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Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats

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Apparent lymphatic recovery of conjugated linoleic acid (CLA) in rats was considerably lower than for linoleic acid, approximately 55% versus 80% for 24 hr, although the distribution in lymph lipoproteins was similar. Not all the CLA constituents were recovered equally, and more tt-isomers were recovered than ct- or tc-isomers in relation to the composition of CLA given. When rats were fed CLA or linoleic acid at the dietary level of 1% for 2 weeks, there were detectable differences in the incorporation of CLA in various tissues, and adipose tissue and lung contained the highest proportion, whereas a limited amount was incorporated into the brain. In general, 9c, 11t/9t,11c isomers were the predominant CLA followed by tt-isomers. Also, CLA was differently incorporated into individual phospholipids in the liver. No effects were observed on serum and liver lipid levels, but the concentration of prostaglandin E2 (PGE2) in serum and spleen tended to be reduced by CLA, the difference in the former was statistically significant. CLA did not increase tissue TBA values. Thus, the metabolic effect of CLA may not be attributed to a single entity. © Elsevier Science Inc. 1997 (J. Nutr. Biochem. 8:38–43, 1997.)

Keywords: conjugated linoleic acid; linoleic acid; lymphatic absorption; tissue distribution; TBA value

Introduction

Conjugated linoleic acid (CLA) is a collective term for positional (positions 9 to 12) and geometrical (*cc, ct, tc,* and *tt*) isomers of linoleic acid. CLA occurs in beef and dairy products at very low concentrations, around 5 mg/g fat.[1,2] However, CLA can exert diverse physiological functions.

A series of studies on the effect of CLA on chemical carcinogen-induced carcinogenesis showed its unique chemopreventive properties.[3,4] CLA, for instance, inhibited mammary cancer incidence very effectively at a dietary levels as low as less than 1%.[5–7] This effective level was definitely low compared with putative anticarcinogenic n-3 polyunsaturated fatty acids.[8] From the study on the timing and duration of CLA feeding, it was suggested that some metabolite(s) of CLA might be involved in suppressing the process of neoplastic promotion and/or progression.[4,7]

CLA was also found to be antihypercholesterolemic and antiatherogenic in rabbits.[9] These effects are also evident when CLA is given at the dietary levels less than 2 energy %. In addition, CLA is a newly recognized nutrient that functions to regulate energy retention and metabolism.[10] The antioxidant properties of CLA are also permissive; although, in some cases it is more susceptible for oxidation than linoleic acid.[11–13]

The observations that CLA exerts the beneficial effects at a low dietary level suggest that CLA is absorbed efficiently like more common fatty acids including linoleic acid. In the studies mentioned, CLA was given as a free fatty acid, and the intestinal absorption rate of long-chain unsaturated fatty acids differs by their forms, either triacylglycerol, free fatty acid or fatty acid ethyl ester.[14,15] However, no information is available as to the mode of intestinal absorption of individual CLA components, although CLA was detected in various tissues of animals.[6,11] Because it seems likely that not all the individual CLA components exert a similar physiological activity,[4] it is crucial to know the absorption specificity of CLA. In addition, it is also expected that CLA may still have additional metabolic function(s).

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The purpose of the present study was to measure the lymphatic absorption and tissue distribution of CLA, its distribution in tissues, and the effect on the metabolism of linoleic acid and hence, eicosanoid production in rats.

Methods and materials

Materials

Linoleic acid and CLA were the products of Sigma Chemical Co., St. Louis, MO USA. CLA was composed of 39.9, 39.5, 18.6, 1.3, and 0.7% of *t*-10, *c*-12-, *c*-9, *t*-11-*t*-9, *c*-11-, *t*-10, *t*-12-*t*-9, *t*-11-, *c*-9, *c*-11- and *c*-10, *c*-12-octadecadienoic acids, respectively. Pentadecanoic acid was purchased from Aldrich, Milwaukee, WI USA. Fatty acid-free bovine serum albumin and sodium taurocholate were purchased from Nacalai Tesque, Kyoto Japan. Pancreatic lipase was the product of Boehringer Mannheim, Tokyo, Japan. BF3-methanol was from Tokyo Kasei, Tokyo Japan. Other reagents were all analytical grades.

