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High dietary levels of a conjugated linoleic acid mixture alter hepatic glycerophospholipid class profile and cholesterol-carrying serum lipoproteins of rats

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To investigate the body composition, hepatic lipids, and serum lipoproteins in response to graded levels of a conjugated linoleic acid (CLA) mixture added to a high linoleate diet, adult male Sprague-Dawley rats were randomly assigned into four dietary groups of 10 rats each and fed for 5 weeks controlled amounts of diets containing 0%, 1%, 3%, or 5% of a CLA mixture in exchange for sunflower oil. The various dietary lipid treatments did not significantly influence growth and body partitioning, although there was a trend toward decreased contents of extractable lipids in carcass (whole bled body without liver and gut) with increasing CLA. When carcass lipids of CLA-treated rats were extracted, a distinct accumulation of total CLA was observed. A dietary level of 1% CLA mixture exhibited only weak effects on hepatic glycerophospholipid levels. CLA levels of 3% and 5% caused distinct changes in phospholipid subclass distribution. These changes were reduced levels of lysophosphatidylethanolamine (LPE) and ethanolamine plasmalogen (EPL) and increased levels of phosphatidylcholine (PC). Further, a 5% level of CLA increased the hepatic concentration of phosphatidylserine (PS) compared with the other treatments. The incorporation of total CLA into individual phospholipids followed a dose-responsive manner. The extent of incorporation of CLA was not the same among the glycerophospholipid species analyzed, the order being cardiolipin > phosphatidylethanolamine and PC > LPE/EPL > phosphatidylinositol > PS. Further, CLA increased the proportions of n-3 fatty acids in the individual glycerophospholipids. High CLA diets containing 3% and 5% of a CLA mixture were associated with increased activity of catalase in the peroxisome-enriched cell fraction of liver and exhibited marked reductions of cholesterol in the low and high density lipoproteins relative to rats receiving no CLA. (J. Nutr. Biochem. 11:184–191, 2000)

Keywords: conjugated linoleic acid mixture; body composition; hepatic lipids; hepatic catalase; serum lipoproteins; rat

Introduction

Conjugated linoleic acids (CLA) are a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. Interest in CLA has been growing as a result of recently reported anticarcinogenic properties of these compounds[1,2] and a beneficial influence on serum cholesterol in rabbits[3] and hamsters.[4] In addition, CLA has been linked to growth development and body composition of animals: Rodents and pigs that were fed CLA-fortified diets deposit less body fat and are leaner than animals fed more ordinary oils such as sunflower oil (SFO) or corn oil.[5–8] However, in these trials on changes in body composition and serum lipids, feed intake was not controlled and the observed effects on body composition and serum lipids, both strongly reacting on energy ingested, may be due at least partially to differences in feed intake between the control group and the CLA-treated animals. This prompted

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this author to reevaluate the effect of CLA on growth development, body composition, and serum lipoproteins of rats by using a feeding regimen with controlled amounts of food and equalized levels of antioxidants in the oils used, an aspect that has not been considered until now. Most of the CLA effects reported were obtained with CLA concentrations between 0.5% and 1.5%. The effect of much higher CLA concentrations are comparatively unknown. Therefore, another objective of the present study was to compare the effects of a CLA dosage that was commonly used in recent animals studies with those of high CLA levels of 3% and 5%.

Some studies, albeit with contradictory results, have shown that only specific CLA isoforms are responsible for physiologic effects.[9,10] Because it has not been clearly established to date which of the CLA isoforms are responsible for the effect, a mixture of CLA including each of these isomers was used in the present study. It is also suspected that the effect of CLA may also be determined by other fatty acids in the diet and by the ratio of n-6 to n-3 fatty acids.[11] This study investigated the effect of CLA in a diet based on the n-6 fatty acid linoleate. This was of interest partly because a previous study had shown that concentrations of n-3 fatty acids in tissues of CLA-treated animals were increased,[12] and the present study design provided an opportunity to test whether this also occurs at extremely low dietary intakes of n-3 fatty acids.

Moreover, it is suggested that CLA is a member of a group of chemicals known as peroxisome proliferators because diets containing 0.5%, 1.0%, or 1.5% CLA have been shown to be associated with six-, nine-, and nine-fold increases, respectively, in hepatic mRNA levels of several enzymes known to be induced during peroxisome proliferation.[13] However, peroxisomes are also indispensible in forming saturated ether lipids and plasmalogens because alkyldihydroxy-acetone phosphate synthase is a membrane enzyme predominantly located in peroxisomes.[14–16] Phospholipids, one of the major component of membranes, not only provide the membrane with its structural integrity and physical properties, but also play an important role in a number of other cell processes that are important for normal cell, tissue, and organism function. Most of the CLA studies have been devoted to the CLA modification of the fatty acyl moieties of phospholipids, but less attention has been paid to the influence of CLA on phospholipid class distribution. Thus, the objectives of the present study were to evaluate the effects of increasing levels of dietary CLA on concentrations of individual glycerophospholipids in liver, which is the major site of lipid metabolism, the extent of incorporation of total CLA into these lipids subclasses, and the influence of CLA on the activity of peroxisome-associated catalase.

