Impact of Dietary Conjugated Linoleic Acid on the Oxidative Stability of Rat Liver Microsomes and Skeletal Muscle Homogenates

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Dietary conjugated linoleic acid (CLA; 0−2.0%) increased CLA concentrations in liver microsomes and skeletal muscle homogenates from rats. Dietary CLA decreased oleic and arachadonic acid concentrations in both liver microsomes and skeletal muscle. The presence of CLA in liver microsomes had no impact on linoleic acid, arachadonic acid, and α-tocopherol oxidation rates. Dietary CLA (2.0%) also did not alter α-tocopherol oxidation rates in liver microsomes or muscle homogenates. Formation of malonaldehyde (MDA) in oxidizing liver microsomes decreased with increasing CLA concentration as determined by measurement of thiobarbituric acid−MDA complexes by HPLC. The ability of CLA to decrease MDA formation without impacting other lipid oxidation markers such as the disappearance of fatty acid and α-tocopherol suggests that decreased MDA concentration was the result of CLA's ability to lower polyenoic fatty acids such as arachadonic acid. While CLA does not appear to act as an antioxidant, its ability to decrease polyenoic fatty acid concentrations could decrease the formation of highly cytotoxic lipid oxidation products such as MDA.

Keywords: *Conjugated linoleic acid; antioxidant; lipid oxidation; fatty acid*

INTRODUCTION

Conjugated linoleic acid (CLA) represents a group of positional and geometric isomers of linoleic acid. CLA is found in high concentrations (3−10 mg/g fat; Shantha et al., 1995, 1994; Chin et al., 1992) in beef, lamb, and dairy foods due to the incomplete biohydrogenation of linoleic acid in the rumen (Kelly et al., 1998). CLA is unique among the fatty acids because of its wide range of bioactivity. Dietary CLA has been reported to decrease the risk of cancer (Ip, 1997) and atherosclerosis (Nicolosi et al., 1997; Lee et al., 1994) and cause a reduction of body fat (West et al., 1998; Park et al., 1997) in laboratory animals.

The mechanism(s) by which CLA impacts such a wide range of different biological pathways is unknown. One property that has been suggested to be responsible for CLA's bioactivity is its ability to act as an antioxidant. CLA was first reported to have antioxidant activity in a linoleic acid model system as determined by the ability of CLA to reduce lipid peroxide formation (Ha et al., 1990). Ip and co-workers (1991) later found that chronic feeding of CLA decreased the concentrations of thiobarbituric acid reactive substances (TBARS) in rat liver and mammary gland tissues. The antioxidant properties of CLA have also been tested in a model where CLA was physically incorporated into phosphatidylcholine liposomes (van den Berg et al., 1995). In the liposome model, CLA was not found to inhibit lipid oxidation promoted by iron or peroxyl radicals as determined by formation of conjugated dienes and oxidation of linoleic acid. Banni and co-workers (1998) found that dietary CLA (2 g/day for 3 day) did not alter oxidation rates of 20:4 and 22:6 in rat liver homogenates. Unfortunately, the work of Banni et al. (1998) did not report if the dietary CLA treatment altered liver CLA concentrations. In canola oil heated to 90 °C, free CLA and CLA methyl esters were found to accelerate lipid oxidation as determined by oxygen uptake and oxidation of linoleic and linolenic acids (Chen et al., 1997).

Dietary CLA can alter liver fatty acid compositions (Belury and Kempa-Steczko, 1997) and can decrease total fat concentrations (West et al., 1998; Park et al., 1997) in mice. These changes in lipid profiles could result in CLA impacting the oxidative stability of tissues. In addition, it is unknown if CLA has any impact on endogenous antioxidant concentrations or activity. Therefore the objective of this research was to determine if dietary CLA can alter the lipid composition of rat liver microsomes and skeletal muscle in a manner which would alter the oxidative stability of these tissues.

