Influence of the Type of Dietary Fat on Developmental Growth of the Mammary Gland in Immature and Mature Female BALB/c Mice

Clifford W. Welsch and Deborah H. O'Connor

Department of Pharmacology and Toxicology, Michigan State University, East Lansing Michigan 48824

ABSTRACT

The purpose of this study was to determine whether or not the type of dietary fat can affect mammary gland growth processes in the immature and mature female BALB/c mouse. Groups of immature and mature mice were fed one of the following purified semisynthetic diets containing different types of fat, i.e., five vegetable oils diets (5% corn oil, 20% corn oil, 20% olive oil, 20% linseed oil, 19% coconut oil-1% corn oil); two animal fat diets (20% lard, 19% beef tallow-1% corn oil); and one fish oil diet (19% Menhaden oil-1% corn oil). In addition, fish-corn oil diets (20%) containing three different levels of corn oil (15%, 10%, 4.5%) and fish oil (5%, 10%, 15.5%) were also examined in these studies. Immature mice were fed these diets from 21 to 45 days of age, ovariectomized at 35 days of age, given injections daily of 17β-estradiol (1 µg) and progesterone (1 mg) on Days 42 to 44, and sacrificed on Day 45. Mammary ductal expansion through the mammary fat-pad (mm, nipple to farthest end bud) was determined on the inguinal (No. 4) mammary glands. Mature mice were treated with mammotrophic hormones; the type or amount of dietary fat can affect mammary gland developmental growth only in mice whose mammae were treated with mammotrophic hormones. The purpose of this study was to determine whether or not the type of dietary fat can affect mammary gland growth processes in the immature and mature female BALB/c mouse. These mice were divided into five groups, i.e., a control group, a group treated with mammotrophic hormones, and three groups treated with different types of fat. The control group was fed a diet consisting of 20% corn oil, 15.5% Menhaden oil-4.5% corn oil, significantly (P < 0.05) reduced mammary gland development was observed when compared to mice fed the 19 to 20% vegetable oil or animal fat diets. No significant difference in mammary gland developmental growth was observed among the groups of mice fed the 19 to 20% vegetable oil or animal fat diets. In immature mice and mammotrophic hormone-treated mature mice, significantly (P < 0.05) reduced mammary gland developmental growth was observed in mice fed the 5% corn oil diet compared with mice fed the 20% corn oil diet. In mature mice not treated with exogenous mammotrophic hormones, no significant effect of diet on mammary development was observed. Mean body weight gains among the dietary groups of mice were not significantly influenced by diet. Thus, among the fat diets examined in this study, only the animals fed the low fat diet (5% corn oil) or the fish oil diet (19% Menhaden oil-1% corn oil, 15.5% Menhaden oil-4.5% corn oil) had altered (suppressed) mammary development. Furthermore, dietary induced suppression of mammary gland developmental growth was observed only in mice whose mammae were in a state of intense proliferation (immature mice and mature mice treated with mammotrophic hormones); the type or amount of dietary fat did not affect mammary development in mice possessing a relatively quiescent mammary gland (during estrous cycle).

INTRODUCTION

It has been reported by numerous laboratories that the amount and type of dietary fat can significantly influence the development and/or growth of mammary tumors in rodents (1). In general, diets rich in certain vegetable oils (e.g., corn oil, safflower seed oil, sunflower seed oil) markedly enhance mammary tumorigenesis, while diets rich in certain animal fats (e.g., beef tallow, lard) are often less efficacious in stimulating this neoplastic process (2–6). In contrast, diets rich in certain fish oils (e.g., Menhaden oil) often do not appear to enhance mammary tumorigenesis in experimental animals (7–11).

In contrast, considerable less effort has been directed toward determining whether or not the amount or type of dietary fat can affect normal mammary gland developmental processes in experimental animals. Recently, we reported (12) that the amount of dietary fat can affect normal mammary gland growth processes in mature female BALB/c mice; i.e., as the fat content (corn oil) of the diet was increased from 0% to 5% to 20%, a significant increase in hormone-induced mammary gland growth was observed. In this paper, we have extended these studies to determine whether or not the amount and type of dietary fat can affect normal mammary gland growth processes in female BALB/c mice. Furthermore, we have examined both the prepubertal (immature) and early postpubertal (mature) female BALB/c mouse as early alterations in developmental growth of the mammary gland may have profound effects upon the susceptibility of this tissue to initiating events in mammary gland neoplasia (13, 14). It is generally recognized that factors which increase normal mammary proliferative processes often enhance the vulnerability of this tissue to dysplasia and/or neoplasia (14, 15). For these studies we have chosen to examine four vegetable oils (corn oil, olive oil, coconut oil, and linseed oil), two animal fats (beef tallow and lard), and one fish oil (Menhaden oil). Each of these fats has marked differences in fatty acid composition and often differs sharply in its ability to influence neoplastic mammary gland growth processes.

