Conjugated Linoleic Acids Exhibit a Strong Fat-to-Lean Partitioning Effect, Reduce Serum VLDL Lipids and Redistribute Tissue Lipids in Food-Restricted Rats

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ABSTRACT Effects of conjugated linoleic acids (CLA) on a series of metabolic events are expected to depend on the feeding regimen and levels of energy ingested. This study was the first examining the mode of action of CLA on body composition, tissue lipids, lipoproteins and hepatic enzymes in situations of enhanced fat store mobilization. Two groups of male growing Sprague-Dawley rats were fed for 3 wk a diet containing 0 (control group) or 3 g/100 g of a CLA mixture at the expense of sunflower oil, and were then subjected to a weight-loss feeding regimen for another 18 d. Rats fed the CLA-fortified diet gained 11% less weight than the control rats (P < 0.05). Rats fed the high CLA diet had less body fat (1.47 ± 0.16 vs. 1.07 ± 0.09 g/100g, P < 0.05) and a higher lean deposition (25.6 ± 0.2 vs. 28.4 ± 0.3 g/100 g, P < 0.05) than control rats. CLA-fed rats had a 41% lower cholesterol concentration in liver than the control rats (P < 0.05). Some differences in glycerophospholipid subclass profile of liver and erythrocyte membrane were observed; the hepatic concentrations of phosphatidylethanolamine (4.76 ± 0.46 vs. 6.86 ± 0.99 μmol/g, P = 0.07) and phosphatidylcholine (12.9 ± 0.5 vs. 15.3 ± 1.2 μmol/g, P = 0.09) tended to be greater and the level of phosphatidylcholine in erythrocyte membranes was significantly greater (1.40 ± 0.12 vs. 1.83 ± 0.16 μmol/g, P < 0.05) in the CLA-treated group than in the control group. The activities of catalase and ornithine decarboxylase in liver did not differ between the groups. Further, CLA-treated rats had significantly lower serum concentrations of VLDL lipids than control rats, whereas concentrations of LDL and HDL lipids were unaffected. The results indicate that a high dose of a CLA mixture is a strong repartitioning agent and a modulator of lipid metabolism under conditions of enhanced fat store mobilization in rats. J. Nutr. 130: 1140–1146, 2000.

KEY WORDS: · conjugated linoleic acids · starvation · body composition · lipoproteins · rats

Conjugated linoleic acids (CLA),1 which refer to a group of positional and geometric isomers of the essential c9, c12 linoleic acid, are suggested to modulate the energy metabolism and body composition of animals because mice, pigs and chicks that were fed CLA-fortified diets deposited less body fat and were leaner than animals fed more ordinary oils, such as sunflower oil and corn oil (Dugan et al. 1997, Pariza et al. 1996, Park et al. 1997 and 1999). Additionally, CLA has been shown to act as a growth factor (Chin et al. 1994) and improve feed conversion efficiency (Chin et al. 1994, Dugan et al. 1997). In all of these studies, growing animals had free access to their diets and the effects were observed with dietary CLA levels ranging between 0.5 and 2%. In contrast, adult swine that were fed a CLA-fortified diet at an energetic maintenance level had similar body weights and thermogenesis as control pigs fed a sunflower oil–based diet (Müller et al. 1999). Additionally, recent data from this laboratory indicated that in adults rats fed controlled amounts of a diet with 1% of a CLA mixture, which provided 25% more energy than was required for maintenance, there was no effect on body composition (Stangl 2000). A slight reduction of the body fat content occurred (P = 0.25), but only at relatively high CLA concentrations of 3 and 5%. Thus, the effect of CLA on body repartitioning is a subject of some controversy and has led to the assumption that CLA respond differently to graded levels of energy intake and physiologic state of the animal species. However, no information is available concerning the mode of action of CLA on body repartitioning in situations of enhanced fat store mobilization.