Lymphatic cannulation

Male Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka Japan) weighing on average of 395g were used for lymph cannulation experiment. The animals were fed a nonpurified diet (type NMF, Oriental Yeast, Tokyo Japan) and drinking water ad libitum for 3 days after arrival. A cannula (single-lumen clear vinyl tube SV35, Dural Plastics, Auburn Australia) was inserted into the left thoracic lymphatic channel of each rat and an indwelling catheter (single-lumen polyethylene tube SP-55, Dural Plastics, Tokyo, Japan) was placed in the stomach under Nembutal anesthesia.[14,15] Normal osmotic 139 mM glucose and 85 mM NaCl mixture was infused continuously at the rate of 3 mL/hr until the end of lymph collection. After surgery, each rat was placed in a restraining cage in a warm recovery room and allowed free access to drinking water containing 130 mM glucose and 85 mM NaCl. During the next morning, after collection of lymph for 2 hr as a blank lymph, each animal was administered 3 mL of a test emulsion via stomach tube, and the lymph was collected for 24 hr beginning at 1000 hr. The test emulsion contained 200 mg of sodium taurocholate, 50 mg of fatty acid-free albumin and 200 mg of CLA or linoleic acid. The emulsion was prepared by sonication in a warm water bath (Ultrasonic disrupter UR-200P, Tomy Seiko, Tokyo Japan), and was immediately administered to rats. Lymph was collected in an EDTA-containing tube at 3-hr intervals during the first 9 hr and at a single collection 9 to 24 hr after administration of a test emulsion. An aliquot of lymph was extracted in 20 volumes of the chloroform:methanol mixture (2:, v/v),[16] and a known amount of pentadecanoic acid was added as an internal standard. After methylating with a BF3-methanol mixture, fatty acid was analyzed by GLC using Shimadzu GC-17 equipped with Supelcowax-10 fused silica capillary column (Supelco Inc., Bellefonte, PA USA, 60 m × 0.32 mm i.d.).[1] The column temperature was kept at 150°C for 2 min and then raised to 220°C at the rate of 4°C/min and kept at this temperature. Identification of individual CLA was done by comparing ECL and by GC-MS (JEOL Auto MS 50, Tokyo Japan) analysis.[1] Fatty acid absorption was calculated by subtracting fatty acid amounts in blank lymph from those of lymph collected after administration of a test emulsion. Another aliquot of lymph was used for the ultracentrifugal separation of lipoproteins (Beckman LS-50 Ultracentrifuge, Beckman, Tokyo Japan).[17] The distribution of CLA in the triacylglycerol and phospholipid moieties was measured after lipase and phospholipase A2 hydrolysis of triacylglycerol.[18]

