed that the timing of dietary fat intake may modify breast cancer risk [9], that breast cancer may have a fetal origin [10–12] and that a maternal high n-6 fat diet results in a greater risk of breast cancer in the female offspring [13]. Our previous study [14] demonstrated that exposure of female rats *in utero* via a maternal high-fat diet containing 20% of safflower oil by weight increases the incidence of DMBA-induced mammary tumors in the female offspring to 60% compared to the 32% seen using chow diet containing 5% of fat by weight. Moreover, the incidence of DMBA-induced mammary tumors in the above study was the same (60%) in female rats exposed to safflower oil *in utero* via maternal intake during pregnancy for only 21 days and in rats exposed to the same diet in postnatal life (Postnatal Days 1–230). In addition, the incidence of DMBA-induced mammary tumor was higher in rats exposed to the safflower oil diet throughout life from *in utero* than in rats exposed to the same diet throughout life except during the *in utero* stage (90% vs. 60%). Thus the timing, but not the duration, of safflower oil diet exposure makes rats more susceptible to DMBA-induced mammary tumors, and exposure *in utero* to a maternal safflower oil diet during pregnancy increases the risk of mammary tumors in the female offspring than exposure of the offspring to the same diet later in life.

In the present study, we used rats exposed to a high n-6 fat diet containing 20% of sunflower oil by weight during the perinatal period via maternal intake to examine the effect of exposure to n-3 fatty acids by fish oil supplementation during the perinatal period, puberty or adulthood. According to our estimate of energy intake in pregnancy or lactation via maternal intake or in the female offspring at puberty or adulthood and analysis of the fish oil, the supplemented n-3 fatty acids provided by fish oil accounted for about 0.35% of the energy source in this study. The incidence of DMBA-induced mammary tumors was no different in rats without fish oil supplementation or with supplementation during puberty or adulthood, but was significantly decreased in rats exposed to fish oil during the perinatal period via maternal intake. These results suggest that the timing of n-3 fatty acids supplementation is important for mammary tumor suppression and that exposure to a high n-6 fat diet with fish oil supplementation during the perinatal period via maternal intake has a greater effect in preventing mammary tumors than fish oil supplementation in later life. It would be interesting to know whether the incidence in the offspring would also be reduced by fish oil supplementation only during pregnancy or using a maternal low-fat diet or low n-6 fatty acid diet with fish oil supplementation.

With the use of the results for the S, $SF_{puberty}$, $S_{in\ utero}$, SF_{adult} and safflower groups as the reference group, the hazard ratio for mammary tumor incidence was 0.483 for the $SF_{perinatal}$ group. If the results for the S, $SF_{puberty}$, $S_{in\ utero}$ and safflower groups were pooled as the reference group, group SF_{adult} tended to have a larger hazard ratio than the other four groups (estimated hazard ratio=1.453, P=.1359), indicating that n-3 fatty acids supplementation during adulthood (Postnatal Days 110–151) might exacerbate DMBA-induced mammary tumor risk in rats. The incidence of DMBA-induced mammary tumors is higher in rats fed during prepuberty (Postnatal Days 5–25) with a high-fat diet with 12% of corn oil and 7% of menhaden oil by weight (estimated 7.5% by weight as n-6 and 2% as n-3 fatty acids) than in those fed 17.5% of corn oil and 1.5% of menhaden oil by weight (containing 9.7% by weight as n-6 and 0.4% as n-3 fatty acids) (89% vs. 75% tumor incidence) [21], indicating that mammary tumor risk may be increased in rats exposed in postnatal life to a high-fat diet with a high n-3 fatty acids content.

The incidence of DMBA-induced mammary tumors was not significantly different in rats exposed to the sunflower oil diet only *in utero* or throughout the perinatal period (*in utero* and lactation) via maternal intake (54% vs. 59%, respectively). We previously found that the incidence of DMBA-induced mammary tumors in rats exposed to a safflower oil diet only *in utero* was 60% [14], whereas, in the present study, the incidence in rats exposed to the safflower oil diet throughout the perinatal period was 59%. This indicates that a maternal high n-6 fat diet during pregnancy is more important for increasing breast cancer risk in the female offspring than a maternal high n-6 fat diet during lactation.

It is interesting to note that the incidence of DMBA-induced mammary tumors was the same in rats exposed during the *in utero* or perinatal period to either the sunflower oil diet (containing 12% of 18:2n-6 by weight) or the safflower oil diet (4.6% of 18:2n-6 by weight). It has been reported that, at 25 weeks after DMBA administration, there is a positive correlation between the incidence of DMBA-induced mammary tumors and the percentage by weight of 18:2n-6 (over the range of 0.5% to 4.4%) in a diet containing 20% of mixed oils by weight fed to female rats during Postnatal Days 53–225, and that the incidence reaches a maximum of 60% in rats fed 4.4–12% of 18:2n-6 by weight [22,23].

It has been suggested that elevated maternal serum estradiol levels are an important factor in increasing susceptibility to subsequent breast cancer development in the female offspring [10,24]. Dizygotic twins, who are exposed to a higher estrogenic environment than single fetuses *in utero*, show an increased breast cancer risk [25–28], and pregnant women who take the synthetic estrogen diethylstilbestrol to prevent miscarriage have a higher breast cancer risk in the offspring [29]. In animal studies, a high-fat corn oil diet resulted in increased plasma estradiol levels in pregnant dams at Gestational Day 14 compared to a low-fat corn oil diet (22 vs. 16 pg/ml) [13], and serum estradiol levels were not changed at Gestation Day 12 (both ~30 pg/ml), but were significantly higher (64 vs. 31 pg/ml) at Gestation Day 19 in pregnant dams fed a high-fat corn oil diet compared to those fed a low-fat corn oil diet [30]. We found that serum estradiol levels on Gestation Day 19 in dams fed the high-fat sunflower oil diet were significantly higher in those without fish oil supplementation than in those with fish oil supplementation (56 vs. 24 pg/ml), suggesting that decreased maternal serum estradiol levels in pregnant rats may be a key factor in reducing susceptibility

to the subsequent development of breast cancer in the female offspring.

Recent evidence indicates that epigenetic changes play an important role in cancer development [31–33]. It has been proposed that gene expression is altered *in utero* by hormones and/or nutrients [12,33]. In human umbilical cord blood, levels of estradiol, estriol, testosterone and insulin growth factor-1 are positively associated with stem cell potential, which may be linked to cancer risk in the offspring [34,35]. Whether a maternal high n-6 fat diet with or without n-3 fatty acids supplementation modifies epigenetic changes or estrogenic effects, resulting in breast cancer susceptibility in the female offspring, requires further study.

Our study provides evidence that exposure of rats during the perinatal period to a maternal high n-6 fat diet with fish oil supplementation significantly reduces the incidence of DMBA-induced mammary tumors in the female offspring compared to either no maternal fish oil supplementation or fish oil supplementation later in life. We found that the *in utero* period is a critical stage for the promoting effect of a high n-6 fat diet on DMBA-induced mammary tumors and that a maternal high n-6 fat diet during pregnancy is more important in increasing the risk of mammary tumors in the female offspring than a maternal high n-6 fat diet during lactation. The incidence of DMBA-induced mammary tumors was the same in rats exposed during the perinatal period to either the sunflower oil diet (containing 12% of 18:2n-6 by weight) or safflower oil diet (4.6% of 18:2n-6 by weight). In addition, the decreased maternal serum estradiol levels in pregnant rats with fish oil supplementation may play a role in reducing susceptibility to later breast cancer development in the female offspring.

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