Moreover, the author assumes that the type of fatty acids supplied by adipose tissue to other tissues and organs, notably in situations of negative energy balance, may possibly enhance specific metabolic effects observed with CLA. Thus, the first objective of this study was to evaluate the effect of a CLA-mixture on body partitioning using a feeding regimen in which rats had almost free access to food for the first 3 wk, followed by an 18-d food-restriction period during which fatty acids were mobilized from fat stores. In addition to the proposed role of CLA as having a fat-to-lean repartitioning effect, it was also established that CLA alter different aspects of the lipid metabolism. These included an increase in carnitine palmitoyl-
transferase activity (Chin et al. 1994, Park et al. 1997), inhibition of proliferation and differentiation of preadipocytes (Brodie et al. 1999), inhibition of lipoprotein lipase activity (Park et al. 1997), a reduction of circulating cholesterol (Lee et al. 1994, Munday et al. 1999, Nicolosi et al. 1997), and enhanced peroxisomal β-oxidation of fatty acids via peroxi-
some proliferation (Belury et al. 1997). However, all of these metabolic events that were altered by CLA, including lipoly-
sis, lipid synthesis, peroxisomal activity and enzyme activities, can also be influenced markedly by energy intake. This prompted the author to undertake an examination of the action of CLA on lipid levels in liver, brain and circulating lipoproteins, the activities of catalase and ornithine decarbox-
ylase (ODC) as a measure for peroxisomal proliferation, and the extent of incorporation of CLA into phospholipids under conditions of the above-mentioned feeding regimen. The recent findings from this laboratory that 1% of a CLA mixture did not induce distinct responses in metabolism, whereas a 3% CLA diet showed effects without influencing other chemical variables that would be indicative of toxic symptoms or severe metabolic disturbances (Stangl 2000), prompted the author to use a high CLA diet with 3% of a CLA mixture in this study.

**MATERIALS AND METHODS**

**Chemicals.** Unless otherwise stated, chemicals were purchased from Sigma Chemical (St. Louis, MO).

**Animals and diets.** In this experiment, 24 male weanling SPF Sprague-Dawley rats (WIGA GmbH, Sulzfeld, Germany) with a mean body weight of 57.8 ± 1.1 g were divided into two groups of 12 and fed equal amounts of a diet based on the AIN-93 formulation (Reeves et al. 1993) containing 0 (control diet) and 3 g/100 g of a CLA-mixture (CLA diet). The CLA-treated rats were fed a diet with 5 g/100 g of a CLA oil, containing 60.5 g/100 g of CLA isomers (Multi-Food GmbH, Buxtehude, Germany). The diet offered to the control rats contained sunflower oil instead of the CLA oil. The fatty acid composition (g/100 g) of the CLA-containing oil, analyzed by the Institute for Biochemistry and Food Chemistry (University of Hamburg, Germany) with the use of a gas chromatographic method (Fritsche and Steinhart 1998), was 16:0 (6.7), 18:0 (2.7), 18:1 (25.2), 18:2, octadecadienoic acid (4.9), c9,t11 CLA (34.6), t10,c12 CLA (18.4), t9,c11 CLA (5.4), and c9,c11 CLA (2.1). CLA isomers, used as standard, were purchased from Sigma or were synthesized as described recently by (Fritsche and Steinhart 1998). The basal diet used consisted of (g/kg) casein (200), cornstarch (308), sucrose (300), sunflower oil (50), sunflower oil or CLA-containing oil (50), minerals (40), fiber (30), vitamins (20) and dl-methionine (2). The concentrations of individual fatty acids in the experimental diets are shown in Table 1. The experimental diets had similar concentrations of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The main difference in dietary fatty acid composition was a 3% reduction of linoleic acid at the expense of CLA isomers in the CLA diet compared with the control diet. The difference in dietary α-tocopherol level between the sunflower oil (1.37 mmol/kg) and the CLA-containing oil (0.14 mmol/kg) was made up by the addition of all-rac-α-tocopherol to the CLA-containing oil (considering that the bioactivity of all-rac-α-tocopherol is 74% of that of α-tocopherol). The diets were stored at −4°C during the experimental period; the amounts of individual fatty acids in each diet were the same at the beginning and at the end of the experiment, indicating no decomposition of unsaturated fatty acids by oxidative processes. The rats were fed these diets in controlled amounts (~90% of the voluntary food intake) to avoid uncontrolled feed for 21 d; rats were then food restricted for the following 18 d. The daily feed allowance during the restriction period was 5.8 g diet dry matter for each rat, which was ~30% below their energy maintenance requirement of 444 kJ metabolizable energy/kg 0.75 (National Research Council 1978).

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-h light:dark cycle and had free access to drinking water. Care and treatment of rats followed recommended guidelines (National Research Council 1985). At the end of the experimental period at d 39, 12 h after the last feeding, rats were killed by decapitation after light anesthesia with diethyl ether.

