The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet

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The objective of the present study was to investigate whether the anticarcinogenic activity of conjugated linoleic acid (CLA) is affected by the amount and composition of dietary fat consumed by the host. Because the anticancer agent of interest is a fatty acid, this approach may provide some insight into its mechanism of action, depending on the outcome of these feeding experiments. For the fat level experiment, a custom formulated fat blend was used that simulates the fatty acid composition of the US diet. This fat blend was present at 10, 13.3, 16.7 or 20% by weight in the diet. For the fat type experiment, a 20% (w/w) fat diet containing either corn oil (exclusively) or lard (predominantly) was used. Mammary cancer prevention by CLA was evaluated using the rat dimethylbenz[a]anthracene model. The results indicated that the magnitude of tumor inhibition by 1% CLA was not influenced by the level or type of fat in the diet. It should be noted that these fat diets varied markedly in their content of linoleate. Fatty acid analysis showed that CLA was incorporated predominantly in mammary tissue neutral lipids, while the increase in CLA in mammary tissue phospholipids was minimal. Furthermore, there was no evidence that CLA supplementation perturbed the distribution of linoleate or other fatty acids in the phospholipid fraction. Collectively these carcinogenesis and biochemical data suggest that the cancer preventive activity of CLA is unlikely to be mediated by interference with the metabolic cascade involved in converting linoleic acid to eicosanoids. The hypothesis that CLA might act as an antioxidant was also examined. Treatment with CLA resulted in lower levels of mammary tissue malondialdehyde (an end product of lipid peroxidation), but failed to change the levels of 8-hydroxydeoxyguanosine (a marker of oxidatively damaged DNA). Thus while CLA may have some antioxidant function \textit{in vivo} in suppressing lipid peroxidation, its anticarcinogenic activity cannot be accounted for by protecting the target cell DNA against oxidative damage. The finding that the inhibitory effect of CLA maximized at 1% (regardless of the availability of linoleate in the diet) could conceivably point to a limiting step in the capacity to metabolize CLA to some active product(s) which is essential for cancer prevention.

Introduction

Conjugated linoleic acid (CLA\(^*\)) is a positional and geometric isomer of linoleic acid (1). It is a minor fatty acid found preferentially in beef and dairy products (2). In contrast to linoleic acid, which has been found consistently to enhance mammary tumorigenesis in rodents over a wide concentration range (3–5), CLA expresses an inhibitory effect at levels of 1% or less in the diet (6,7). Recently, we described two distinct activities of CLA in mammary cancer prevention with the use of the methylnitrosourea (MNU) model (8). First, exposure to CLA during the early post-weaning and peripubertal period only (21–42 days of age) is sufficient to block subsequent tumorigenesis induced by a single dose of MNU given at 56 days of age. This observation suggests that CLA is able to effect certain changes in the immature mammary gland and render it less susceptible to neoplastic transformation later in life. Second, CLA is also active in suppressing tumor promotion/progression. However, this mode of action is different from the first in that once the mammary cells have been initiated by a carcinogen, a continuous intake of CLA is necessary to achieve maximal inhibition.

The above cited studies on CLA chemoprevention (6–8) were carried out in rats fed a 5% (w/w) fat diet formulated with corn oil. Currently, there is no information as to whether an increase in the level of fat or a substitution of the type of fat in the diet might affect the cancer inhibitory efficacy of CLA. The experiments described in this paper were designed to address this question. Because the anticancer agent of interest is a fatty acid, it is anticipated that the approach will provide some insight into its mechanism of action, depending on the outcome of these feeding experiments. For the fat level experiment, a custom formulated fat blend was used that simulates the fatty acid composition of the US diet. The idea was to examine the efficacy of CLA in the context of a fat consumption habit (10–20% by weight) that is relevant to humans. For the fat type experiment, a 20% (w/w) fat diet containing either corn oil (exclusively) or lard (predominantly) was used. Corn oil and lard differ significantly in their content of linoleate. Therefore, changes in the inhibitory activity of CLA in the presence of these two fat types may point to a possible interaction between CLA and linoleic acid in modulating tumor growth. Mammary cancer prevention by CLA under these various dietary conditions was evaluated using the rat dimethylbenz[a]anthracene (DMBA) model.