Feeding experiment

Male Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka Japan), 4 weeks old, were acclimated in an air-conditioned room (23–25°C, lights on 0800 to 2000) for 7 days after arrival. The animals were then separated into two groups of five to six rats each and freely assessed AIN-93 type diets[19] for 2 weeks. Diets contained in g/kg, casein 200, L-cystine 3.0, choline bitartrate 2.5, sucrose 100, corn starch 397, 48, dextranized corn starch 132, soybean oil 60, cellulose 50, vitamin mixture 10, mineral mixture 35, *tert*-butylhydroquinone 0.02 and linoleic acid or CLA 10. Vitamin and mineral mixtures (AIN-93) were purchased from Oriental Yeast Co., Tokyo Japan. Food intake and body weight were recorded every other day. On the last day of the feeding period, rats were killed by withdrawing blood from the abdominal aorta under light diethylether anesthesia. After blood collection, tissues were excised immediately, weighed and stored frozen at −80°C until analysis. Fatty acid compositions of tissue lipids were measured as described. Serum total and HDL-cholesterol, triacylglycerol and phospholipid were analyzed by enzymatic kits (Test Wako™ lipid analysis kits, Wako Pure Chemicals, Osaka Japan). Liver cholesterol, triacylglycerol, and phospholipid were analyzed as described elsewhere.[30] Serum and liver TBA values were measured by an enzymatic kit (Wako Pure Chemicals, Osaka Japan). Tissue lipids were extracted according to the method of Folch et al.[16] and the phospholipid subclasses were separated by thin-layer chromatography on Silica gel-G (Merck, Darmstadt, Germany) for fatty acid analysis.[21] For measurement of prostaglandin E2 (PGE2) in spleen and serum, an enzymatic immunoassay kit (PGE2 Monoclonal EIA Kit, Cayman Chemical, Ann Arbor, MI USA) was used. PGE2 was measured under the condition of production being linear with respect to tissue weight or serum volume and incubation time at 25°C.[22]

Statistical analysis

Data were analyzed by Student's *t*-test in each experiment.

Results

Lymphatic recovery of CLA

As shown in *Figure 1*, the apparent lymphatic recovery rate of CLA was slower than that of linoleic acid, and the cumulative recovery rate for 24 hr was significantly lower in CLA than in LA (mean ± SE, 78.5 ± 2.5 versus 53.4 ± 4.1%,

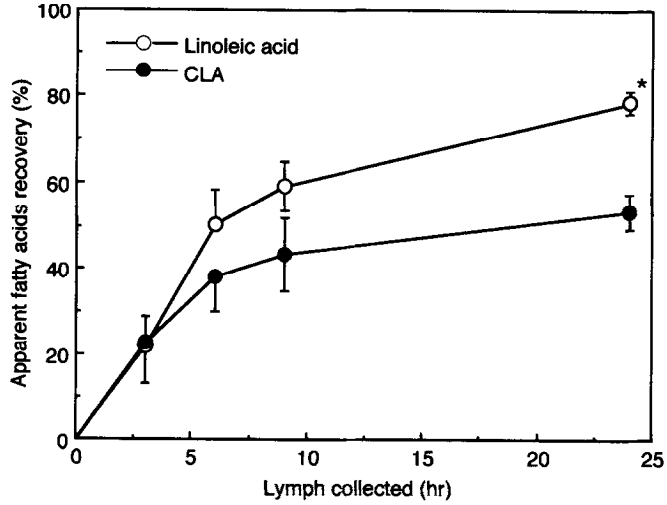

Figure 1 Lymphatic Recovery of CLA and linoleic acid. Mean ± SE of four rats per group. *Significantly different from the linoleic acid group at $P < 0.05$.

$P < 0.05$). The lymph flow rate was the same between two groups. Similar to the case of linoleic acid, approximately 80% of CLA was carried as chylomicrons and remaining 20% as very low-density lipoproteins, and approximately 95% of CLA was incorporated into triacylglycerol and 5%, phospholipids. Distribution of individual CLA in the triacylglycerol was similar to that in the phospholipids. There were detectable differences in the compositions of CLA between the gavage and lymph CLA (*Figure 2*). When the lymph collected for 24 hr was analyzed, more *tt*-isomers while less *ct*- and *tc*-isomers were detected in the lymph than in CLA given intragastrically. The tendency was similar in the lymph collected in the first 3 hr after the fat meal. Approximately the same proportion of *tc*- and *ct*-octadecadienoic acids was incorporated into *sn*-2 and *sn*-1,3 positions of lymph triacylglycerol similar to the case of linoleic acid (each approximately 50%), whereas *tt*-18:2 was exclusively distributed in sn-1,3 positions.