MATERIALS AND METHODS

Materials. 1,1,3,3-Tetraethoxypropane, propyl gallate, ethylenediaminetetracetic acid (EDTA), and (+) α-tocopherol were obtained from Sigma Chemical Co. (St. Louis, MO). CLA was obtained from Nu-Chek Inc. (Elysian, MN). All other chemicals were of analytical grade or purer.

Female Sprague Dawley rats (eight animals/treatment; 21 days old) were fed AIN-76A diets containing 5% lipid as corn oil or CLA (substituted for corn oil at 0, 0.5, 1.0, 1.5, and 2.0%) at the Roswell Park Cancer Institute (Buffalo, NY). Rats were sacrificed after 30 days, and the liver and thigh muscle from the hind legs were excised, frozen, and shipped overnight on

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10.1021/jf991343n CCC: $19.00
Published on Web 08/10/2000

dry ice to the University of Massachusetts, Amherst. Upon delivery, the samples were immediately stored at −80 °C.

Liver Microsome and Muscle Homogenate Preparation. Liver microsomes were isolated by a modified method of Player and Hultin (1977). Rat livers were thawed at room temperature, minced, and mixed with 4 parts 0.12 M KCl−5 mM phosphate buffer at pH 7.25. The mixture was homogenized in a Waring Blender for 1 min. Homogenates were centrifuged in a Sorvall RC2−B centrifuge (Wilmington, DE) for 30 min at 15 000*g*. The supernatant was filtered through four layers of cheesecloth and recentrifuged in a Sorval Ultra80 ultracentrifuge (Wilmington, DE) for 60 min at 100 000*g*. The resulting pellet was suspended in 20 mL of 0.6 M KCl−5 mM phosphate buffer (pH 7.25) and centrifuged at 100 000*g* for 60 min. The final pellet, representing the microsomal fraction, was resuspended in 0.12 M KCl−5 mM phosphate buffer (pH 7.25) to a final volume of 20 mL. Protein concentration of the microsomal suspension was determined using the Lowrey method (Lowrey et al., 1951), and the microsomes were frozen at −80 °C until further analysis.

Thigh muscle was thawed at room temperature and trimmed of all excess fat and connective tissue. After the muscle was minced with a knife, 4 parts of 5 mM phosphate buffer (pH 7.4) was added (1:4), and the mixture was homogenized in a Waring Blender for 45 s.

Analysis of Lipid Oxidation. Microsomes (1 mg protein/mL of microsome) and muscle homogenate (20 mL) were oxidatively stressed in a shaking water bath at 37 °C. Homogenates and microsomes were placed in 25 mL Erlenmeyer flasks and sealed with Parafilm to limit water loss. Samples were removed and analyzed for lipid oxidation products, fatty acids, and α-tocopherol for up to 48 h of incubation.

Malonaldehyde−thiobarbituric acid (MDA−TBA) adducts were prepared by mixing muscle homogenate (1.0 mL) or microsomes (0.5 mL) with 1 mL of a solution containing 7.5% trichloroacetic acid, 0.1% propyl gallate, and 0.1% EDTA. The sample was vortexed for 5 s and centrifuged at 2000*g* for 5 min. Supernatant (1 mL) was removed, mixed with 1 mL of 0.01 M TBA, and placed in a boiling water bath for 20 min (Srinivasan and Xiong, 1996). After being cooled, (10 min) the resulting solution was filtered through an end tip syringe filter (0.45 μm).

MDA−TBA adducts were analyzed by HPLC separation (Richard et al., 1992) using an Econosil C18 10 μm column (250 mm × 4.6 mm) from Alltech Associated Inc. (Deerfield, IL). The mobile phase contained 50 mM phosphate buffer (pH 6.0) and methanol (58:42) and was run at 1.0 mL/min. Fluorometric detection of the MDA−TBA adduct was performed on a Waters 470 scanning fluorescence detector (Millipore Corp., Milford, MA) using an excitation wavelength of 532 nm and an emission wavelength of 553 nm. Concentrations were calculated from standard curves prepared using MDA generated from 1,1,3,3-tetraethoxypropane.

