Korean J Nutrition 30(4) : 442~450, 1997

Conjugated Linoleic Acid Changes Fatty Acid Composition by Decreasing Monounsaturated Fatty Acids in Rabbits and Hep G2 Cells

Nam, Kisun

Department of Nutritional Sciences Food Research Institute University of Wisconsin-Madison Madison, WI 53706, USA

ABSTRACT

Conjugated dienoic derivatives of linoleic acid(CLA) are a mixture of positional and geometric isomers of linoleic acid(LA). We previously found that CLA changes the fatty acid profile in chicken eggs and serum by decreasing monounsaturated fatty acids. Studies were conducted to explore the effects of CLA on fatty acid composition. Rabbits were fed a semisynthetic diet with or without CLA(0.5g CLA/rabbit/day) for 22 weeks. Compared to the control, rabbits fed CLA had significantly lower monounsaturated fatty acid levels(palmitoleic acid C16 : 1 by 50% and oleic acid C18 : 1, by 20%) in plasma lipids. We found similar differences in fatty acid composition in the liver and the aorta. The inhibitory effect of CLA on Δ9 desaturation was confirmed in a human hepatoma cell line, Hep G2. CLA significantly decreased Δ9 desaturation in 4 - 5 hours as shown by an increase in the ratio of C16 : 0 to C 16 : 1. This is apparently due to a decrease in Δ9 desaturase(stearoyl-CoA desaturase, SCD) activity ; it was decreased more than 50%. These results, along with our previous findings, indicate that CLA is an inhibitor of Δ9 desaturase in the liver. (*Korean J Nutrition* 30(4) : 442~ 450, 1997)

KEY WORDS : conjugated linoleic acid(CLA) · monounsaturated fatty acids · Δ9 desaturase (stearoyl-CoA desaturase, SCD) · rabbits · Hep G2 cells.

Introduction

Conjugated dienoic derivatives of linoleic acid(CLA) are a series of positional and geometric isomers of linoleic acid(LA), some of which occur naturally in foods[1)2)]. CLA is an anticarcinogen in several animal models[3-6)], reduces the adverse catabolic effects induced by immune stimulation in rats, mice, and chickens[7)], and enhances growth performance in rats[8)]. CLA reduces plaque formation in rabbits[9)] and hamsters[10)] fed an atherogenic diet. It also reduces body fat content and increases lean body mass[11)]. These observations appear to be a consequence of effects of CLA on lipid metabolism.

Quantitatively, the most significant components of lipids for human biochemistry and nutrition are the fatty acids. To achieve the desired physical properties of lipids in cells, both saturated and unsaturated fatty acids are required. Monounsaturated fatty acids are formed in the microsomes by direct oxidative desaturation of preformed long chain saturated fatty acids. Several tissues, especially liver, are considered to be responsible for their formation[12)]. Stearoyl CoA desaturase(SCD, Δ9 desaturase) catalyzes the conversion of stearic(C18 : 0) and palmitic(C16 : 0) acids to oleic (C18 : 1) and palmitoleic(C16 : 1) acids[13)].

Accepted : May 15, 1997

We previously found that CLA changes the fatty acid profile in chicken eggs ; feeding a diet containing CLA to laying hens resulted in the reduction of C18 : 1 levels by 30% in serum and by 50% in eggs[14]. Studies were conducted to explore the effects of CLA on fatty acid composition. We now report that dietary CLA significantly decreases the proportions of monounsaturated fatty acids in rabbit tissues as well as in Hep G2 cells(a human hepatoma cell line). This is apparently due to a decrease in Δ9 desaturase activity.

Materials and Methods

Animals and Diet. All protocols for animal use were approved by the Research Animal Resources Center at the University of Wisconsin-Madison. Two groups of New Zealand white rabbits(six rabbits per group) were fed a semi-purified diet containing high fat and 0.1% cholesterol(this experiment was initiated to study the effects of CLA on atherosclerosis : Atherosclerosis 108(1994) : 19 – 25). The composition of the diet was as follows : 25% vitamin free casein, 20% sucrose, 20% corn starch, 15% cellulose, 12% hydrogenated coconut oil, 2% corn oil, 1% vitamin mixture, 5% mineral mixture with methionine and arginine, and 0.1% cholesterol. For one group, the diet was augmented with CLA(0.5g CLA/rabbit/day). Food consumption and body weights were recorded daily and weekly, respectively. Blood samples were collected from animals after 17 hours fasting. At the end of 22 weeks, the rabbits were sacrificed using an overdose of sodium phenobarbital and fatty acid analyses were conducted on the plasma, liver, and aorta.