MATERIALS AND METHODS

A total of 599 nulliparous female BALB/c mice were used in these studies. The mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. They were housed in a temperature-controlled (25.5 ± 0.5°C) and light-controlled (14 h/day) room.

Diet Composition

The diets used in these studies are purified semisynthetic diets prepared in our laboratory every 2 wk (fish diets weekly), stored in plastic bags containing nitrogen gas, and kept frozen prior to feeding. The composition of the diets and percentage by weight are as follows. The low fat diet consists of corn oil (5%), casein (17.10%), DL-methionine (0.30%), dextrin (45.50%), sucrose (22.70%), AIN salt mixture (3.50%), AIN vitamins (1.00%), and cellulose (5.00%). The high fat diets (20%) consist of 4 vegetable oils, i.e., corn oil (20%), olive oil (20%), linseed oil (20%), coconut oil (19%)-corn oil (1%); 2 animal fats, i.e., lard (20%), beef tallow (19%)-corn oil (1%); and a series of fish oil (Menhaden oil)-corn oil diets, i.e., fish (19%)-corn oil (1%), fish (15.5%)-corn oil (4.5%), fish (10%)-corn oil (10%), and fish (5%)-corn oil (15%). The components of the high fat diets (20%) included casein (20.1%), DL-methionine (0.35%), dextrin (32.18%), sucrose (16.09%), AIN salt mixture (4.13%), AIN vitamins (1.18%), and cellulose (5.00%). One very high fish oil-corn oil diet (30%) was used, i.e., fish oil (19.75%) and corn oil (10.25%); the components of this diet included casein (22.23%), DL-methionine (0.39%), dextrin (23.47%), sucrose (22.70%), AIN salt mixture (3.50%), AIN vitamins (1.00%), and cellulose (5.00%).
Dietary Fat and Mammary Gland Developmental Growth in Immature Mice

Experiment 1. The experimental design of this study is provided in Table 2. Diets were fed to mice 21 to 45 days of age. At 35 days of age, each mouse was ovariectomized. Commencing at 42 days of age, each mouse received 3 consecutive days of hormone treatment (17β-estradiol plus progesterone, injected s.c.) for the purpose of providing a uniform ovarian hormonal milieu. The steroids (Sigma Chemical Co., St. Louis, MO) were mixed with gum arabic (Sigma Chemical Co.); the hormone: gum arabic mixture was dissolved in 0.9% NaCl solution. At 45 days of age, all mice were sacrificed, and their inguinal (No. 4) mammary glands were excised, fixed in glacial acetic acid: 100% ethanol (1:3, v/v), and stained with alum carmine (Sigma Chemical Co.) for whole-mount evaluation. The extent of mammary duct expansion through the mammary fat-pad of each gland was determined.

Experiment 2. The experimental design of this study is provided in Table 3. The design of this study is identical to that described in Experiment 1 except that only 2 dietary fats (oils) are examined (corn oil and Menhaden oil); such fats were blended together in 4 different ratios. Certain diets were formulated to ensure equal linoleic acid levels. Table 3 Interaction between dietary corn oil and Menhaden oil on mammary gland development in immature female BALB/c mice

Table 1 Predominant fatty acids in oils and fats (percentage)