α-Tocopherol was analyzed using a modified method of Liu et al. (1996). Two milliliter of muscle homogenate or microsomal suspension was placed in a screw-capped tube containing 0.25 g of ascorbic acid and 7.3 mL of KOH in ethanol (11 g of KOH dissolved in 55 mL of ethanol and 45 mL of deionized water). The samples were vortexed until the ascorbic acid was completely dissolved. The samples were saponified in a water bath (80 °C) for 15 min (Pfalzgraf et al., 1995) followed by cooling in ice water and addition of 4 mL of hexane by vortexing for 2 min. The samples were placed in ice water for 5 min after which 3.8 mL of the hexane layer was removed and evaporated under nitrogen. The samples were reconstituted with 0.5 mL of hexane and filtered through an end tip syringe filter (0.45 μm) prior to HPLC analysis.

The analytical column used for tocopherol analysis was an Econosil C18, 10 μm column (250 mm × 4.6 mm) from Alltech Associates Inc. (Deerfield, IL). The mobile phase contained acetonitrile (75%) and methanol (25%) and was run at 1.5 mL/min. A Waters 470 scanning fluorescence detector (Millipore Corp., Milford, MA) was used for α-tocopherol detection and quantification. Excitation and emission wavelengths were 298 and 360 nm, respectively (Hatam and Kayden, 1979). Authentic α-tocopherol was for identification and quantitation.

Table 1. Total Fat Concentration of Rat Thigh Muscle from Animals Fed Varying Levels of Conjugated Linoleic Acid (CLA)

treatment	total fat (mg/g muscle)
control	20.7 ± 0.26[a]
0.5%	20.5 ± 2.87[a]
1.0%	20.6 ± 1.16[a]
1.5%	21.9 ± 3.24[a]
2.0%	21.4 ± 3.05[a]

[a] Means were not significantly different ($p > 0.05$).

Total Lipid and Fatty Acid Analysis. Thigh muscle lipids for total lipid determination and fatty acid analysis were extracted using the Bligh and Dyer (1959) procedure. Muscle lipid concentrations were determined gravimetrically after evaporation of solvent under nitrogen. Microsomal lipids for fatty acid analysis were isolated by vortexing 1 mL of the microsomal suspension (1 mg of protein/mL) with 5 mL of choloroform/methanol (1:1, v/v) containing 100 ppm butylated hydroxytoluene for 1 min. Aqueous saturated sodium chloride solution (1 mL) was vortexed into the solvent for 20 s and centrifuged at 2000*g* for 5 min. The bottom choloroform layer (4 mL) was removed and dried under nitrogen. Separation of polar and neutral thigh muscle lipids was performed by thin-layer chromatography using silica gel plates (Fisher Scientific, Pittsburgh, PA) and a hexane/diethyl ether/acetic acid (85:15:1) solvent system. After separation, samples were scrapped off the plates and lipids were extracted with chloroform/methanol (1:1) followed by solvent collection and subsequent evaporation using nitrogen (Ackman, 1991).

Isolated lipids were methylated with tetramethylguanidine (5%) in methanol (1 mL) at 100 °C for 10 min (Shantha et al., 1993). Hexane (1 mL) and saturated sodium chloride solution (1 mL) were mixed for 20 s (by vortexing) with the cooled solution containing the methylated fatty acids. The top hexane layer was then mixed with 5 mL of aqueous, saturated NaCl solution by vortexing for 1 min, and the hexane was again isolated by 5 min of centrifugation at 2000*g*. Fatty acids in the hexane layer were analyzed on a Hewlett-Packard 5890 gas chromatograph (GC) equipped with a Carbowax fused silica capillary column (30 m × 0.3 mm, 0.2 μm film thickness). Oven temperature was programmed as follows: 70 °C for 2 min; 70−180 °C at 20 °C/min; 180−230 °C at 2 °C/min; 230 °C for 10 min. Other parameters were split injection, helium carrier gas (11 psi), injector and flame-ionization detector temperatures of 250 °C. Fatty acids were identified using a fatty acid methyl ester kit from Sigma Chemical Co. (St. Louis, MO). Under these conditions CLA isomers were resolved as three peaks, and these three peaks were quantitated as total CLA. The purity of conjugated linoleic acid in each of the three peaks was verified by mass spectral analysis on a Hewlett-Packard gas chromatography/mass spectrometer (Ratnayake et al., 1986). Percentage of each fatty acid was calculated by normalization of the total fatty acid methyl exters.