Cell culture. Hep G2 cells were obtained from ATCC. Cell stocks were maintained in Dulbecco's modified Eagle's medium(DMEM) containing antibiotics with non-essential amino acids and 10% fetal bovine serum(FBS) and were incubated at 37℃ in a humidified atmosphere of 95% air, 5% CO_2. Subcultures for the experiments were obtained from stock monolayers using trypsin. The medium was changed every 2 – 3 days. For the experiments, confluent monolayers were washed several times with phosphate buffered saline(PBS) and incubated with serum free DMEM for 24 hours. This was followed by DMEM containing albumin as carrier, with or without corresponding fatty acids. Fatty acid was complexed with fatty acid free bovine serum albumin(BSA) in a 2 : 1 molar ratio according to Mahoney et al[15]. After 20 – 24 hours fatty acid treatment, cell monolayers were washed and harvested. Cytotoxicity was assessed by trypan blue exclusion and MTT-dye(dimethylthiazol diphenyltetrazolium bromide) reduction[16].

Lipid extraction and fatty acid analysis. Total lipids were extracted from cell suspensions and from culture media with ethylacetate/acetone(2 : 1, by vol)[17], or from rabbit tissues with chloroform/methanol(2 : 1, by vol)[18]. Phospholipids were separated either by thin layer chromatography(TLC)[19] or by Sep-Pak cartridge [20]. Aliquots of the lipid extracts were dried under nitrogen and were converted to fatty acid methyl esters using 4% HCl in methanol[21]. Fatty acid methyl esters were separated by gas chromatography[1].

Assay of SCD activity in Hep G2 cells. Activity of stearoyl-CoA desaturase(EC 1.14.99.5) was measured by a modification of the method of Legrand et al[22]. Microsomal fraction isolated from Hep G2 cells was added to reaction mixture in a shaking water bath at 37℃. Each reaction mixture contained $MgCl_2$, ATP, Coenzyme A, NADH and [1-^{14}C] C16 : 0. Reactions were initiated by the addition of microsomes and stopped by the addition of 10% KOH/MeOH followed by heating at 80℃ for 45 min. After acidification, fatty acids were recovered and converted to methyl esters for analysis by argentation TLC. The quantitative distribution of radioactivity between the substrate and product was measured by a scintillation counter.

Conjugated linoleic acid. CLA was prepared from linoleic acid by alkali isomerization as previously described[2]. The purity of CLA exceeded 95%, and consisted of 2 major and several minor isomers. The two major isomers were c9, t11-CLA(42%) and t10, c12-CLA(44%).

Statistical analysis. Results are expressed as mean ± standard error unless otherwise stated. Data were analyzed by the Student's t-test or one-way ANOVA and Tukey's multiple range test. The difference was considered significant if the p value was less than 0.05.

Results

Animal experiment(rabbit). There were no differences in food intake and body weight between the groups. The addition of CLA resulted in substantial increases in the content of CLA in the total lipids of the plasma(Table 1), liver(Table 2), and aorta(Table 3). The plasma fatty acid composition in the total and the phospholipid fractions are shown in Table 1. Compared to the control rabbits, rabbits fed CLA had significantly lower monounsaturated fatty acid levels (palmitoleic acid C16 : 1, by 50% and oleic acid C 18 : 1, by 20%) in both the total lipid and phospholipid fractions. Similar results were seen for the total lipid fraction from the liver(Table 2) and from the aorta(Table 3), but less so for liver phospholipids, and not at all for aorta phospholipids.

Cell culture experiment(Hep G2 cells). The fatty acid compositions of total lipids and phospholipids in Hep G2 cell were modified by both LA and CLA(Table 4). The proportions of monounsaturated fatty acids in cellular lipids were significantly decreased by LA or CLA. However, the effect of CLA on monounsaturated fatty acids in total cellular lipids was stronger than that of LA ; the difference in the proportion of monoun-

Table 1. Fatty acid composition of total lipids and phospholipids from plasma in rabbits

Fatty acid	Total Lipids		Phospholipids	
	Control	CLA	Control	CLA
C12 : 0	2.13±0.37	1.98±0.45	0.23±0.02	0.16±0.03
C14 : 0	4.37±0.41	3.84±0.54	1.43±0.10	1.31±0.15
C16 : 0	19.48±0.56	19.86±0.54	22.20±0.91	22.40±0.77
C16 : 1	4.39±0.39	2.46±0.20*	1.31±0.16	0.67±0.13*
C18 : 0	16.21±0.49	18.28±0.48*	27.92±1.13	30.54±0.86
C18 : 1	23.49±0.81	18.61±0.81	13.76±0.67	11.37±0.42*
C18 : 2	26.36±0.51	29.16±0.49*	27.94±1.09	29.23±0.72
C18 : 3	0.85±0.21	1.18±0.21	3.88±0.66	2.73±0.98
C20 : 4	2.62±0.10	2.44±0.16	3.27±0.40	2.42±0.11
CLA	0.25±0.20	2.81±0.52*	ND	0.19±0.08
Δ9 desaturation index				
C16 : 0/C16 : 1	4.57±0.31	8.38±0.78*	18.04±1.89	29.08±2.82*
C18 : 0/C18 : 1	0.70±0.03	0.99±0.05*	2.07±0.17	2.71±0.16*

Values are mean±SE. Fatty acid composition is expressed as percentage of the total recovered fatty acids. ND ; not detected. Two groups of animals were fed an atherogenic diet with or without CLA(0.5g CLA/rabbit/day) for 22 weeks. See Materials and Methods for experimental details. *The difference is significant at $p<0.05$ by the Student's t-test