<table>
<thead>
<tr>
<th>Fatty aci</th>
<th>Corn oil (%)</th>
<th>Olive oil (%)</th>
<th>Coconut oil (%)</th>
<th>Lard (%)</th>
<th>Beef tallow (%)</th>
<th>Menhaden oil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprylic (8:0)</td>
<td>5.8</td>
<td>6.6</td>
<td>3.5</td>
<td>1.5</td>
<td>3.4</td>
<td>8.0</td>
</tr>
<tr>
<td>Capric (10:0)</td>
<td>6.6</td>
<td>53.5</td>
<td>18.4</td>
<td>101.13</td>
<td>7.9</td>
<td>5.4</td>
</tr>
<tr>
<td>Lauric (12:0)</td>
<td>10.1</td>
<td>11.3</td>
<td>7.9</td>
<td>5.4</td>
<td>25.3</td>
<td>25.5</td>
</tr>
<tr>
<td>Myristic (14:0)</td>
<td>2.5</td>
<td>2.8</td>
<td>7.9</td>
<td>1.6</td>
<td>2.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>31.4</td>
<td>78.5</td>
<td>5.0</td>
<td>9.0</td>
<td>44.5</td>
<td>35.7</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>56.3</td>
<td>7.2</td>
<td>1.0</td>
<td>24.0</td>
<td>9.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>47.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>10.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linolenic (18:3)</td>
<td>12.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Influence of dietary fat on mammary gland development in immature female BALB/c mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Body wt (terminal)</th>
<th>Mammary gland developmental growth (nipple to end bud distance, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil (5%)</td>
<td>20</td>
<td>19.1 ± 0.4 (a)</td>
<td>8.94 ± 0.4 (a)</td>
</tr>
<tr>
<td>Corn oil (20%)</td>
<td>20</td>
<td>20.1 ± 0.3 (a)</td>
<td>10.59 ± 0.43 (a)</td>
</tr>
<tr>
<td>Olive oil (20%)</td>
<td>20</td>
<td>19.8 ± 0.8 (a)</td>
<td>9.51 ± 0.79 (a)</td>
</tr>
<tr>
<td>Linseed oil (20%)</td>
<td>20</td>
<td>19.8 ± 0.7 (a)</td>
<td>9.31 ± 0.58 (a)</td>
</tr>
<tr>
<td>Coconut oil (19%) + corn oil (1)</td>
<td>20</td>
<td>20.2 ± 0.5 (a)</td>
<td>9.18 ± 0.59 (a)</td>
</tr>
<tr>
<td>Lard (20%)</td>
<td>20</td>
<td>19.9 ± 0.6 (a)</td>
<td>8.99 ± 0.61 (a)</td>
</tr>
<tr>
<td>Beef tallow (19%) + corn oil (1)</td>
<td>18</td>
<td>20.0 ± 0.3 (a)</td>
<td>10.62 ± 0.33 (a)</td>
</tr>
<tr>
<td>Menhaden oil (19%) + corn oil (1)</td>
<td>20</td>
<td>18.0 ± 0.4 (b)</td>
<td>6.18 ± 0.60 (b)</td>
</tr>
</tbody>
</table>

* Female BALB/c mice were fed the diets from 28 days of age to 118 to 128 days of age. All mice were sacrificed in estrus between 118 and 128 days of age, and both inguinal mammary glands (No. 4) were excised and examined for developmental growth.

** Numbers in parentheses, percentage.

i.e., the 5% corn oil/4.5% corn oil-15.5% Menhaden oil diets and the 20% corn oil/19.75% corn oil-10.25% Menhaden oil diets. Dietetic Fat and Mammary Gland Developmental Growth in Mature Mice: No Hormone Treatment

Experiment 1. The experimental design of this study is provided in Table 4. Diets were fed from 21 to 45 days of age. At 35 days of age, all mice were ovariectomized. From 42 to 44 days of age, all mice were given injections s.c. daily of 17β-estradiol (1 μg) and progesterone (1 mg). The mice were sacrificed at 45 days of age, and both inguinal mammary glands (No. 4) were excised and examined for developmental growth.

** Numbers in parentheses, percentage.

* Mean ± SE.

** Numbers in brackets, range.

a/b, P < 0.05.

See "Results."

Dietary Fat and Mammary Gland Developmental Growth in Mature Mice: Mammatropic Hormone Treatment

Experiment 1. The experimental design of this study is provided in Table 5. Diets were fed from 28 to 128 days of age. Commencing at 118 days of age, 17β-estradiol plus progesterone was injected s.c. daily for 10 days; all mice were sacrificed at 128 days of age. At sacrifice, the inguinal (No. 4) mammary glands were excised and prepared for whole-mount evaluation. Developmental growth of the mammary glands was assessed on each gland by ascription of mammary gland development scores, determination of mammary gland epithelial area, and determination of mammary gland DNA levels.

** Numbers in parentheses, percentage.

No. Mean ± SE.

* Female BALB/c mice were fed the diets from 28 days of age to 118 to 128 days of age. All mice were sacrificed in estrus between 118 and 128 days of age, and both inguinal mammary glands (No. 4) were excised and examined for developmental growth.