Previous work by Ha \textit{et al.} (9) suggested that CLA is a potent antioxidant. At a molar ratio of 1 part CLA to 1000 parts linoleic acid, peroxide formation was reduced by >90% in a test tube assay. In fact, CLA was superior to tocopherol in this regard. In order to investigate whether interference with oxidative processes in cells might be implicated in cancer prevention by CLA, we examined the effect of CLA on two markers of cellular oxidative damage in the mammary tissue of rats fed either a high corn oil (unsaturated fat) or high lard (saturated fat) diet. These markers were malondialdehyde.

\(\text{CLA}^*\)
(MDA), an end product of lipid peroxidation, and 8-hydroxy-
deoxyguanosine (8-OHdG), an oxidized base isolated from
DNA. Lipid peroxidation products have been implicated in
mediating the formation of 8-OHdG in DNA (10). A recent
publication from Thompson's laboratory has also reported that
the number of 8-OHdG residues in mammary gland DNA
increased in proportion to the degree of fatty acid unsaturation
(as determined by iodine value) in the diet oils (11). More
importantly, the rate of increase was sensitive to the presence
or absence of nutritional levels of antioxidants such as vitamin
E and selenium. Because of the above findings, we felt that
these markers would be appropriate in assessing whether the
antioxidant activity of CLA is manifest in vivo. Our goal was
to investigate the possible relationship between the modulation of
oxidative damage and the efficacy of cancer protection
by CLA.

Materials and methods

Source and composition of CLA and other dietary fats

The method of CLA synthesis from >99% pure linoleic acid was detailed in
our earlier publication (6). CLA was custom ordered from Nu-Chek Inc.
(Elysian, MN). Gas chromatographic analysis showed that three particular
isomers, C9:11-, C9:11- and t10:12-CLA, constituted ~90% of the total.
From our experience over several years, we have found that there were
minimal variations in isomer distribution from batch to batch.

A 'vegetable fat blend' was prepared by Kraft Foods Inc. at their Technology
Center (Glenview, IL). This fat blend was designed specifically to
summate the fatty acid composition in the average US diet. It consisted of 39.5%
soybean oil, 22% palm oil, 12.5% high oleic sunflower oil, 9% cottonseed
oil, 8.5% coconut oil and 8.5% cocoa butter. The reason that plant oils were
used exclusively was to minimize the CLA content of the fat blend. Gas
chromatographic analysis showed the following composition: C8:0, 0.9%;
C10:0, 0.7%; C12:0, 5.1%; C14:0, 2.3%; C16:0, 18.8%; C16:1, 0.2%; C18:0,
5.6%; C18:1, 31.8%; C18:2, 29.5%; C18:3, 3.4%; C20:0, 0.4%; C22:0, 0.3%;
CLA, not detectable. The above 'vegetable fat blend' has a polyunsaturate/
monounsaturate/saturated fatty acid ratio of 1:1:1, which provided a fatty acid
profile similar to that found in the typical US diet.

Two other commercial fats were used in this study: Mazola brand corn
oil was obtained from Best Foods (Somerset, NJ) and lard was purchased from
Harlan Teklad (Madison, WI). Lard contains ~0.3 mg CLA/g fat.

Design of mammary cancer chemoprevention experiments

Pathogen-free female Sprague–Dawley rats were purchased from Charles
River Breeding Laboratories (Raleigh, NC) and housed in an environmentally
controlled room with a 12 h light/12 h dark cycle. Mammary tumors were
induced by a single i.g. dose of 7.5 mg DMBA at 50 days of age. Animals
were palpated weekly to determine the time of appearance and location of
tumors. At necropsy the mammary glands were exposed for the detection of
non-palpable or microscopic tumors. Only histologically confirmed adenocarcin-
omas were reported in the results. In general between 10 and 15% of the
tumors found in all groups (with or without CLA) were fibroadenomas. Tumor
incidences at the final time point were compared by $x^2$ analysis and the total
mammary tumor yield between groups was compared by frequency distribution analysis
as described previously (2).