Materials and methods

Animals and diets

Forty male SPF Sprague-Dawley rats (WIGA GmbH, Sulzfeld, Germany) with an average body weight of 256 ± 1 g were fed semisynthetic cholesterol-free diets based on the AIN-93 formulation[17] containing either SFO or different levels of a CLA-containing oil with 60.5% CLA isomers (Multi-Food GmbH, Buxtehude, Germany) for 5 weeks. Rats were divided into four groups of 10 rats each and fed diets containing 0% (SFO control diet), 1%, 3%, and 5% levels of a CLA mixture (by weight) at the expense of SFO. The semisynthetic diet used for all groups consisted of (in g/kg) fat-free casein (200), corn starch (308), sucrose (300), SFO/CLA oil (83.3), minerals (40), fiber (30), vitamins (20), coconut oil (16.7), and DL-methionine (2). The concentrations of individual fatty acids in the experimental diets are shown in *Table 1*. The predominant fatty acid in the control diet was the linoleate (18:2 n-6). The main differences between the CLA-fortified diets and the control diet was a concomitant increase of CLA isomers at the expense of linoleate. The isomeric composition (measured in percent by weight) of the CLA-containing oil was analyzed by the Institute for Biochemistry and Food Chemistry (University of Hamburg, Hamburg, Germany) using a gas chromatographic method.[18] Data from this analysis gave the following isomer composition: 34.6% *c*9,*t*11 plus *t*9,*c*11 CLA, 18.4% *t*10,*c*12 CLA, 5.4% *t*9,*t*11 CLA, and 2.1% *c*9,*c*11 CLA. The difference in dietary α-tocopherol level between the SFO (640 mg/kg) and the CLA-containing oil (21 mg/kg) was made up by adding 619 mg α-tocopherol/kg CLA-containing oil. The daily feed allowance that was 11.4 g diet dry matter for each rat exceeded the energetic maintenance requirement of 444 kJ metabolizable energy/kg $W^{0.75}$ by approximately 25%.[19] The diets were stored at −4°C during the experimental period. The fatty acid pattern of the diets remained constant during storage.

Table 1 Fatty acid concentrations (mg/g) of the experimental diets (average of three determinations)

Fatty acid	SFO	1% CLA	3% CLA	5% CLA
12:0	6.51	6.84	6.55	6.57
14:0	2.51	2.68	2.54	2.51
16:0	6.25	5.89	5.37	4.67
18:0	4.09	3.67	2.83	1.98
22:0	0.14	0.15	0.15	0.15
SFA	19.5	19.2	17.4	15.9
14:1	0.02	ND	ND	ND
16:1	0.16	0.13	0.13	0.11
18:1	16.6	16.9	18.5	19.7
22:1	0.88	0.74	0.60	0.45
MUFA	17.7	17.8	19.2	20.3
18:2 n–6	55.2	45.0	25.1	6.8
18:3 n–6	0.69	0.40	0.26	0.13
18:3 n–3	0.33	0.07	0.05	ND
20:2 n–6	0.12	0.10	0.08	0.06
Total CLA	0.06	9.99	29.8	48.8
PUFA	56.8	55.6	55.3	55.0
Total fatty acids	93.6	92.6	91.9	92.0

SFO–sunflower oil. CLA–conjugated linoleic acids. SFA–total saturated fatty acids. MUFA–total monounsaturated fatty acids. PUFA–total polyunsaturated fatty acids. ND, not detectable.

Rats were housed individually in a controlled environment, in Macrolon cages (Becker GmbH, Castrop-Ruxel, Germany), in a room maintained at 24°C with 60% humidity. All rats were kept under conditions of controlled lighting with a daily 12-hr light: dark cycle and had free access to drinking water. Care and treatment of rats followed recommended guidelines.[20] At the end of the experimental period of 5 weeks, 12 hr after the last feeding, the rats were sacrificed by decapitation after light anesthesia with diethyl ether.

Analytical determinations

Blood for determination of serum lipids, lipoproteins, and clinical chemical variables was collected in untreated test tubes. The liver

was promptly excised. For analysis of body composition, the whole bled body without liver and gut (defined as carcass) was used. Serum, liver, and carcass samples were stored at −80°C until analyzed. Frozen carcasses were chopped, ground, and freeze-dried to determine total nitrogen and fat content. Total nitrogen in the carcasses was analyzed in triplicate by a Macro N apparatus (Heraeus, Hanau, Germany). Crude protein was calculated as N × 6.25. The content of extractable lipids from carcass was measured gravimetrically by extraction with diethyl ether overnight using a Soxhlet apparatus. Fat analyses were run in triplicate.