** Numbers in parentheses, percentage.

* Mean ± SE.

** Numbers in brackets, range.

a/b, P < 0.05.

See "Results."
Mature Mice—Determination of Mammary Gland Epithelial Area

The following criteria were used:
1. Few ducts, few or no end ducts;
2. Moderate ductal-alveolar growth as in late pregnancy;
3. Numerous ducts and branches, minimal duct growth, moderate number of end ducts;
4. Numerous ducts and branches, dense lobuloalveolar growth; and
5. Numerous ducts and branches, dense lobuloalveolar growth through the mammary fat-pad, from the nipple to the most distant mammary end bud (see Fig. 1). Distance of duct expansion (penetration) through the mammary fat-pad was determined by a computer-assisted image analysis system (R & M Biometrics Corp., Nashville, TN).

Mature Mice—Ascription of Mammary Gland Development Scores

Development of the mammary ductal-alveolar epithelium was assessed in whole-mount preparations by measuring the linear distance (mm) of duct expansion through the mammary fat-pad, from the nipple to the most distant mammary end bud. Distance of duct expansion (penetration) through the mammary fat-pad was determined by a computer-assisted image analysis system (R & M Biometrics Corp., Nashville, TN).

Mature Mice—Determination of Mammary Gland DNA Levels

DNA content of the mammary gland in the mature rodent is an index of a state of late pregnancy, increases approximately 27 times (19). Thus, the DNA of the gland is derived from the ductal-alveolar component.

Mature Mice—Ascription of Mammary Gland Development Scores

Development of the mammary ductal-alveolar epithelium was assessed in whole-mount preparations by a standard procedure (16). Mammary glands were coded and rated for development according to the following criteria: 1. Few ducts, few or no end ducts; 2. Moderate duct growth, moderate number of end ducts; 3. Numerous ducts and branches, many end ducts; 4. Numerous ducts and branches, minimal lobuloalveolar growth; 5. Numerous ducts and branches, moderate lobuloalveolar growth; and 6. Numerous ducts and branches, dense lobuloalveolar growth as in late pregnancy.

Mature Mice—Determination of Mammary Gland Epithelial Area

Mammary ductal-alveolar epithelial area (mm²) was determined in coded whole mounts of mouse mammary glands by a computer-assisted image analysis system (R & M Biometrics Corp.) (12). A television camera attached to a low-power microscope generates an image of the tissue section consisting of light (fat-pad) and dark (epithelium) points. This image is relayed to a computer (Zenith Data Systems), which counts the number of dark points. These data are then converted to area (mm²) measurements. The total epithelial area for each gland was determined.

Mature Mice—Ascription of Mammary Gland Development Scores

Development of the mammary ductal-alveolar epithelium was assessed in whole-mount preparations by a standard procedure (16). Mammary glands were coded and rated for development according to the following criteria: 1. Few ducts, few or no end ducts; 2. Moderate duct growth, moderate number of end ducts; 3. Numerous ducts and branches, many end ducts; 4. Numerous ducts and branches, minimal lobuloalveolar growth; 5. Numerous ducts and branches, moderate lobuloalveolar growth; and 6. Numerous ducts and branches, dense lobuloalveolar growth as in late pregnancy.

Mature Mice—Determination of Mammary Gland DNA Levels

Mammary gland DNA levels were determined on coded whole mounts of mouse mammary glands by a standard procedure (17). The lymph nodes were excised from each gland, and water and lipids were extracted from trichloroacetic acid-precipitated materials by sequential washings (4°C) with 1 M sodium acetate in methanol, methanol:chloroform (2:1, v/v), absolute ethanol, and ethyl ether. The resulting dry fat-free tissue was washed with cold 10% perchloric acid and then treated with hot 5% perchloric acid (30 min); the DNA content in the supernatant was determined by a diphenylamine reaction. Total DNA content of the mammary gland in the mature rodent is an index of mammary growth when the ducial structures are in an active state of proliferation and expansion, i.e., after the administration of exogenous mammatrophic hormones or during the physiological state of pregnancy (18). In the virgin mouse, only ≈20% of the DNA in the whole mammary gland (inguinal minus lymph nodes) is derived from ductal epithelium; after hormonal stimulation (e.g., late pregnancy), >90% of the DNA of the gland is derived from the ductal-alveolar component. The amount of mammary epithelial DNA, from the virgin state to the state of late pregnancy, increases approximately 27 times (19). Thus, this method of quantitatively assessing mammary ductal-alveolar developmental growth is useful when examining the mammary of hormonally stimulated mature female mice.

