Effects of Type of Dietary Fat on Phorbol Ester-elicited Tumor Promotion and Other Events in Mouse Skin

Julius Leyton, Marilyn L. Lee, Mary Locniskar, Martha A. Belury, Thomas J. Slaga, David Bechtel, and Susan M. Fischer

ABSTRACT

Based on the biological activity of arachidonic acid metabolites, we hypothesized that alterations in the consumption of linoleic acid, the precursor to arachidonic acid, would result in a modification in tumor development when fed during the tumor promotion stage of the mouse skin initiation-promotion model. The effects of seven different levels of dietary linoleic acid (LA), supplied as corn oil in a 15% fat diet, on the incidence and rate of papilloma and carcinoma development were determined. SENCAR mice were placed on one of the experimental diets, containing 1.0, 3.6, 6.0, 7.9, 9.9, 12.5, or 15.0% corn oil, 1 week after initiation with 10 nmol of 7,12-dimethylbenz[a]anthracene and 3 weeks prior to the start of twice weekly promotion with 1 μg 12-O-tetradecanoylphorbol-13-acetate (TPA). At 15 weeks of TPA treatment there were significant differences in papilloma number among diet groups, such that an inverse correlation (r = 0.92) was observed between tumor number and level of corn oil; the lowest corn oil diet group had an average of 11.7 tumors/mouse, while the highest corn oil group had 5.4 tumors/mouse. However, there was little difference in tumor incidence among diet groups. A general relationship between diet and carcinoma incidence was also found, such that the highest corn oil diet group had the lowest carcinoma incidence. In an experiment performed with DBA/2 mice, the average number of papillomas/mouse at 17 weeks was 4.5 (1.0% corn oil), 5.6 (7.9% corn oil), and 23.0 (15.0% corn oil). Papilloma incidence was also affected by diet, with a 79% incidence for the 15.0% corn oil and an incidence of 93% for the 1.0% corn oil group. Analyses of the fatty acid composition of epidermal phospholipids in mice fed the experimental diets reflected the dietary LA levels, in that an accumulation of phospholipid LA, accompanied by an overall decrease in arachidonic acid, occurred with increasing dietary corn oil. In spite of the high membrane content of LA, no measurable amount of epidermal conjugated dienes of LA could be detected. Epidermal prostaglandin E₂ levels in acetonetreated mice were similar for all diet groups (approximately 3 pg/mg DNA). However, 6 h after topical application with 4 μg of TPA, prostaglandin E₂ levels were elevated 5- to 10-fold; an inverse correlation (P < 0.05) was seen with increasing dietary LA, although the concordance with decreased phospholipid arachidonic acid was not strong. The extent of the hyperplastic response of the epidermis to 1 μg TPA showed no correlation with increasing dietary LA, in that no differences were seen among the diet groups. These studies indicate that increasing dietary intake of corn oil (or decreasing saturated fat) in the tumor promotion stage of multistage carcinogenesis in mouse skin results in significant suppression of tumor development. The observations from this study also suggest that, while such diets may offer protection against tumor development by reducing prostaglandin synthesis, it is probable that other mechanisms are operative as well.

INTRODUCTION

The majority of human cancers are currently thought to be caused by environmental factors (1), with diet being one of the most important modifying agents (2). To understand the relationship of dietary fat to tumor development, both quantitative and qualitative aspects have been considered. With regard to total fat levels, studies from several laboratories have shown that elevated levels of dietary fat increased the incidence of both spontaneous and chemically induced mammary tumors in rats (3-8). Using the initiation-promotion model in mouse skin, Birt et al. (9) also found an increase in tumor incidence and yield in mice fed high levels of fat.

When fat level is held constant and type of fat is considered, there appears to be a relationship between the degree of saturation and tumor incidence. Carroll and Khor (10) reported that mammary tumor incidence was greater with diets containing PUFA's, when compared to diets with saturated fat, i.e., tallow or coconut oil. The nature of the PUFA also appears to be important. Dietary consumption of ω-3-PUFA's, as found in fish oils, provided a protective effect against mammary carcinogenesis (11) when compared to corn oil, which contains relatively high levels of ω-6 PUFA's, principally as LA. However, such protection was not observed in the tumor promotion stage of skin carcinogenesis, using either TPA or benzoyl peroxide as the promoter (12). Differences in activity between ω-3 and ω-6 fatty acids are believed to be related to the involvement in, or effect on, arachidonic acid metabolism (13). The metabolites derived from the ω-3 fatty acids are produced to a lesser extent and are biologically less active, when compared to arachidonate metabolites (14).