Feeding study

There were no significant differences in food intake (21.6 ± 0.8 versus 21.9 ± 0.3 g/day for the linoleic acid and CLA groups) and weight gain (112 ± 6 versus 120 ± 4 g/2 week) between rats fed linoleic acid and CLA. The weights of liver, heart, kidney, lung, spleen, brain, and perirenal adipose tissue were also comparable between two groups.

Essentially no statistically significant differences were observed in serum concentrations of total and HDL-cholesterol, triacylglycerol, and phospholipid, and also liver lipids (data not shown). These values were all in the ranges reported for rats fed cholesterol-free diets.

Table 1 shows the concentration of serum and spleen PGE2 together with the serum and liver TBA values. Feeding CLA resulted in a trend toward a decreasing concentration of PGE2 in serum and spleen compared with linoleic acid, and the difference in the serum was statistically significant. The concentration of serum and liver TBA was not significantly influenced by the type of dietary octadecadienoic acids.

The extent of incorporation of CLA in various tissues is summarized in *Figure 3*. The incorporation of CLA depends on the tissues, and the adipose tissue and lung contained most and the brain the least. The composition of CLA also was tissue-dependent, and it did not necessarily resemble that of dietary CLA, although in general *9t,11c*-/*9c,11t*-18:2, the major component in dietary CLA, was the predominant component in all tissues. However, the compositions of the major fatty acids in these tissues were not significantly different in two groups of rats (data not shown).

Table 2 shows the fatty acid composition of liver individual phospholipids. The incorporation of CLA was not the same among the phospholipid species analyzed, the order being phosphatidyllinositol > cardiolipin > phosphatidylethanolamine > phosphatidylserine > phosphatidylcholine. Although there were no differences in the compositions of major fatty acids in each phospholipid between the linoleic acid and CLA groups, the ratio of (20:3n-6 + 20:4n-6)/18:2n-6, an index for linoleic acid desaturation, tended to be higher in rats fed CLA than in those fed linoleic acid, and the difference was significant in phosphatidylinositol and cardiolipin.

Table 1 Prostaglandin E2 concentration and TBA value in tissues

Eicosanoid and TBA value	Linoleic acid ($n = 6$)	CLA ($n = 5$)
PGE2		
Serum (pmol/L)	284 ± 41	150 ± 22*
Spleen (pmol/g)	8.54 ± 0.79	5.31 ± 0.73
TBA value		
Serum (nmol/L)	373 ± 39	529 ± 80
Liver (nmol/g)	5.67 ± 0.12	5.43 ± 0.11

Values are means ± SE. Significantly different at $^*P < 0.05$ (Student's *t*-test).

Discussion

Our results showed that apparent lymphatic recovery of linoleic acid (78.5%) was significantly higher than that of CLA (53.4%). It has been reported that the feeding of fats to

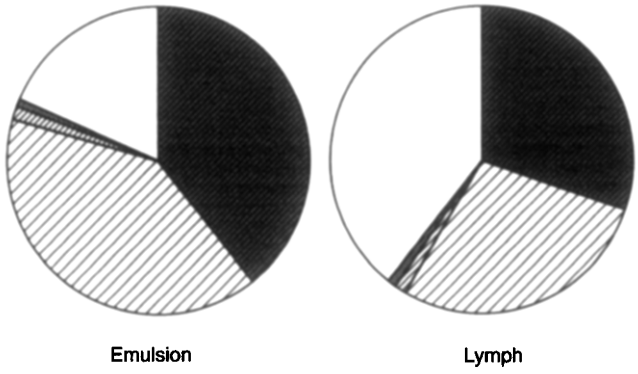

Figure 2 Comparison of the distribution of individual CLA components between emulsion given and lymph collected for 24 hr. Mean of six rats. ▦, 9*c*,11*t*/9*t*,11*c*; ▨, 10*t*,12*c*/10*c*,12*t*; ▨, 9*c*,11*c*; ▩, 10*c*,12*c*, and □, 9*t*,11*t*/10*t*,12*t*.