The first experiment involved feeding rats a diet containing different levels
of the 'vegetable fat blend' at 10, 13.3, 16.7 and 20% by weight, with or
without 1% CLA. Thus there were a total of eight dietary treatment groups
between 2.5 and 4.0. The MDA-TBA adduct was separated using a 4.6X 150
mm C18 column (Beckman Ultrasphere ODS) and a mobile phase consisting
of 0.1% phosphoric acid (pH 3.7). The detector was at 6500 nm. The MDA
was quantified as its thiobarbituric acid (TBA) derivative with
reverse phase HPLC and photometric absorbance detection at 535 nm based
on an extensive modification of the method described by Draper and Hadley
(15). Mammary gland samples were homogenized with a Polytron in water
containing a 1% antioxidant solution (AOS; 0.3 M dipyridyl and 2% butylated
hydroxyanisole in ethanol), 1 part mammary tissue to 9 parts water (w/v).
The samples were centrifuged at 6500 g and the fat plugs were removed,
followed by further homogenization to resuspend the pellet. Since optimal
reaction conditions were found to vary with protein concentration, an amount
of homogenate containing ~1.2 mg protein was prepared for hydrolysis.
The homogenate was combined with 7.5 ml 5 N HCl, 7.5 µl AOS and enough
water to bring the volume to 1.5 ml. The covered tubes were heated to 96°C
for 3 h. They were cooled quickly in tap water and 30 µl/m unit sodium tungstate
(Na2WO4) was added to facilitate precipitation of protein. After centrifugation
at 6500 g for 10 min, 1 ml supernatant was then transferred to a clean glass
tube. An aliquot of 0.75 ml TBA solution (11% TBA in 74 mM KOH) was
added to each tube, followed by heating for 90 min to form the MDA–TBA
adduct. Samples were quickly cooled and the pH adjusted, if necessary, to
bring the samples to pH 1.5. Photometric absorbance detection was at 535 nm. MDA
was quantified by comparison of sample peak heights to those of the standard
prepared from 1,1,3,3-tetramethoxypropane. The final results are expressed as
nmol MDA/mg protein. Protein in tissue homogenates was quantified by the
Bradford method using a commercial dye reagent (BioRad Protein Assay;
BioRad Laboratories, Richmond, CA).

For the assay of 8-OHdG, the various procedures of DNA purification from
the mammary gland, the enzymatic digestion of DNA to deoxynucleosides,
the isotopic separation of 8-OHdG and deoxyguanosine (dG) by HPLC
and the quantitation of 8-OHdG with an electrochemical detector were described
detail in a recent publication from Thompson's laboratory (11). The only
modification introduced here was the elimination of phenol from the DNA
isolation procedure. Detector response was linear from 10 to >800 µg/
mg DNA for 8-OHdG and from <500 to 6000 µg for dG. Results are reported
as residues 8-OHdG/10^6 residues dG. The simultaneous analysis of both
deoxynucleosides on a single HPLC injection abrogated the need for a
recovery standard.

Results

Table 1 summarizes the mammary cancer chemoprevention data of CLA in rats fed different levels of fat. Tumor incidence at the
at the time of necropsy was significantly reduced ($P < 0.05$) by CLA treatment in each of the four fat groups. In the absence of
CLA supplementation, the total number of tumors increased
by ~40% (from 71 to 98) in the range 10–20% dietary fat
that CLA was equally effective in suppressing tumorigenesis supplementation perturbed the distribution of linoleate or other activity of CLA in rats fed either an unsaturated fat (corn oil) incorporation in different classes of lipid. Again there was no evidence that CLA acid profile found in phospholipids was different from that distinctive characteristic of phospholipids. Thus the fatty acids. In particular, the high level of C20:4 incorporation was substantial way. These findings suggest that there is selectivity of CLA feeding of 1% CLA in diets containing 10 and 20% fat (P < 0.05) in the 20% fat group. The in C18:2 incorporation from selected groups were processed for fatty acid analysis rats from selected groups were processed for fatty acid analysis in the neutral lipid fraction, the three predominant fatty acids were C16:0, C18:1 and C18:2. In rats fed 10 and 20% 56% reduction on the 13.3% fat diet, 51% reduction on the 16.7% fat diet and 50% reduction on the 20% diet. This observation suggests that the efficacy of CLA in mammary cancer prevention is independent of the level of fat in the diet. The number of fibroadenomas found across all groups was very low and CLA did not affect the formation of these benign lesions.