The importance of arachidonic acid release and metabolism in the tumor promotion stage of multistage carcinogenesis in mouse skin has been well established (reviewed in ref. 15). The conclusions of several studies indicate that (a) arachidonic metabolites or eicosanoids are capable of eliciting inflammation, an event induced by tumor promoters (16), (b) exogenous application of prostaglandins with the phorbol ester tumor promoter TPA can modify tumor development (prostaglandin F₂₀ enhances while PGE₂ inhibits), and (c) use of inhibitors of various parts of the arachidonate cascade causes an inhibition of tumor incidence (15).

Based on the biological activity of arachidonate and the requirement for its metabolites in the skin tumor promotion model, we hypothesized that alterations in the consumption of LA (18:2, n-6), the precursor to AA (20:4, n-6), would result in changes in tumor development. Specifically, it was predicted that higher dietary levels of LA, supplied in the form of corn oil, would be associated with a higher tumor rate (9). The first part of this study, therefore, was conducted to determine the effects of seven different ratios of corn oil to coconut oil, in a 15% fat diet, on the incidence and rate of papilloma and carcinoma development.

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15% fat diet, on the incidence and rate of papilloma and carcinoma development.
carcinoma development; the second part was to determine the disposition of the dietary linoleate with regard to changes in LA and AA content in epidermal phospholipids, particularly phosphatidylcholine, and whether changes in dietary LA would alter phorbol ester-induced PGE2 production or hyperplasia. Also, the possible conversion of LA to its conjugated forms was evaluated, since Pariza and co-workers (17, 18) have shown that topical application of CLA inhibited the development of tumors in the mouse skin model and inhibited TPA-induced ornithine decarboxylase in mouse forehead.

METHODS AND MATERIALS

Chemicals. 12-O-Tetradecanoylphorbol-13-acetate was purchased from LC Services (Woburn, MA). 7,12-Dimethylbenz(a)anthracene, phospholipid standards, and snake venom phospholipase A2 were obtained from Sigma Chemical Co. (St. Louis, MO). Solvents were obtained from Fisher Scientific (Fair Lawn, NJ). TLC plates were obtained from EM Science (Gibbstown, NJ). Prostaglandin E2 radioimmunoassay kits were purchased from Advanced Magnetics, Inc. (Cambridge, MA). Authentic CLA was kindly provided by Dr. Michael W. Pariza (Food Research Institute, University of Wisconsin, WI). All diets were obtained from Teklad (Madison, WI). The corn oil used in the diets was research grade Mazola, supplied to Teklad by Best Foods, Inc. (Union, NJ).

Tumor Study in SENCAR Mice. Groups of 30 female SENCAR mice (4 weeks of age), purchased from the NCI-Frederick Cancer Research Facility (Frederick, MD), were randomized and housed 10/cage prior to assignment to one of seven diet groups. All animals were maintained in climate-controlled, pathogen-free quarters with a 12-h light/dark cycle. All groups were placed on diet 1 (5% fat, 1.7% corn oil, as shown in Table 1), which contained 15% fat. All diets were obtained from Teklad (Madison, WI). The corn oil used in the diets was research grade Mazola, supplied to Teklad by Best Foods, Inc. (Union, NJ).

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Table 1 Composition of experimental diets

<table>
<thead>
<tr>
<th></th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>Diet 6</th>
<th>Diet 7</th>
<th>Diet 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil (%)</td>
<td>1.7</td>
<td>1.0</td>
<td>3.6</td>
<td>6.0</td>
<td>7.9</td>
<td>4.5</td>
<td>5.6</td>
<td>4.0</td>
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<tr>
<td>Linoleate (%)</td>
<td>1.0</td>
<td>0.8</td>
<td>2.2</td>
<td>3.5</td>
<td>4.5</td>
<td>4.5</td>
<td>5.6</td>
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</tr>
<tr>
<td>Dextrose</td>
<td>65.0</td>
<td>51.5</td>
<td>51.5</td>
<td>51.5</td>
<td>51.5</td>
<td>51.5</td>
<td>51.5</td>
<td>51.5</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>3.3</td>
<td>14.0</td>
<td>11.4</td>
<td>9.0</td>
<td>7.1</td>
<td>5.1</td>
<td>2.5</td>
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</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
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<tr>
<td>n-6 Methylone</td>
<td>0.3</td>
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<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td>Choline bitartrate</td>
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<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
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<td>0.2</td>
</tr>
<tr>
<td>Mineral mix AIN-76</td>
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<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
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<tr>
<td>Vitamin mix AIN-76A</td>
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<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.2</td>
</tr>
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</table>

Diet 4 is prepared according to the AIN-76 formulation. Diet 1 contains 5% fat; all other diets contain 15% fat. Diet components are expressed as g/100 g and diets are referred to by either corn oil content or linoleic acid content.
performance liquid chromatograph. The conjugated dienes were measured at 234 nm, and the nonconjugated fatty acids LA and AA were measured at 205 nm. The recovery of authentic CLA was greater than 73% when added to epidermal samples.