Statistical Analysis. All experiments utilized samples from eight animals. Analysis of variance was performed using StatMost (Dataxiom Software, Los Angles, CA). Pairwise multiple comparison of means was performed using the least significant difference procedure.

RESULTS AND DISCUSSION

Influence of Dietary CLA on Rat Tissue Composition. Dietary CLA is capable of modifying fat metabolism as observed by reduction of adipose fat depot weight in mice (West et al., 1998) and lower body fat and higher lean body mass in mice (Park et al., 1997). The total fat content of skeletal muscle from rats in this study was not influenced by dietary CLA (Table 1). Lack of changes in fat content could be due to

Table 2. Area Percent Fatty Acid Composition of Rat Liver Microsomes Isolated From Animals Fed Varying Levels of Conjugated Linoleic Acid (CLA)

	treatment				
fatty acid	control	0.5%	1.0%	1.5%	2.0%
CLA	0.00 ± 0.00[a]	0.55 ± 0.06[a]	1.09 ± 0.12[a]	1.26 ± 0.11[a]	2.01 ± 0.39[b]
14:0	0.16 ± 0.01[a]	0.23 ± 0.04[a]	0.27 ± 0.09[a]	0.28 ± 0.06[a]	0.23 ± 0.06[a]
16:0	11.42 ± 0.18[a]	13.71 ± 0.71[b]	14.03 ± 1.02[a]	14.22 ± 0.68[b,c]	12.72 ± 0.27[b,d]
18:0	27.03 ± 2.13[a]	27.25 ± 1.42[a]	26.73 ± 1.93[a]	29.30 ± 0.07[a]	27.89 ± 1.04[a]
16:1	0.63 ± 0.06[a]	0.74 ± 0.19[a]	0.59 ± 0.02[a]	0.53 ± 0.04[a]	0.37 ± 0.10[b]
18:1	3.59 ± 1.10[a]	3.48 ± 1.47[a]	3.05 ± 1.47[a]	2.60 ± 1.28[b]	2.64 ± 1.15[b]
18:2	10.49 ± 0.73[a]	9.52 ± 0.73[a]	11.13 ± 0.76[a]	10.29 ± 0.78[a]	11.05 ± 2.51[a]
18:3	0.30 ± 0.30[a]	0.22 ± 0.03[a]	0.21 ± 0.03[a]	0.196 ± 0.06[a]	0.20 ± 0.26[a]
20:3	0.61 ± 0.10[a]	0.44 ± 0.05[b]	0.49 ± 0.05[a]	0.44 ± 0.08[a]	0.44 ± 0.42[a]
20:4	30.41 ± 0.32[a]	28.05 ± 1.08[a]	25.95 ± 0.86[b]	26.69 ± 1.44[b]	27.45 ± 0.55[b]
21:4	1.11 ± 0.15[a]	0.89 ± 0.09[a]	0.90 ± 0.12[a]	0.85 ± 0.15[a]	0.83 ± 0.12[a]
21:5	5.39 ± 0.49[a]	6.85 ± 0.83[a]	7.64 ± 0.39[a]	6.52 ± 1.92[a]	7.10 ± 2.98[a]
22:5	0.19 ± 0.20[a]	0.05 ± 0.08[a]	0.20 ± 0.15[a]	0.11 ± 0.11[a]	0.20 ± 0.21[a]
22:6	3.87 ± 0.49[a]	3.73 ± 0.33[a]	3.97 ± 0.25[a]	3.41 ± 0.28[a]	3.35 ± 0.29[a]

[a−c] Means with different letters within the same row are significantly different ($p < 0.05$).