The uninvolved (non-tumor-bearing) mammary glands of rats from selected groups were processed for fatty acid analysis in the neutral lipid and phospholipid fractions. The results from four dietary treatment groups (10 and 20% fat ± CLA) are presented in Table II. The data are expressed as percentages of total fatty acids. As indicated in footnote b, each value represents the mean of seven to eight samples, but since the standard error of the group mean is generally within 5% of the mean, the SEM is omitted from the table in order to make it more readable.

In the neutral lipid fraction, the three predominant fatty acids were C16:0, C18:1 and C18:2. In rats fed 10 and 20% fat without CLA the most significant change was an increase in C18:2 incorporation (P < 0.05) in the 20% fat group. The feeding of 1% CLA in diets containing 10 and 20% fat increased the neutral lipid CLA content by 17.5-fold (from 0.2 to 3.5%) and 12-fold (from 0.2 to 2.4%) respectively, but did not alter the proportion of the other fatty acids in any substantial way.

Analysis of the phospholipid fraction showed that C16:0, C18:0, C18:2 and C20:4 accounted for >90% of total fatty acids. In particular, the high level of C20:4 incorporation was a distinctive characteristic of phospholipids. Thus the fatty acid profile found in phospholipids was different from that found in neutral lipids. Again there was no evidence that CLA supplementation perturbed the distribution of linoleate or other fatty acids in phospholipids. Interestingly, the increase in CLA incorporation in phospholipids (~0.3%) was much smaller in magnitude compared with that observed in neutral lipids (~2–3%). These findings suggest that there is selectivity of CLA incorporation in different classes of lipid.

Table III shows the mammary cancer chemopreventive activity of CLA in rats fed either an unsaturated fat (corn oil) or a saturated fat (lard) diet. It was apparent from the data that CLA was equally effective in suppressing tumorigenesis regardless of the type of dietary fat intake. Furthermore, the magnitude of tumor inhibition seen in this experiment was very similar to that described in the first experiment (Table I). In other words, with a constant dose of DMBA, feeding of 1% CLA reduced the number of mammary tumors by ~50% and this activity was evidently unaffected by the fat content (level or type) in the diet.

The efficacy of CLA in inhibiting lipid peroxidation and oxidative damage in mammary tissue was assessed by measuring MDA in mammary gland homogenate and 8-OHdG in mammary gland DNA. The results are presented in Table IV. In this experiment rats were fed the same corn oil or lard diet with or without 1% CLA as in the mammary carcinogenesis experiment shown in Table III. However, the animals were not treated with DMBA and they were sacrificed after 2 months of feeding. MDA levels were significantly elevated in rats fed the corn oil versus the lard diet (P < 0.001), this finding thus confirms the increased susceptibility of unsaturated fat to peroxidation. Feeding of CLA was associated with a reduction in MDA in the mammary tissue in both fat groups (P < 0.001). This effect was somewhat greater in rats fed a rich unsaturated fat diet (corn oil, 35% reduction; lard, 25% reduction; P = 0.02). Diet-associated differences in tissue levels of 8-OHdG were less remarkable. A 10–15% increase in 8-OHdG levels was detected with feeding the corn oil versus the lard diet (P = 0.08), however, tissue levels of this oxidized base were unaffected by CLA (P = 0.42).

