Cyclic Fatty Acid Monomers from Heated Oil Modify the Activities of Lipid Synthesizing and Oxidizing Enzymes in Rat Liver1,2

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ABSTRACT Cyclic fatty acid monomers purified from a heated linseed oil were given for 2 wk to adult rats as triacylglycerol at two dose levels, i.e., 0.1 and 1 g/100 g diet, to determine their effect on some aspects of lipid metabolism. Indirect evidence of a peroxisome proliferator–like effect was observed, as determined by an elevation of some characteristic enzyme activities, such as peroxisomal acyl-CoA oxidase, and the microsomal ω- but also (ω-1)-laurate hydroxylase (CYP4A1 and CYP2E1, respectively). The dietary cyclic fatty acids induced a coordinated regulation between the activities of the lipogenic enzymes studied (Δ9-desaturase, phosphatidate phosphohydrolase) and peroxisomal oxidation, but not with mitochondrial β-oxidation. The dose-dependent decrease of Δ9-desaturase activity (P < 0.05) with cyclic fatty acid monomer intake was accompanied by a similar decrease of the monounsaturated fatty acid level in liver. The increase in the γ-linolenic acid level also suggested an increase in Δ6-desaturase activity with cyclic fatty acid intake (P < 0.05). In addition, our results strongly suggested that the altered liver levels of eicosapentaenoic and arachidonic acids were due to the peroxisomal retroconversion process in rats fed cyclic acids. Finally, an effect of these cyclic compounds on the carbohydrate metabolism cannot be disregarded because they decreased liver glycerogen concentration. We conclude that cyclic fatty acid monomers affect different aspects of lipid metabolism, including a phenotypic peroxisome proliferator response. This provides the ground for further studies investigating the biochemical pathways that underlie the nutritional effect of such molecules. J. Nutr. 130: 1524–1530, 2000.

KEY WORDS: • rats • liver • cyclic fatty acid monomer • lipogenic enzymes • peroxisome proliferator

Cyclic fatty acid monomers (CFAM)4 are formed from the unsaturated 18-carbon fatty acids of the edible oils as a result of domestic frying and industrial refining (Sébédió et al. 1989). CFAM generated from 18:1(n-9) are composed of at least eight different saturated cyclic fatty acids with a C5- or a C6-membered ring (Dobson et al. 1996). Thirteen identified monomeric CFAM are formed from 18:2(n-6), containing mostly a C5-membered ring (Christie et al. 1993b, Sébédió et al. 1989). Finally, 18:3(n-3) gives rise to 16 identified dienoic CFAM, with a mixture of C5- and C6-membered rings with some bicyclic acids (Dobson and Christie, 1996, Mossoba et al. 1995, Sébédió et al. 1989) (Fig. 1). Although they are usually present at low levels in oils (from 0.01 to 0.66 g/100 g of the total fatty acids) (Frankel et al. 1984, Sébédió et al. 1991), some investigators, using a purified preparation of CFAM, suggested that they might have adverse effects in animal models. However, these studies are scarce; when CFAM were administered orally to mice, a higher death rate was observed (Saito and Kaneda 1976). Weight gain in weaning rats was decreased and the liver weight (Iwaoka and Perkins, 1976, Sébédió and Grandgirard, 1989) and death rate was increased in rat pups from mothers fed CFAM. CFAM feeding also reduced the number of pups per litter (Sébédió and Grandgirard 1989, Sébédió et al. 1995). CFAM are also incorporated into cultured heart cells (Ribot et al. 1992) where they alter the electrophysiologic properties (Athias et al. 1992). However, they do have a positive effect on prostacyclin release by cultured pig endothelial cells (Flickinger et al. 1997). Few studies have addressed the effect of CFAM on the major biochemical pathways of the intermediary metabolism. Recently, several investigators described the effect of CFAM on some rat liver enzyme activities (Lamboni et al. 1998). In that instance, they restricted their observations to only a few enzymes involved in lipid metabolism (carnitine palmitoyltransferase-I, in particular). Therefore, our first goal was to identify which important lipid-related biochemical pathways could be modified by CFAM ingestion.

