Induction of Apoptosis by Conjugated Linoleic Acid in Cultured Mammary Tumor Cells and Premalignant Lesions of the Rat Mammary Gland

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Abstract

Conjugated linoleic acid (CLA) is an effective agent in preventing mammary cancer in rats treated with a carcinogen. The appearance of a tumor mass is the net result of cell proliferation minus cell death. Thus, apoptosis could be an important mechanism in controlling clonal expansion of the early premalignant lesions. The overall objective of this report was to determine whether CLA stimulated apoptosis. In the first part of the study, CLA was found to increase chromatin condensation (visualized through fluorescent 4',6-diamidino-2-phenylindole staining to DNA) and to induce DNA ladderization, both evidence of apoptosis, in a rat mammary tumor cell line. The second part was to investigate the effect of CLA feeding on the development of histologically identifiable premalignant lesions in the rat mammary gland, as well as on the quantification of apoptosis (by terminal uridyltransferase nick end labeling assay) and the expression by immunohistochemistry of apoptosis regulatory proteins (bcl-2, bak, and bax) in normal versus premalignant mammary structures. CLA inhibited the formation of premalignant lesions by ~50%. It also significantly increased apoptosis and reduced the expression of bcl-2 in these lesions, but it did not modulate the levels of bak or bax. In contrast, neither apoptosis nor any of the apoptosis regulatory proteins was affected by CLA in normal mammary gland alveoli or terminal end buds. The data suggest that early pathological lesions may be particularly sensitive to CLA. In addition to providing a molecular basis for elucidating the mechanism of action of CLA in cancer prevention, the research on CLA-responsive biomarkers also has a practical side because these assays can be applied to biopsied human tissue samples in future CLA intervention trials.

Introduction

Previous research showed that CLA is very effective in protection against tumor development in the rat mammary gland (1–3). CLA has been found to inhibit the proliferation of TEB cells, which are the primary target sites for chemical induction of mammary carcinogenesis in the rodent model (4). Tumor growth, however, is the net result of cell proliferation minus cell death (5); thus, the appearance of a tumor mass is likely to be suppressed by enhancing cell death in addition to decreasing cell proliferation. The scenario predicts a slow down in the expansion of the transformed clones, thereby delaying the onset of clinical signs of malignancy and reducing the number of tumors observed over a defined period of time. These desirable attributes are the hallmark of cancer prevention.

Using a mammary epithelial organoid primary culture system derived from enzymatically digested mammary gland of untreated young virgin rats, we recently reported that CLA is able to induce apoptosis, or programmed cell death, within the differentiated epithelial colonies (6). Cultures that were exposed to CLA had significantly more pyknotic nuclei, and the lumen of the organoids was full of apoptotic bodies and cell debris. Two different CLA preparations were evaluated in the above in vitro experiment: one was enriched with the 9,11-CLA isomer, and the other contained a mixture of 8,10-, 9,11-, 10,12- and 11,13-isomers. Both preparations were equally effective in enhancing apoptosis in organoids cultivated from normal non-transformed mammary epithelial cells.

In the first part of this report, we extended the in vitro apoptosis studies to a rat mammary tumor cell line. The objective was to determine whether neoplastic cells would respond similarly to CLA for induction of apoptosis which, in the present experiments, was assessed by the techniques of (a) fluorescent DAPI binding to DNA, and (b) DNA ladderization. The second part of the report is focused on in vivo studies that were designed to investigate the effect of CLA feeding on the development of histologically identifiable premalignant lesions in the rat mammary gland as well as on the quantification of apoptosis and the expression of apoptosis regulatory proteins in normal versus premalignant mammary structures. Rats in these experiments were given either the enriched 9,11-CLA isomer or the mixture of CLA isomers as described in our recent publication (7).

