Dietary Energy and Fat Effects on Tumor Promotion

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Abstract

We investigated dietary modulation, by energy level and energy source, of two-stage skin tumorigenesis initiated with 7,12-dimethylbenz[a]anthracene and promoted with 12-O-tetradecanoylphorbol-13-acetate in SENCAR mice. Studies comparing the influence of dietary calorie restriction (feeding less carbohydrate and less fat) with diet restriction and with ad libitum control feeding indicated an inhibition of papillomas and carcinomas in both restricted groups. The inhibition was greatest in the calorie-restricted group. We reported an increase in the number and incidence of papillomas and the earlier appearance of carcinomas in mice fed a high-fat diet during promotion, in comparison with control groups fed the same caloric allotment. Recent work compared restriction of fat calories (high carbohydrate, restricted fat) with restriction of carbohydrate calories (high fat, restricted carbohydrate), and both protocols resulted in fewer papillomas and carcinomas. Restriction of fat calories resulted in a greater inhibition of papillomas, whereas carcinoma rates were comparable with both protocols. Protein kinase C activity in epidermal cells from mice fed the high-fat diet was higher than activity from mice fed the control diet. Calorie restriction reduced protein kinase C activity. Phosphatidylinositol-inositol phosphate labeling studies suggest alteration of inositol lipid turnover in epidermal cells from mice fed a calorie-restricted diet.

Introduction

Tannenbaum (1, 2) demonstrated the importance of both calorie intake and source of calories as modifiers of skin carcinogenesis. He suggested interactions between fat and calories in carcinogenesis, with calories from fat enhancing carcinogenesis more than calories from carbohydrate (1, 2). Recent studies demonstrated a reduction of mammary (3), colon (4), and skin (5) cancer in animals fed calorie-restricted diets. Evidence from the skin-cancer (5) and mammary-cancer models (6) suggests that fat and calories interact to modify carcinogenesis at these sites. Calories from fat appear to enhance cancer more than do calories from carbohydrate.

This paper reviews work from our laboratory on the influence of calorie intake and level of dietary fat in the two-stage model of skin carcinogenesis in female SENCAR mice. Cancer was initiated with a single application of 10 nmol DMBA (5) and was promoted with twice-weekly treatments with 3.2 nmol TPA throughout the 20-week TPA treatment period and until the end of the experiment, for their influence on promotion.

The results of these studies were presented previously (12) and are summarized here. The high-fat diet fed during initiation reduced development of papilloma, but carcinoma development was not influenced. The high-fat diet fed during and after promotion elevated papilloma numbers (Fig. 3) and elevated promotion by TPA. Studies of dietary fat in skin tumorigenesis, conducted by Leyton et al. (15), did not show effects of calorie intake on skin carcinogenesis.

Effects of Dietary Fat on Skin Carcinogenesis

High-fat diets have enhanced carcinogenesis in a number of tumor systems, including breast (8), colon (9), and pancreas (10). Skin tumorigenesis was elevated in models using polycyclic hydrocarbon carcinogens (11, 12) and UV light-induced skin cancer (13, 14). Our studies compared the influence of control diets, containing 10% of calories from fat, and high-fat diets, containing 40% of calories from fat, on skin cancer initiation with DMBA or promotion with TPA (12). Corn oil was the fat used in all diets in this study. Mice were trained to consume equivalent calorie intakes of the control and high-fat diets. One group was fed the high-fat diet before and during treatment with DMBA, to study the influence of dietary fat on initiation. Another group was fed a high-fat diet during and after treatment with TPA, to study the influence of the diets on tumor promotion.

The results of these studies were presented previously (12) and are summarized here. The high-fat diet fed during initiation reduced development of papilloma, but carcinoma development was not influenced. The high-fat diet fed during and after promotion elevated papilloma numbers (Fig. 3) and elevated development of carcinomas (Table 2). The high-fat diet alone after DMBA initiation, without TPA promotion, did not result in tumors. Thus, the high-fat diet was not a promoter but enhanced promotion by TPA. Studies of dietary fat in skin tumorigenesis, conducted by Leyton et al. (15), did not show effects of calorie intake on skin carcinogenesis.