PGE₂ Analysis. Groups of 16 SENCAR mice on each of the experimental diets were dorsally shaved and topically treated with either 200 μl acetone (8 mice/diet group) or 4 μg TPA in acetone (8 mice/diet group). Six h later, the mice were sacrificed (this time point was previously determined to represent a peak of PGE₂ production; data not shown) and the dorsal surface was quickly frozen on dry ice, followed by immersion in liquid nitrogen and storage at −70°C. For assay, a 1.5-cm² area of epidermis was chipped from the frozen skin into 1 ml ice-cold methanol. Following homogenization for 30 s at 4°C, a 200-μl aliquot was removed for determination of DNA content, and the remainder was extracted according to the method of Chang et al. (23) for PGE₂ determination by dilution to 20% methanol with acidified water (pH 3–4). The supernatant from a 10-min centrifugation at 2000 × g was applied to a preconditioned SPICE C18 column (Analytech, Newark, DE), the column was washed with 5 ml petroleum ether:diethyl ether (9:1), and the prostaglandins were eluted with 1 ml methanol. Following solvent evaporation, the prostaglandins were reconstituted in 1 ml buffer (supplied in the radioimmunoassay kit) and stored at −20°C. PGE₂ levels were determined by radioimmunoassay (cross-reactivity with prostaglandin E₂ may occur). Recovery of PGE₂ was greater than 60%. The DNA content was determined using the method of Schmidt and Thannhouser (24), and the level of epidermal PGE₂ was expressed as pg PGE₂/μg DNA.

Histologies. Groups of 12 SENCAR mice on each of the experimental diets were dorsally shaved 3 days prior to topical treatment with either 200 μl acetone or 1 μg TPA in acetone. Acetone-treated mice were sacrificed after 48 h. TPA-treated mice (3/diet group) were sacrificed 24, 48, or 72 h later. Sections of dorsal skin were fixed in formalin and embedded in paraffin, and 3.5–4-μm thick sections were stained with hematoxylin and eosin. The number of nucleated cells in the epidermis was counted in 10 different fields, using a 0.125-mm length of basement membrane/field. The data are expressed as the mean number of nucleated cells/0.125 mm².

Statistics. Analysis of variance was performed on all sets of data and, where a treatment effect was observed, the Fisher protected least significance analysis was used to determine significance between groups. For the average number of papillomas/mouse, analysis of variance of the regression equations over diet groups was performed using BMDP-1R multiple linear regression (BMDP Statistical Software, Inc., Los Angeles, CA).

RESULTS

Food Consumption and Body Weight. The effect of the experimental diets on body weight is shown in Fig. 1. A steady weight gain was observed in all the groups. Body weights were not determined after 23 weeks because of the large tumor burden. A slight but not statistically significant reduction in body weight was found in the 1.0% corn oil diet (lowest level of corn oil). Food consumption, calculated as kcal/mouse/day, was determined to be the same across all diet groups (data not shown), with an average of 28.6 ± 2.1 (mean ± SE) kcal/mouse/day at 15 weeks.

Tumor Development in SENCAR Mice. The papilloma data were calculated in terms of incidence (percentage of mice bearing tumors) and yield (average number of tumors/mouse). Papillomas were first observed during week 5 after promotion was started and they continued to appear through weeks 12 to 13, after which the incidence remained constant (Fig. 2, upper). Little difference in latency was noted between diet groups, although, for those animals fed the highest levels of corn oil, tumors appeared 1 week later, compared to those animals fed lower levels of corn oil. Early in the experiment (9 weeks of promotion), the tumor incidence was higher in the four groups fed the lowest levels of corn oil (70 to 90%, compared to 56 to 60% for the highest corn oil groups). The final papilloma incidence at 13 to 15 weeks was 90–100%, with no apparent relationship between dietary corn oil level and incidence. The papilloma incidence observed was that expected with the doses of DMBA and TPA employed.