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Materials and Methods

In Vitro Assays of Apoptosis and Cell Growth. The NMU cell line, established from a rat mammary adenocarcinoma induced by NMU, was purchased from American Type Culture Collection (Rockville, MD) and passaged routinely in DMEM (DMEM/F12; Life Technologies, Grand Island, NY) containing 10% (v/v) FBS. For evaluation of the effect of CLA and LA on nuclear morphology, NMU cells were plated on coverslips (10^5 cells/coverslip), which were placed in each well of a 6-well plate and allowed to adhere in DMEM/F12 medium containing 10% FBS for 2 h. Five ml of the appropriate culture medium (DMEM/F12 containing 2% FBS, with or without CLA or LA) were then added to each well, and the cells were cultured for 72 h. Both fatty acids, obtained from Nu-Chek Prep (Elysian, MN), were added as sodium salts. They were prepared and diluted into 2% FBS as described previously (6). The CLA used in these in vitro experiments contained a mixture of isomers, the composition of which is given in the next subsection. At the end of the culture period, the slides were fixed in ice-cold acetone for 5 min, rehydrated in PBS for ~10 min, then stained with 1 µg/ml DAPI (Sigma, St. Louis, MO) in PBS for 5 min. The slides were then rinsed in PBS and water, and mounted on glass slides with aqueous Polymount (Polysciences, Inc., War rington, PA). Slides were viewed on an Olympus BX40 epifluorescence microscope, and the percentage of apoptotic cells was calculated by counting a total of ~1200 cells on each of triplicate slides for each group.

For evaluation of the effect of CLA and LA on DNA laddering, NMU cells (2 × 10^6 cells) were plated in 100-mm dishes and allowed to adhere overnight in DMEM/F12 containing 10% FBS, and the media was then replaced with DMEM/ F12 containing 2% FBS, with or without CLA or LA. Cells were cultured for 15, 24, 48, 72, or 96 h, both adherent and floating cells were collected, and DNA was extracted, labeled with α-32P-dCTP (NEN, Boston, MA), and evaluated using the TACS apoptotic DNA laddering kit ( Trevigen, Inc., Gaithersburg, MD).

For evaluation of the effect of CLA and LA on cell growth, NMU cells (2 × 10^4 cells/well) were plated in each well of a 24-well plate, allowed to adhere for 4 h in DMEM/F12 containing 10% FBS, then cultured for 24, 72, or 96 h in media containing 2% FBS with or without CLA or LA. Viable cell number was quantitated in triplicate wells by a modification of the MTT assay that we have described previously (8, 9).

Experimental Protocol for Rat CLA Feeding Studies. Pathogen-free female Sprague Dawley rats were purchased from Charles River Breeding Laboratories (Raleigh, NC) at 45 days of age. On arrival, they were fed a basal diet with no CLA to acclimatize them to a powdered ration. This diet consisted of 5% corn oil, 20% casein, 65% dextrose, 3.5% AIN-76 mineral mix, 1% AIN-76A vitamin mix, 5% alphacel, 0.3% methionine, and 0.2% choline bitartrate. After 1 week, all rats were given an i.p. injection of NMU at a dose of 50 mg/kg of body weight. Immediately after carcinogen treatment, they were divided into three dietary groups of six rats each: (a) basal diet; (b) basal diet + 1% enriched 9,11-CLA; and (c) basal diet + 1% CLA mixture. Animals were sacrificed at 6 weeks after CLA supplementation. The enriched 9,11-CLA isomer (cis 9, trans 11-CLA) and the CLA mixture were purchased from Matreya (Pleasant Gap, PA) and Nu-Chek (Elysian, MN), respectively. The Matreya product contained >95% 9,11-CLA, whereas the Nu-Chek product contained a mixture of 17.6% 11,13-CLA; 36.5% 10,12-CLA; 25.3% 9,11-CLA; and 15.3% 8,10-CLA.

The detailed methodology for quantifying the different CLA isomers was reported previously (7).

Quantification of Premalignant Lesions in the Mammary Gland. As noted in the “Introduction,” TEBs are the primary sites for chemical induction of mammary adenocarcinomas in the rat mammary gland. Within 2–3 weeks after carcinogen dosing, enlargement of the TEB, characterized by a localized piling up of intraductal cells, is detectable in histological sections. These early transformed lesions, which are known as IDPs, are the precursors for the eventual formation of palpable carcinomas. At necropsy, the abdominal-inguinal mammary gland chains on both sides were excised in one piece, fixed in methacarn, and processed in a Tissue-Tek Vacuum Infiltration Processor (Miles Scientific, Elkhart, IN). Each mammary gland whole mount was divided into six segments and embedded into paraffin blocks. Ribbons of 5-µm thickness were cut from each block and placed on slides that had been treated with 3-aminopropyltriethoxysilane. Every tenth section was heat-immobilized, deparaffinized in xylene, rehydrated in descending grades of ethanol (100% to 70%), and stained with H&E. These H&E slides were examined under the microscope for the appearance of IDP lesions using the criteria described by Russo et al. (10). Once a section showing the pathology of an IDP was found, the in-between slides were similarly stained to confirm the histology. The size of each IDP lesion could thus be estimated operationally by the number of serial sections showing the same pathology.