Statistics

Multigroup mean comparisons were analyzed by one-way analysis of variance in concert with the Newman-Keuls multiple comparison test. Student’s t test was used when comparing paired group means. Mammary gland development score means were analyzed by the Wilcoxon signed rank test. Unless otherwise noted, significance was set at P < 0.05.
Fig. 1. Diagramatic sketch of an inguinal (No. 4) mammary gland from an immature female mouse. The length (mm) of the curved line, from the nipple, through the lymph node and extending to the farthest end bud was determined for each inguinal (No. 4) mammary gland (immature mice).

Fig. 2. Representative whole mount of an inguinal (No. 4) mammary gland from a fish oil (19% Menhaden oil-1% corn oil)-fed immature mouse. Distance from nipple to farthest end bud is 7.4 mm.

Fig. 3. Representative whole mount of an inguinal (No. 4) mammary gland from a vegetable oil (20% corn oil)-fed immature mouse. Distance from nipple to farthest end bud is 10.9 mm.

Fig. 4. Whole mount of an inguinal (No. 4) mammary gland from a fish oil (19% Menhaden oil-1% corn oil)-fed immature mouse. Distance from nipple to farthest end bud is 3.4 mm. Approximately 15% of the fish oil-fed immature mice had profound inhibition of mammary duct expansive growth as shown in this photograph. Such inhibition of duct growth was observed only in fish oil-fed mice.
RESULTS

Effect of Dietary Fat on Developmental Growth of the Mammary Gland in Immature Mice. Developmental growth of the mammary gland in immature mice was assessed by measuring the expansive growth of the ductal system through the fat-pad. This was accomplished by determining the distance (mm) from the nipple to the farthest extended end bud (see Fig. 1). There was no significant difference among the groups of mice fed the vegetable oil and/or animal fat diets in mean nipple to end bud distance (range, 8.99 to 10.59 mm) (Table 2). Ductal expansive growth in mice fed the fish oil diet, however, was significantly ($P < 0.05$) reduced (mean, 6.18 mm) when compared with ductal growth in animals fed the vegetable oil or animal fat diets (Table 2) (Fig. 2 to 4). In mice fed the low fat diet (corn oil, 5%), ductal growth was less (mean, 8.94 mm) than that of mice fed the high fat diet (corn oil, 20%) (mean, 10.59 mm), but this difference just missed the 5% level of statistical probability ($P < 0.07$) (Table 2).

The corn oil/fish oil diet group components of this study were repeated (10 mice/group). Nipple to end bud distances (mm) were $7.80 \pm 0.49$, $11.31 \pm 0.47$, and $9.50 \pm 0.52$ in the 5% corn oil, 20% corn oil, and 19% Menhaden oil-1% corn oil dietary groups, respectively. Mean body weights (g) at the termination of the study were $16.7 \pm 0.4$, $17.0 \pm 0.6$, and $17.8 \pm 0.6$, respectively, for the mice in these dietary groups. Nipple to end bud distance was significantly ($P < 0.05$) less in mice fed the 5% corn oil diet and fish oil diet compared with mice fed the 20% corn oil diet. Combining both of these experiments, nipple to end bud distances in the 5% corn oil diet group and fish oil diet groups were significantly ($P < 0.05$) less than that observed in the 20% corn oil group; no significant difference in nipple to end bud distance was observed between the 5% corn oil and fish oil diet groups.

The effects of different ratios of dietary corn oil and fish oil on expansive growth of the ductal system in immature mice are shown in Table 3. Corn oil/fish oil dietary ratios of 10%/10% or 4.5%/15.5%, respectively, significantly ($P < 0.05$) decreased nipple to end bud distance when compared to mice fed a 20% corn oil diet. Mice fed the 5% corn oil diet had significantly ($P < 0.05$) reduced nipple to end bud distances when compared to mice fed the 20% corn oil diet.

Effect of Dietary Fat on Developmental Growth of the Mammary Gland in Mature Mice: No Hormone Treatment. Developmental growth of the mammary gland in mature mice having normal estrous cycles was assessed by ascription of mammary gland development scores and determination of mammary gland epithelial area. There was no significant difference among the groups of mice fed the vegetable oil and/or animal fat diets in development scores or epithelial areas (Table 4). Mammary gland development scores were significantly ($P < 0.05$) reduced in mice fed the fish oil diet compared with mice fed the 20% corn oil diet, olive, oil, and lard diets although no significant difference in mammary gland epithelial areas was observed among any of the groups of mice.