**Analyzes.** Blood for determination of serum lipids, lipoproteins and clinical chemical variables was collected into untreated tubes. For the analysis of the carcass composition, gut, liver, brain, heart and both kidneys were excised. Serum, liver, brain and carcass samples were stored at −80°C until analyzed. Frozen carcasses were chopped, ground, and freeze-dried to determine total nitrogen and the fat content. Total protein was assessed by the macro-Kjeldahl method (Association of Official Analytical Chemists 1984) with a nitrogen-to-protein conversion factor of 6.25. Total nitrogen in the carcasses was analyzed in triplicate. The weight of residue obtained after exhaustive Soxhlet extraction of a 3-g aliquot of the freeze-dried carcass with petroleum ether, followed by evaporation of the solvent, provided the measure of lipid content. Fat analyses were conducted in triplicate.

Diet, liver, brain, erythrocyte membrane and carcass lipids were extracted with a hexane/isopropanol mixture (3:2, v/v, containing BHT as antioxidant) (Harra and Radin 1978). Preparation of erythrocyte membranes for glycerophospholipid and fatty acid analyses was done as described previously (Andail et al. 1990). Hepatic cardiolipin (CL), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and lysophos-
phatidylethanolamine plus ethanolamine plasmalogens (LPE + EPL) were separated by HPLC (Eder et al. 1992) and collected with a fraction collector (model 201, Gilson, Villiers-le-Bel, France). The CL, PC, PE, PI, PS and LPE + EPL separated by HPLC were methylated with boron fluoride/methanol reagent (Morrison and Smith 1964). Fatty acid methyl esters (FAME) were separated by gas chromatography using a Hewlett-Packard HP 5890 gas chromatographic system (Hewlett-Packard, 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 i.d., film thickness 0.2 μm; Chrompack, Middelburg, The Netherlands). The oven temperature program used was as described by Eder and Kirch-
gessner (1996). The detector temperature was 300°C. FAME were identified by comparing their retention times with those of individual purified standards, and quantified with heptadecanoic acid methyl

### Table 1

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>3% CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg fresh diet</td>
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<tr>
<td>14:0</td>
<td>0.09</td>
<td>0.12</td>
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<tr>
<td>16:0</td>
<td>5.76</td>
<td>4.97</td>
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<td>18:0</td>
<td>4.25</td>
<td>3.94</td>
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<tr>
<td>22:0</td>
<td>0.68</td>
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<tr>
<td>Total SFA</td>
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<td>8.56</td>
</tr>
<tr>
<td>16:1(n-7) + (n-9)</td>
<td>0.16</td>
<td>0.17</td>
</tr>
<tr>
<td>18:1(n-7) + (n-9)</td>
<td>21.1</td>
<td>21.1</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>21.3</td>
<td>21.3</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>60.6</td>
<td>31.4</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.66</td>
<td>0.30</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.96</td>
<td>0.44</td>
</tr>
<tr>
<td>Total CLA</td>
<td>0.04</td>
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</tr>
<tr>
<td>Total PUFA</td>
<td>62.3</td>
<td>61.7</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>94.3</td>
<td>92.8</td>
</tr>
</tbody>
</table>

1 Values are means of two determinations.  
2 CLA, conjugated linoleic acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
ester as internal standard (Eder et al. 1991). The total CLA concentration (sum of the CLA isomers) was determined by using a standard mixture consisting of octadecadienoic acid methyl esters with cis and/or trans double bonds in the 9,11- and 10,12-positions (Sigma Chemical). Because it was not possible to separate completely all peaks obtained from the single CLA isomers, total CLA were determined by adding the relevant peaks. The amounts of CL, PC, PE, PI, PS and LPE + EPL were calculated by the amount of their bound fatty acids.

For measurement of liver total triacylglycerol 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 (Stangl and Kirchgesner 1998). After evaporation of 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 catalase (EC 1.11.1.6), a large granule fraction that was enriched mainly in lysosomes and peroxisomes was obtained (Goldenberger et al. 1976, Leighton et al. 1975). Therefore, liver homogenates were centrifuged at 600 × g for 10 min to remove nuclei, unbroken cells and cell debris. For stepwise preparation of the large granule fraction, the 600 × g supernatant was centrifuged for 10 min at 3000 × g; the supernatant was then centrifuged at 20,000 × g for another 10 min. The pellet obtained was then suspended in 2 mL of 0.25 mol/L sucrose, and was termed the large granule fraction. This procedure yielded a complete sedimentation of peroxisomes and a supernatant fraction with some microsomes (Leighton et al. 1975). Total catalase activity was measured spectrophotometrically according to Aebi (1970) 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 conversion of 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 according to Smith et al. (1975) using bicinchoninic acid, and bovine serum albumin as a standard. One unit catalase is defined as 1 μmol hydrogen peroxide substrate decomposed per minute at 25°C.