Immunohistochemical Staining of Apoptosis, bcl-2, bak, and bax. Apoptotic cells in mammary gland sections were determined by the TUNEL assay with the Apoptag kit (Intergen, Purchase, NY). Fragmentation of DNA is known to occur during apoptosis. The assay is based on the addition of digoxigenin-labeled nucleotides to the numerous 3′-hydroxyl ends generated as a result of DNA breaks. The reaction is catalyzed by the terminal deoxynucleotidyl transferase enzyme. Detection of apoptosis by this method is commonly referred to as the TUNEL assay. Immunohistochemical staining was accomplished by exposure to antidigoxigenin antibody following the procedure supplied with the kit. Apoptotic cells were identified by a brown stain over the nuclei. All hard copy images were coded so that the persons analyzing the data were blinded to the group assignment to avoid bias.

Antibodies for immunohistochemical staining of apoptosis regulatory proteins were obtained commercially: mouse monoclonal anti-bcl-2 from Beckman-Coulter (Miami, FL), rabbit polyclonal anti-bak from Upstate Biotechnology (Lake Placid, NY), and rabbit anti-bax from Santa Cruz Biotechnology (Santa Cruz, CA). These antibodies were applied at the following dilutions and exposure times: anti-bcl-2 at 1:100 for 1 h at ambient temperature, and anti-bak and anti-bax at 1:1000 for overnight at 4°C. After the tissue sections were incubated with the primary antibody, they were treated with a biotinylated goat antirabbit or rabbit antimouse secondary antibody (Chemcon, Temecula, CA). This was followed by the addition of streptavidin horseradish peroxidase (Dako, Carpenteria, CA), which binds to biotin. Diaminobenzidine (Research Genetics, Huntsville, AL) was used as the chromogen to generate a brown precipitate attributable to its reaction with peroxidase. All slides were counterstained with hematoxylin, rinsed, dehydrated, and mounted with Permount (Fisher Scientific, Fair Lawn, NJ). Cells expressing the antigen were identified by a brown stain in the cytoplasm. The signal was scored using a method adapted from Allred et al. (11). The score is based on estimating both the percentage of positively stained cells on a slide (proportion...
Results

In Vitro Growth Inhibition and Apoptosis Induction of NMU Rat Mammary Tumor Cells by CLA. Previous studies demonstrated that CLA inhibited the growth of normal rat mammary epithelial cells in primary culture and that this effect was exerted, at least in part, by stimulating apoptosis (6). This suggested that CLA might also be effective in inhibiting the growth of rat mammary tumor cells. To examine this, the effect of CLA was compared with that of LA for its effect on the growth of NMU rat mammary tumor cell line. To analyze the size distribution data, a repeated measures option was added to the Poisson regression model (13). For the immunohistochemistry of bcl-2, bak, and bax, differences in score were analyzed using a Kruskal-Wallis rank test (14).

Reduction of Premalignant Lesions in Mammary Gland by CLA Feeding. A composite histological micrograph showing the pathology of IDP progression from TEBs was presented in a recent publication (15). At a few weeks after NMU dosing, cells at the tip and neck of the TEB begin to proliferate and invade into the cavity of the ductal structure. The development of the IDP lesion continues until the lumen of the duct is either partially or completely occupied. As noted in “Materials and Methods,” the size of each IDP lesion was estimated by the number of serial sections exhibiting this typical pathology. Fig. 4 illustrates the quantitative IDP count from NMU-treated rats that were given either the CLA mixture or 9,11-CLA. All of the lesions were categorized into five size classes with each containing ≥10, 11–20, 21–30, 31–40, or >40 serial sections, respectively. First, to analyze the size distribution data, a repeated measures option was added to the Poisson regression (13) because most animals presented lesions in more than one size class. No significant decreases were found by either CLA.
treatment within a given size class, probably because of the small sample number in each category when the data were segregated; this reduced statistical power to detect significant differences attributable to treatment. Next, the total number of lesions across all size classes was added up arithmetically, and the data were analyzed. There were 56 lesions found in a total of six rats in the control group. Treatment with either the CLA mixture or 9,11-CLA reduced the number of IDPs to 30 and 27, respectively \((P < 0.05)\). Thus CLA was able to inhibit the formation of premalignant lesions in the mammary gland.