Table 3. Area Percent of Fatty Acids From Polar and Neutral Lipids Isolated From the Hind Thigh Muscle of Rats Fed 0 (control) and 2.0% Conjugated Linoleic Acid (CLA)

fatty acid	polar lipids		neutral lipids	
	control	2.0% CLA	control	2.0% CLA
CLA	0.00 ± 0.00[a]	2.72 ± 0.17[b]	0.00 ± 0.00[a]	10.54 ± 1.24[b]
14:0	0.19 ± 0.03[a]	0.19 ± 0.03[a]	2.37 ± 0.01[a]	2.62 ± 0.10[a]
16:0	22.31 ± 0.12[a]	23.15 ± 0.41[a]	23.01 ± 1.19[a]	26.02 ± 2.29[a]
18:0	13.56 ± 0.21[a]	12.98 ± 0.94[a]	3.05 ± 0.16[a]	3.25 ± 0.06[a]
16:1	0.36 ± 0.37[a]	0.17 ± 0.16[b]	5.81 ± 1.37[a]	3.09 ± 0.59[b]
18:1	4.77 ± 0.40[a]	3.31 ± 0.18[b]	28.40 ± 0.43[a]	21.10 ± 0.44[b]
18:2	21.43 ± 1.35[a]	25.39 ± 0.44[a]	29.81 ± 2.36[a]	28.51 ± 1.59[a]
18:3n6	0.11 ± 0.00[a]	0.06 ± 0.01[a]	0.15 ± 0.01[a]	0.09 ± 0.14[a]
20:4	19.84 ± 1.32[a]	18.50 ± 1.00[b]	1.11 ± 0.03[a]	0.43 ± 0.14[b]
21:4	1.67 ± 0.30[a]	1.49 ± 0.51[a]	0.31 ± 0.10[a]	0.43 ± 0.14[a]
21:5	5.03 ± 0.04[a]	4.81 ± 1.66[a]	0.27 ± 0.06[a]	0.12 ± 0.04[a]
22:5	0.64 ± 0.09[a]	0.59 ± 0.01[a]	0.22 ± 0.00[a]	0.10 ± 0.01[a]
22:6	4.74 ± 0.10[a]	3.89 ± 0.59[a]	0.10 ± 0.06[a]	0.06 ± 0.02[a]

[a−c] Means with different letters within the same row are significantly different ($p < 0.05$).

differences in animal species, a shorter CLA feeding period or the presence of minimal amounts of adipose fat in the skeletal muscle.

CLA was not detected in liver microsomes or in skeletal muscle lipids from animals not fed CLA (Tables 2 and 3). In animals fed CLA, CLA concentrations increased with increasing dietary CLA levels in both liver and skeletal muscle. In skeletal muscle, CLA was found in both polar and neutral lipid fractions (Tables 3). Increasing dietary CLA to 2.0% increased total CLA 3.6-fold in liver microsomes and 3.1-fold in muscle homogenates as compared to the 0.5% CLA treatment.

Dietary CLA also caused a change in the fatty acid profile of the rat tissues. In the rat liver microsomes, all levels of dietary CLA were observed to significantly ($p \leq 0.05$) increase 16:0, and 1.5 and 2.0% CLA decreased 18:1 and 20:4 (Table 2). In the polar and neutral lipid fractions of rat skeletal muscle, 2.0% CLA was observed to significantly ($p \leq 0.05$) decrease 16:1, 18:1, and 20:4 (Table 3). Belury and Kempa-Steczko (1997) found that 1.5% dietary CLA decreased both 18:1 and 20:4 in mouse liver neutral lipids but had no effect on mouse polar lipids. Hayek et al. (1999) reported that 1% dietary CLA decreased $n-3$ fatty acids in the neutral lipids of young rats. Banni et al. (1999) observed that dietary CLA decreased in 18:3, 20:3, and 20:4 in lipids from rat mammary tissue.

