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

Clement Ip, Stephanie P. Briggs, Albert D. Haegeli, Henry J. Thompson, Jayne Storkson and Joseph A. Scimeca

Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY 14263, 1Division of Laboratory Research, AMC Cancer Research Center, Denver, CO 80214, 2Food Research Institute, Department of Food Microbiology, University of Wisconsin—Madison, Madison, WI 53706 and 3Nutrition Department, Kraft Foods Inc., Glenview, IL 60025, USA

*To whom correspondence should be addressed

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 fat 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 treatment with CLA may have some antioxidant function 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 fat 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 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

*Abbreviations:* CLA, conjugated linoleic acid; MNU, methylnitrosourea; DMBA, 7,12-dimethylbenz[a]anthracene; MDA, malondialdehyde; 8-OhdG, 8-hydroxydeoxyguanosine; TBA, thiobarbituric acid; AOS, antioxidant solution; dG, deoxyguanosine.
(MDA), an end product of lipid peroxidation, and 8-hydroxydeoxyguanosine (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. (Elyssian, MN). Gas chromatographic analysis showed that there were minimal variations in isomer distribution from batch to batch. A vegetable fat blend prepared by Kraft Foods Inc. at their Technology Center (Glenview, IL). This fat blend was designed specifically to simulate 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 polyunsaturated/monounsaturated/saturated fatty acid ratio of 1:1:1, which provided a fatty acid profile similar to that found in the typical US diet.

Determination of MDA and 8-OHdG in mammary tissue

A separate experiment was set up to evaluate the effect of CLA on markers of lipid peroxidation and cellular oxidative damage in the mammary gland. Rats were fed the same corn oil or lard diet with or without 1% CLA as described in the above section. However, they were not treated with DMBA and the feeding period only lasted 2 months. At necropsy, the abdominal inguinal mammary gland chains (glands 4–6) were excised and immediately frozen in liquid nitrogen.

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 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 excised for the detection of non-palpable or microscopic tumors. Only histologically confirmed adenocarcinomas 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 χ² analysis and the total tumor yield between groups was compared by frequency distribution analysis as described previously (13).

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 diet treatment groups in this design. All diets, which were prepared according to the AIN-76 formulation (6), were started 1 week before DMBA and continued until sacrifice (32 weeks post-DMBA). It has previously described the method of nutrient adjustment for diets containing different levels of fat so that the intake of protein, vitamins, minerals and calories was similar among the different groups (13).

At necropsy, the uninvolved (non-tumor-bearing) mammary glands from selected groups were excised and immediately dropped into liquid nitrogen. Upon removal from storage at −80°C, the frozen samples were pulverized and total fat was extracted with chloroform/methanol. The separation of phospholipids and neutral lipids was achieved with the use of a Sep-Pak silica cartridge as described in our earlier publication (6). Gas chromatographic analysis of the fatty acid methyl esters (including CLA) was determined by the method reported previously by Chin et al. (2).

The second experiment involved feeding a diet containing either 20% corn oil or a mixture of 8% corn oil + 12% lard, both with or without 1% CLA. Lard was chosen over tallow because of the much lower CLA content in lard (4 mg CLA/g fat in tallow versus 0.3 mg CLA/g fat in lard). The 12% lard in the diet therefore contributed <4 mg CLA/100 g diet, an amount that was insignificant compared with the level of 1% CLA used in this experiment. It should be noted that the lard diet also contained 8% corn oil. The reason was the previous finding of a high linoleate requirement for mammary tumorigenesis in the DMBA model (3,14). Similar to the above protocol, the feeding of the corn oil or lard diet ± 1% CLA was started 1 week before DMBA and continued until sacrifice.

The third experiment involved feeding a 20% corn oil diet with either 0.5, 1 or 1.5% CLA. The purpose was to determine the dose–response characteristics with respect to CLA in the presence of a linoleate–rich diet and to compare the results obtained here with our previous study of CLA efficacy (also at 0.5, 1 or 1.5%) in rats fed a 5% corn oil diet (6). Corn oil consists of ~68% linoleate. Thus the 5 and 20% corn oil diets contain ~3 and 12 g linoleate/100 g diet, respectively. The purpose was to find out whether a diet that was in linoleate would require more CLA to achieve a maximal inhibitory effect.