Diet, liver, and carcass lipids were extracted with a hexane: isopropanol mixture (3:2, v/v, containing butylated hydroxytoluene as antioxidant).[21] The hepatic glycerophospholipids cardiolipin (CL), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), and lysophospatidylethanolamine (LPE) and the ethanolamine plasmalogen (EPL) were separated by high performance liquid chromatography (HPLC)[22] and collected with a fraction collector (model 201, Gilson, Villiers-le-Bel, France). CL, PE, PC, PI, PS, and LPE/EPL separated by HPLC were methylated with boron fluoride/methanol reagent.[23] Fatty acid methyl esters (FAMEs) were separated by gas chromatography using a Hewlett-Packard HP 5890 gas chromatographic system (Taufkirchen, Germany), fitted with an automatic on-column injector, a flame ionization detector, and a CP-Sil 88 capillary column (50 m × 0.25 mm internal diameter, film thickness 0.2 μm; Chrompack, Middleburg, The Netherlands). The oven temperature program used was as described by Eder and Kirchgessner.[24] The detector temperature was 300°C. FAMEs were identified by comparing their retention times with those of individual purified standards and quantified with heptadecanoic acid methyl ester as internal standard.[25] The total CLA concentration was determined by using a standard mixture, which consisted of octadecadienoic acid methyl esters with *cis* and/or *trans* double bonds in the 9,11 and 10,12 positions. Because it was not possible to separate all peaks obtained from the single CLA isomers in the tissue phospholipids, total CLA was determined by adding the relevant peaks. The amounts of CL, PE, PC, PI, and PS were calculated by the amount of their bound fatty acids. Quantitative analysis of the LPE and EPL was done by a HPLC method using 1-dimethylamino-naphthalene-S-sulfonyl (Dns) chloride as derivatization reagent.[26] Hepatic PE, LPE, and EPL were determined as Dns derivatives. The Dns derivatives of PE and LPE were separated by a silica gel column with gradient elution (Si 60 Lichrospher column, 25 m × 4 mm internal diameter, film thickness 5 μm; Merck, Darmstadt, Germany). The eluate was monitored by fluorescence detection at 342 nm (excitation) and 500 nm (emission). EPL was determined indirectly by converting their derivatives into Dns-LPE with exposure to HCl fumes.

For measurement of liver total triacylglycerols and total cholesterol, liver lipids were extracted with hexane/isopropanol (3:2, v/v) and then dissolved in a chloroform/Triton X-100 mixture (1:1, v/v) as described previously.[27] After evaporating the chloroform under vacuum, total triacylglycerols and total cholesterol were determined by adding test reagents for fully enzymatic spectrophotometric assay of triacylglycerols (Merck, ref. 14354) and cholesterol (Boehringer, Mannheim, Germany, ref. 816302).

For determination of hepatic peroxisome-associated catalase (EC 1.11.1.6), liver homogenates (25%, w/v), prepared in 0.25 M sucrose (containing 0.1% ethanol), were fractioned by differential centrifugation to obtain a particle-bound fraction that is mostly enriched in lysosomes and peroxisomes.[28,29] Therefore, liver homogenates were centrifuged at 600 × g for 10 min to remove nuclei, unbroken cells, and cell debris. Then supernatant was centrifuged for 10 min at 3,000 × g. The supernatant from this centrifugation step was centrifuged at 20,000 × g for another 10 min. This procedure gives a complete sedimentation of peroxisomes.[28] The pellet obtained was then suspended in 2 mL 0.25 M sucrose and used for the determination of the catalase activity. Total catalase activity was measured spectrophotometrically,[30] after pretreatment of the enzyme source with Triton X-100, to a final concentration of 1% to disrupt the peroxisomal membranes. The determination of the enzyme activity was based on the measurement of the rate of converting hydrogen peroxide at 240 nm and a temperature of 25°C in the presence of the enzyme. Protein in the large granule fraction used for enzyme determination was measured by a method of Smith and coworkers[31] using bicinchoninic acid and bovine serum albumin as a standard.

The lipoproteins very low density lipoprotein (VLDL; density < 1.019 kg/L), low density lipoprotein (LDL; density 1.019–1.063 kg/L), and high density lipoprotein (HDL; density > 1.063 kg/L) were isolated by step-wise ultracentrifugation (230,000 × g for 20 hr at 8°C).[32] The concentrations of triacylglycerols, total cholesterol, and PC in serum and the individual lipoproteins were measured enzymatically using an auto analyzer (model 704, Hitachi, Tokyo, Japan) and kit reagents (Boehringer).

Total protein, albumin, creatinine, urea, glucose, aspartate amino transferase (ASAT; EC 2.6.1.1), and alanine amino transferase (ALAT; EC 2.6.1.19) in serum were determined by standardized procedures using an auto analyzer (model 704, Hitachi) and Boehringer kit reagents (Boehringer).

Statistical analysis

The effect of dietary CLA was evaluated by analysis of variance and compared for statistical significance ($P < 0.05$) by the Student Newman Keuls (SNK) test. All data in the present text are expressed as means ± SEM.

Results

Table 2 shows that body weights of adult rats fed the CLA-supplemented diets were not different from those of the SFO-fed controls. Hence, despite similar body weights, feeding 3% and 5% CLA resulted in a trend toward decreasing carcass fat and carcass fatty acid content. The various dietary lipid treatments did not influence the proportions of dry matter and protein in rat carcasses. In rats fed the CLA-fortified diets, the concentrations of individual fatty acids in carcass were different than those in rats fed SFO (*Table 2*). When total carcass lipids were extracted, a positive association between increasing amounts of dietary CLA mixture and total CLA per gram carcass was observed. The increase in CLA content primarily occurred at the expense of the linoleate (18:2 n-6) and was accompanied by a dose-responsive decrease of the elongation and desaturation products 18:3 n-6, 20:4 n-6, 22:4 n-6, and 22:5 n-6. Essentially, no statistical significant differences were observed with the concentrations of the saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA).