Effects of Calorie Intake on Skin Carcinogenesis

Previous studies of the influence of calorie intake on carcinogenesis generally fed smaller amounts of a complete diet as a means of calorie restriction (7). This approach results in feeding less of all dietary components, rather than just fewer calories. Our investigations compared feeding less total diet (total-diet restriction) with feeding fewer calories supplied by fat and carbohydrate (5). With the latter approach, the diet contains a higher ratio of other nutrients to calories supplied from carbohydrate and fat. These restricted diets either were fed before and with DMBA treatment, for their influence on initiation, or were fed from the time of the first treatment with TPA throughout the 20-week TPA treatment period and until the end of the experiment, for their influence on promotion.
similar effects of dietary corn oil on skin tumorigenesis. However, those workers used lower levels of dietary fat, kept total fat constant, and followed different treatment protocols (15).

Effects of Restriction of Fat or Carbohydrate Calories on Skin Carcinogenesis

This study compared restriction of fat calories with restriction of carbohydrate calories. The control diet was compared with a diet formulated to allow restriction of either carbohydrate or fat calories. We designated this diet balanced high-fat (because of its high fat content and the balance between fat and carbohydrate calories). Two restricted groups were compared with the balanced high-fat diet, high fat with carbohydrate restricted and high carbohydrate with fat restricted. With both restricted dietary treatments, only the calories from the designated source were reduced. The diets were fed during and after TPA treatment, to study the influence of restriction on tumor promotion. We did not conduct studies to determine the influence of these diets on initiation, because our previous studies showed no influence of calorie restriction on initiation of skin tumors.

The results have not been published, and we will provide a preliminary report here. Restriction of fat or carbohydrate calories reduced papilloma and carcinoma yield, as shown in Table 3. The inhibition was greatest with the high-carbohydrate, calorie-restricted diet. Papillomas were inhibited more by reduction of fat calories than of carbohydrate calories. The diets were fed during and after TPA treatment, to study the influence of restriction on tumor promotion. We did not conduct studies to determine the influence of these diets on initiation, because our previous studies showed no influence of calorie restriction on initiation of skin tumors.

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Effects of Calories and Fat on Epidermal Cell Protein Kinase C

PKC has been identified as a cellular receptor for the phorbol ester tumor promoters (16). PKC is loosely associated with the inner surface of the plasma membrane and is activated by diacylglycerol and calcium (Fig. 4). PKC activity also requires a phospholipid, such as phosphatidylserine (17). We became interested in PKC because of its potential importance in tumor promotion and because of its regulation by membrane lipids.

It is not entirely clear how the interaction of promoters, such as TPA with PKC, affects promotion. Inhibitors of PKC have been studied as potential inhibitors of tumor promotion, with somewhat limited success. In studies with palmitoylcarnitine, inhibition of PKC was correlated with the inhibition of skin-tumor promotion (18); however, the inhibition of PKC by sphingosine3 or staurosporine (19, 20) did not correlate with skin-tumor promotion, because these PKC inhibitors enhanced skin tumor promotion. In addition, overexpression of PKC-B1 in some fibroblast cell lines increased susceptibility to malignant transformation by oncogenes, whereas overexpression of this gene in HT29 human colon cancer cells did not enhance tumorigenicity in nude mice (21). Weinstein (21) suggested that multistage carcinogenesis may be described as a "progressive disorder in signal transduction." Understanding the relationship between TPA and PKC and the impact of this relationship on tumor promotion has priority in many laboratories.

The influence of dietary restriction and restriction of fat or carbohydrate calories on epidermal PKC activity was investigated in two experiments. In the first, mice were restricted by feeding 40% of the control diet, compared with a freely fed control group. In the second experiment, mice were fed control and balanced high-fat diets ad libitum, compared with mice fed 35% fewer calories from fat or calories from carbohydrate, as described above. These diets were fed for 10–13 weeks. Epidermal cells were isolated, lysed, and fractionated into particulate and soluble fractions, and the particulate fraction was resolubilized with Triton X-100 (Rohm and Haas Co., Philadelphia, PA). The fractions were subjected to DEAE-
cellulose acetate chromatography, with a 0–0.3 M KCl gradient. The fractions were collected and PKC activity was measured by using [γ-32P]ATP phosphorylation of lysine-rich histone, in the presence and absence of calcium (22). In general, total PKC activity was reduced in epidermal cells from mice in each of the restricted-diet groups. The restriction of calories from fat and carbohydrate or restriction of calories from fat resulted in higher ratios of soluble to particulate activity than did restriction of total diet or restriction of calories from carbohydrate, respectively.

Results with the particulate fraction from an individual experiment are shown in Fig. 5. They suggest that the particulate form of PKC, which is believed to be the active form, was more profoundly reduced by these dietary restrictions.