Oxidative Stability of Microsomal Preparations. The ability of lipids to resist oxidation is dependent on factors such as level of fatty acid unsaturation and antioxidant concentrations. Fatty acid oxidation rates can be compared by determining changes in the ratio of an oxidizable unsaturated fatty acid to a nonoxidizable saturated fatty acid. When oxidation in the liver microsomes was monitored for up to 48 h, 18:2/18:0 or 20:4/18:0 ratios decreased similarly for all CLA treatments (Figure 1). In the 2.0% CLA treatment, 18:2/18:0 and 20:4/18:0 ratios decreased 10 and 12%, respectively, after 48 h of oxidation compared to a decrease in total CLA/18:0 ratio of 42%. Increased oxidation rates of CLA compared to 18:2 has also been reported by Chen et al. (1997) and van den Berg et al. (1995).

The influence of 2.0% dietary CLA on rat liver microsome and skeletal muscle α-tocopherol concentrations was measured to determine if CLA was impacting the concentration or activity of this important tissue antioxidant. If CLA was capable of impacting α-tocopherol oxidation, then differences in α-tocopherol disappearance rates would be observed. CLA did not alter initial α-tocopherol concentrations in either liver microsomes or muscle homogenates (Table 4). After 9 h of oxidation. α-tocopherol concentrations were observed to decrease in both liver microsomes and muscle homogenates (Table 4). α-Tocopherol concentrations decreased during oxidation in a similar manner in both microsomes and muscle homogenates regardless of whether CLA was present, suggesting that CLA was neither sparing nor regenerating α-tocopherol.

Malonaldehyde (MDA) concentrations increased in both liver microsomes and muscle homogenates during incubation (Tables 5 and 6). MDA formation was lower in liver microsomes from rats in the high (≥1.0%) CLA treatments. MDA formation was lower in muscle homogenates originating from animals fed 2.0% CLA compared to control animals; however, these differences were not significant ($p \geq 0.05$). Malonaldehyde is primarily formed from the oxidation of fatty acids with three or more double bonds, with the amount of MDA formation increasing with increasing double bond number (Sinnhuber and Yu, 1977; Janero, 1990). Since dietary CLA decreases the concentration of polyenoic fatty acids such as 20:4 (Table 2), it is possible that CLA decreases the yield of MDA produced from oxidizing liver microsomes without decreasing lipid oxidation rates. This hypothesis is supported by the observation