Table 2. Fatty acid composition of total lipids and phospholipids from liver in rabbits

Fatty acid	Total Lipids		Phospholipids	
	Control	CLA	Control	CLA
C12 : 0	1.62±0.34	1.58±0.64	0.07±0.01	0.08±0.01
C14 : 0	5.39±0.63	4.81±1.13	0.58±0.08	0.55±0.08
C16 : 0	19.50±0.68	21.40±0.45*	15.90±1.44	16.34±0.78
C16 : 1	4.03±0.18	2.33±0.35*	1.23±0.31	1.15±0.13
C18 : 0	13.78±1.08	17.76±1.51	34.86±1.37	34.37±1.02
C18 : 1	26.26±1.35	22.78±1.48	19.07±0.69	15.27±0.78*
C18 : 2	24.42±3.01	22.95±1.18	24.36±0.61	27.20±0.97*
C18 : 3	0.67±0.16	0.55±0.09	0.51±0.02	0.76±0.27
C20 : 4	4.11±0.57	4.15±0.65	3.58±0.45	4.62±0.75
CLA	0.21±0.02	1.71±0.25*	ND	0.56±0.23
Δ9 desaturation index				
C16 : 0/C16 : 1	4.86±0.17	10.07±1.20*	11.60±1.13	15.29±1.92
C18 : 0/C18 : 1	0.53±0.04	0.81±0.12	1.85±0.13	2.29±0.16*

Values are mean±SE. Fatty acid composition is expressed as percentage of the total recovered fatty acids. ND ; not detected. Two groups of animals were fed an atherogenic diet with or without CLA(0.5g CLA/rabbit/day) for 22 weeks. See Materials and Methods for experimental details. *The difference is significant at $p<0.05$ by the Student's t-test

Table 3. Fatty acid composition of total lipids and phospholipids from aorta in rabbits

Fatty acid	Total Lipids		Phospholipids	
	Control	CLA	Control	CLA
C12 : 0	6.68±0.78	7.11±0.26	3.33±0.72	3.48±0.47
C14 : 0	10.43±0.85	11.73±0.43	6.08±0.94	6.52±0.80
C16 : 0	28.24±0.88	27.88±0.43	34.75±1.44	33.16±1.61
C16 : 1	3.33±0.22	2.30±0.20*	2.28±0.43	1.56±0.12
C18 : 0	7.47±0.47	8.82±0.34*	22.31±2.07	22.79±1.29
C18 : 1	24.27±0.63	21.25±0.48*	14.75±1.05	13.79±0.70
C18 : 2	17.65±0.60	18.33±1.03	12.69±1.66	12.65±1.12
C18 : 3	1.41±0.34	0.99±0.10	0.13±0.13	ND
C20 : 4	0.56±0.09	0.45±0.03	5.53±0.80	6.31±1.27
CLA	ND	1.16±0.19	ND	ND
Δ9 desaturation index				
C16 : 0/C16 : 1	8.62±0.44	12.47±0.77*	17.86±3.09	21.83±1.09
C18 : 0/C18 : 1	0.31±0.02	0.42±0.01*	1.59±0.23	1.70±0.17

Values are mean±SE. Fatty acid composition is expressed as percentage of the total recovered fatty acids. ND : not detected. Two groups of animals were fed an atherogenic diet with or without CLA(0.5g CLA/rabbit/day) for 22 weeks. See Materials and Methods for experimental details. *The difference is significant at $p<0.05$ by the Student's t-test

Table 4. Fatty acid composition of total lipids and phospholipids in Hep G2 cells incubated with LA and CLA for 20 – 24 Hours

	C14 : 0	C16 : 0	C16 : 1 (n-7)	C18 : 0	C18 : 1 (n-9)	C18 : 1 (n-7)	C18 : 2	C20 : 4	CLA
Total lipids									
Control	4.7±0.1^a	26.2±0.1^a	18.5±0.0^a	5.7±0.0^a	20.9±0.4^a	19.9±0.2^a	1.2±0.0^a	2.9±0.1^a	ND
150μM LA	3.9±0.5^b	21.8±0.3^b	12.2±0.2^b	5.1±0.1^b	13.3±0.4^b	12.4±0.1^b	25.4±0.5^b	6.0±0.2^b	ND
150μM CLA	4.8±0.1^a	24.5±0.1^c	10.1±0.2^c	5.9±0.1^a	13.5±0.3^b	11.9±0.1^b	1.7±0.0^a	2.1±0.0^c	25.7±0.0
Phospholipids									
Control	3.6±0.3	22.1±0.7^a	15.6±0.4^a	12.4±1.3	22.7±0.4^a	18.0±0.6^a	1.4±0.2^a	4.4±0.5^a	ND
150μM LA	3.2±0.2	19.0±0.6^b	11.3±0.0^b	10.1±0.6	16.8±0.6^b	13.8±0.1^b	19.7±0.6^b	6.2±0.5^b	ND
150μM CLA	4.1±0.3	22.3±0.7^a	11.0±0.3^b	11.3±0.6	17.8±0.4^b	13.3±0.1^b	1.8±0.2^a	3.0±0.2^a	15.5±0.7