*Presented at the 90th AOCS meeting and exposition, May 9–12, 1999, Orlando, FL [Martin, J.C., Joffre, F., Siess, M.H., Vernevaut, M.F. & Sébédió, J.L. (1999) Cyclic fatty acid monomers from heated oil modify lipogenic enzyme activities and induce an peroxisome proliferator-like response in rat liver. p. 59 (abs.)].
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‡F. J. is a recipient of a INRA and Nestlé fellowship.
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4 ACO, acyl-CoA oxidase; CFAM, cyclic fatty acid monomers; CPT, carnitine palmitoyltransferase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; GLC, gas-liquid chromatography, PAP, phosphatidate phosphohydrolase; PUFA, polyunsaturated fatty acids; TG, triacylglycerol.
This study focused on the effect of CFAM, purified from a heated linseed oil and administrated in the triacylglycerol form, on some aspects of the lipid-associated metabolism in the liver of rats. Hepatic enzymes involved in glycerolipid synthesis (phosphatidate phosphohydrolase) and fatty acid metabolism [Δ9-desaturase, carnitine palmitoyltransferase, acyl-CoA oxidase, ω and (ω-1)-laurate hydroxylase] were assayed after CFAM administration. The data suggest that CFAM acted as peroxisome proliferator analogs.

MATERIALS AND METHODS

Chemicals. Solvents were from SDS (Peyrin, France) and were distilled before use. Scintillation solutions Ecoscint A and Ecoscint B were purchased from National Diagnostic (Atlanta, GA), and Floscint II and Ultima Flo were obtained from Packard Bioscience (Groningen, The Netherlands). [1-14C]-lauryc acid (2 GBq mmol⁻¹), [1-14C]-palmitic acid (2 GBq mmol⁻¹) and [1-14C]-stearic acid (2 GBq mmol⁻¹) were purchased from Amersham (Amersham, Courtaboeuf, France). All the other chemicals were from Sigma-Aldrich (Sigma, L’Ile d’Abeau-Chène, France).

Animals and diet. Official French regulations (n° 87/848) for care and use of laboratory animals were followed (n° 30356). Weanling male Wistar rats (n = 18), weighing 266–300 g, were obtained from the Center d’elevage DEPRE (Saint Doulchard, France). They were housed at a constant humidity and temperature, with a 12-h light:dark cycle. They had free access to water and received the same amount of a purified diet daily (Table 1). Rats were divided into three groups (n = 6/group) and were fed for 2 wk a 10 g/100 g fat diet (by weight) containing either 0.1 or 1 g/100 g CFAM isolated from heated linseed oil (Sébédo et al. 1987) and in the form of triacylglycerols [TG; synthesized as described by Martín et al. (1997)]. The control group received the same soybean oil based–diet (10 g/100 g). The Δ9-desaturase activity was determined by adding 1.6 MBq of [1-14C]-18:0 dissolved in 3 μL of ethanol (30 nmol/assay) to the microsome preparation (0.4 mL, 4–5 mg protein), incubated with 0.3 mL of cytosol (4–5 mg protein) for 15 min at 37°C under gentle shaking in a pH 7.4 phosphate buffer made up of 0.15 mol/L Na2HPO4, 0.15 mol/L KH2PO4, 7.2 mmol/L ATP, 6 mmol/L MgCl2, 1.2 mmol/L NADPH and 0.54 mmol/L CoA. The reaction was terminated by saponification of the fatty acid esters while adding 18 mL of 2 mol/L KOH and heating at 70°C for 30 min. After acidification with HCl, the free fatty acids released were extracted with 25 × 4 mL of diethyl ether. The solvent was removed under a stream of nitrogen and the fatty acids were methylated with boron trifluoride (140 g/L) in methanol as described by Morrison and Smith (1964). After hexane extraction, the residue was redissolved in 100 μL of acetonitrile and analyzed by radiodetection. The fatty acid methyl esters (FAME) (0.5–1 mg) were separated by HPLC (model 600, Waters, Saint Quentin en Yvelines, France) using a reversed-phase column (Nucleosil C18, 5 μm particle size, 250 mm length × 4.6 mm i.d.) (Interchim, Montluçon, France) and isocratic elution with pure acetonitrile at 1 mL/min. The radioactivity was detected using a radiochromatographic detector Flo-one β (serie A-100, Radiomatic).

Enzyme activity. The peroxisomal acyl-CoA oxidase (ACO) and mitochondrial carnitine palmitoyltransferase (CPT) activities were both assessed in the peroxisomal + mitochondrial fractions. The ACO activity was determined according to Lazaro (1976) and the CPT activity according to the method of Bieber et al. (1972).