**Stimulation of Apoptosis by CLA in IDP Lesions.** In view of the evidence that CLA is able to induce apoptosis in cultured rat mammary tumor cells, we proceeded to examine the effect of CLA on apoptosis in both normal and premalignant mammary gland structures (Table 1). This was done by using the TUNEL assay in paraffin-embedded sections. The sections containing the different structures all came from rats treated with NMU. CLA did not cause any change in apoptosis in the normal alveoli and TEBs. In contrast, both CLA preparations were effective in enhancing apoptosis in the IDP lesions \((P < 0.05)\).

**Table 1** Effect of CLA feeding on apoptosis in different mammary gland structures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Alveoli</th>
<th>TEB</th>
<th>IDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.22 ± 0.01</td>
<td>0.46 ± 0.03</td>
<td>0.47 ± 0.03</td>
</tr>
<tr>
<td>CLA mixture</td>
<td>0.24 ± 0.02</td>
<td>0.52 ± 0.03</td>
<td>1.03 ± 0.07(^b)</td>
</tr>
<tr>
<td>9,11-CLA</td>
<td>0.20 ± 0.01</td>
<td>0.54 ± 0.04</td>
<td>0.98 ± 0.08(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Because of the low rate of apoptosis, \(\sim 20,000–25,000\) cells were counted for each type of structure per group. The slides were obtained from six rats for each treatment. The results are expressed as mean ± SE \((n = 40–60 fields)\).

\(^b\) \(P < 0.05\) compared with the corresponding control value.

**Fig. 3.** Increase in DNA laddering in NMU rat mammary tumor cells by CLA. NMU cells were cultured for 15, 24, 48, 72, or 96 h with 32 \(\mu\)M CLA or vehicle, and DNA was extracted, radiolabeled, and electrophoresed on a 1.5% agarose gel. This figure, which is representative of three separate experiments, demonstrates chromatin fragmentation in the CLA group, but not in the control group.

**Fig. 4.** Effect of CLA feeding on the formation of IDP lesions in the mammary gland at 6 weeks after NMU. The size of the IDP lesions was classified operationally according to the number of serial sections \((\leq 10, 11–20, 21–30, 31–40, \text{and} >40)\) showing the same pathology. The total number of IDP lesions in each treatment group \((n = 6\text{ rats/group})\) was calculated based on the sum of the five size classes.

**SIZE DISTRIBUTION**

**Fig. 5.** A and B, shows representative pictures of an IDP slide from the control group and the Nu-Chek CLA group, respectively. The arrows highlight the chromogen-generated brown stain, which is indicative of apoptotic cells. It should be noted that in almost every case, the brown stain overlaps the condensed chromatin of apoptotic bodies, thus confirming that the TUNEL assay correlates well with the morphological appearance of apoptosis. The rate of apoptosis is generally very low in the IDP lesions as well as in other structures of the mammary gland. Typically, for an IDP slide from the control group, we found an average of 3–4 apoptotic cells in a field of about 700 cells (Fig. 5A). This number increased to an average of eight to nine stained cells in the CLA group (Fig. 5B).

**CLA Modulation of Apoptosis Regulatory Proteins in Mammary Gland.** The expression of three apoptosis regulatory proteins was also evaluated by immunohistochemistry. They included bcl-2, which is known as a repressor of apoptosis, as well as bak and bax, which are inducers. Table 2 summarizes the score (see “Materials and Methods”) for bcl-2 expression in different mammary gland structures. There was no difference in the bcl-2 score between the control and CLA groups in either alveoli or TEBs. However, the expression of bcl-2 was markedly reduced by both CLA preparations in the IDP lesions. It is important to realize that although the score in the two CLA groups was approximately half of that of the control, it does not mean that the level of bcl-2 was decreased...
This is attributable to the fact that the stoichiometry of antibody and antigen interaction in the immunohistochemical assay is not known. Representative staining pictures from a control and a CLA slide of an IDP section are presented in Fig. 5, C and D, respectively. The patterns show an exclusive cytoplasmic localization of the antibody. The staining intensity was considerably more robust in the control than in the CLA sample, although the proportion of positively stained cells was roughly the same between the two.