For determination of the putrescine content and the activity of ODC (EC 6.4.1.17), a 105,000 × g cytosolic fraction of liver was prepared, ~1 g of liver was homogenized in 4 mL of 0.25 mol/L sucrose buffer (in 0.1 mol/L phosphate buffer, pH 7.4) using a Potter-Elvehjem-homogenizer. Homogenates were centrifuged at 105,000 × g for 1 h at 4°C, and the supernatants were used for the spectrophotometric assay of putrescine and the ODC activity (Ngo et al. 1987). The spectrophotometric assay is based on the reaction of putrescine with 2,4,6-trinitrobenzenesulfonic acid to give a colored product soluble in 1-pentanol; this is not the case with ornithine. Basal putrescine concentration and the amount of putrescine produced by the enzyme were determined by measuring the absorbance of the 1-pentanol extract of the reaction mixture at 420 nm. ODC activity was stopped with 10% trichloracetic acid. Protein in the 105,000 × g fraction of liver used for the putrescine and enzyme determination was measured according to Smith et al. (1975) using bicinchoninic acid, and bovine serum albumin as a standard. One unit of ornithine decarboxylase activity is defined as the amount that catalyzes the formation of 1 nmol of putrescine per minute under the assay conditions described.

The lipoproteins, VLDL (d < 1.019 kg/L), LDL (d = 1.019–1.063 kg/L) and HDL (d > 1.063 kg/L) were isolated by stepwise ultracentrifugation (230,000 × g for 20 h at 8°C), according to Tiedink and Katan (1989). The lipoprotein fractions were analyzed enzymatically for the concentrations of triacylglycerols (Boehringer, ref. 1488872), total cholesterol (Boehringer, ref. 1489232) and PC, the major phospholipid of lipoproteins (Boehringer, ref. 691844) using an autoanalyzer (model 704, Hitachi, Tokyo, Japan).

Concentrations of serum components were determined by standardized procedures using an autoanalyzer (model 704, Hitachi) and Boehringer kit reagents (Boehringer, total protein, ref. 1553836; albumin, ref. 1489143; creatinine, ref. 1489291; urea, ref. 816361; glucose, ref. 1447513).

Statistics. The effect of dietary CLA was evaluated for statistical significance (P < 0.05) by Student’s t test. All data in the text are expressed as means ± SEM.

RESULTS

Body weight and body repartitioning. Rats fed the two experimental diets gained comparable amounts of weight during the first phase of the experiment (Table 2). All rats that were food-restricted for the following 18 d lost weight, but CLA-treated rats tended to lose more weight than the control rats (P = 0.09). At the end of the experiment at d 39, CLA-fed rats had lower body weights than their controls; growth gain from d 0 to 39 was also lower in CLA-treated rats than in the control rats. The effect of CLA on body weight was accompanied by an alteration in body composition (Table 2). Feeding CLA to rats changed body composition from fat toward lean deposition. Relative to their respective controls, the percentage of carcass fat of CLA-fed rats was reduced by 27%; in contrast, the percentage of carcass protein was enhanced by 11%, giving a 45% higher protein/fat ratio in CLA-fed rats compared with rats fed no CLA. When carcass fatty acids were extracted, CLA-treated rats had lower concentrations of almost all individual fatty acids compared with the controls, but the most profound alteration was observed with linoleate [18:2 (n-6)], which was reduced by 60% in rats fed the CLA-fortified diet relative to the control rats (Table 2). In view of the experimental feeding regimen used in this study, the proportion of total CLA (sum of all CLA isomers) in the carcass of rats fed no CLA was 1.74 ± 0.15 mol/100 mol fatty acids, and that of CLA-fed rats was 7.09 ± 0.34 mol/100 mol fatty acids. The higher CLA proportion in the carcass of CLA-treated rats