Significant differences in papilloma number between diet groups occurred, such that a strong inverse correlation (r = 0.92) was observed between tumor number and dietary level of corn oil (Fig. 2, lower). At 15 weeks, the groups fed 1.0% corn oil, the lowest level of corn oil, had an average of 11.7 tumors/mouse, while the groups fed 1.5, 3.6, and 7.9% corn oil had 5.4 tumors/mouse. Although a dose-response relationship was found, it was not linear. Comparison of the regression equations for the average number of papillomas/mouse over time for each diet group revealed a statistically significant difference between the 1.0, 3.6, 6.0, and 7.9% corn oil diet groups, compared to the groups fed 9.9, 12.5, and 15.0% corn oil diets (P ≤ 0.0002). The data thus appeared to fall into two groupings, with the 1.0, 3.6, 6.0, and 7.9% corn oil diets clustered at the high tumor number end of the spectrum and the 9.9, 12.5, and 15.0% corn oil diets clustering at the lower end. When these two groups, termed low and high corn oil diet groups, respectively, were compared, the overall tumor yield was approximately 2 times lower for the high corn oil diet group.

Tumor size, measured as the mean diameter, was determined for all tumors in all groups at 15 weeks of TPA treatment (Table 2), the time point at which tumor number had plateaued. Overall tumor size at 15 weeks did not show a strong relationship to dietary corn oil. Some interesting trends were apparent, however. The diet groups fed the intermediate levels of corn oil (3.6, 6.0, and 7.9%) had the highest percentage of tumors in the large category (>6 mm), whereas the 12.5 and 15.0% groups had the least.

Carcinomas began to appear at approximately 20 weeks after the initial treatment with TPA. The cumulative carcinoma incidence, calculated as a percentage of mice with carcinomas, was determined at monthly intervals thereafter (Table 3). The high mortality rate between 20 and 28 weeks for the lowest (1.0 and 3.6%) corn oil diet groups, due primarily to ulcerative
DIETARY FAT EFFECTS ON TUMOR PROMOTION

Fig. 2. Effect of diet on papilloma incidence and yield in SENCAR mice. Groups of 30 SENCAR mice were initiated with 10 nmol of DMBA, placed on one of the seven experimental diets 1 week later, and promoted twice weekly with 1 μg of TPA after an additional 3 weeks. Tumors were counted weekly and incidence was calculated as the percentage of animals bearing tumors in each diet group (top). Tumor yield was calculated as the average number of papillomas/mouse for each diet group (bottom). At 16 weeks, the SE for tumor number was ≤10% of the mean. Q, 1.0%; •, 3.6%; D, 6.0%; $r, 7.9%; •, 9.9%; G, 12.5%; A, 15.0% corn oil.

dermatitis, confounded the calculation of cumulative carcinoma incidence, since a significant number of animals died prior to the time of expected appearance of carcinomas. In general, there was an association between level of dietary corn oil and survival (Fig. 3), such that the mortality rate was highest for the lowest corn oil diet groups. The principal cause of death for all groups after 25 weeks was carcinoma.

If the 1.0 and 3.6% corn oil groups are omitted from further consideration of carcinoma incidence, a general relationship between diet and incidence is observed, with the highest LA diet group producing both a delay in appearance and the lowest incidence. Carcinoma multiplicity often occurred but was not calculated or considered further because of frequent coalescence of tumors. In addition, animals were sacrificed when any carcinoma reached a diameter of approximately 2 cm, precluding the appearance of subsequent carcinomas.

Tumor Development in DBA/2 Mice. Because of concern that the inverse relationship between dietary LA level and tumor yield might represent a unique feature of the SENCAR mouse, an additional tumor experiment was performed using DBA/2 mice fed high (15.0%), low (1.0%) and intermediate (7.9%) levels of corn oil. As shown in Fig. 4, upper, papilloma incidence was affected by the level of dietary LA, with the group consuming the highest levels of corn oil producing the lowest incidence. The overall reduced incidence, compared to SENCAR mice, is

Table 2 Effect of diet on papilloma size

<table>
<thead>
<tr>
<th>Diet (% corn oil)</th>
<th>Tumor size distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-3 mm</td>
</tr>
<tr>
<td>1.0</td>
<td>67.4</td>
</tr>
<tr>
<td>3.6</td>
<td>75.0</td>
</tr>
<tr>
<td>6.0</td>
<td>67.4</td>
</tr>
<tr>
<td>7.9</td>
<td>79.7</td>
</tr>
<tr>
<td>9.9</td>
<td>77.4</td>
</tr>
<tr>
<td>12.5</td>
<td>82.7</td>
</tr>
<tr>
<td>15.0</td>
<td>74.3</td>
</tr>
</tbody>
</table>

Table 3 Cumulative carcinoma incidence

Groups of 30 SENCAR mice were initiated and promoted as described in "Materials and Methods." Beginning at 23 weeks after TPA was started, carcinomas were counted monthly and cumulative incidence was calculated as the percentage of animals bearing carcinomas in each diet group.