Downloaded from https://academic.oup.com/carcin/article-abstract/17/5/1045/2475679 by guest on 12 February 2019
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

---

**Table I. Mammary cancer prevention by CLA in rats fed different levels of fat**

<table>
<thead>
<tr>
<th>Dietary fat level</th>
<th>CLA</th>
<th>Tumor incidence</th>
<th>Total no. of tumors</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>1%</td>
<td>68.8%</td>
<td>71</td>
<td>56%</td>
</tr>
<tr>
<td>10%</td>
<td>0.5%</td>
<td>81.3%</td>
<td>74</td>
<td>56%</td>
</tr>
<tr>
<td>13.3%</td>
<td>1%</td>
<td>46.9%</td>
<td>40</td>
<td>46%</td>
</tr>
<tr>
<td>16.7%</td>
<td>1%</td>
<td>87.5%</td>
<td>94</td>
<td>51%</td>
</tr>
<tr>
<td>16.7%</td>
<td>0.5%</td>
<td>90.6%</td>
<td>98</td>
<td>50%</td>
</tr>
<tr>
<td>20%</td>
<td>1%</td>
<td>59.4%</td>
<td>49</td>
<td>50%</td>
</tr>
</tbody>
</table>

*The fat used in this experiment was the 'vegetable fat blend' as described in Materials and methods. There were 32 rats/group.

**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
this paper tend to suggest that CLA is unlikely to interfere with oxygenase or lipoxygenase pathways. The data presented in this study perturbation of eicosanoid biosynthesis (19,20).

In vivo, polyunsaturated fatty acids has been postulated to be through perturbation of eicosanoid biosynthesis (19,20). In vivo, linoleic acid is converted to arachidonic acid, which is the precursor for the various eicosanoids produced via either the cyclooxygenase or lipoxygenase pathways. The data presented in this paper tend to suggest that CLA is unlikely to interfere with the metabolic cascade involved in converting linoleic acid to eicosanoids. First, the antitumorogenic efficacy of CLA was not affected by variations in linoleic acid intake, as demonstrated by the experiments reported in Tables I and III.

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>+CLA</td>
</tr>
<tr>
<td>C12:0</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>C14:0</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>C16:0</td>
<td>24.3</td>
<td>24.7</td>
</tr>
<tr>
<td>C16:1</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.9</td>
<td>3.6</td>
</tr>
<tr>
<td>C18:1</td>
<td>42.3</td>
<td>38.9</td>
</tr>
<tr>
<td>C18:2</td>
<td>20.9</td>
<td>21.0</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>C20:4</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>CLA</td>
<td>0.2</td>
<td>3.5</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>83.3%</td>
<td>68</td>
<td></td>
<td></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>80.0%</td>
<td>60</td>
<td></td>
<td></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 control group without CLA.

Table IV. Effect of CLA feeding on MDA and 8-OHdG levels in mammary gland<sup>a</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</td>
<td>-CLA</td>
</tr>
<tr>
<td></td>
<td>+CLA</td>
<td>+CLA</td>
</tr>
<tr>
<td>Corn oil</td>
<td>1.39 ± 0.08</td>
<td>0.90 ± 0.14</td>
</tr>
<tr>
<td>Lard</td>
<td>0.43 ± 0.03</td>
<td>0.32 ± 0.02</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 ratio 88.903; 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.

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
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.

Acknowledgements

The authors thank Todd Parsons and Cathy Russin for technical assistance with the experiments. This work was supported by grant no. CA 61763 from the National Cancer Institute, National Institutes of Health, to Clement Ip, grant no. AIBS 2423 from the Department of Defense to Henry Thompson and a gift from Kraft Foods Inc., Glenview, IL.

References

3. Ip,C., Carter,C.A. and Ip,M.M. (1985) Requirement of essential fatty acid 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).


Received on September 13, 1995; revised on November 17, 1995; accepted on December 4, 1995