Our first studies of dietary fat effects on PKC were in Swiss Webster mice fed control diets (5%) or diets high in corn oil (24.5%) (22). These diets were fed for 4–6 weeks before the epidermal cells were isolated. Epidermal cells from mice fed high-fat diets had elevated PKC activity in the particulate fraction (Fig. 6). We also determined epidermal phospholipid composition and fatty acids in phospholipid fractions (22). In more recent studies, we fed these low- and high-fat diets to SENCAR mice, because our tumorigenesis studies were conducted in this model. The results with SENCAR mice were similar to our observations with Swiss Webster mice; however, both particulate and soluble PKC activities were elevated in the cells from mice fed the high-fat diet.

Effects of Calories and Fat on Epidermal Phosphatidylinositol Metabolism

Phosphatidylinositol metabolism in the plasma membrane was identified as important in receptor-mediated signal transduction pathways (23). Phosphatidylinositol is phosphorylated in the plasma membrane, and hydrolysis by phospholipase C releases diacylglycerol, IP3, and other phosphorylated forms of inositol. IP3 and diacylglycerol function as bifurcating activa-

Fig. 2. Papilloma size in mice fed restricted diets after DMBA treatment. Data are presented as the proportion of papillomas in each size category. Columns, mean; bars, SE. Statistically different values in comparing diet groups within a time interval are indicated by superscripts above the error bars, a < b (P < 0.05). Reprinted by permission of the American Association for Cancer Research from Ref. 5.

Fig. 3. Number of skin papillomas on SENCAR mice fed the high-fat diet during initiation with DMBA (A) or promotion with TPA (B) of skin tumorigenesis. ○, low-fat diet; ●, high-fat diet. In A, a significant inhibition of the number of skin tumors per effective animal was observed in mice fed the high-fat diet. In B, mice fed the high-fat diet had a significantly elevated number of papillomas per effective animal. Reprinted with permission of the American Association for Cancer Research from Ref. 12.
Table 2 Carcinoma incidence and multiplicity in mice fed high-fat diets during initiation or promotion

<table>
<thead>
<tr>
<th>Diets fed during</th>
<th>Week 25–34</th>
<th>Week 30–40</th>
<th>Week 41–44</th>
<th>Cumulative carcinoma yield, week 25–44</th>
<th>Av. survival TBA α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EFA* (no.)</td>
<td>EFA (no.)</td>
<td>EFA (no.)</td>
<td>EFA (no.)</td>
<td>EFA (no.)</td>
</tr>
<tr>
<td></td>
<td>Incidence (%)</td>
<td>CRC/EFA</td>
<td>Incidence (%)</td>
<td>CRC/EFA</td>
<td>Incidence (%)</td>
</tr>
<tr>
<td>Control 0-5</td>
<td>40 3 0.03*</td>
<td>35 23 0.37</td>
<td>27 52 0.89</td>
<td>40 58 0.95</td>
<td>41.2 ± 0.8*</td>
</tr>
<tr>
<td>Control 6-44</td>
<td>26 0 0.00*</td>
<td>25 28 0.40</td>
<td>16 50 0.75</td>
<td>26 58 0.85</td>
<td>38.8 ± 0.9*</td>
</tr>
<tr>
<td>Control High-fat</td>
<td>36 14 0.25*</td>
<td>27 26 0.41</td>
<td>16 44 0.69</td>
<td>36 53 0.86</td>
<td>42.0 ± 1.1</td>
</tr>
</tbody>
</table>

α EFA, effective number of animals; TBA, tumor-bearing animals; CRC, carcinoma.

Table 3 Papilloma yield and carcinoma incidence in mice initiated with DMBA and fed diets restricted in fat (HCR) or carbohydrate (HFR) calories during and after promotion with TPA

<table>
<thead>
<tr>
<th>Diet group</th>
<th>No. of papillomas/mouseα</th>
<th>Carcinoma incidenceb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 14</td>
<td>Wk 24</td>
</tr>
<tr>
<td>BHF*</td>
<td>38</td>
<td>4.3 ± 0.01*</td>
</tr>
<tr>
<td>HCR</td>
<td>40</td>
<td>0.4 ± 0.01*</td>
</tr>
<tr>
<td>HFR</td>
<td>42</td>
<td>1.9 ± 0.01*</td>
</tr>
<tr>
<td>Control</td>
<td>38</td>
<td>4.1 ± 0.01*</td>
</tr>
</tbody>
</table>

α Values presented as mean ± SE. Statistical differences in each column determined by t tests on the basis of the analysis of variance; c > d > e (P < 0.05).

β Statistical differences in each column determined by χ² tests and shown with superscripts c > d for individual values and e > f for freely fed (BHF and control), compared with restricted (HCR and HFR), groups (P < 0.05).