Values are mean±SE. Fatty acid composition is expressed as percentage of the total recovered fatty acids. ND : not detected. Hep G2 cells were incubated in media containing fatty acid-free BSA(control) or BSA complexed with 150μM LA or CLA. See Materials and Methods for experimental details. One-way ANOVA and Tukey's multiple range test were performed on SPSS to compare means

Values with different superscripts are significantly different at $p<0.05$

saturated fatty acids between LA and CLA-treated groups was significant. Moreover, according to the ratio of C16 : 0/C16 : 1 or C18 : 0/C18 : 1, the effects of CLA on Δ9 desaturation are more potent than those of LA on the total lipid and phospholipid fractions, as well as cellular microsomes(Table 5). In addition, monounsaturated fatty acids in the culture medium, which are secreted from Hep G2 cells after a 20 hour exposure, were decreased by CLA but not LA(Table 6). The proportion of C18 : 1(n-7) was also significantly decreased by CLA. The inhibitory effect of CLA on Δ9 desaturation seems to occur at concentrations as low as 15μM and is significantly different from that of LA(Fig. 1). Fig. 2 shows the changes in Δ9 desaturation as a function of time of exposure to CLA supplementation. CLA decreased Δ9 desaturation as shown by an increase in the ratio of C 16 : 0 to C16 : 1 in 4 – 5 hours. We report here only the ratio of C16 : 0 to C16 : 1 because the change in the ratio of C18 : 0 to C18 : 1 follows the same pattern. According to this result, CLA seems to affect the Δ9 desaturase enzyme(SCD) because the half-life of this enzyme is 4 hours[23]. In order to test this hypothesis, we measured SCD enzyme activity in cellular microsomes. As shown in Table 7, the activity of Δ9 desaturase was decreased more than 50% by CLA in Hep G2 cells.

CLA content in Hep G2 cells increased with the

Table 5. Effect of CLA on the ratios of C16 : 0/C16 : 1 and C18 : 0/C18 : 1 in Hep G2 cells

			Δ9 desaturation index	
			C16 : 0/ C16 : 1	C18 : 0/ C18 : 1
Cell	Total lipids	Control	1.42 ± 0.03^a	0.28 ± 0.01^a
		LA	1.79 ± 0.02^b	0.38 ± 0.01^b
		CLA	2.44 ± 0.04^c	0.44 ± 0.01^c
	Phospholipids	Control	1.42 ± 0.05^a	0.55 ± 0.06
		LA	1.69 ± 0.10^a	0.61 ± 0.06
		CLA	2.03 ± 0.21^b	0.63 ± 0.02
	Microsomes	Control	2.07 ± 0.01^a	0.45 ± 0.02^a
		LA	2.33 ± 0.04^b	0.47 ± 0.01^a
		CLA	3.33 ± 0.04^c	0.62 ± 0.00^b
Medium	Total lipids	Control	2.97 ± 0.03^a	1.11 ± 0.03^a
		LA	2.81 ± 0.05^a	1.06 ± 0.01^a
		CLA	4.79 ± 0.09^b	1.44 ± 0.04^b

Values are mean±SE. Fatty acid composition is expressed as percentage of the total recovered fatty acids ND : not detected. Hep G2 cells were incubated in media containing fatty acid-free BSA(control) or BSA omplexed with 150μM LA or CLA. See Materials and Methods for experimental details. One-way ANOVA and Tukery's multiple range test were performed on SPSS to compare means

Values with different superscripts are significantly different at $p<0.05$

Table 6. Fatty acid composition of culture medium of Hep G2 cells incubated with LA and CLA for 20 – 24 hours

	Control	Linoleic acid	CLA
C12 : 0	5.8±0.5	4.7±0.4	6.2±0.4
C14 : 0	4.1 ± 0.2^a	4.1 ± 0.1^a	5.9 ± 0.3^b
C16 : 0	20.3 ± 0.4^a	20.8 ± 0.3^a	22.7 ± 0.2^b
C16 : 1 (n-7)	6.9 ± 0.2^a	7.4 ± 0.1^a	4.8 ± 0.1^b
C18 : 0	11.3 ± 0.2^a	9.8 ± 0.3^b	11.6 ± 0.3^a
C18 : 1 (n-9)	10.2 ± 0.4^a	9.2 ± 0.3^a	8.1 ± 0.2^b
C18 : 1 (n-7)	6.1 ± 0.3^a	6.0 ± 0.1^a	4.2 ± 0.1^b
C18 : 2	3.3 ± 0.0^a	17.0 ± 0.8^b	3.4 ± 0.0^a
C20 : 2	32.0 ± 1.1^a	19.0 ± 2.2^b	25.4 ± 0.6^c
C20 : 4	ND	1.9±0.1	ND
CLA	ND	ND	7.7±0.1