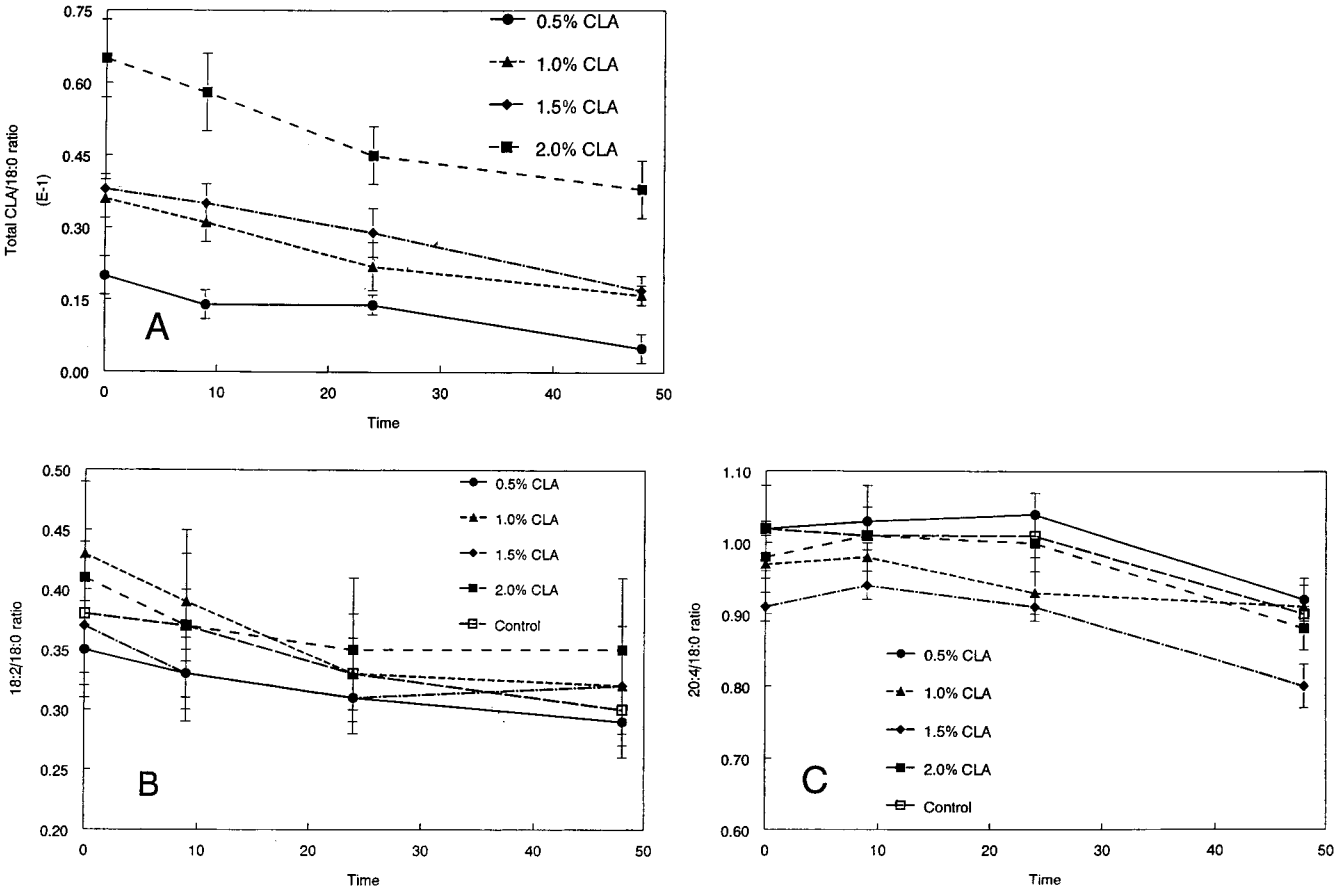

Figure 1. Oxidation of fatty acids in rat liver microsomes during incubation at 37 °C for 48 h as determined by total CLA/18:0 (A), 18:2/18:0 (B), and 20:4/18:0 (C) ratios.

Table 4. α-Tocopherol Concentrations (ng/mg protein) in Rat Liver Microsomes and Liver Homogenates† During Oxidation at 37 °C for up to 9 h

	liver microsomes		muscle homogenates	
time (h)	control	2.0% CLA	control	2.0% CLA
0.25	$44.3 \pm 2.5^{a,x}$	$42.7 \pm 4.3^{a,x}$	$1676.3 \pm 241.5^{a,x}$	$1238.2 \pm 162.2^{a,x}$
1.0	$32.5 \pm 5.3^{a,x}$	$32.3 \pm 13.5^{a,x}$	nd‡	nd
4.5	$31.7 \pm 6.1^{a,x}$	$31.5 \pm 2.2^{a,x}$	$1036.4 \pm 117.1^{a,x}$	$1084.2 \pm 185.3^{a,x}$
9.0	$22.5 \pm 4.0^{a,y}$	$26.2 \pm 5.3^{a,y}$	$836.5 \pm 182.1^{a,y}$	$801.3 \pm 43.1^{a,y\dagger}$

† Muscle and liver was obtained from rats fed 0 (control) or 2.0% CLA. ‡ nd = not determined. $^{a-c}$ Means with different letters within the same row and tissue sample are significantly different ($p < 0.05$). $^{x-z}$ Means with different letters with the same column are significantly different ($p < 0.05$).