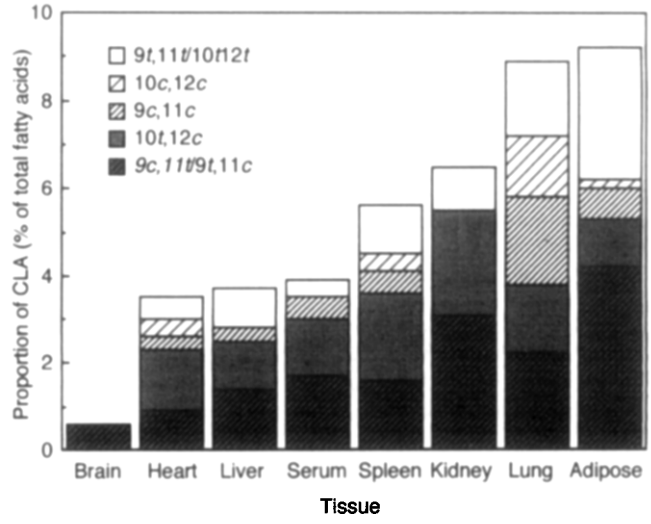

Figure 3 Incorporation of CLA into tissue total lipids. Mean of five rats per group.

Table 2A Polyunsaturated fatty acid composition of liver phosphatidylcholine

Fatty acid	Linoleic acid (n = 6)	CLA (n = 5)
	mol/100 mol	
18:2n-6	12.0 ± 0.6	10.8 ± 0.6
20:4n-6	31.9 ± 0.4	31.9 ± 0.3
22:5n-6	0.7 ± 0.1	1.0 ± 0.1
22:6n-3	6.4 ± 0.3	7.0 ± 0.2
CLA		
9*t*,11*c*/9*c*,11*t*	0.1 ± 0.1	0.3 ± 0.1
10*t*,12*c*	0.1 ± 0.1	0.4 ± 0.1**
9*c*,11*c*	nd	0.1 ± 0.1
10*c*,12*c*	nd	nd
9*t*,11*t*,10*t*,12*t*	nd	0.0 ± 0.0
Ratio		
(20:3 + 20:4)/18:2	2.76 ± 0.16	2.94 ± 0.18

Values are means ± SE. Fatty acids, except for CLA, less than 1 mol/100 mol were omitted. nd, not detected. Significantly different at $^{**}P < 0.01$ (Student's *t*-test).

Table 2C Polyunsaturated fatty acid composition of liver phosphatidylinositol

Fatty acid	Linoleic acid (n = 6)	CLA (n = 5)
	mol/100 mol	
18:2n-6	5.1 ± 1.2	4.4 ± 1.2
18:3n-6	0.6 ± 0.3	1.7 ± 1.1
18:3n-3	0.1 ± 0.1	2.3 ± 1.0
20:3n-6	1.5 ± 0.2	1.3 ± 0.2
20:4n-6	38.5 ± 1.4	34.7 ± 4.2
22:5n-6	1.1 ± 0.1	1.4 ± 0.2
22:6n-3	3.1 ± 0.4	2.3 ± 0.3
CLA		
9*t*,11*c*/9*c*,11*t*	nd	1.4 ± 0.6*
10*t*,12c	nd	1.2 ± 1.1
9c,11c	nd	nd
10c,12c	nd	nd
9*t*,11*t*/10*t*,12*t*	0.2 ± 0.2	1.9 ± 1.3
Ratio		
(20:3 + 20:4)/18:2	9.58 ± 1.55	10.53 ± 2.93

Values are means ± SE. Fatty acids, except for CLA, less than 1 mol/100 mol were omitted. nd, not detected. Significantly different at $^{*}P < 0.05$ (Student's *t*-test).

lymph duct-cannulated rats may increase endogenous linoleic acid in chyle due to the incorporation of pre-existing mucosal linoleoyl phosphatidylcholine species into chyle lipoproteins.[23] Therefore, our results might overestimate the linoleic acid absorption. Analyses of the chyle collected after CLA feeding revealed that 17 mg of linoleic acid was estimated to be originated from endogenous linoleic acid during 24 hr lymph collection. If the same amount of endogenous linoleic acid was secreted into chyle in rats given linoleic acid, absorption of linoleic acid is estimated to be 70%. The value is still higher than CLA absorption rate. More precise information will be obtained by using the labeled fatty acid.