Values are mean±SE. Fatty acid composition is expressed as percentage of the total recovered fatty acids. ND : not detected. Hep G2 cells were incubated in media containing fatty acid-free BSA(control) or BSA complexed with 150μM LA or CLA. See Materials and Methods for experimental details. One-way ANOVA and Tukey's multiple range test were performed on SPSS to compare means. Values with different superscripts are significantly different at $p<0.05$

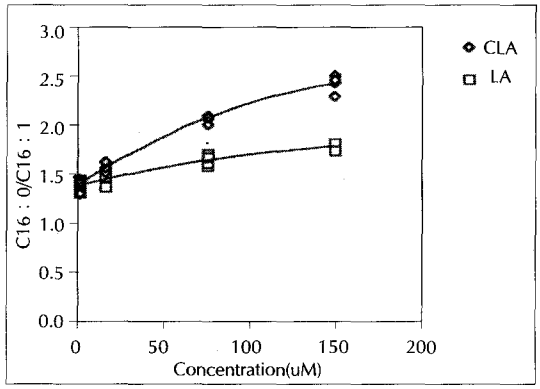

Fig. 1. The ratio of C16 : 0/C16 : 1 in cellular total lipids as a function of CLA or linoleic acid concentration in the culture medium. Eath line represents 2nd order polynomial regression($r^2=0.983$ for CLA, 0.904 for LA). Difference between CLA and LA is significant($p<0.001$).

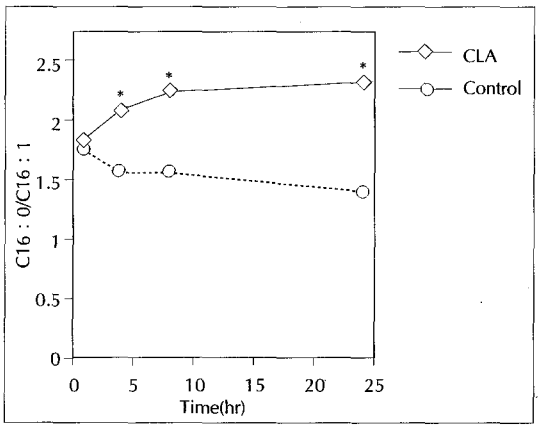

Fig. 2. The ratio of C16 : 0/C16 : 1 in total lipids from Hep G2 cells over the time of incubation in medium containing 150μM CLA. The values are mean± SE(SEs are too small to be shown in this graph). *Differences are significant at $p<0.001$

concentration of CLA and the time of exposure, showing a typical response curve with a plateau. However, the ratio of these two isomers in the lipids from cells and from media increased with time of exposure (Table 8).

Discussion

The results confirm our previous finding that CLA significantly decreases monounsaturated fatty acids in rabbits as well as in Hep G2 cells. Throughout the experiments, we used the ratios of C16 : 0/C16 : 1 or

Table 7. Effect of CLA on stearoyl CoA desaturase(SCD, Δ9 desaturase) activity in Hep G2 cells

	SCD (nmol/min/mg Protein)
Control	0.135±0.019
150μM CLA	0.060±0.006*

Values are mean±SE. After 20 – 24 hours fatty acid treatment, cell monolayers were washed and harvested. Stearoyl CoA desaturase activity was measured in cellular microsomes

*The difference is significant at $p<0.01$ by t-test

Table 8. The ratio of c9, t11-CLA to t10, c12-CLA in Hep G2 cells with time of incubation

Time(hr)	c9, t11-CLA/t10, c12-CLA	
	Cells	Culture media
0(1)		0.955±0.001
1	0.932±0.016[a]	1.034±0.011[a]
4	0.980±0.003[a]	1.243±0.044[b]
8	1.026±0.004[b]	1.510±0.024[c]
24	1.140±0.004[c]	1.495±0.062[c]
48(2)	1.485±0.040[d]	1.610±0.075[c]

Values are mean±SE

(1) CLA was in experimental media at 0 point

(2) Hep G2 cells were incubated in the medium containing 150μM CLA for 24 hours and then followed by fatty acid-free albumin-containing media for 24 hours

Values with different superscripts in a column are significantly different at $p<0.05$

C18 : 0/C18 : 1 as an Δ9 desaturation index to show Δ9 desaturation ; increasing these ratios represents decreased Δ9 desaturation. Although this ratio seems to provide indirect evidence of Δ9 desaturation, it has been used for the measurement of Δ9 desaturation with cyclopropene fatty acid, a well-known Δ9 desaturase inhibitor[24]. It was also reported[25] that Δ9 desaturase activity in the liver is positively correlated with the ratio of C18 : 1 to C18 : 0 in plasma total lipids (note that we use the opposite ratio). In addition, the assessment of Δ9 desaturation using these ratios was consistent with the result of the proportion of C18 : 1(n-7). The level of C18 : 1(n-7), the elongation product of C16 : 1(n-7), was also used to reflect Δ9 desaturation activity of tissues when animals are challenged by dietary manipulations[26][27]. In some reports[28][29] Δ9 desaturation was assessed by either C16 : 1 or C18 : 1 content. However we believe that the ratios of C16 : 0/C16 : 1 or C18 : 0/C18 : 1(substrate to product) better suggest an effect on Δ9 desaturase since the relative percentages of C16 : 0, C18 : 0, C 16 : 1, and C18 : 1 are expected to change with an increase in any supplemented fatty acids.