Tables 3 and 4 summarize the data on bak and bax expression. Neither protein appeared to be affected by CLA in any of the mammary gland structures, including IDP lesions. Fig. 6 presents a composite of bak and bax staining, respectively, in alveoli, TEBs, and IDP. Although there was no difference between the control and treatment groups (hence no need to display a comparison), the pictures are included to show (a) the distinctive morphology of the three types of mammary gland structures examined in this study; (b) the cytoplasmic localization of bak and bax; (c) the fairly abundant expression of these two positive apoptosis regulatory proteins despite the very low rate of apoptosis detected in the mammary gland (Table 1); and (d) the rather uniform level of expression of bak and bax among the three kinds of structures (as reflected by the scores summarized in Tables 3 and 4).

![Representative immunohistochemistry pictures (magnification, 40×) of TUNEL apoptotic cells (A and B) and bcl-2 expression (C and D) in IDP lesions. Arrows, apoptotic cells.](Image)
mean bcl-x proteins homologous to bcl-2 that either repress (e.g., Extensive research has since revealed that bcl-2 is just one contributing factor to neoplastic growth by blocking apoptosis (16).

proteins act as molecular switches in either a positive manner. Bcl-2 was originally recognized as a con-proliferation of bcl-2 is associated with the nonapoptotic outer layer of lesions of the mammary gland. Interestingly, no stimulation of apoptosis by CLA was seen in alveoli and TEBs, which represent two of the normal structural components of the mammary tree. The data seem to indicate that early pathological lesions may be particularly sensitive to CLA-mediated arrest of their clonal expansion, a finding that is consistent with the decreased number of IDPs found in the mammary gland. Apoptotic cell death is governed by a large number of genes whose protein products act as molecular switches in either a positive or negative manner. Bcl-2 was originally recognized as a contributing factor to neoplastic growth by blocking apoptosis (16).

Extensive research has since revealed that bcl-2 is just one member of what is now a multigene family that consists of proteins homologous to bcl-2 that either repress (e.g., bcl-2 and bcl-xL) or induce (e.g., bak, bax, and bad) apoptosis. These two classes of proteins are known to heterodimerize in counteracting each other (17). In the present study, we did not observe an effect of CLA on bak and bax, suggesting that these two apoptosis inducer proteins may not play a critical role in the action of CLA. However, we did detect a down-regulation of bcl-2 by CLA in the IDP cells. Whether this effect is causally related to apoptosis remains to be clarified.

Is there evidence of a functional role for bcl-2 in mammary gland biology or neoplastic progression? Information in this area is meager but suggestive. Humphreys et al. (18) proposed that apoptosis is an important mechanism in ductal morphogenesis from the highly proliferative TEBs during pubescent mammary gland development in mice. The proportion of cells undergoing apoptosis depends on their location in the TEB structure, with more apoptotic cells in the inner few layers than in the outer layer. Consistent with this, a high level of expression of bcl-2 is associated with the nonapoptotic outer layer of cells. Additionally, it is generally accepted that mammary gland involution after the end of lactation is a result of an increase in apoptosis. Merlo et al. (19) reported that the expression of bcl-2 is decreased, with little accompanying change in bax, in the involuting gland, suggesting that bcl-2 might serve as an intracellular mediator of signals that influence the apoptotic activity of mammary epithelial cells and mammary gland remodeling. In human breast cancer, a recent study of a subset of 49 tumor samples showed that a low level of bcl-2 is strongly correlated with a high rate of apoptosis (20). Variations in bcl-2 expression have also been documented in ductal hyperplasia of the breast (21). Further research will be needed to determine whether a down-regulation of bcl-2 in premalignant and malignant breast cells is likely to have an impact on the development of a less aggressive phenotype, which in turn may lead to a more favorable prognostic outcome. Atypical ductal hyperplasia, a purported precursor to invasive carcinoma of the breast, is equivalent to IDP in the rat model. The concept of CLA modulation of bcl-2 in IDP lesions is novel and exciting. Potentially, it provides a molecular anchor to elucidate the significance of CLA nutrition in cancer prevention that is targeted at an early stage of the disease. Finally, it is tempting to speculate that CLA may exert a therapeutic effect against some breast tumors through its ability to lower bcl-2, thus rendering them more sensitive to chemotherapy.