<table>
<thead>
<tr>
<th>Diet (% corn oil)</th>
<th>Cumulative carcinoma incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 23</td>
</tr>
<tr>
<td>1.0</td>
<td>15</td>
</tr>
<tr>
<td>3.6</td>
<td>18</td>
</tr>
<tr>
<td>6.0</td>
<td>19</td>
</tr>
<tr>
<td>7.9</td>
<td>37</td>
</tr>
<tr>
<td>9.9</td>
<td>15</td>
</tr>
<tr>
<td>12.5</td>
<td>11</td>
</tr>
<tr>
<td>15.0</td>
<td>7</td>
</tr>
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</table>

Fig. 3. Survival rate of SENCAR mice on experimental diets under the initiation and promotion protocol. For each group of 30 mice in the tumor experiment shown in Fig. 2, the percentage of animals surviving is plotted as a function of time. Q, 1.0%; •, 3.6%; D, 6.0%; $r, 7.9%; •, 9.9%; G, 12.5%; A, 15.0% corn oil.
was the phosphatidylcholine fraction, since it is the phospho-

epidermal membranes is shown in Table 4. Of principal interest
the LA and AA composition of phospholipids isolated from

0.05).

dietary corn oil demonstrated suppressed tumor development,
the same as observed in SENCAR mice; the group fed the high
initiation-promotion protocol (25). The relationship of papil-
a reflection of strain differences in sensitivities to this particular
initiation-promotion protocol (25). The relationship of papil-
oma number to dietary corn oil (Fig. 4, lower) was essentially
the same as observed in SENCAR mice; the group fed the high
dietary corn oil demonstrated suppressed tumor development,
compared to the intermediate and low dietary LA groups (P <

Fatty Acid Composition. The effect of dietary linoleic acid on
the LA and AA composition of phospholipids isolated from

lipid that undergoes the highest turnover rate in response to

TPA (26). For the 1.0 and 3.6% corn oil groups, the percentages
of LA were similar, but they increased significantly (P < 0.05)
in the 6.0% and higher corn oil groups, reaching a maximum
in the 15.0% corn oil group. Regression analysis showed a
significant positive correlation (P < 0.01) between dietary LA
levels (as supplied in the corn oil) and accumulation of LA in
epidermal phosphatidicholine. In contrast, there was an over-
all reduction in the phosphatidicholine AA with increasing
dietary LA; regression analysis showed a negative correlation
(P < 0.22) between dietary LA and epidermal phosphatidichlo-
one AA. As a result of increasing LA and decreasing AA, the
ratio of LA to AA increased at least 2-fold, from 3.4 in the
1.0% corn oil group to 8.1 in 12.5% corn oil group.

Similar changes were seen for LA and AA in the other
phospholipids. The LA content of phosphatidylethanolamine
was similar to that of phosphatidicholine; the AA content,
however, was approximately double. Regression analysis
showed a positive correlation (P < 0.03) between dietary corn
oil (and thus LA) levels and accumulation of LA in epidermal
phosphatidylethanolamine. The percentage of AA increased
from 9.9% in the lowest corn oil group to 14.4% in the next
highest group but then remained relatively unchanged for the
remainder of the diet groups. The changes in LA in the com-
bined phosphatidylserine and -inositol fractions were similar to
those in the other phospholipids, in that a positive correlation
(P = 0.22) was observed with level of dietary LA. AA, on the
other hand, showed very overall change.

Few changes were noted in the other fatty acids (data not
shown). The major saturated fatty acids, i.e., palmitic (16:0)
and stearic (18:0), and the monounsaturated oleic acid (18:1)
did not follow any discernable pattern or trend. The overall
ratio of polyunsaturated to saturated fatty acids remained rel-
atively constant across all the diet groups (data not shown).

CLA Analysis. Because of the report that CLA has anticar-
cinogenic activity (17, 18), it was necessary to determine if the
feeding of high LA diets would result in the formation of
measurable amounts of CLA in the epidermis. Standard curves
constructed for authentic CLA and LA indicated a minimum
level of detection of 50 ng for CLA and 20 ng for LA. High
performance liquid chromatography analysis was unable to
detect CLA in the epidermis of mice from any of the diet groups
(Fig. 5). However, in the same samples LA was readily meas-
urable.