We observed similar changes in the ratios of C16 : 0/C16 : 1 and C18 : 0/C18 : 1 between plasma and liver in rabbits. In contrast to those of total lipids, the ratios in rabbit aorta phospholipid fractions seemed unaffected by CLA. The phospholipid turnover in rabbit aorta seems to be very slow since CLA was not detected in the phospholipid fraction in rabbits fed CLA. In view of cell turnover, it may not be surprising that the fatty acid composition of aorta phospholipids was similar between control and CLA groups. The phospholipid composition of membranes varies markedly between different tissues and organs within an individual animal and in the same tissues and organs between different species, and is a specific characteristic of the membranes[30].

The inhibitory effect of CLA on Δ9 desaturation in Hep G2 cells was more potent than that of LA, which also decreased Δ9 desaturation in total lipids (Table 5). It is not clear whether the changes in monounsaturated fatty acids by LA are due to LA itself or to arachidonic acid(its metabolite by elongation and desaturation), which has been reported to decrease Δ9 desaturation[31]. Arachidonic acid content was increased by the supplementation of LA in this experiment(Table 4 and 6). Unlike monounsaturated fatty acids in total lipids extracted from cell suspensions, those in a culture medium secreted from Hep G2 cells were significantly decreased by CLA but not LA. The modulation of fatty acid composition in a culture medium is important since fatty acids in culture medium under these experimental conditions come from secreted lipoproteins(VLDL). In this sense, it may be a model for the plasma fatty acid profile in animals. Therefore, the results are consistent with others from intact animals such as rabbits and chickens[14].

The microsomal activity of this enzyme was significantly decreased in CLA-treated cells, which is consistent with the results shown by the ratios of saturated fatty acids to monounsaturated fatty acids. Hence, with all our results, it can be postulated that the effect of CLA on fatty acid composition in rabbits as well as chickens[14] is due to the decreased formation of monounsaturated fatty acids caused by reduced activity of Δ9 desaturase(SCD) in the liver.

The reduction of monounsaturated fatty acid levels

may not be due solely to inhibition of the desaturase system, but possibly also to direct competition with CLA via selective binding to acyltransferases in animals. It is unlikely, however, that this phenomenon plays a role in the reduction of monounsaturated fatty acids in Hep G2 cells ; there is no fatty acid in the experimental medium other than CLA. It is also possible that if fatty acid synthesis is increased for any reason, it results in accumulated palmitic acid(C16 : 0). However, Δ9 desaturase is usually predominant, and the activity increases dramatically with fatty acid synthesis. Moreover, increasing saturated fatty acid intake can also raise the level of desaturase activity[13].

Synthetically prepared CLA, which was used in all of our experiments, consists of two major isomers and several minor isomers[1]. The two major isomers are c9, t11-CLA(42%) and t10, c12-CLA(44%). The result (Table 8) shows that the ratio of these two isomers(c9, t11-CLA/t10, c12-CLA) was increased with time of exposure which means more c9, t11 than t10, c12 was found. It is likely that cells take up both isomers at the same rate, depending upon their concentrations in experimental media at the beginning. It seems that c9, t11-CLA was preferentially incorporated into the cells or that t10, c12-CLA was more readily metabolized. Although it is not presently possible to account for this phenomenon, it may indicate that each CLA isomer has a different activity in biological functions.

The synthesis of long chain monounsaturated fatty acids in animal cells depends on the microsomal Δ9 desaturase(SCD) system. SCD is regulated by dietary deprivation and alteration[32], hormones[33], and the composition of dietary fat[31][34]. Diets rich in polyunsaturated fatty acids typially depress the activity of SCD[26][34-36]. However, the effect of CLA on Δ9 desaturation seems to be more potent than that of other fatty acids because the amount of other polyunsatu-rated fatty acids must be much higher to show similar effects. For example, Field et al[37] showed that 10% menhaden oil (high in n-3 fatty acids) decreased mono-unsaturated fatty acids in rabbits. Δ9 desaturase was inhibited by diets containing 20% linseed oil, sunflower oil, and fish oil[34].

The consequences of decreased production of monounsaturated fatty acids, especially oleic acid, may be relevant to lipoprotein metabolism. It has been suggested that changing monoenoic fatty acids into liver lipids by Δ9 desaturase would facilitate their incorporation into nascent VLDL, their secretion from the liver, and subsequent transport to adipose tissue[38]. Indeed, there is evidence suggesting that the activity of liver SCD is positively associated with body fat[39-41]. It is of interest that CLA reduces body fat content[11]. The hepatic secretion of VLDL requires sufficient amounts of monounsaturated fatty acids generated by Δ9 desaturase and hepatic synthesis of apoB100, the major apolipoprotein of VLDL[42]. Studies using cultured hepatocytes or livers showed that oleic acid stimulated both the synthesis and secretion of VLDL[43][44]. Recent studies performed on Hep G2 cells demonstrated that exogenous oleic acid could be a physiological modulator of apoB secretion by increasing the intracellular stability of this apoprotein[45]. These studies provide a possible explanation of the specific importance of Δ9 desaturase, among other lipogenic enzymes, in the control of liver VLDL secretion. The reduction of plasma TG by CLA in rabbits[9] might be related to the tendency of CLA to decrease monounsaturated fatty acid production by the SCD enzyme in the liver. Many mechanisms may be involved in the process of regulating Δ9 desaturase activity. The most convincing evidence indicates that changes in desaturase activity represent changes in enzyme synthesis[31]. Dietary polyunsaturated fatty acids, especially AA, decrease the abundance of SCD mRNA[46]. Indeed, in another study we found that CLA also reduced hepatic SCD1 mRNA levels. This data will be presented elsewhere.