For CLA-treated rats, the liver wet weights and their triacylglycerol contents were not different from those of SFO-fed control rats (*Table 3*). CLA exhibited some slight but inconsistent effects on cholesterol level in liver. However, the most striking differences between the groups occurred with the individual glycerophospholipids. A dietary CLA level of 1% exhibited only weak effects on phospholipid levels in liver. CLA levels of 3% and 5% caused distinct changes in phospholipid subclass distribution. These changes were reduced concentrations of LPE and EPL and increased levels of PC compared with rats

Table 2 Effect of CLA on body weight and carcass composition[1,2]

	SFO	1% CLA	3% CLA	5% CLA
Initial body weight (g)	256 ± 2	256 ± 2	256 ± 2	256 ± 2
Final body weight (g)	317 ± 4	325 ± 3	327 ± 3	317 ± 3
Carcass dry matter (%)	32.9 ± 0.4	33.3 ± 0.4	32.6 ± 0.3	32.7 ± 0.4
Carcass protein content (%)[3]	23.5 ± 0.1	23.6 ± 0.2	23.8 ± 0.2	23.5 ± 0.2
Carcass fat content (%)[3]	6.27 ± 0.50	6.38 ± 0.33	5.92 ± 0.27	5.66 ± 0.48
Carcass fatty acid content (μmol/g)[3]	224 ± 15	237 ± 12	211 ± 11	201 ± 19
Individual fatty acids (μmol/g)[3]				
SFA	65.5 ± 4.8	71.7 ± 3.4	69.1 ± 3.8	72.4 ± 6.8
MUFA	58.3 ± 4.2	59.8 ± 3.1	55.9 ± 2.7	59.5 ± 5.3
PUFA	100 ± 7^{a}	105 ± 5^{a}	86 ± 5^{a,b}	69 ± 7^{b}
n–6 PUFA	97.3 ± 6.7^{a}	92.7 ± 4.9^{a}	58.7 ± 3.2^{b}	32.8 ± 3.1^{c}
18:2	86.9 ± 6.4^{a}	83.2 ± 4.7^{a}	49.5 ± 3.0^{b}	23.9 ± 2.6^{c}
18:3	1.03 ± 0.07^{a}	0.95 ± 0.05^{a}	0.60 ± 0.01^{b}	0.41 ± 0.03^{c}
20:4	6.03 ± 0.12^{a}	5.30 ± 0.10^{b}	4.53 ± 0.07^{c}	3.89 ± 0.09^{d}
22:4	0.90 ± 0.03^{a}	0.82 ± 0.03^{b}	0.72 ± 0.03^{c}	0.54 ± 0.02^{d}
22:5	0.52 ± 0.03^{a}	0.44 ± 0.03^{a}	0.37 ± 0.01^{c}	0.27 ± 0.01^{d}
Others	1.60 ± 0.09^{a}	1.48 ± 0.05^{b}	1.44 ± 0.10^{b}	1.22 ± 0.10^{c}
n–3 PUFA	2.05 ± 0.11^{a}	2.13 ± 0.10^{a}	1.69 ± 0.08^{b}	1.80 ± 0.06^{b}
18:3	0.65 ± 0.07^{a}	0.67 ± 0.08^{a}	0.33 ± 0.05^{b}	0.32 ± 0.04^{b}
Others	1.41 ± 0.05	1.46 ± 0.04	1.36 ± 0.04	1.48 ± 0.03
Total CLA	1.31 ± 0.06^{d}	11.1 ± 0.4^{c}	26.6 ± 1.5^{b}	36.0 ± 3.6^{a}

[1]Carcass is defined as the whole bled body without liver and gut.
[2]Data are represented as means ± SEM for 10 rats in each group.
[3]Referring to carcass wet weight.
[a,b,c,d]Values without a common superscript letter are significantly different at $p < 0.05$ (Student Newman Keuls test); values in the same row with no superscript are not different ($p > 0.05$).
SFO–sunflower oil. CLA–conjugated linoleic acids. SFA–total saturated fatty acids. MUFA–total monounsaturated fatty acids. PUFA–total polyunsaturated fatty acids.

receiving no CLA. Additionally, a 5% level of dietary CLA increased the concentration of PS in liver. Further, diets containing 3% and 5% CLA were associated with a higher activity of catalase in the peroxisome-enriched cell fraction of liver by 16% and 54%, respectively, relative to the SFO diet. Increasing levels of dietary CLA exhibited dose-responsive incorporation of total CLA into the different glycerophospholipid species (*Tables 4* and *5*). The extent of incorporation of total CLA was not the same among the phospholipid species analyzed, the order being CL > PE and PC > LPE/EPL > PI > PS. Slight differences in total SFA were observed among diet groups. No definite changes to proportions of total MUFA were observed for rats fed either the control or the CLA-fortified diets. The most profound alterations of fatty acid composition occurred in total polyene proportion. A reduction of n-6 polyunsaturated fatty acids (PUFA) in all glycerophospholipid species analyzed was observed in rats fed diets high in CLA. These changes seemed to be offset by a greater proportion of long-chain n-3 PUFA, primarily through significant elevation to docosahexaenoic acid (22:6 n-3).