PGE2 Production. Epidermal PGE2 levels were measured 6 h
following topical treatment with either acetone or TPA. This
time point has been previously determined to be one of the
several peaks that occur after TPA treatment (27, 28) and,
under our conditions, was larger than the peak at 24 h (data
not shown). The effect of the seven experimental diets on
PGE2 levels is shown in Fig. 6. TPA treatment caused a large
increase in all diet groups, although the extent was in general
negatively correlated with dietary corn oil (P < 0.05). The 1.0
and 3.6% corn oil groups differed significantly (P < 0.05) from
all the other diet groups, which did not differ among themselves.

Histological Analysis. The effect of dietary corn oil on the
number of nucleated cells in the epidermis 48 h after acetone
(solvent control) or TPA treatment is shown in Fig. 7. His-

istological analysis was also performed for treatment times of 24
and 72 h (data not shown) to assure that, under the dietary
conditions used in this study, 48 h was the time of maximum
hyperplasia, as previously reported (29). The hyperplastic re-
response in mouse skin epidermis to TPA (1 μg) was significantly greater (P < 0.05) than in the acetone-treated mice in all the diet groups at 48 h. However, among the TPA-treated groups there was no correlation between hyperplasia and level of dietary corn oil.

**DISCUSSION**

It was hypothesized that increasing dietary LA would result in an increased tumor yield in the mouse skin carcinogenesis model. This hypothesis was based both on previous work in this model system, in which arachidonic acid metabolism has been shown to be positively correlated with tumor development, and on the work of Ip et al. (29, 30) in the rat mammary model, where increasing dietary linoleate increased tumor incidence to a maximum with 4.4% dietary LA, with no additional increase at higher LA levels. Similar increases in tumor number with increasing dietary LA were reported by Roebuck et al. (31) in the rat pancreas. As reported here, however, in the skin model the relationship between dietary LA levels and tumor development is quite different. When the effects of various levels of dietary LA on tumor development are compared between incidence in the mammary model (29) and papilloma yield in the skin model, they appear to be opposite. When tumor size and carcinoma incidence are considered in the skin model, some similarity with the mammary system is apparent for the lower corn oil diets (those containing 0.8 to 4.5% LA), in that within that range increasing dietary LA is associated with a slight increase in size and incidence. Above 7.9% corn oil (4.5% LA), however, the effect of increasing dietary corn oil is different in the skin model, in that both papilloma number and carcinoma incidence are reduced, a phenomenon that does not occur in the mammary model (29). It is possible that in both the mammary and skin models maximum tumor growth requires approximately 4.5% dietary LA. Higher levels of dietary LA may have an inhibitory effect in the skin that is not seen in the mammary gland because of differences in disposition or metabolism of LA in the two tissues.

In order to elucidate the possible mechanism(s) by which dietary corn oil influences the skin tumor promotion process, alterations in specific tumor promoter-induced changes as a function of the corn oil (linoleic acid) content of the diet were measured, including skin prostaglandin levels and hyperplasia. Evidence from a number of studies has shown that TPA induces the release of free AA from membrane phospholipids (32), greatly increasing the synthesis of prostaglandins and lipoxygenase products (32–34). Inhibitors of AA metabolism have been shown to inhibit both tumor promotion in the skin model (32) and the incidence of chemically induced mammary tumorigenesis in the rats (35, 36), indicating an involvement of eicosanoids in tumor development in both organ systems. Cohen et al. (37) have, in particular, shown that increasing dietary LA correlated with enhanced mammary tumor PGE2 production. In the skin model, however, we observed an inverse correlation between LA and TPA-induced epidermal PGE2 levels.

PGE2 synthesis can be controlled at several different points in the pathway. First, the availability of the precursor fatty acid, AA, is rate limiting. AA is not provided directly by the diet but is instead synthesized from LA by a series of elongation and desaturation reactions. Liver is the primary site for this conversion (38), and it is probable that mouse skin lacks this ability, based on the work of Chapkin and Ziboh (39) in which rat and guinea pig epidermis were found to be deficient in Δ6- and Δ5-desaturase activity. In some tissues, the rate at which LA is converted to AA can also be regulated by the amount of LA present (40). Galli et al. (41) reported that the conversion of LA to AA in rat platelet lipids was increased when dietary LA was reduced. In the study reported here, an inverse correlation was found between increasing dietary LA and AA levels in epidermal phosphatidylcholine. Whether the reduction in AA was due to decreased activity of the desaturase and elongase enzymes in skin or liver by increasing levels of LA is not known. Clearly one or both mechanisms occurred, since the level of LA in epidermal cells increased with increasing levels of dietary LA. McGregor and Renaud (42) have reported similar observations with respect to platelet lipids.