The ω- and (ω-1)-laurate hydroxylations were determined as a marker of the cytochrome P450A1 and cytochrome P4502E1 activities, respectively (Amet et al. 1994, Pacot et al. 1993). Measurements were determined in the microsomal fraction according to Orton and Parker (1982) as modified by Laignelet et al. (1989). The reaction products were extracted with diethyl ether, separated by reversed-phase HPLC and quantified with a radiochromatographic detector Flo-one β (serie A-100, Radiomatic Instruments, Tampa, FL) by peak integration (HPLC conditions: ODS column 25 cm length × 4.6 mm i.d., scintillation solution of Floscint II, 2:1, v/v). The mobile phase was made up of ammonium acetate (A, 270 g/L), acetonitrile (B, 320 g/L and H2O (C, 410 g/L); the samples were eluted with this mixture for 4 min. The mobile phase was then changed within a 2-min gradient to A (0 g/L) and B (900 g/L) and held for a further 4.5 min.

The phosphatidate phosphohydrolase (PAP) total activity (i.e., Mg2⁺-dependent and Mg2⁺-independent) was assessed in both the microsomal fraction and the cytosol, essentially as described by Wall and Pomer (1985) and modified by Surette et al. (1992). The Δ9-desaturase activity was determined by adding 1.6 MBq of [1-14C]-18:0 dissolved in 3 μL of ethanol (30 nmol/assay) to the microsome preparation (0.4 mL, 4–5 mg protein), incubated with 0.3 mL of cytosol (4–5 mg protein) for 15 min at 37°C under gentle shaking in a pH 7.4 phosphate buffer made up of 0.15 mol/L Na2HPO4, 0.15 mol/L KH2PO4, 7.2 mmol/L ATP, 6 mmol/L MgCl2, 1.2 mmol/L NADPH and 0.54 mmol/L CoA. The reaction was terminated by saponification of the fatty acid esters while adding 18 mL of 2 mol/L KOH and heating at 70°C for 30 min. After acidification with HCl, the free fatty acids released were extracted with 25 × 4 mL of diethyl ether. The solvent was removed under a stream of nitrogen and the fatty acids were methylated with boron trifluoride (140 g/L) in methanol as described by Morrison and Smith (1964). After hexane extraction, the residue was redissolved in 100 μL of acetonitrile and analyzed by radiodetection. The fatty acid methyl esters (FAME) (0.5–1 mg) were separated by HPLC (model 600, Waters, Saint Quentin en Yvelines, France) using a reversed-phase column (Nucleosil C18, 5 μm particle size, 250 mm length × 4.6 mm i.d.) (Interchim, Montluçon, France) and isocratic elution with pure acetonitrile at 1 mL/min. The radioactivity was detected using a radiochromatographic detector Flo-one β (serie A-100, Radiomatic).

TABLE 1

Composition of the experimental diets

<table>
<thead>
<tr>
<th>g/kg diet</th>
<th>Casein</th>
<th>Sucrose</th>
<th>Cornstarch</th>
<th>Cellulose</th>
<th>Mineral mixture1</th>
<th>Vitamin mixture2</th>
<th>Oil3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180</td>
<td>220</td>
<td>420</td>
<td>20</td>
<td>50</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

1 Contained CaCO3 (12 g), K2HPO4 (10.75 g), CaHPO4 (10.75 g), MgSO4, 7H2O (5 g), NaCl (3 g), Mgo (2 g), FeSO4 7H2O (400 mg), ZnSO4 7H2O (350 mg), MnSO4 H2O (100 mg), CuSO4 5H2O (50 mg), Na2SiO3 * 3H2O (25 mg), SiO2 (10 mg), K2Cr2O7 (5.7 mg), NaF (5 mg), NiSO4 6H2O (5 mg), H2BO3 (5 g), CoSO4 7H2O (2.5 mg), KIO3 (2 mg), NH4H2MoO4 2H2O (1 mg), LiCl (0.75 mg), Na2SeO3 (0.75 mg), NaH2CO3 (0.5 mg), sucrose (5.5 mg).

2 Contained retinyl acetate (1.72 kg), cholecalciferol (30 μg), DL-α-tocopherol acetate (100 mg), phylloquinone (1 mg), thiamin chloride (10 mg), riboflavin (10 mg), nicotinic acid (50 mg), Ca2+ pantothenate (25 mg), pyridoxine chloride (10 mg), β-biotin (0.2 mg), folic acid (2 mg), cyanocobalamin (25 μg), choline chloride (1 g), DL-methionine (2 g), p-amino benzoic acid (50 mg), inositol (100 mg), sucrose (5.5 mg).