CLA supplementation led to a number of considerable alterations in serum lipids and lipoproteins (*Table 6*). Animals fed the CLA-supplemented diet exhibited a con-

Table 3 Effect of CLA on lipid concentrations and catalase activity of liver[1]

	SFO	1% CLA	3% CLA	5% CLA
Wet weight (g)	7.74 ± 0.19	7.80 ± 0.12	8.09 ± 0.13	8.18 ± 0.13
Lipids (μmol/g)				
Triacylglycerols	10.8 ± 0.9	10.5 ± 0.7	9.73 ± 0.40	9.54 ± 0.29
Total cholesterol	5.37 ± 0.11^{a,b}	5.26 ± 0.08^{a,b}	5.06 ± 0.05^{b}	5.55 ± 0.17^{a}
Phosphatidylethanolamine	7.36 ± 0.30	7.18 ± 0.45	6.79 ± 0.35	6.15 ± 0.23
Ethanolamine plasmalogen	0.54 ± 0.05^{a}	0.49 ± 0.07^{a,b}	0.36 ± 0.03^{b,c}	0.30 ± 0.02^{c}
Lysophosphatidylethanolamine	0.55 ± 0.06^{a}	0.51 ± 0.05^{a}	0.34 ± 0.03^{b}	0.22 ± 0.01^{c}
Phosphatidylserine	1.09 ± 0.04^{b}	0.92 ± 0.04^{c}	1.15 ± 0.03^{b}	1.39 ± 0.07^{a}
Cardiolipin	1.72 ± 0.13	1.69 ± 0.12	1.63 ± 0.13	1.44 ± 0.10
Phosphatidylinositol	0.70 ± 0.06	0.72 ± 0.07	0.66 ± 0.04	0.56 ± 0.05
Phosphatidylcholine	14.9 ± 1.3^{b}	19.7 ± 1.5^{a,b}	25.3 ± 3.2^{a}	22.0 ± 3.4^{a,b}
Catalase (U/mg protein)[2]	527 ± 29^{c}	520 ± 27^{c}	613 ± 18^{b}	809 ± 30^{a}

[1]Data are represented as means ± SEM for 10 rats in each group.
[2]1 U of catalase is defined as 1 μmol hydrogen peroxide substrate decomposed/min at 25°C.
[a,b,c]Values without a common superscript letter are significantly different at $p < 0.05$ (Student Newman Keuls test); values in the same row with no superscript are not different ($p < 0.05$).
SFO–sunflower oil. CLA–conjugated linoleic acids.

Table 4 Effect of CLA on fatty acid composition (mol/100 mol) of cardiolipin, phosphatidylethanolamine, and phosphatidylcholine of rat liver[1]

Fatty acids	SFO	1% CLA	3% CLA	5% CLA
Cardiolipin				
SFA	9.9 ± 0.4	11 ± 1	10 ± 1	11 ± 1
MUFA	15 ± 1[a]	11 ± 1[b]	12 ± 1[b]	17 ± 1[a]
n–6 PUFA	75 ± 1[a]	77 ± 1[a]	74 ± 2[a]	66 ± 1[b]
18:2 n–6	65 ± 1[a]	68 ± 1[a]	65 ± 2[a]	58 ± 1[b]
20:4 n–6	3.9 ± 0.3	4.2 ± 0.4	4.2 ± 0.4	3.9 ± 0.4
Others	5.6 ± 0.4	5.0 ± 0.3	5.0 ± 0.3	5.0 ± 0.3
n–3 PUFA	ND	ND	ND	ND
Total CLA	ND	1.0 ± 0.1[c]	3.1 ± 0.2[b]	5.9 ± 0.3[a]
Phosphatidylethanolamine				
SFA	43 ± 1[b]	42 ± 0[b]	44 ± 1[a,b]	45 ± 1[a]
MUFA	7.8 ± 0.3[a]	6.6 ± 0.3[b]	5.9 ± 0.3[b]	7.7 ± 0.4[a]
n–6 PUFA	46 ± 1[a]	46 ± 1[a]	43 ± 1[b]	36 ± 1[c]
18:2 n–6	8.8 ± 0.4[a]	8.6 ± 0.4[a]	6.4 ± 0.3[b]	5.8 ± 0.4[b]
20:4 n–6	30 ± 1[a]	30 ± 0[a]	29 ± 1[a]	25 ± 1[b]
22:5 n–6	4.8 ± 0.3[a,b]	5.4 ± 0.4[a]	5.7 ± 0.4[a]	3.9 ± 0.3[b]
Others	2.1 ± 0.1[a]	2.1 ± 0.1[a]	1.6 ± 0.1[b]	1.2 ± 0.1[c]
n–3 PUFA	3.8 ± 0.1[c]	4.6 ± 0.3[b]	4.7 ± 0.2[b]	7.5 ± 0.3[a]
Total CLA	0.4 ± 0.0[a]	1.4 ± 0.1[b]	2.8 ± 0.2[c]	3.4 ± 0.3[d]
Phosphatidylcholine				
SFA	42 ± 0[b]	41 ± 0[b]	42 ± 1[b]	44 ± 1[a]
MUFA	7.6 ± 0.3[b]	6.5 ± 0.2[b]	7.0 ± 0.4[b]	11 ± 1[a]
n–6 PUFA	48 ± 1[a]	49 ± 0[a]	46 ± 1[b]	39 ± 1[c]
18:2 n–6	12 ± 0[b]	13 ± 1[b]	12 ± 1[b]	15 ± 1[a]
20:4 n–6	32 ± 1[a]	32 ± 0[a]	29 ± 1[a]	20 ± 2[b]
Others	4.3 ± 0.2	4.4 ± 0.2	4.4 ± 0.2	4.1 ± 0.2
n–3 PUFA	1.8 ± 0.1[c]	2.1 ± 0.1[b,c]	2.3 ± 0.1[b]	3.0 ± 0.2[a]
Total CLA	0.5 ± 0.0[d]	1.3 ± 0.1[c]	2.9 ± 0.2[b]	3.3 ± 0.3[a]