The corn oil/fish oil diet group components of this study were repeated (10 mice/group). Mean mammary gland development scores were 2.05 (1.5 to 2.5), 2.10 (1.5 to 2.5), and 1.9 (1.5 to 2.5) for the 5% corn oil, 20% corn oil, and 19% Menhaden oil-1% corn oil dietary groups, respectively. Mean mammary gland epithelial areas (mm²) were 28.7 ± 1.3, 27.0 ± 2.0, and 30.3 ± 1.8, respectively, for these dietary groups. Mean body weights (g) at the termination of the study were 25.5 ± 0.7, 23.2 ± 1.0, and 24.7 ± 0.8, respectively. No significant effect of these diets on mammary gland development scores or epithelial areas was observed. Combining both of these studies, no significant effect on developmental growth of the mammary gland was observed among any of these groups of mice, irrespective of the amount or type of dietary fat.

Effect of Dietary Fat on Developmental Growth of the Mammary Gland in Mature Mice: Treatment with Mammotrophic Hormones. Developmental growth of the mammary gland in mature mice treated with mammotrophic hormones (17β-estradiol and progesterone) was assessed by ascription of development scores (Figs. 5 to 8), determination of epithelial area, and/or determination of total DNA levels. Mean mammary gland development scores or mean mammae epithelial areas were not significantly different among the groups of mice fed the vegetable oil or animal fat diets (Table 5). Mice fed the fish oil diet had significantly ($P < 0.05$) reduced mammary gland development scores when compared to mice fed the vegetable oil or animal fat diets; mean mammary gland epithelial area was significantly ($P < 0.05$) less in mice fed the fish oil diets compared with mice fed the 20% corn oil or coconut oil diets. Mean total mammary gland DNA level was numerically the lowest in mice fed the fish oil diet; however, no significant differences among the dietary groups were observed when utilizing this growth parameter. Mammary gland development scores were significantly ($P < 0.05$) reduced in mice fed the 5% corn oil diet compared with mice fed the 20% corn oil diet; mean mammae epithelial area was less in the 5% corn oil diet group compared with the 20% corn oil group, but this difference did not reach the 5% level of statistical probability.

The corn oil/fish oil diet group components of this study were repeated (10 mice/group). Mean mammary gland development scores for the 5% corn oil, 20% corn oil, and 19% Menhaden-1% corn oil dietary groups were 2.60 (2.0 to 3.0), 3.40 (2.5 to 4.5), and 2.45 (2.0 to 3.5), respectively. The mean mammary gland epithelial areas (mm²) for these dietary groups were 59.2 ± 2.9, 69.7 ± 5.8, and 59.8 ± 3.6, respectively. Mean mammary gland DNA (pg/gland) levels for these dietary groups were 235.4 ± 17.1, 234.9 ± 31.4, and 184.3 ± 20.1, respectively. Mean body weights (g) at the termination of the study were 28.2 ± 0.8, 28.5 ± 0.6, and 27.5 ± 0.5 Mean mammary gland development scores were significantly ($P < 0.05$) reduced in the 5% corn oil and fish oil diet groups, compared with the 20% corn oil group; mammary gland epithelial areas were also reduced in these groups, compared with the 20% corn oil group, but this difference did not quite reach the 5% level of statistical probability ($P < 0.10$). The mean mammae DNA level was numerically less in the fish oil diet group compared with the 20% corn oil group, but this difference was not statistically significant. Combining both of these experiments, mean mammary gland development scores and mean mammae epithelial areas were significantly ($P < 0.05$) less in the 5% corn oil and fish oil diet groups when compared with the 20% corn oil diet group.
Fig. 5. Whole mount of an inguinal (No. 4) mammary gland from a mammotrophic hormone-treated mature female mouse. Mammary gland development score is 2.0. This gland is characterized as having moderate duct growth and a moderate number of end ducts. This gland was obtained from a fish oil (19% Menhaden oil:1% corn oil)-fed mouse.

Fig. 6. Whole mount of an inguinal (No. 4) mammary gland from a mammotrophic hormone-treated mature female mouse. Mammary gland development score is 3.0. This gland is characterized as having numerous ducts and branches and many end ducts. This gland was obtained from a corn oil (5%)-fed mouse.