3 Contained 100% soybean oil (control group), or 99% soybean oil and 1% CFAM (0.1% CFAM group), or 90% soybean oil and 10% CFAM (1% CFAM group).

CFAM (general structure)

[Image of cyclic fatty acid monomer]

FIGURE 1 General structure of cyclic fatty acid monomers (CFAM) formed from linolenic acid (the carboxyl-side chain is monounsaturated; x = 1 to 4; m = 1 or 2; total carbons number = 18).

ACYO (general structure)
Liver Food efficiency, g/d Food intake, g/d Food efficiency, g gain/g food Liver Weight, g/g/100 g body weight Liver Glycogen, µg/g Liver Protein, mg/g Microsomal protein, mg/g Lipid, mg/g 14.85 ± 0.23a 3.83 ± 0.08b 3.89 ± 0.05b 2.0 ± 0.05 19.9 ± 0.9 41.8 ± 4.3 43.7 ± 7.0 Control CFAM CFAM 0.1 g/100 g 1 g/100 g 106.6 ± 2.0 101.2 ± 3.3 97.5 ± 4.0 35.9 ± 1.6 36.2 ± 1.9 35.8 ± 1.9 0.47 ± 0.11 0.41 ± 0.05 0.40 ± 0.05 14.85 ± 0.23a 18.82 ± 0.63a 18.47 ± 0.35a 12.82 ± 0.63a 3.83 ± 0.08b 3.89 ± 0.05b 4.28 ± 0.16a 2.0 ± 0.05 19.1 ± 1.3 21.0 ± 1.1 21.0 ± 1.1 19.1 ± 1.3 21.0 ± 1.1 21.0 ± 1.1 19.1 ± 1.3 21.0 ± 1.1

Table 2 Food efficiency, liver weight and liver glycogen, protein and lipid concentrations in rats fed diets containing 0, 0.1 or 1.0 g/100 g cyclic fatty acid monomers (CFAM)

<table>
<thead>
<tr>
<th>Control</th>
<th>CFAM</th>
<th>CFAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 g/100 g</td>
<td>1 g/100 g</td>
<td></td>
</tr>
<tr>
<td>Weight gain, g/2 wk</td>
<td>106.6 ± 2.0</td>
<td>101.2 ± 3.3</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>35.9 ± 1.6</td>
<td>36.2 ± 1.9</td>
</tr>
<tr>
<td>Food efficiency, g gain/g food</td>
<td>0.47 ± 0.11</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>Liver Weight, g</td>
<td>14.85 ± 0.23a</td>
<td>18.82 ± 0.63a</td>
</tr>
<tr>
<td>Liver Glycogen, µg/g</td>
<td>714 ± 6.8a</td>
<td>494 ± 10.3b</td>
</tr>
<tr>
<td>Liver Protein, mg/g</td>
<td>133 ± 7</td>
<td>137 ± 7</td>
</tr>
<tr>
<td>Liver Microsomal protein, mg/g</td>
<td>19.9 ± 0.9</td>
<td>19.1 ± 1.3</td>
</tr>
<tr>
<td>Liver Lipid, mg/g</td>
<td>41.8 ± 4.3</td>
<td>43.7 ± 7.0</td>
</tr>
</tbody>
</table>

1 Values are means ± sem; n = 5 or 6. Means with a different superscript in a row are different, P < 0.05.

Instruments) after addition of Ultima Flo (2:1, v/v). The results were expressed as the percentage of conversion of [1-14C]-stearic acid to [1-14C]-oleic acid.

**Fatty acid oxidation.** The total fatty acid oxidation was measured in the peroxisomal + mitochondrial fraction as adapted from Anderson (1968) and Clouet et al. (1989), using albumin-bonded [1-14C]-palmitate (1:2, mol/mol; 50 μmol/L with a specific activity of 0.34 GBq/mmol).

**Lipid analysis.** The total lipids of a portion aliquot of the liver were extracted with chloroform/methanol (2:1; v/v) according to Folch et al. (1957) and weighed after removal of the solvent under reduced pressure. Total FAME of 5 mg of the lipid residue were then prepared using boron trifluoride in 140 g/L methanol, as described by Folch et al. (1957) and weighed after removal of the solvent under reduced pressure. The results were collected fractions were then dried at reduced pressure, dissolved in a minimum of solvent and stored at -20°C before GLC analysis under the same conditions as above. Linoleic acid contained in samples served as an internal standard in both the total fatty acid profile and the CFAM fraction to recalculate the relative content of the CFAM in the total lipid. CFAM identification was performed by comparison with a heated linseed oil CFAM standard whose peak characterization has been detailed elsewhere (Christie et al. 1993a).