CLA is a mixture of several octadecadienoic acids, and it is likely that all of these components have a similar physiological function as putatively suggested by Ip et al. To explore this possibility the measurement of intestinal absorbability and tissue distribution of each component may be helpful. The present study showed that not all the individual octadecadienoic acids were absorbed to a similar extent, and the 9*t*,11*t*-10*t*,12*t*-isomers were most preferentially absorbed (*Figure 2*). Because 9*c*,11*t*-isomer is the predominant CLA in fats from ruminant animals[1,2] and because it is considered to be the active component,[4] the physiological significance of this observation is hardly explained. In addition, not only the magnitude of deposition, but also the composition of CLA differed depending on the tissues, and in general the most predominant CLA was 9*t*,11*c*/9*c*,11*t*-isomers followed by the *tt*-isomers in most of the tissues as reported previously.[8,11,24] Thus, it is difficult to predict which isomer(s) is the putative candidate, although 9*c*,11*t*-isomer is postulated as the most biologically

Table 2B Polyunsaturated fatty acid composition of liver phosphatidylethanolamine

Fatty acid	Linoleic acid (n = 6)	CLA (n = 5)
	mol/100 mol	
18:2n-6	6.6 ± 0.4	5.1 ± 0.2
20:4n-6	29.1 ± 0.8	28.9 ± 0.6
22:5n-6	1.5 ± 0.1	2.1 ± 0.2
22:6n-3	14.6 ± 0.4	16.0 ± 0.4*
CLA		
9*t*,11*c*/9*c*,11*t*	0.1 ± 0.0	0.4 ± 0.1**
10*t*,12*c*	0.0 ± 0.0	0.4 ± 0.1
9*c*,11*c*	nd	nd
10*c*,12*c*	nd	0.2 ± 0.1
9*t*,11*t*/10*t*,12*t*	nd	0.4 ± 0.3
Ratio		
(20:3 + 20:4)/18:2	4.60 ± 0.29	5.78 ± 0.22

Values are means ± SE. Fatty acids, except for CLA, less than 1 mol/100 mol were omitted. nd, not detected. Significantly different at $^{*}P < 0.05$, $^{**}P < 0.01$ (Student's *t*-test).

Table 2D Polyunsaturated fatty acid composition of liver phosphatidylserine

Fatty acid	Linoleic acid (n = 6)	CLA (n = 5)
	mol/100 mol	
18:2n-6	5.0 ± 0.6	4.2 ± 0.3
20:4n-6	34.0 ± 0.7	32.1 ± 0.6
22:4n-6	1.0 ± 0.4	0.8 ± 0.0
22:5n-6	7.1 ± 0.4	0.8 ± 0.0
22:6n-3	6.7 ± 0.6	8.7 ± 0.2
CLA		
9*t*,11*c*/9*c*,11*t*	nd	0.1 ± 0.1
10*t*,12*c*	nd	0.1 ± 0.1
9c,11c	nd	nd
10c,12c	nd	nd
9*t*,11*t*/10*t*,12*t*	nd	0.7 ± 0.5
Ratio		
(20:3 + 20:4)/18:2	7.38 ± 0.78	7.91 ± 0.58

Values are means ± SE. Fatty acids, except for CLA, less than 1 mol/100 mol were omitted. nd, not detected.