It was necessary to ascertain whether increased dietary corn oil resulted in increased levels of LA or AA in the membranes of the dorsal epidermis, particularly in phosphatidylcholine, the phospholipid whose turnover is most enhanced by TPA (43). Fatty acid analysis of skin phospholipids revealed an increase in LA, particularly in phosphatidylcholine, which correlated with dietary levels. A reduction in phosphatidylcholine AA was observed with increasing dietary LA, that was generally associated with a decrease in TPA-induced PGE2 levels. A reduction in substrate (AA) would be expected to result in diminished product formation. In addition, it has been reported that the metabolism of AA to PGE2 can be inhibited by several fatty acids, including LA, linolenic acid, and oleic acid (44, 45). Multiple regulatory mechanisms may thus account for the imperfect correlation observed between AA and PGE2 levels.

### Table 4 Percentage of arachidonic acid and linoleic acid in the phospholipid fractions of mouse epidermis

<table>
<thead>
<tr>
<th>LA and AA levels (% of total fatty acids)</th>
<th>1.0%*</th>
<th>3.6%</th>
<th>6.0%</th>
<th>7.9%</th>
<th>9.9%</th>
<th>12.5%</th>
<th>15.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylcholine 18:2</td>
<td>16.5 ± 4.3</td>
<td>17.6 ± 1.9</td>
<td>20.9 ± 3.6</td>
<td>23.8 ± 3.9</td>
<td>22.3 ± 2.8</td>
<td>22.7 ± 3.7</td>
<td>24.2 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>4.9 ± 0.8</td>
<td>5.5 ± 2.4</td>
<td>3.7 ± 1.1</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.6</td>
<td>2.8 ± 1.0</td>
<td>4.4 ± 1.2</td>
</tr>
<tr>
<td>Phosphatidylethanolamine 18:2</td>
<td>13.2 ± 3.7</td>
<td>15.0 ± 1.7</td>
<td>18.5 ± 3.3</td>
<td>15.4 ± 5.4</td>
<td>18.2 ± 2.9</td>
<td>18.4 ± 4.6</td>
<td>18.5 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>9.9 ± 1.7</td>
<td>14.4 ± 5.8</td>
<td>13.7 ± 3.8</td>
<td>14.8 ± 5.0</td>
<td>8.9 ± 1.2</td>
<td>11.2 ± 2.1</td>
<td>12.9 ± 1.0</td>
</tr>
<tr>
<td>Phosphatidylserine/inositol 18:2</td>
<td>6.6 ± 4.7</td>
<td>14.4 ± 4.5</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>15.4 ± 3.3</td>
<td>15.7 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>8.1 ± 2.6</td>
<td>1.6 ± 4.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>6.7 ± 0.5</td>
<td>9.2 ± 1.7</td>
</tr>
<tr>
<td>Phosphatidylethanolamine 20:4</td>
<td>18:2</td>
<td>6.6 ± 4.7</td>
<td>14.4 ± 4.5</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20:4</td>
<td>8.1 ± 2.6</td>
<td>1.6 ± 4.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Diet (% corn oil).  
* ND, not detected.
The relationship between epidermal PGE$_2$ levels and tumor number is such that reduction in PGE$_2$ is associated with reduction in tumor number, a relationship previously observed with anti-inflammatory agents (15). However, in this study the relationship is not ideal, indicating that reduction of eicosanoids is probably not the only mechanism by which high dietary corn oil inhibits tumor promotion.

It was expected that the reduction in PGE$_2$ with increasing dietary AA would be accompanied by a reduction in the extent of hyperplasia following TPA treatment, since Furstenberger and Marks (46) reported that TPA-induced PGE$_2$ synthesis in mouse skin was an obligatory event for the subsequent hyperplastic response. It is possible that the level of POE? produced, even under the highest dietary LA conditions, was sufficient to support the increased cell proliferation. The lack of a correlation between extent of hyperplasia and tumor yield suggests that this short term parameter may be of limited usefulness in predicting the outcome of tumor studies employing dietary fats.

Pariza and co-workers have recently shown that initiation of mouse epidermal carcinogenesis (17, 47) and induction of ornithine decarboxylase by TPA in forestomach (18) are inhibited by treatment with synthetically prepared CLA. This suggested the possibility that high levels of dietary LA might lead to measurable levels of conjugated dienes in the skin, thereby explaining the protective effect of high levels of dietary LA during promotion. However, under our dietary conditions we were unable to measure detectable quantities of CLA. It is, therefore, unlikely that the level of CLA that may be produced endogenously would approach that used to inhibit tumor initiation (47) or TPA effects (18) or would be sufficient to account
for the observed inhibition of tumor yield seen in the group fed high dietary LA.