[1]Data are represented as means ± SEM for 10 rats in each group.
[a,b,c,d]Values without a common superscript letter are significantly different at $p < 0.05$ (Student Newman Keuls test); values in the same row with no superscript are not different ($p > 0.05$).
SFO–sunflower oil. CLA–conjugated linoleic acids. SFA–total saturated fatty acids. MUFA–total monounsaturated fatty acids. PUFA–total polyunsaturated fatty acids. ND, not detectable.

sistent reduction of serum cholesterol and PC concentrations by week 5 compared with the SFO-fed controls. The reduction of cholesterol and PC level was primarily due to a 20% decrease in LDL lipids in the group fed 3% CLA and a more than 60% decrease in the group fed 5% CLA. A dose-dependent response of dietary CLA also was observed for HDL cholesterol concentration, which was reduced by 14%, 23%, and 22% for 1%, 3%, and 5% CLA levels, respectively, compared with that of the SFO group. Triacylglycerol concentrations in serum and lipoproteins were unaffected by CLA consumption. Rats fed the 5% CLA mixture exhibited higher concentrations of serum albumin, creatinine, and glucose than did control rats receiving no CLA (*Table 6*). Concentrations of total protein and urea, and the activities of ASAT and ALAT, which are indicative of tissue cell damage, remained unaffected by dietary treatment.

Discussion

Dietary supplementation with 1% CLA, a widely used concentration in the literature and one reported to exhibit maximum activity and induce numerous responses.[4,33–35] In the present study, which used an experimental design of controlled feed intake, adult animals, and equalized levels of vitamin E in the SFO and the CLA-containing oil (by adding α-tocopherol to the CLA-containing oil), 1% CLA had no effect on body composition, catalase activity in the liver, or serum levels of LDL cholesterol. Weak effects were observed on the concentrations and fatty acid composition of individual glycerophospholipids. The principal effects of 1% CLA treatment were marked accumulation of CLA in the extractable lipids of carcass in favor of n-6 fatty acids and reduced HDL cholesterol concentration in the serum. The nonexistent or weak effects of the diet with 1% CLA used in the present study are at variance with numerous findings in literature. This suggests that the selection of the feeding regimen, the composition of the CLA mixture, the composition of the basal diet, specifically the "non-CLA fatty acids," and possibly the species as well might have a crucial impact on the magnitude of the CLA effect and on the type of response. The CLA effects described in the literature such as reduction of total serum cholesterol and LDL fraction,[3,4] reduced body fat content,[5,8,34,35] and changes in catalase activity[36,37] occurred, either significantly or as a trend, only at the relatively high CLA concentrations of 3% and 5%. These are concentrations that markedly exceeded the levels of CLA that are normally ingested by humans. The altered concentrations of clinicochemical serum parameters at 5% CLA are potentially indicative of major metabolic changes.

The finding that dietary CLA increases the concentrations of the n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid, and decreases the ratio of n-6:n-3

Table 5 Effect of CLA on fatty acid composition (mol/100 mol) of phosphatidylinositol, phosphatidylserine, and lysophosphatidylethanolamine plus ethanolamine plasmalogen of rat liver[1]