Fig. 7. Whole mount of an inguinal (No. 4) mammary gland from a mammotrophic hormone-treated mature female mouse. The mammary gland development score is 4.0. This gland is characterized as having numerous ducts and branches with minimal lobuloalveolar development. This gland was obtained from a corn oil (20%)-fed mouse.

Fig. 8. Whole mount of an inguinal (No. 4) mammary gland from a mammotrophic hormone-treated mature female mouse. The mammary gland development score is 5.0. This gland is characterized as having numerous ducts and branches with moderate lobuloalveolar development. This gland was obtained from a corn oil (20%)-fed mouse.
Body Weight Gains. There was no significant difference in body weight gains among any of the dietary groups of mice (immature mice, mature mice, or mammotrophic hormone-treated mature mice) with but one exception. The group of immature mice fed the fish oil diet in Experiment 1 (Table 2) had significantly \( P < 0.05 \) less body weight gains than did the groups of mice fed the 20\% vegetable or animal fat diets. Body weight gains among the groups of mice fed the fish oil diets in each of the other experiments were not significantly altered.

DISCUSSION

In this study, high levels of corn oil, olive oil, coconut oil, linseed oil, lard, beef tallow, and fish oil were fed to groups of immature and mature female BALB/c mice. Only the mice fed the fish oil diets showed altered (suppressed) mammary gland development. Furthermore, fish oil-induced, suppressed mammary development was observed only in mice whose mammary epithelium was in a state of intense proliferation (immature mice and mammotrophic hormone-treated mature mice). Importantly, no significant difference in mammary development was observed in groups of immature or mature mice fed the other fat (oil) diets; mammary gland development and growth among these groups of mice were indistinguishable.

The first laboratory to study and report the effect of type of dietary fat on normal mammary gland developmental processes was that of Abraham et al. (20). They reported that female BALB/c mice fed a high level of corn oil had considerably more mammary gland ductal epithelial growth than did mice fed a comparable level of hydrogenated cottonseed oil. The hydrogenated cottonseed oil is deficient in essential fatty acids; essential fatty acid deficiency in mice results in suppressed mammary gland development (21, 22), a phenomenon that can be reversed by the sole administration of linoleic acid (21). To circumvent this problem, we added a small amount of corn oil, which is rich in linoleic acid, to each of our high fat diets (coconut oil, beef tallow, and Menhaden oil) that contain marginal levels of this essential nutrient. It is our experience that mice fed beef tallow or fish oil diets (20\%), as their sole source of dietary fat, have significantly reduced body weight gains. In the present study, body weight gains were comparable among all dietary groups, in immature mice as well as mature mice. Thus, the significant inhibitory effect of dietary fish oil on mammary gland development in immature and mature mice was observed in animals with a normal rate of body weight gain. In our studies we also observed a suppression of mammary gland development in mice fed a low fat diet (5\% corn oil) compared with mice fed a high fat diet (20\% corn oil). This growth differential was observed only in mice bearing a proliferating mammary gland (immature mice and mammotrophic hormone-treated mature mice) and is consistent with a previous report from our laboratory (12) and by others (23).

The mechanism by which dietary fish oil suppresses mammary gland developmental processes is uncertain. The results of our study clearly demonstrate that the rate of ductal proliferation (expansive growth through the mammary fat-pad) is significantly reduced in mice fed a diet rich in fish oil when compared with mice fed, e.g., a diet rich in corn oil. Thus, our data clearly support the concept that changes in dietary fat composition act by influencing mammary ductal cell proliferation. It is germane to point out that Abraham et al. could not demonstrate any significant difference in DNA synthesis or cell cycle kinetics (3, 11, 24) of mouse mammary tumors as a function of the type of dietary fat (e.g., corn oil versus hydrogenated cottonseed oil or fish oil); diet was shown only to affect the rate of tumor cell loss (11, 24). Thus, according to the concept proposed by Abraham et al., increased mammary tumor size, as a function of type of dietary fat, is a reflection primarily of cell loss, not proliferative processes. This is a very important concept, one that clearly needs to be confirmed and is indirectly supported by a number of laboratories who report that the fat content of the diet can significantly affect immune system mechanisms (25–28). In our study, utilizing the normal mammary gland, dietary fish oil clearly suppressed mammary proliferative processes. It is unlikely that immune system dynamics would modulate normal mammary development; an interaction with neoplastic mammary, in contrast, is far more likely.