It has been reported previously that 1% CLA produced a maximal inhibitory effect on mammary carcinogenesis in rats fed a 5% corn oil diet (6). No further protection was detected at levels above 1% CLA. In order to find out whether the dose–response characteristics with respect to CLA might be different in rats fed a 20% corn oil diet, an experiment was carried out to evaluate such a possibility (the protocol was otherwise identical to the previous 5% corn oil experiment with CLA supplemented at 0.5, 1 or 1.5%). As pointed out in Materials and methods, the difference in linoleate intake is substantial between a 5 and a 20% corn oil diet. If the action of CLA is totally dissociated from the availability of linoleic acid, the dose–response characteristics with respect to CLA are likely to be the same in rats consuming either a 5 or 20% corn oil diet. The results in Table V clearly show that maximal tumor inhibition was obtained with 1% CLA in rats fed a 20% corn oil diet. Increasing the concentration of CLA to 1.5% did not lead to a greater benefit in cancer protection.

**Discussion**

CLA is not the only fatty acid known to inhibit carcinogenesis. Eicosapentaenoic acid and docosahexaenoic acid, which are representative of the n-3 polyunsaturated fatty acids in fish oil, also fit this category (16). However, CLA differs from the fish oil fatty acids in two distinct aspects as far as their efficacies are concerned. Whereas fish oil is usually required at levels of ~10%, CLA at levels of 1% or less is sufficient to produce a significant cancer protective effect (7). Additionally, there are a number of papers which have indicated that an optimal ratio of fish oil to linoleate in the diet is critical in achieving maximal tumor inhibition (17–19). As can be seen from the present study, the potency of CLA in cancer prevention is largely dissociated from the quantity and type of dietary fats consumed by the host.

A possible mechanism of cancer prevention by fish oil n-3
The experiments reported in Tables I and II tend to suggest that CLA is unlikely to interfere with the metabolic cascade involved in converting linoleic acid to eicosanoids. The data presented in Tables II and III for the various eicosanoids produced via either the cyclooxygenase or lipoxygenase pathways. In vivo, perturbation of eicosanoid biosynthesis (19,20) by factorial analyses of variance the following effects on 8-OHdG were noted. Type of fat, F = 3.18, P = 0.024. Ratio, F = 5.62, P = 0.001; interaction between fat type and CLA, F = 0.37, P = 0.54.

Table II. CLA incorporation in neutral lipid and phospholipid fractions of mammary gland

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Neutral lipid&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Phospholipid&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10% fat</td>
<td>20% fat</td>
</tr>
<tr>
<td></td>
<td>-CLA +CLA</td>
<td>-CLA +CLA</td>
</tr>
<tr>
<td>C12:0</td>
<td>1.2 1.2</td>
<td>1.6 1.6</td>
</tr>
<tr>
<td>C14:0</td>
<td>1.7 1.9</td>
<td>1.7 1.6</td>
</tr>
<tr>
<td>C16:0</td>
<td>24.3 24.7</td>
<td>21.3 20.5</td>
</tr>
<tr>
<td>C16:1</td>
<td>3.9 3.9</td>
<td>3.0 1.5</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.9 3.6</td>
<td>3.7 4.8</td>
</tr>
<tr>
<td>C18:1</td>
<td>42.3 38.9</td>
<td>40.4 38.8</td>
</tr>
<tr>
<td>C18:2</td>
<td>20.9 21.0</td>
<td>26.2 27.0</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.9 0.9</td>
<td>1.3 1.2</td>
</tr>
<tr>
<td>C20:4</td>
<td>0.7 0.4</td>
<td>0.6 0.6</td>
</tr>
<tr>
<td>CLA</td>
<td>0.2 3.5</td>
<td>0.2 2.4</td>
</tr>
<tr>
<td>100</td>
<td>100 100</td>
<td>100 100</td>
</tr>
</tbody>
</table>

<sup>a</sup>The samples were processed from uninvolved glands of rats reported in Table I.
<sup>b</sup>Results are expressed as percent of total fatty acids. The sum of each column is equal to 100%. Each value represents the mean of 7-8 samples, the SEM generally being within 5% of the mean.