Fatty acids	SFO	1% CLA	3% CLA	5% CLA
Phosphatidylinositol				
SFA	49 ± 1[b]	50 ± 1[b]	51 ± 0[b]	59 ± 1[a]
MUFA	5.1 ± 0.6[a]	3.3 ± 0.3[b]	3.4 ± 0.4[b]	4.5 ± 0.2[a,b]
n–6 PUFA	46 ± 1[a]	47 ± 1[a]	45 ± 1[a]	36 ± 1[b]
18:2	12 ± 1[a]	12 ± 1[a]	10 ± 0[a,b]	9.2 ± 0.6[b]
20:4	32 ± 1[a]	33 ± 1[a]	32 ± 0[a]	24 ± 1[b]
Others	2.1 ± 0.2	2.2 ± 0.2	2.4 ± 0.2	2.6 ± 0.1
n–3 PUFA	ND	ND	ND	ND
Total CLA	ND	ND	0.6 ± 0.1[b]	1.0 ± 0.1[a]
Phosphatidylserine				
SFA	51 ± 1	51 ± 1	50 ± 1	53 ± 1
MUFA	12 ± 2[a]	6.8 ± 0.9[b]	8.0 ± 1.2[b]	7.5 ± 0.9[b]
n–6 PUFA	37 ± 2[a,b]	40 ± 1[a]	40 ± 1[a]	33 ± 1[b]
18:2	8.9 ± 0.6[a]	8.2 ± 0.2[a]	8.0 ± 0.4[a]	6.6 ± 0.3[b]
20:4	24 ± 2[a,b]	27 ± 1[a]	27 ± 1[a]	22 ± 1[b]
Others	4.0 ± 1.0	4.7 ± 0.6	4.5 ± 0.3	4.8 ± 0.4
n–3 PUFA	ND	2.0 ± 0.4[b]	2.6 ± 0.4[b]	6.5 ± 0.5[a]
Total CLA	nD	ND	ND	ND
Lysophosphatidylethanolamine plus ethanolamine plasmalogen				
SFA	36 ± 1	35 ± 1	35 ± 1	38 ± 1
MUFA	7.5 ± 0.6[a]	5.8 ± 0.5[b]	5.6 ± 0.3[b]	7.5 ± 0.7[a]
n–6 PUFA	54 ± 1[a]	56 ± 1[a]	54 ± 1[a]	48 ± 1[b]
18:2	9.5 ± 0.4[a,b]	9.6 ± 0.4[a]	8.5 ± 0.3[b,c]	8.2 ± 0.3[c]
20:4	36 ± 1[a,b]	38 ± 1[a]	38 ± 1[a]	34 ± 1[b]
22:4	4.0 ± 0.2[a]	3.9 ± 0.3[a]	3.0 ± 0.1[b]	1.7 ± 0.1[c]
22:5	4.2 ± 0.3[a]	4.7 ± 0.3[a]	4.8 ± 0.3[a]	3.2 ± 0.2[b]
n–3 PUFA	3.2 ± 0.2[c]	3.8 ± 0.2[b]	3.8 ± 0.2[b,c]	5.6 ± 0.3[a]
Total CLA	ND	ND	1.3 ± 0.1[b]	1.8 ± 0.2[a]

[1]Data are represented as means ± SEM for 10 rats in each group.
[a,b,c]Values without a common superscript letter are significantly different at $p < 0.05$ (Student Newman Keuls test); values in the same row with no superscript are not different ($p > 0.05$).
SFO–sunflower oil. CLA–conjugated linoleic acids. SFA–total saturated fatty acids. MUFA–total monounsaturated fatty acids. PUFA–total polyunsaturated fatty acids. ND, not detectable.

fatty acids in liver, brain, spleen, and serum of rats,[12] was re-examined in the present study by using a diet with high levels of n-6 fatty acids and extremely low levels of n-3 fatty acids. The use of an extremely n-6 fatty acid-rich and n-3 fatty acid-poor diet was possible because adult animals have a very low n-3 fatty acid requirement, the body fat stores of the test animals at the beginning of the trial had a sufficiently high n-3 fatty acid concentration in excess of 2 Mol%, and the 5-week duration of the trial is very short. The n-6 fatty acid-rich and n-3 fatty acid-poor diet used in the present study confirmed the results of Li and Watkins[12] who observed an increase in n-3 fatty acids of 22:5 n-3 and 22:6 n-3 in rat tissues with CLA treatment. In this study, the proportion of n-3 fatty acids in the phospholipids PS, LPE/EPL, PE, and PC of CLA-treated animals was in some cases considerably increased compared with control animals. The relative sparing of n-3 fatty acids in rats given CLA was explained as either an increase in utilization of n-6 or a conservation of n-3 fatty acids.[12] It can also be suggested that the increased incorporation of n-3 fatty acids may counteract the high CLA proportion and the diminished levels of long-chain n-6 PUFA in the phospholipid species to maintain membrane integrity. However, one must not forget that the difference in the fatty acid composition of the diets, principally the reduced proportion of linoleic acid in the CLA diets, also might have some effect on this phenomenon.

Data from this study show a marked dose-dependent accumulation of CLA in the various phospholipids with increasing amounts of ingested CLA, which has apparently not peaked even at a concentration of 5% CLA, although it is known that the phospholipid fatty acids in general are much more resistant to diet-induced modification than triacylglycerol-esterified fatty acids. The presence of small amounts of CLA in rats fed the control diet may be attributable to the traces of CLA found in the casein-based diet and the previously reported fact that the intestinal tract of non-ruminants is capable of isomerizing free linoleic acid to CLA.[38] However, it is quite accepted that phospholipid fatty acid composition could be modified rapidly by dietary fat, whereas phospholipid distribution would not be modified as readily. However, a few studies on particular membrane fractions have shown that the phospholipid profile could be influenced by dietary fat.[39–42] The results from the present study also indicate that high CLA diets may alter the glycerophospholipid profile in liver, in which the most striking alterations were an increase of PC and PS and a reduction of the ethanolamine phospholipids. Similar changes have been observed in mitochondrial membranes of rats fed a high-eruic acid rapeseed oil diet[39] and in erythrocyte membranes of n-3 fatty acids-treated rats.[42] The fatty acid pattern of membrane phospholipids together with the class distribution of phospholipids are major lipid-related factors that may be involved in regulation of the physical