BHF, balanced high-fat; HCR, high-carbohydrate, fat-restricted; HFR, high-fat, carbohydrate-restricted.

Fig. 4. Major pathways for modulation of PKC by extracellular signals and TPA. PI, phosphatidylinositol; PIP, phosphatidylinositol-4-phosphate; PIP2, phosphatidylinositol-4,5-biphosphate; PLC, phospholipase C; DAG, diacylglycerol; ER, endoplasmic reticulum; IP3, inositol triphosphate; IP2, inositol biphosphate; IP1, inositol phosphate.

Fig. 5. PKC activity in the solubilized particulate fraction from epidermal cells isolated from mice on calorie-restricted and total-diet-restricted protocols. The kinase activity was measured in the presence of 500 μM CaCl2 (C) or 100 μM ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (O). All samples contain phosphatidylserine.

Tors of PKC. Diacylglycerol directly activates PKC. IP3 has been identified as a second messenger; it stimulates the release of calcium from intracellular stores, such as the endoplasmic reticulum, and this calcium is required for activation of PKC (23). Because of the importance of inositol lipid metabolism in the regulation of PKC, we initiated studies on the influence of dietary fat and calorie intake on phosphatidylinositol turnover.

Mice were fed diets restricted in calories from fat and carbohydrate in our first experiment. Epidermal cells were isolated and cultured in inositol-free medium before being labeled with [3H]inositol. Epidermal cells from mice fed control and calorie-restricted diets were incubated until the cells were uniformly labeled with inositol. They were then washed and incubated in medium containing lithium chloride, to block many of the phosphatases that act on the phosphorylated inositides and effectively prevent much reuse of inositol. The results of this experiment indicated that phosphatidylinositol levels remained higher in mice fed calorie-restricted diets. This suggests that inositol metabolism may be slower in calorie-restricted mice than in control mice. Thus, reduced calorie intake may reduce phosphatidylinositol turnover and thereby reduce PKC activation. The influence of high-fat diets on the metabolism of inositol lipids is under investigation.

10 E. S. Kris, M. Choe, H. Pinch, T. Barnett, and D. F. Birt, unpublished observations.
aminoethyl ether)-A',/V,A'',A"-tetraacetic acid ( ). All samples contain phos- 
phatidylserine. Reprinted with permission of Oxford University Press from Ref. 
measured in the presence of 500 UMCaCl2( ) or 100 ^M ethylene glycol bis(/i-
isolated from mice on a low-fat diet or a high-fat diet. The kinase activity was 

carbohydrate) modify cancer promotion. Calorie restriction is 
Discussion 
Our studies on two-stage skin tumorigenesis in SENCAR 
mice indicate that both calorie intake and calorie source (fat or 
the effects of diet on skin tumorigenesis in SENCAR mice indicate that both calorie intake and carbohydrate source (fat or carbohydrate) modify cancer promotion. Calorie restriction is a strong inhibitor, and feeding a high-carbohydrate, calorie-restricted diet causes greater inhibition than does feeding a high-fat, calorie-restricted diet. These results agree with observations reported by Ip (3) from studies of DMBA-induced mammary cancer in rats. 
The effects of diet on epidermal-cell PKC correlate well with the effects of diet on skin tumorigenesis in SENCAR mice. Groups with the lowest incidence of skin papilloma and carcinoma yields had the greatest reduction in epidermal PKC, particularly in the particulate fractions. Lipids were also shown to modify colonic crypt PKC activity (24). Saturated fatty acids or unsaturated fatty acids were administered to cultured crypt-cell preparations or given to animals by gavage. PKC activity shifted toward the particulate fraction in the cells treated with 
Modification of inositol lipid metabolism by calorie restriction 
Modification of inositol lipid metabolism by calorie restriction may involve several aspects of the cellular effects of diet restriction. Restricted animals may have a lowered hormonal responsiveness because of a reduction in this critical aspect of the signal-transduction pathway (23). It is possible that reduced levels of phosphorylated forms of phosphatidylinositol may reduce PKC activity in epidermal cells from mice fed calorie-restricted diets. This biochemical aspect of dietary restriction merits further investigation.

References

Fig. 6. PKC activity in the solubilized particulate fraction from epidermal cells isolated from mice on a low-fat diet or a high-fat diet. The kinase activity was measured in the presence of 500 ^M CaCl2( ) or 100 ^M ethylene glycol bis(β-aminoethyl ether)-N,N',N''-tetraacetic acid ( ——— ). All samples contain phosphatidylserine. Reprinted with permission of Oxford University Press from Ref. 22.
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