TABLE 2

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Growth development and body composition of rats fed a high CLA diet or a control diet with sunflower oil.1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3% CLA</td>
</tr>
<tr>
<td>Initial body weight, g</td>
<td>57.6 ± 1.3</td>
</tr>
<tr>
<td>Body weight after feeding period, g</td>
<td>254 ± 5</td>
</tr>
<tr>
<td>Weight gain during feeding period, g/1–21 d</td>
<td>197 ± 5</td>
</tr>
<tr>
<td>Weight loss during food restriction, g/22–39 d</td>
<td>45.1 ± 3.0</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>209 ± 5</td>
</tr>
<tr>
<td>Total weight gain, g/1–39 d</td>
<td>152 ± 5</td>
</tr>
<tr>
<td>Body dry matter, g/100 g</td>
<td>30.9 ± 0.3</td>
</tr>
<tr>
<td>Body protein, g/100 g</td>
<td>25.6 ± 0.2</td>
</tr>
<tr>
<td>Body fat, g/100 g</td>
<td>1.47 ± 0.16</td>
</tr>
<tr>
<td>Protein/fat ratio</td>
<td>18.4 ± 2.3</td>
</tr>
<tr>
<td>Body fatty acid content, μmol/g</td>
<td>66.1 ± 8.2</td>
</tr>
<tr>
<td>Individual fatty acids, μmol/g</td>
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</tr>
<tr>
<td>18:0</td>
<td>11.1 ± 1.3</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>5.65 ± 0.37</td>
</tr>
<tr>
<td>Total SFA</td>
<td>17.6 ± 1.8</td>
</tr>
<tr>
<td>16:1(n-7) + (n-9)</td>
<td>1.67 ± 0.24</td>
</tr>
<tr>
<td>18:1(n-7) + (n-9)</td>
<td>14.4 ± 2.5</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>16.1 ± 2.7</td>
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<tr>
<td>18:2(n-6)</td>
<td>21.8 ± 3.4</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>5.24 ± 0.11</td>
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<tr>
<td>Total CLA</td>
<td>1.08 ± 0.05</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>32.3 ± 3.7</td>
</tr>
</tbody>
</table>

1 CLA, conjugated linoleic acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. 
2 Values are means ± SEM, n = 12; * Significantly different from the control group, P < 0.05. 
3 Referring to carcass wet weight.
Liver, erythrocyte and brain lipids. For CLA-treated rats, the liver weights and triacylglycerol concentrations were not different from those of the control rats (Table 3). Rats fed the CLA-fortified diet exhibited a 41% lower cholesterol concentration in liver than the control rats (P < 0.05). Some differences also occurred with the individual glycerophospholipids in liver. Hepatic concentrations of PE (P = 0.07) and PC (P = 0.09) in livers of the CLA-treated rats tended to be higher than in livers of control rats. The concentrations of liver PS, CL, PI and LPE + EPL of rats consuming the CLA-fortified diet were not different from those of the controls. The type of fat in the diet also influenced the phospholipid levels in the erythrocyte membranes (Table 3). Rats fed the high CLA diet had a 31% higher PC concentration in erythrocyte membranes than rats fed no CLA (P < 0.05). The concentration of erythrocyte LPE + EPL was not appreciably affected by dietary CLA. Control rats and rats fed the CLA-fortified diet had activities of peroxisome-associated catalase and cytosolic ornithine decarboxylase, and concentrations of putrescine in liver that did not differ (Table 3).

The dietary treatments exhibited only slight effects on the fatty acid composition of the individual glycerophospholipids in liver (Table 4 and 5). CL, PC and PE of rats fed the CLA-fortified diet had significantly higher proportions of total CLA than those of control rats. The extent of incorporation of CLA was highest for the CL subclass. Major changes of other fatty acids included reduced proportions of the sum of (n-6) PUFAs and most of the individual (n-6) PUFAs in CL, PC, PE and LPE of CLA-fed rats relative to the control rats. Rats fed the high CLA diet had a significantly higher proportion of docosapentaenoic acid [22:5 (n-6)] in PC, PE and PS of liver compared with rats fed the sunflower oil–based diet. The fatty acid composition of PI in liver did not differ between the two groups (data not shown). PC of the erythrocyte membranes from CLA-treated rats was characterized by significantly higher proportions of docosapentaenoic acid [22:5 (n-6)].

### Table 3

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Control</th>
<th>3% CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, g</td>
<td>5.13 ± 0.14</td>
<td>5.04 ± 0.14</td>
</tr>
<tr>
<td>Triacylglycerols, µmol/g</td>
<td>5.27 ± 0.32</td>
<td>5.22 ± 0.10</td>
</tr>
<tr>
<td>Total cholesterol, µmol/g</td>
<td>11.8 ± 1.1</td>
<td>6.92 ± 0.13*</td>
</tr>
<tr>
<td>Phosphatidylethanolamine, µmol/g</td>
<td>4.76 ± 0.46</td>
<td>6.86 ± 0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatidylcholine, µmol/g</td>
<td>0.75 ± 0.06</td>
<td>0.64 ± 0.06</td>
</tr>
<tr>
<td>Phosphatidylethanolamine, µmol/g</td>
<td>1.29 ± 0.5</td>
<td>1.53 ± 1.2</td>
</tr>
<tr>
<td>Total SFA</td>
<td>7.22</td>
<td>6.6</td>
</tr>
</tbody>
</table>

1 Conjugated linoleic acids.
2 Values are means ± SEM, n = 12; * Significantly different from the control group, P < 0.05.
3 Unit of catalase is defined as 1 µmol hydrogen peroxide substrate decomposed per minute at 25°C.
4 Unit of ornithine decarboxylase is defined as 1 pmol putrescine synthesized per minute at 37°C.

occurred primarily at the expense of linoleic acid (control, 32.4 ± 1.4 mol/100 mol fatty acids; CLA, 19.5 ± 0.8 mol/100 mol fatty acids; P < 0.05).