Table III. Mammary cancer prevention by CLA in rats fed either an unsaturated fat or a saturated fat diet<sup>a</sup>

<table>
<thead>
<tr>
<th>Dietary fat</th>
<th>CLA</th>
<th>Tumor incidence</th>
<th>Total no. of tumors</th>
<th>Inhibition (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>1%</td>
<td>83.3%</td>
<td>68</td>
<td>49%</td>
</tr>
<tr>
<td>Corn oil</td>
<td>1%</td>
<td>40.0%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49%</td>
</tr>
<tr>
<td>Lard</td>
<td>1%</td>
<td>80.0%</td>
<td>60</td>
<td>47%</td>
</tr>
<tr>
<td>Lard</td>
<td>1%</td>
<td>40.0%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47%</td>
</tr>
</tbody>
</table>

<sup>a</sup>The unsaturated fat diet contained 20% corn oil, while the saturated fat diet contained 8% corn oil + 12% lard. There were 30 rats per group.
<sup>b</sup>Percent inhibition was calculated using the tumor number data.
<sup>c</sup>P < 0.05 compared with the corresponding control group without CLA.

Table IV. Effect of CLA feeding on MDA and 8-OHdG levels in mammary gland<sup>a,b</sup>

<table>
<thead>
<tr>
<th>Dietary fat</th>
<th>Malondialdehyde (nmol/mg protein)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>8-OHdG (residues/10&lt;sup&gt;6&lt;/sup&gt; dG)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-CLA +CLA</td>
<td>-CLA +CLA</td>
</tr>
<tr>
<td>Corn oil</td>
<td>1.39 ± 0.08 0.90 ± 0.14</td>
<td>4.00 ± 0.26 4.05 ± 0.20</td>
</tr>
<tr>
<td>Lard</td>
<td>0.43 ± 0.03 0.32 ± 0.02</td>
<td>3.38 ± 0.26 3.75 ± 0.30</td>
</tr>
</tbody>
</table>

<sup>a</sup>Rats were fed either the corn oil or lard diet with or without 1% CLA for 2 months.
<sup>b</sup>Results are expressed as mean ± SE (n = 9).
<sup>c</sup>By factorial analyses of variance the following effects on malondialdehyde were noted. Type of fat, F = 8.903, P < 0.001; CLA, F ratio 13.76, P = 0.001; interaction between fat type and CLA, F ratio 5.62, P = 0.024.
<sup>d</sup>By factorial analyses of variance the following effects on 8-OHdG were noted. Type of fat, F ratio 3.18, P = 0.08; CLA, F ratio 0.42, P = 0.42; interaction between fat type and CLA, F ratio 0.37, P = 0.54.

The results indicate that CLA is unlikely to interfere with the metabolic cascade involved in converting linoleic acid to eicosanoids. First, the anticarcinogenic efficacy of CLA was not affected by variations in linoleate intake, as demonstrated by the experiments reported in Tables I and III. Second, similar dose–response characteristics with respect to CLA at 1% and below were noted in rats fed either a 5 or 20% corn oil diet (6; Table V). No further protection was evident with supplementation of CLA above 1% in both cases. The fact that the effect of CLA maximizes at 1% may indicate a limiting step in the capacity to metabolize CLA to some active product(s) which is essential for inhibition of carcinogenesis. Suffice it to note that absorption of CLA is probably not a confounding factor here, because tissue accumulation of CLA continues to rise with dietary levels above 1% (unpublished data).

In all the carcinogenesis experiments included in this paper, CLA was given to the animals starting 1 week before DMBA and continuing until termination of the experiment. We adopted this protocol initially with the experiment shown in Table I, and in order to maintain uniformity, followed the same protocol in subsequent experiments reported in Tables III and V. However, we have observed that CLA does not affect DMBA binding to mammary cell DNA (7) nor does it affect phase II conjugating enzymes, such as glutathione S-transferase and UDP-glucuronoyl transferase (6). In other words, CLA is expected to have little influence on DMBA activation or detoxification. It can thus be conjectured that the major impact of CLA on mammary carcinogenesis with the above protocol is due to its inhibitory effect on tumor promotion or progression.