Table 6 Effect of CLA on concentrations of serum lipids, lipoproteins, and clinico-chemical variables[1]

	SFO	1% CLA	3% CLA	5% CLA
Triacylglycerols (mmol/L)				
Serum	0.60 ± 0.06	0.54 ± 0.04	0.56 ± 0.07	0.56 ± 0.06
VLDL	0.40 ± 0.04	0.38 ± 0.03	0.37 ± 0.04	0.35 ± 0.03
LDL	0.14 ± 0.02	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.02
HDL	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
Total cholesterol (mmol/L)				
Serum	1.99 ± 0.14^{a}	1.76 ± 0.10^{a,b}	1.49 ± 0.09^{b,c}	1.31 ± 0.06^{c}
VLDL	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.13 ± 0.02
LDL	0.49 ± 0.05^{a}	0.45 ± 0.04^{a}	0.39 ± 0.03^{a}	0.19 ± 0.01^{b}
HDL	1.52 ± 0.09^{a}	1.31 ± 0.07^{b}	1.17 ± 0.05^{b}	1.18 ± 0.05^{b}
Phosphatidylcholine (mmol/L)				
Serum	1.77 ± 0.10^{a}	1.64 ± 0.06^{a}	1.43 ± 0.04^{b}	1.35 ± 0.05^{b}
VLDL	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
LDL	0.25 ± 0.02^{a}	0.22 ± 0.02^{a}	0.20 ± 0.02^{a}	0.09 ± 0.01^{b}
HDL	1.45 ± 0.08	1.32 ± 0.05	1.28 ± 0.03	1.34 ± 0.05
Total protein (g/L)	64.3 ± 0.8	64.7 ± 0.9	64.1 ± 0.7	65.1 ± 0.3
Albumin (g/L)	35.5 ± 0.4^{b}	35.1 ± 0.4^{b}	36.0 ± 0.3^{b}	38.7 ± 0.4^{a}
Creatinine (μmol/L)	53.9 ± 1.2^{b}	54.4 ± 0.9^{b}	55.7 ± 1.2^{a,b}	58.5 ± 0.7^{a}
Urea (mmol/L)	5.05 ± 0.28	4.96 ± 0.17	4.81 ± 0.19	5.28 ± 0.15
Glucose (mmol/L)	6.00 ± 0.23^{b}	6.41 ± 0.11^{a,b}	6.53 ± 0.20^{a,b}	6.86 ± 0.16^{a}
ASAT (U/L)	201 ± 12	201 ± 11	206 ± 13	196 ± 9
ALAT (U/L)	44.0 ± 2.3	49.0 ± 1.7	45.5 ± 2.0	49.6 ± 2.0

[1]Data are represented as means ± SEM for 10 rats in each group.
[a,b,c]Values without a common superscript letter are significantly different at $p < 0.05$ (Student Newman Keuls test); values in the same row with no superscript are not different ($p > 0.05$).
SFO–sunflower oil. CLA–conjugated linoleic acids. VLDL–very low density lipoprotein. LDL–low density lipoprotein. HDL–high density lipoprotein. ASAT–aspartate amino transferase. ALAT–alanine amino transferase.

state of membranes. The results demonstrate in vivo that dietary CLA is a significant determinant of the phospholipid class content and phospholipid composition. In light of this observation, this topic would deserve further investigation, because phospholipids, lyso phospholipids, and phospholipid-bound fatty acids not only provide the membrane with its structural integrity and physical properties, but also play an important role as signal transducers during cell processes.[43]

Some properties of CLA observed in this study suggest that it will act as an hepatic peroxisome proliferator: (1) the hypocholesterolemic effect in serum lipoproteins, (2) the increase in catalase activity, an enzyme that exists in the matrix of peroxisomes, and (3) the slight increase of the total glycerophospholipid content. Such changes have also been observed with peroxisome proliferators such as lipid-lowering drugs such as clofibrate.[44,45] The present results, therefore, support the finding that dietary CLA displays a typical peroxisome proliferation response in rodent liver by inducing peroxisome-specific accumulation in mouse liver.[13] Various fatty acids such as very long-chain fatty acids are shortened to a medium chain length via the peroxisomal β-oxidation system.[46] Because this mechanism of chain shortening does not involve coupling to oxidative phosphorylation, the energetic efficiency of adenosine triphosphate formation is lower when compared with mitochondrial β-oxidation. Because CLA is suggested to be associated with a proliferation of peroxisomes, dietary CLA might shift energy production toward thermogenesis, which in turn might explain the trend toward reduced carcass fat in rats fed the high CLA diets.

In conclusion, these results clearly demonstrate that high dietary CLA paralleled with controlled amounts of food exhibited alterations of phospholipid-specific fatty acid composition and phospholipid class distribution and some of the typical peroxisome proliferation responses.

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