### Table 4

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Cardiolipin</th>
<th>Phosphatidylcholine</th>
<th>Phosphatidylethanolamine</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>3% CLA</td>
<td>Control</td>
</tr>
<tr>
<td>16:0</td>
<td>4.57 ± 0.20</td>
<td>5.02 ± 0.19</td>
<td>24.0 ± 0.4</td>
</tr>
<tr>
<td>18:0</td>
<td>2.65 ± 0.11</td>
<td>2.86 ± 0.14</td>
<td>17.6 ± 0.6</td>
</tr>
<tr>
<td>Total SFA</td>
<td>7.22 ± 0.29</td>
<td>7.87 ± 0.29</td>
<td>41.6 ± 0.5</td>
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<tr>
<td>16:1(n-7) + (n-9)</td>
<td>1.48 ± 0.19</td>
<td>2.35 ± 0.23</td>
<td>1.20 ± 0.08</td>
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<tr>
<td>18:1(n-7) + (n-9)</td>
<td>9.03 ± 0.29</td>
<td>9.60 ± 0.33</td>
<td>7.87 ± 0.22</td>
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<tr>
<td>Total MUFA</td>
<td>10.5 ± 0.4</td>
<td>11.9 ± 0.5</td>
<td>9.07 ± 0.28</td>
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<tr>
<td>18:2(n-6)</td>
<td>74.3 ± 0.6</td>
<td>71.8 ± 0.7*</td>
<td>18.0 ± 0.4</td>
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<td>20:4(n-6)</td>
<td>2.92 ± 0.10</td>
<td>2.57 ± 0.10*</td>
<td>25.8 ± 0.4</td>
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<td>22:4(n-6)</td>
<td>0.46 ± 0.04</td>
<td>0.34 ± 0.03*</td>
<td>0.37 ± 0.02</td>
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<td>22:5(n-6)</td>
<td>2.03 ± 0.07</td>
<td>1.53 ± 0.07*</td>
<td>1.48 ± 0.12</td>
</tr>
<tr>
<td>Other (n-6)</td>
<td>1.87 ± 0.10</td>
<td>0.92 ± 0.09*</td>
<td>0.98 ± 0.05</td>
</tr>
<tr>
<td>Total (n-6) PUFA</td>
<td>81.6 ± 0.7</td>
<td>77.1 ± 0.7</td>
<td>46.6 ± 0.4</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>0.65 ± 0.03</td>
<td>0.47 ± 0.02*</td>
<td>2.02 ± 0.07</td>
</tr>
<tr>
<td>Other (n-3)</td>
<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
</tr>
<tr>
<td>Total (n-3) PUFA</td>
<td>0.65 ± 0.03</td>
<td>0.47 ± 0.02*</td>
<td>2.16 ± 0.07</td>
</tr>
<tr>
<td>Total CLA</td>
<td>ND</td>
<td>ND</td>
<td>2.57 ± 0.13</td>
</tr>
</tbody>
</table>

1 CLA, conjugated linoleic acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
2 Values are means ± SEM, n = 12; * Significantly different from the control group, P < 0.05.
3 ND, not detectable.
![Image of a page from a document with text and tables]

### TABLE 5

<table>
<thead>
<tr>
<th>Fatty acid composition of phosphatidylserine and plasmalogen- and lysophosphatidylethanolamine in liver of rats fed a high CLA diet or a control diet with sunflower oil$^{1,2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fatty acid</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>mol/100 mol fatty acids</td>
</tr>
<tr>
<td>16:0</td>
</tr>
<tr>
<td>18:0</td>
</tr>
<tr>
<td>Total SFA</td>
</tr>
<tr>
<td>18:1(n-7) + (n-9)</td>
</tr>
<tr>
<td>Total MUFA</td>
</tr>
<tr>
<td>18:2(n-6)</td>
</tr>
<tr>
<td>20:4(n-6)</td>
</tr>
<tr>
<td>22:4(n-6)</td>
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<tr>
<td>22:5(n-6)</td>
</tr>
<tr>
<td>Total (n-6) PUFA</td>
</tr>
<tr>
<td>Total CLA</td>
</tr>
<tr>
<td>18:1(n-7)</td>
</tr>
</tbody>
</table>

$^1$ CLA, conjugated linoleic acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

$^2$ Values are means ± SEM, n = 12; * Significantly different from the control group, P < 0.05.