Some explanation is called for here about the finding that in rats which were maintained on the ‘vegetable fat blend’ diet there was a small but detectable amount of CLA in the mammary tissue even though the animals did not receive an...
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exogenous supply of CLA. In an attempt to determine whether the bacterial flora in the colon of rats could be the source of CLA, Chin et al. (21) have recently examined the tissue levels of CLA between conventional and germ-free rats which were fed diets with or without free linoleic acid. With the conventional rats, tissue CLA concentrations were 5–10 times higher in those animals given a 5% linoleic acid supplement. In contrast, CLA concentrations in tissues of germ-free rats were not affected by the addition of linoleic acid. These findings strongly suggest that the intestinal bacterial flora of rats is capable of converting linoleic acid to CLA.

As shown by the data in Table II, there might be some selectivity in the incorporation of CLA into different lipids following ingestion of a diet rich in CLA. When expressed as a percentage of total fatty acids, CLA is more abundant in neutral lipids than in phospholipids. It is unclear whether this uneven distribution of CLA in various lipid fractions has any relevance to cancer risk modulation. Because of the configuration of the trans double bond(s) in CLA, the incorporation of CLA in membrane phospholipids could conceivably diminish the fluidity of the lipid bilayer. On the other hand, the small amount of CLA in phospholipids tends to argue against the significance of a membrane effect. The storage of CLA in neutral lipids could portend the importance of this pool in providing a continuous supply of CLA for generation of some active metabolite(s). Further research is needed to examine the rate of turnover of CLA in neutral lipids and the possible oxidative modification of CLA, similar to that observed with linoleic acid (22–24).

The ability of CLA to suppress lipid peroxidation was first described by Pariza's laboratory (9). In that work linoleic acid was exposed to air and moderate heat with or without a very small amount of CLA for an extended period of time. Under those conditions the degree of linoleic acid oxidation (peroxide value) was determined by the thiocyanate method (25). It was hypothesized that an oxidized derivative of CLA might be the active antioxidant species, rather than CLA itself (9). According to the proposed scheme, which is supported by spectrophotometric evidence, a β-hydroxy acrolein moiety would be introduced across the conjugated double bond of CLA following reaction with a hydroxyl or peroxyl radical and molecular oxygen. Antioxidant activity would result from chelation of iron by the β-hydroxy acrolein functional group, thereby interfering with the Fenton reaction. A recent paper by van den Berg (26), however, contradicted the above conclusion. These investigators studied whether CLA could protect membrane vesicles composed of 1-palmitoyl-2-linoleoyl phosphatidylcholine from oxidative modification under various conditions. Oxidation was determined by direct spectrophotometric measurement of conjugated diene formation and by gas chromatographic/mass spectrometric analysis of fatty acids. It was found that CLA neither acts as a radical scavenger nor is it converted into a metal chelator in the Fe2+/ion-dependent oxidative reaction. Thus, at least in a model membrane system, CLA does not function as an effective antioxidant or antioxidant precursor.

The results presented in Table IV may provide new clues as to the effect of CLA on oxidative events in vivo. MDA levels were lower in mammary tissue of CLA-treated rats and the suppressive effect was somewhat greater in rats fed the more unsaturated dietary fat. Since MDA was measured in whole mammary gland homogenate, it is likely to represent the peroxidation of neutral lipids, which are found predominantly in the mammary gland adipocytes. As shown in Table II, CLA is also preferentially incorporated in the neutral lipid fraction. On the other hand, the levels of 8-OHdG, which are only marginally affected by the type of dietary fat and not at all by CLA supplementation, are probably a better indicator of DNA oxidative damage that may be causally related to tumor promotion/progression. The presence of 8-OHdG has been implicated in mismatching errors and base substitutions in DNA replication (27,28). The absence of a detectable effect of CLA on 8-OHdG is also consistent with the lack of a significant accumulation of CLA in the phospholipid fraction, which is likely to originate from mammary epithelial cells. In summary, based on the information obtained in this study, we believe that the ability of CLA to inhibit mammary carcinogenesis is not mediated by protecting the target cell DNA against damage induced by reactive oxygen species. Current research is focused on using a mammary epithelial cell culture model (29,30) to generate new insights into potential mechanisms of CLA in regulating growth and differentiation.

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