Table 5. MDA−TBA Adduct (ng MDA/mg protein) Concentration in Rat Liver Microsomes† Incubated at 37 °C for up to 9 h

	% CLA				
time (h)	control	0.5	1.0	1.5	2.0
0.25	$0.0 \pm 0.0^{a,x}$	$0.0 \pm 0.0^{a,x}$	$0.0 \pm 0.0^{a,x}$	$0.0 \pm 0.0^{a,x}$	$0.0 \pm 0.0^{a,x}$
1.0	$22.7 \pm 11.3^{a,x}$	$24.5 \pm 4.3^{a,x}$	$0.0 \pm 0.0^{b,x}$	$11.3 \pm 10.0^{a,x}$	$0.0 \pm 0.0^{b,x}$
4.5	$53.7 \pm 4.5^{a,y}$	$55.0 \pm 10.0^{a,y}$	$43.7 \pm 12.7^{b,y}$	$63.5 \pm 12.7^{a,y}$	$13.5 \pm 2.2^{b,y}$
9.0	$135.3 \pm 22.1^{a,z}$	$130.2 \pm 5.2^{a,z}$	$82.7 \pm 5.5^{b,z}$	$88.3 \pm 11.7^{b,z}$	$53.5 \pm 14.5^{b,z\dagger}$

† Microsomes were isolated from rats fed conjugated linoleic acid (CLA) at concentrations ranging from 0−2.0%. $^{a-c}$ Same as in Table 2. $^{x-z}$ Means with different letters within the same column are significantly different ($p < 0.05$).

that CLA does not impact other lipid oxidation markers such as oxidation of specific fatty acids (Figure 1) and α-tocopherol (Table 4). Since MDA is the primary lipid oxidation product measured by the TBA reaction, this could explain why earlier studies found that dietary CLA could impact the amount of TBA reactive substances in rat liver and mammary glands (Ip et al., 1991).

CONCLUSIONS

The results of this research suggests that dietary conjugated linoleic acid does not inhibit lipid oxidation in rat liver microsomes and skeletal muscle homogenates, thus suggesting that CLA is not an antioxidant. However, dietary CLA does cause small alterations in the fatty acid composition of rat tissues. The type and

Table 6. MDA−TBA Adduct (ng MDA/g muscle) Concentrations in Rat Muscle Homogenates Incubated at 37 °C for up to 9 h

time (h)	control	2.0% CLA
0.25	$0.00 \pm 0.0^{a,x}$	$0.00 \pm 0.0^{a,x}$
4.5	$103.2 \pm 72.1^{a,x}$	$23.7 \pm 20.2^{a,x}$
9.0	$173.5 \pm 55.2^{a,y}$	$83.5 \pm 21.1^{a,y\dagger}$

† Livers were obtained from rats fed 0 (control) or 2.0% conjugated linoleic acid (CLA). $^{a-c}$ Same as in Table 2. $^{x-z}$ Same as in Table 5.

concentration of breakdown products arising from oxidizing lipids is dependent on fatty acid composition. Such an example is malonaldehyde which is produced in higher concentrations from the oxidation of polyenoic fatty acids. Since lipid oxidation products have been implicated in the development of diseases such as atherosclerosis and cancer (Kubow, 1992; 1993), it is possible that CLA's ability to alter fatty acid profiles could also alter the formation of cytotoxic lipid oxidation products, thereby protecting tissues from the detrimental effects of these compounds.

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Received for review December 9, 1999. Accepted May 7, 2000.

JF991343N