The investigation of CLA-responsive biomarkers also has a practical side because these assays can be applied to biopsied human tissue samples in future CLA intervention trials. A variety of methodology is available to evaluate multiple biomarkers in a small amount of biopsied material. They include immunohistochemistry, flow cytometry, PCR amplification of cDNA, and so forth. As noted in the results, the technique of immunohistochemistry has a major pitfall in that it does not provide precise quantitative data. It only gives an estimation of either “more” or “less” than what is present in the control. On the other hand, immunohistochemistry is the best way to detect distinctive expression patterns associated with a specific cell population because the morphology can be unequivocally identified under the microscope. To characterize phenotypic changes in defined structures of the mammary gland, i.e., alveoli, TEBs, and IDP lesions, there is little choice but to use histological methods in the present experimental design.

Based on the results of this study, we were unable to distinguish the efficacy of the 9,11-CLA isomer and the mixture of CLA isomers in decreasing the formation of IDP lesions or in modulating apoptosis and the expression of apoptosis regulatory proteins. These observations are reminiscent of the findings from a recent study in the same rat model in which we demonstrated that these two CLA preparations, when present at 1% level in the diet, were equally effective in reducing mammary epithelial mass, in suppressing cell proliferation, and in blocking mammary tumorigenesis (7). There are a number of explanations that may account for the similarity in efficacy. At the 1% level of enriched 9,11-CLA, we might already have reached a plateau effect, and as a result, we could not discriminate this dose from the 0.25% 9,11-CLA dose provided by the Nu-Chek preparation (one-fourth of the total CLA in the Nu-Chek material is represented by the 9,11-isomer; see “Materials and Methods”). Alternatively, it is possible that in addition to the 9,11-CLA isomer, one or more of the other CLA isomers (e.g., 8,10-, 10,12- and 11,13-CLA) are also capable of altering the various biological end points that we have studied. Moreover, these latter isomers may have a greater activity than the 9,11-isomer, and that is the reason why the mixture of CLA isomers is equipotent to the enriched 9,11-CLA isomer. In support of this hypothesis, we have in vitro data indicating that 10,12-CLA is 20 times more powerful than 9,11-CLA in inhibiting the proliferation of the NMU rat mammary tumor cell line. There is a growing realization that specific CLA isomers may have unique biological effects. For example, the 10,12-CLA and not the 9,11-CLA is believed to be the active isomer responsible for reducing body fat accretion in mice (22) and milk fat synthesis in lactating cows (23). To answer the question of isomer specificity, further experimentation will have to be carried out in characterizing the dose-response profile of each CLA isomer under both in vitro and in vivo situations and with respect to a defined set of biological end points.

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**Table 4** Effect of CLA feeding on bax expression in different mammary gland structures

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Immunohistochemical score for bax*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Alveoli</td>
</tr>
<tr>
<td>Control</td>
<td>8.2 ± 0.6</td>
</tr>
<tr>
<td>CLA mixture</td>
<td>8.5 ± 0.7</td>
</tr>
<tr>
<td>9,11-CLA</td>
<td>9.3 ± 0.8</td>
</tr>
</tbody>
</table>

* Each score represents the results from six slides per rat, six rats per group, mean ± SE.

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Chek material is represented by the 9,11-isomer; see “Materials and Methods”). Alternatively, it is possible that in addition to the 9,11-CLA isomer, one or more of the other CLA isomers (e.g., 8,10-, 10,12- and 11,13-CLA) are also capable of altering the various biological end points that we have studied. Moreover, these latter isomers may have a greater activity than the 9,11-isomer, and that is the reason why the mixture of CLA isomers is equipotent to the enriched 9,11-CLA isomer. In support of this hypothesis, we have in vitro data indicating that 10,12-CLA is 20 times more powerful than 9,11-CLA in inhibiting the proliferation of the NMU rat mammary tumor cell line. There is a growing realization that specific CLA isomers may have unique biological effects. For example, the 10,12-CLA and not the 9,11-CLA is believed to be the active isomer responsible for reducing body fat accretion in mice (22) and milk fat synthesis in lactating cows (23). To answer the question of isomer specificity, further experimentation will have to be carried out in characterizing the dose-response profile of each CLA isomer under both in vitro and in vivo situations and with respect to a defined set of biological end points.

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Unpublished data.
Acknowledgments

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References


Fig. 6. Representative immunohistochemistry pictures (magnification, 40×) of bak (A, B, and C) and bax (D, E, and F) expression in different structures of the mammary gland.


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