Altered mammary proliferative processes, as a function of the fat content of the diet, can be explained by a number of mechanisms. Potential mechanisms include the generation of lipid peroxides and/or oxygen radicals (29), alteration in membrane fluidity (30), changes in intercellular communication (31), alterations in mammotrophic hormone secretion (32, 33), and enhancement of hormone and/or growth factor responsiveness (12). In recent years, Abraham and coworkers (3, 11, 20, 22, 24, 34) and others (35–38) have offered the hypothesis that dietary fat, at least in part, may affect normal (and neoplastic) mammary gland growth processes by influencing, directly or indirectly, prostaglandin biosynthesis. Certain dietary fish oils, e.g., Menhaden oil, are especially rich in long chain n-3 fatty acids such as EPA\(^2\) (20:5) and DHA (22:6). EPA and DHA modify linoleic acid (18:2) and arachidonic acid (20:4) metabolism, thus sharply interfering with prostaglandin biosynthesis (39–41). It is conceivable, therefore, that the inhibitory effect of dietary Menhaden oil on the developmental growth of the mouse mammary gland, as observed in our study, is manifested via an inhibition of linoleic acid utilization. Although our Menhaden oil diet was supplemented with corn oil (1\%), ensuring adequate recommended daily allowance levels of linoleic acid, the rather large amounts of EPA and DHA in this diet could interfere with linoleic acid utilization. Indeed, in our study, suppressed normal mammary developmental growth was observed in mice fed dietary corn oil/Menhaden ratios of 1:3 (4.5% corn oil:15.5% Menhaden oil) and even 2:1 (19.75% corn oil:10.25% Menhaden oil). Thus, if the EPA-DHA/linoleic acid utilization concept is correct, then EPA and/or DHA appears to be effective even when diets contain an abundance of linoleic acid. It is important to point out, in addition, that our observed inhibitory effect of dietary Menhaden oil on mouse mammary developmental growth cannot solely be attributed to the broad class of n-3 fatty acids, as high dietary levels of linseed oil did not, whatever, affect this developmental process. Linseed oil is rich (47\%) in linolenic acid (18:3, n-3). Although linolenic acid can inhibit the metabolism of arachidonic acid, via cyclooxygenase, and the conversion of linoleic acid to arachidonic acid, this n-3 fatty acid does not appear to be as effective as EPA/DHA in the suppression of eicosanoid formation from arachidonic acid (42).

It is important to point out that we could find no correlation between the linoleic acid content of the diet and developmental growth of the mouse mammary gland. Widely varying linoleic acid levels (percentage) of \( \approx 11.2, 1.4, 0.8, 4.8, 1.9, \) and 0.9 are found in our corn oil, olive oil, coconut oil, linseed oil, lard, and beef tallow, respectively, high fat diets; such diets did not differentially affect the developmental growth of the mouse mammary gland. These results are in contrast with those of a

---

\(^2\) The abbreviations used are: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
number of laboratories that report a direct relationship between the level of dietary linoleic acid and rodent mammary tumor developmental growth stimulation (36, 43–47). It is conceivable, therefore, that the neoplastic mammary gland is more sensitive to dietary linoleic acid than the proliferating mouse mammary gland. It is also interesting to note that mice fed the low fat diet (5% corn oil) had less mammary gland developmental growth than did mice fed any of the high (20%) fat diets (except Menhadan oil). The linoleic acid content of the low fat diet is 2.8%, a level of linoleic acid that is higher than most of the high fat diets used in our study. It is difficult to explain this difference on the basis of calories, as these diets were formulated to be isocaloric. The mechanism by which diets low in fat but containing adequate levels of essential fatty acids suppress mammary developmental processes remains to be determined.

Factors which influence developmental growth processes of the normal mammary gland can be significantly affected (suppressed) by diet, but by only rather extreme dietary intervention, i.e., by reducing fat consumption by 75% (20% to 5%) or by feeding very high levels in n-3 polyunsaturated fatty acids such as EPA and/or DHA. The feeding of high dietary levels of four different vegetable consumption by 75% (20% to 5%) or by feeding very high levels in n-3 polyunsaturated fatty acids such as EPA and/or DHA.


Influence of the Type of Dietary Fat on Developmental Growth of the Mammary Gland in Immature and Mature Female BALB/c Mice

Clifford W. Welsch and Deborah H. O’Connor


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/49/21/5999

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.