Table 2E Polyunsaturated fatty acid composition of liver cardiolipin

Fatty acid	Linoleic acid (n = 6)	CLA (n = 5)
18:2n-6	69.6 ± 1.5	54.7 ± 7.4**
18:3n-3	1.4 ± 0.3	1.5 ± 0.9
20:3n-6	2.8 ± 0.2	3.0 ± 0.6
20:4n-6	3.0 ± 0.2	5.5 ± 1.0
22:6n-3	2.1 ± 0.2	2.5 ± 0.5
CLA		
9*t*,*c*-11/9*c*,11*t*	nd	0.5 ± 0.3
10*t*,12*c*	0.1 ± 0.1	nd
9*c*,11*c*	nd	0.6 ± 0.6
10*c*,12*c*	nd	nd
9*t*,11*t*/10*t*,12*t*	nd	1.3 ± 1.3
Ratio		
(20:3 + 20:4)/18:2	0.08 ± 0.01	0.18 ± 0.06*

Values are means ± SE. Fatty acids, except for CLA, less than 1% were omitted. nd, not detected. Significantly different at $^*P < 0.05$, $^{**}P < 0.01$ (Student's *t*-test).

active form of CLA.[4] However, in this context the peculiar function of 9*t*,12*t*-octadecadienoic acid is of interest, because it markedly interfered with the metabolic conversion of linoleic acid to arachidonic acid and hence, eicosanoid production.[25] The reducing tendency of PGE2 levels in serum and spleen by CLA coincides with this possibility (*Table 2*). Because PGE2 is one of the most possible determinants for promoting the growth of several cancer cells including mammary and colon cancer cells,[26,27] the marked suppressive effect of CLA on mammary carcinogenesis[5–7] can at least, in part, be explained by its depressing effect on PGE2 production in addition to the previously proposed mechanisms.[4] Alternately, it is plausible that CLA is converted to an active substance(s) that gives a cancer protective effect. The antioxidative effect of CLA may also be involved in carcinogenesis.[5,13,23] Ha et al.[24] also showed that CLA was a potent inhibitor of DMBA metabolism in vitro to its ultimate carcinogen.

There was a tissue-dependent difference in the incorporation of CLA (*Figure 3*). The incorporation of dietary CLA was analyzed mainly on serum, liver, and adipose tissue, and the content of CLA in the total lipids in the latter two tissues was comparable.[11] The different patterns of the incorporation of individual CLA molecules in liver phospholipid species may also suggest the specific role of CLA in the metabolic function when one considers different functions of phospholipid.[28] Although it is difficult to explain the significance of differential tissue retention of various CLA, it is at least likely that these differential distribution may reflect the availability of CLA in the tissues. Analysis of the CLA distribution in colon and mammary tissues will provide additional information regarding its specific action on carcinogenesis of these tissues.

Dietary CLA decreased linoleic acid in liver cardiolipin (*Table 2E*). It was reported that the decrease in linoleic acid in cardiolipin of heart mitochondrial membrane diminished heart mitochondrial cytochrome C oxidase activity that required cardiolipin as an activator.[29] Therefore, our results suggest a possibility that modified fatty acid composition in liver cardiolipin might affect mitochondrial respiratory function in liver.

In the present study, CLA did not show a cholesterol-lowering effect on serum and liver, in contrast to the hypocholesterolemic observation in rabbits.[9] In our experiment, rats were fed CLA at the level similar to the reported study, approximately 2.3 energy %. The short feeding period and the species difference should be the plausible reason to cause such a discrepancy.

CLA did not influence serum and liver TBA values. Although still controversial, the susceptibility of CLA to oxidation is regarded, in part, as its untoward property, because peroxidized lipids are the potential inducer of various metabolic disorders.[30,31] The effect of CLA as an inducer of lipid peroxidation may depend on the compositions of diets simultaneously ingested.

In conclusion, although rats used in this study were young and feeding periods were a little short, the present study suggested that not all the constituents of CLA are similarly effective to exert beneficial functions. The reducing tendency of PGE2 levels may be one of the putative factors expressing diverse functions as it is a potential regulator of a wide range of metabolic pathways.

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