$^3$ ND, not detectable.

### TABLE 6

<table>
<thead>
<tr>
<th>Fatty acid composition of phosphatidylcholine from erythrocyte membranes and total lipids from brain of rats fed a high CLA diet or a control diet with sunflower oil$^{1,2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fatty acid</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>mol/100 mol fatty acids</td>
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<tr>
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<td>16:0</td>
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<tr>
<td>18:0</td>
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<tr>
<td>Total SFA</td>
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<tr>
<td>16:1(n-7) + (n-9)</td>
</tr>
<tr>
<td>18:2(n-6)</td>
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<tr>
<td>Total MUFA</td>
</tr>
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<td>18:2(n-6)</td>
</tr>
<tr>
<td>20:4(n-6)</td>
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<tr>
<td>22:4(n-6)</td>
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<tr>
<td>22:5(n-6)</td>
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<td>Other (n-6)</td>
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<tr>
<td>Total (n-6) PUFA</td>
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<tr>
<td>22:6(n-3)</td>
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<tr>
<td>Other (n-3)</td>
</tr>
<tr>
<td>Total (n-3) PUFA</td>
</tr>
<tr>
<td>Total CLA</td>
</tr>
</tbody>
</table>

$^1$ CLA, conjugated linoleic acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

$^2$ Values are means ± SEM, n = 12; * Significantly different from the control group, P < 0.05.

$^3$ ND, not detectable.
by a relatively high level of CLA in both the control and CLA-treated groups. Between the two groups, the CLA proportion did not differ.

**Serum lipoproteins and clinical chemical variables.** The influence of a high CLA diet on lipoproteins was examined in rats killed at the end of the second phase, during which all animals lost weight. The concentrations of triglycerides, total cholesterol and PC of serum LDL and HDL were not affected by the dietary treatment (data not shown). There was a great influence of the high CLA diet on the VLDL lipids. The VLDL concentrations of triglycerides (control, 191 ± 20 µmol/L; CLA, 129 ± 5 µmol/L; P < 0.05), total cholesterol (control, 0.29 ± 0.06 µmol/L; CLA, 0.60 ± 0.01 µmol/L; P < 0.05), and PC (control, 0.08 ± 0.01 mmol/L; CLA, 0.04 ± 0.00 mmol/L; P < 0.05) were significantly lower in the CLA-treated rats than in the control rats.

Total lipids in the VLDL fraction were reduced by 59% in rats fed the CLA-fortified diet compared with the control rats (control, 0.56 ± 0.07 mmol/L; CLA, 0.23 ± 0.01 mmol/L; P < 0.05). Rats fed the CLA-fortified diet had lower serum concentrations of total protein (control, 62.0 ± 0.7 g/L; CLA, 59.8 ± 0.8 g/L; P = 0.05) and higher serum concentrations of glucose (control, 5.53 ± 0.20 mmol/L; CLA, 6.08 ± 0.18 mmol/L; P = 0.06) than the control rats. Concentrations of albumin (control, 35.1 ± 0.6 g/L; CLA, 34.9 ± 0.5 g/L), creatinine (control, 49.5 ± 1.0 µmol/L; CLA, 48.0 ± 1.3 µmol/L) and urea (control, 7.39 ± 0.50 mmol/L; CLA, 8.06 ± 0.51 mmol/L) were unaffected by CLA treatment.