■ Acknowledgements

The author thank Dr. Michael Pariza for reviewing this manuscript and Jayne Storkson for technical assistance.

Literature cited

1) Ha YL, Grimm NK, Pariza MW. Newly recognized anticarcinogenic fatty acids : Identification and quantification in natural and processed cheeses. *J Agric Food Chem* 37 : 75-81, 1989

2) Chin SF, Liu W, Storkson JM, Ha, YL, Pariza MW. Dietary Sources of conjugated dienoic isomers of linoleic

acid, a newly recognized class of anticarcinogens. *J Food composition and Analysis* 5 : 185-197, 1992

3) Ha YL, Grimm NK, Pariza MW. Anticarcinogens from fried ground beef : Heat altered derivatives of linoleic acid. *Carcinogenesis* 8 : 1881-1887, 1987

4) Ha YL, Storkson JM, Pariza MW. Inhibition of benzo(a) pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Res* 50 : 1097-1101, 1990

5) Ip C, Chin SF, Scimeca JA, Pariza MW. Mammary cancer prevention by conjugated linoleic acid. *Cancer Res* 51 : 6118-624, 1991

6) Ip C, Singh M, Thompson HJ, Scimeca JA. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Res* 54 : 1212-1215, 1994

7) Miller CC, Park Y, Pariza MW, Cook ME. Feeding conjugated linoleic acid to animals partially overcomes catabolic response due to endotoxin injection. *Biochem Biophys Res Comm* 198 : 107-1112, 1994

8) Chin SF, Storkson JM, Albright KJ, Cook ME, Pariza MW. Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *J Nutr* 124 : 2344-2349, 1994

9) Lee KN, Kritchevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108 : 19-25, 1994

10) Nicolosi RJ, Courtemanche KV, Laitinen L, Scimeca JA, Huth PJ. Effect of feeding diets enriched in conjugated linoleic acid on lipoproteins and aortic atherogenesis in hamsters. *Circulation 88 Suppl* : 2458, 1993

11) Park Y, Albright KJ, Liu W, Cook ME, Pariza MW. Dietary conjugated linoleic acid(CLA) reduces body fat content and isomers of CLA are incorporated into phospholipid fraction. Abstract for 1995 Annual Meeting of the Institute of Food Technologists. 64-10, p183, 1995

12) Gurr MI. Lipid metabolism in man. *Proc Nutr Soc* 47 : 277-285, 1988

13) Jeffcoat R, James AT. The regulation of desaturation and elongation of fatty acids in mammals. In Numa S. ed. Fatty Acid Metabolism and Its Regulation, pp.85-112, Elsevier, Amsterdam, 1984

14) Lee KN, Cook ME, Pariza MW. Conjugated linoleic acid alters fatty acid composition and hatchability in chicken eggs. *J Nutr* submitted for publication, 1996

15) Mahoney EM, Hamill AL, Scott WA, Zanvil AC. Response of endocytosis to altered fatty acyl composition of macrophage phospholipids. *Pro Natl Sci USA* 74 : 4895-4899, 1977

16) Plumb JA, Milroy R, Kaye SB. Effects of the PH dependence of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide-formazan absorption on chemosensitivity determination by a novel tetrazolium-based assay. *Cancer Res* 49 : 4435-4440, 1989

17) Slayback JR, Cheung LWY, Geyer RP. Quantitative extraction of microgram amounts of lipid from cultured human cells. *Anal Biochem* 83 : 372-384, 1977

18) Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* 226 : 497-509, 1957

19) Peters TJ, de Duve C. Lysosomes of the arterial wall. II. Subcellular fractionation of aortic cells from rabbits with experimental atheroma. *Exp Mol Pathol* 20 : 228-256, 1974

20) Juaneda P, Rocquelin G. Rapid and convenient separation of phospholipids and non phospholipids lipids from rat heart using silica cartridges. *Lipids* 20 : 40-41, 1985

21) Luddy FE, Barford RA, Riemenschneider RW. Direct conversion of lipid components to their fatty acid methyl esters. *J Am Oil Chem Soc* 37 : 447-450, 1960

22) Legrand P, Bensadoun A. Stearyl-CoA desaturase activity in cultured rat hepatocytes. *Biochim Biophys Acta* 1086 : 89-94, 1991