In addition to its effects, direct and indirect, on eicosanoid production, the LA content of skin can have profound effects on the regulation of cellular proliferation/differentiation and on the integrity of the permeability barrier. There is substantial evidence (48, 49) that LA can be metabolized by 15-lipoxygenase (normally responsible for the metabolism of AA to 15-hydroxyeicosatetraenoic acid) to HODEs. The finding that HODEs have biological activity has only recently been demonstrated; activity includes 13-HODE inhibition of tumor cell adhesion (50, 51) and the elevation of 13-HODE levels in epidermis associated with psoriasis (52, 53). Application of 13-HODE has also been reported to completely reverse the hyper-proliferation of essential fatty acid deficiency (54). The barrier function of the skin is principally due to the ceramides, which are rich in LA. Since the composition and concentration of lipids making up the lamellar bodies of the barrier affect the ability of topicaly applied agents to penetrate the stratum corneum or horny layer of the skin (55, 56), it is possible that increasing dietary LA decreases the ability of TPA to penetrate. Further investigation is clearly warranted to determine if one or more of these mechanisms are involved in the suppression of tumor promotion provided by high levels of dietary LA.

Another aspect that must be considered is that the mechanisms involved in the promotion stage of skin carcinogenesis are sufficiently different from the mechanisms involved in chemical carcinogenesis of rat mammary tissue. The multistage carcinogenesis model in skin differs from mammary carcinogenesis in several important aspects. Foremost, the mammary model does not employ exogenous or xenobiotic tumor promoters. Prolactin as well as estrogen may act as endogenous promoting agents in the mammary model, but they do not produce an inflammatory state in the mammary gland. In the mouse skin, topical application of tumor promoters, and especially the phorbol esters, causes inflammation, a condition that involves production of high levels of eicosanoids. There is some evidence from the skin model that one role of TPA-induced inflammation during promotion is to cause the overlying epidermis to become hyperplastic (57), an event essential for promotion (58). In the mammary model, increased proliferation is also required, but the cause is not due to inflammation but rather is a result of gland development in the young female. In this model, a role for eicosanoids in the growth of rodent mammary tumors has been implicated. In a review on potential mechanisms by which dietary fat enhances mammary tumorigenesis, Welsch (59) has suggested that the enhancement by high dietary levels of unsaturated fatty acids is through increased prostaglandin synthesis. He further suggested that prostaglandins may be critical growth-stimulatory factors in the tumorigenesis process. In this respect, the mammary and skin models appear to have in common the two elements of increased proliferation and elevated eicosanoids. The extent to which these or other factors are the major determinants or mechanistic processes involved in tumorigenesis in either organ remains uncertain.

There are many examples of organ specificity with regard to types or effects of promoting agents or their modifiers. Because it is also possible that there are species differences in the disposition of essential fatty acids between rat and mouse, studies are currently being conducted to determine the influence of these corn oil diets on mammary carcinogenesis in the SENCAR mouse. This should establish more clearly whether the findings from the skin model represent species or organ site differences.

A final consideration is the effect of saturated fat on tumor promotion. In the experimental diets used in this study, the saturated fat level decreased with increasing corn oil. Although the data here have been interpreted primarily from the point of view of tumor suppression by high levels of dietary LA, they may also be interpreted as enhancement of tumor yield by high levels of saturated fat, although this would not agree with work from other organ models. There are several reports (30, 60, 61) showing that maintenance of animals on diets high in saturated fats prolongs the latent period of tumor appearance and inhibits tumor incidence, compared to corn oil. Although alterations in membrane fluidity have been implicated (59), it remains to be shown how this alters critical cellular processes.

Two conclusions emerge from this study. First, it emphasizes the need to better understand the mechanisms of tumorigenesis in the different organ models. It is difficult to explain the current disparity in the effects of dietary fats on tumorigenesis at different organ sites in the absence of such understanding. Second, caution and more studies are needed before recommendations are made to alter the human diet for cancer prevention.

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REFERENCES


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DIETARY FAT EFFECTS ON TUMOR PROMOTION


Effects of Type of Dietary Fat on Phorbol Ester-elicited Tumor Promotion and Other Events in Mouse Skin

Julius Leyton, Marilyn L. Lee, Mary Locniskar, et al.


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