**DISCUSSION**

The results of this study demonstrate for the first time that a high CLA diet can exhibit a strong fat-to-lean partitioning effect in food-restricted growing rats with fat store mobilization and a feeding regimen in which possible differences in total energy intake were eliminated by matching the quantity of food given to the two experimental groups, an experimental measure that has often been unconsidered in many experiments on the metabolic effects of CLA. Additionally, isonitrogenous and essentially isocaloric diets with equalized levels of vitamin E equivalents were used in this study to limit observed differences in the actions of the fatty acids provided with the diets alone. Under these experimental conditions, dietary CLA can be referred to as a repartitioning agent. The observed effect of CLA on body repartitioning is similar to that reported for carnitine (Ji et al. 1996) and β-agonists such as phenethanolamine clenbuterol (Greife et al. 1989) that have been shown to depress fat deposition and stimulate protein accretion consistent with improved nitrogen retention through accelerated fat oxidation. Ji et al. (1996) considered the possibility that by increasing the ratios of acetyl CoA:CoA-SH and ATP:ADP through accelerated β-oxidation, the repartitioning agent carnitine might stimulate flux through pyruvate carboxylase and reduce flux through the branched-chain α-keto acid dehydrogenase complex, thereby increasing the supply of carbon for synthesis of nonessential amino acids and the supply of branched-chain α-keto acids for reamination to leucine, isoleucine and valine. It was also suggested that insulin counterregulatory mechanisms, acting through the β-adrenergic system, may be the mechanism responsible for body repartitioning (Greife et al. 1989). In searching for a biochemical explanation for the observed changes in body composition of CLA-fed rats, it was our working hypothesis that CLA, in exchange for linoleate, might also improve the supply of amino acids through accelerated β-oxidation. However, it is not clear what regulates the partitioning of fatty acids between storage or mitochondrial oxidation. It has been demonstrated that PUFA undergo more rapid oxidation than SFA (Beynen and Katan 1985, Jones et al. 1985, Leyton et al. 1987). The fact that carnitine palmitoyl transferase, which is rate limiting for fatty acid β-oxidation, is increased by dietary CLA supplementation in both fat pad and skeletal muscle (Park et al. 1997) indicates that CLA may increase β-oxidation of fatty acids. Uncoupling proteins 2 and 3 (UCP2 and UCP3) are two newly cloned genes that have been implicated in the regulation of lipids as fuel substrate in skeletal muscle. Recent results suggest that circulating free fatty acids may function as an interorgan signal between adipose tissue fat metabolism and skeletal muscle UCP gene expression (Samec et al. 1998). The mRNA expressions of the UCP are up-regulated during starvation when fat stores are being rapidly mobilized. The up-regulation of UCP2 and UCP3 mRNA expression in skeletal muscle during food deprivation parallels the increased mobilization of the fat stores and the well-known increased uptake and utilization of lipids by muscles under such starvation conditions (Boss et al. 1997, Gong et al. 1997, Millet et al. 1997). This could be the reason for the distinct fat-to-lean partitioning effect of CLA under conditions of starvation in which substrate utilization in all muscle types shifts toward lipids. The reduced level of VLDL observed in serum of CLA-treated rats may also be indicative of an accelerated decomposition of triglycerol-bound fatty acids for oxidation because an important function of triglycerol-rich lipoproteins is the delivery of triglycerol-derivated fatty acids to tissue in which this lipid functions as a fuel. Consequently, further studies will be required to gain a better understanding of the mechanism(s) by which CLA exerts the body-partitioning effect.

Moreover, CLA has been shown to display typical peroxisomal and cell proliferation responses (Belury et al. 1997), which also could be responsible for the body fat loss. The shortening of various fatty acids such as very long-chain fatty acids, trans fatty acids, and branched-chain fatty acids via the peroxisomal β-oxidation system (Kramer 1986, Mannaerts and Van-Veldhoven 1993) does not involve coupling to oxidative phosphorylation; therefore, the energy efficiency of ATP formation is lower when compared with mitochondrial β-oxidation. The increased levels of the glycerophospholipids PC and PE, and the concomitant reduction of cholesterol observed with CLA-treated rats in this study, may be indicative of a peroxisome proliferation, although an increased activity of the peroxisome-associated enzyme catalase was not demonstrable. This can be deduced from the fact that an enhanced synthesis of PC and PE (Adinehazadeh and Reo 1998, Adinehazadeh et al. 1999, Kawashima et al. 1994), and suppression of the 3-hydroxymethylglutaryl-CoA reductase, the key enzyme of cholesterol synthesis (Hayashi and Takahata 1991), are typical responses to peroxisome proliferator agents. In light of this observation, this topic would deserve further investigation because 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.

In conclusion, CLA in exchange for linoleate exhibit a strong fat-to-lean repartitioning effect in growing rats with enhanced fat store mobilization. In food-restricted rats, the response of tissue lipids, lipoproteins and hepatic enzymes to high amounts of CLA seems to be different from those in rats with positive energy balance. However, some effects on lipid metabolism may possibly result from the difference in the amounts of linoleate provided with the diets. It may be supposed that the feeding regimen, the composition of the basal
diet, specifically the “non-CLA fatty acids,” and possibly the physiologic state of the animals used, might well have a crucial effect on the magnitude of the CLA effect and on the type of response. Consequently, further studies are warranted to gain a better understanding of the mechanism by which CLA exerts body-repartitioning and lipid-remodeling effects.

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LITERATURE CITED