23) Joshi VC, Aranda LP. Hormonal regulation of the terminal enzyme of microsomal stearoyl coenzyme A desaturase in cultured avian liver explants. *J Biol Chem* 254 : 11779-11782, 1979

24) Phelps RA, Shenstone FS, Kemmerer AR, Evans RJ. A review of cyclopropenoid compounds : Biological effects of some derivatives. *Poultry Sci* 44 : 358-394, 1965

25) de Antueno RJ, Cantrill RC, Huang Y-S, Elliot M, Horrobin DF. Relationship between mouse Δ9 desaturase activity and plasma lipids. *Lipids* 28 : 285-290, 1993

26) Garg ML, Wierzbicki AA, Thomson ABR, Clandinin MT. Dietary cholesterol and/or n-3 fatty acid modulatd Δ9 desaturase activity in rat liver microsomes. *Biochim Biophys Acta* 962 : 330-336, 1988

27) Spence MW. The effect of a fat free diet on esterified monoenoic fatty acid isomers in rat tissues. *Lipids* 6 : 831-853, 1971

28) Cunnane SC, Horrobin DF, Manku MS. Contrasting effects of low or high copper intake on rat tissue lipid essential fatty acid composition. *Ann Nutr Metab* 29 : 103-110, 1985

29) Evans RJ, Bandermer SL, Anderson M, Davidson JA. Fatty acid distribution in tissues from hens fed cottonseed oil or sterculia foetida seeds. *J Nutr* 76 : 314-319, 1962

30) Wahle KWJ. Fatty acid modification and membrane lipids. *Proc Nutr Soc* 42 : 273-287, 1983

31) Ntambi JM. Dietary regulation of stearoyl CoA desaturase 1 gene expression in mouse liver. *J Biol Chem*

267 : 10925-10930, 1992

32) Cook HW, Spence MW. Formation of monoenoic fatty acids by desaturation in rat brain homogenate. Effect of age, fasting and refeeding and comparisons with liver enzyme. *J Biol Chem* 248 : 1793-1796, 1973

33) Brenner RR. Endocrine control of fatty acid desaturation. *Biochem Soc Trans* 18 : 773-775, 1990

34) Christiansen EN, Lund JS, Rortveit T, Rustan AC. Effect of dietary n-3 and n-6 fatty acids on fatty acid desaturation in rat liver. *Biochim Biophys Acta* 1082 : 57-62, 1991

35) de Antueno RJ, Cantrill RC, Huang Y-S, Raha SK, Elliot M, Horrobin DF. Effect of n-3 and n-6 fatty acids on hepatic microsomal lipid metabolism : A time course study. *Mol Cell Biochem* 118 : 153-161, 1992

36) de Schrijver R, Privett OS. Effect of dietary long chain fatty acids on the biosynthesis of unsaturated fatty acids in the rat. *J Nutr* 112 : 619-626, 1982

37) Field FJ, Albright EJ, Mathur SN. Effect of dietary n-3 fratty acids on HMG-Co A reductase and ACAT activities in liver and intestine of the rabbit. *J Lipid Res* 28 : 50-58, 1987

38) Jeffcoat R. The biosynthesis of unsaturated fatty acids and its control in mammalian liver. *Essays Biochem* 15 : 1-36, 1979

39) Enser M. The role of insulin in the regulation of stearic acid desaturase activity in liver and adipose tissue from obese-hyperglycemic(ob/ob) and lean mice. *Biochem J* 180 : 551-558, 1979

40) Lerand P, Mallard J, Bernard-Griffiths MA, Douaire M, Lemarchal P. Hepatic lipogenesis in genetically lean and fat chickens. In Vitro studies. *Comp Biochem Physiol* 87B : 789-792, 1987

41) Wahle KWJ, Radcliffe JD. Effect of a diet rich in sunflower oil on aspects of lipid metabolism in the genetically obese rat. *Lipids* 12 : 135-139, 1977

42) Gibbons GF. Assembly and secretion of hepatic very low density lipoprotein. *Biochem J* 268 : 1-13, 1990

43) Moberly JB, Cole TG, Alpers DH, Schonfeld G. Oleic acid stimulation of apolipoprotein B secretion from Hep G2 cells and Caco2 cells occurs post transcriptionally. *Biochim Biophys Acta* 1042 : 70-80, 1990

44) Salam WH, Wilcox HG, Heimberg M. Effects of oleic acid on the biosynthesis of lipoprotein apoproteins and distributions into the very low density lipoprotein by isolated perfused rat liver. *Biochem J* 251 : 809-816, 1988

45) Dixon JL, Furukawa S, Ginsberg HN. Oleate stimulats secretion of apolipoprotein B-containing lipoproteins from Hep G2 cells by inhibiting early intracellular degradation of apolipoprotein B. *J Biol Chem* 266 : 5080-5086, 1991

46) Tebbey P, Buttke TM. Arachidonic acid regulates unsaturated fatty acid synthesis in lymphocytes by inhibiting stearoyl-CoA desaturase gene expression. *Biochim Biophys Acta* 1171 : 27-34, 1992