**Other determinations.** The protein content of the total liver homogenate as well as of each subfraction was determined according to Lowry et al. (1951) and the glycogen content as described by Lo et al. (1970).

Statistics. Results were computed and analyzed with the use of SigmaStat software (Jandel Scientific, San Rafael, CA). Comparisons were made using one-way ANOVA or ANOVA on Ranks when the normality test failed. Student-Newman-Keuls test was used when heterogeneity among groups was demonstrated. Regressions were established using the linear-regression fit model. The level of significance was set at P ≤ 0.05.

**RESULTS**

Weight gain, food intake and feed efficiency did not differ among groups (Table 2) and no significant weight differences were observed at the end of the feeding period (data not shown). Relative liver weight was higher in the rats fed 1 g/100 g CFAM than in the other two groups (P < 0.05) (Table 2). The rats fed both levels of CFAM had liver glycogen concentrations 30–40% lower than those in the control group, whereas the protein and lipid concentrations did not differ (Table 2). In contrast, microsomal and cytosolic PAP activities (a rate-limiting enzyme in the glycerolipid synthesis) were lower (P ≤ 0.05) in rats fed 1 g/100 g CFAM than in controls (Table 3).

CFAM consumption caused a significant stepwise increase of peroxisomal ACO activity (Table 3). The peroxisomal ACO activity was accompanied by a similar rise of microsomal -ω- and (ω-1)-laurate hydroxylases (CYP4A1 and CYP2E1, respectively) (Table 3 and Table 4). Conversely, no effects of CFAM intake on mitochondrial β-oxidation were detected, as evaluated by measurements of the CPT-I activity and the radioactive oxidation products arising from [1-14C]-palmitate (Table 3). It is interesting to note that enzymes involved in peroxisomal lipid oxidation (ACO, CYP2E1 and CYP4A1) were negatively correlated with enzymes involved in lipid peroxidation (Table 3).

**Table 3**

Enzyme activities involved in lipid metabolism and in vitro [1-14C]-palmitic acid oxidation in the liver of rats fed diets containing 0, 0.1 or 1.0 g/100 g cyclic fatty acid monomers (CFAM)

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Control</th>
<th>CFAM</th>
<th>CFAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomal PAP2</td>
<td>17.3 ± 1.5a</td>
<td>15.6 ± 1.1a,b</td>
<td>11.9 ± 1.5a,b</td>
</tr>
<tr>
<td>Cytosolic PAP2</td>
<td>58.4 ± 2.2a</td>
<td>57.8 ± 2.9a</td>
<td>48.0 ± 2.6ap</td>
</tr>
<tr>
<td>ACO, nmol/min</td>
<td>7.4 ± 0.3c</td>
<td>9.3 ± 0.7b</td>
<td>16.5 ± 0.8a</td>
</tr>
<tr>
<td>(ω-1)-Laurate hydroxylase (CYP2E1), nmol/min</td>
<td>1.2 ± 0.2c</td>
<td>2.0 ± 0.2b</td>
<td>2.9 ± 0.2a</td>
</tr>
<tr>
<td>(ω-1)-Laurate hydroxylase (CYP4A1), nmol/min</td>
<td>2.9 ± 0.6c</td>
<td>4.7 ± 0.4b</td>
<td>6.8 ± 0.6a</td>
</tr>
<tr>
<td>CPT-I, nmol/min</td>
<td>4.8 ± 1.1</td>
<td>5.1 ± 0.4</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>Oxidation products, % of total radioactivity</td>
<td>52.2 ± 1.9</td>
<td>55.0 ± 4.9</td>
<td>58.5 ± 2.6</td>
</tr>
<tr>
<td>Δ9-Desaturase, pmol/min</td>
<td>18.5 ± 0.2a</td>
<td>19.7 ± 0.07a</td>
<td>13.7 ± 0.01b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM; n = 5–6. Means in a row with different superscripts differ, P < 0.05.
2 Total PAP activity (Mg2+-dependent and Mg2+-independent).
synthesis (PAP and \(\Delta^9\)-desaturase) after CFAM consumption (Table 4). This was not the case between the enzyme associated with mitochondrial oxidation (CPT-I) and the above lipogenic enzymes (Table 4).

In all groups, the main saturated and monounsaturated fatty acids were the 16- and 18-carbon fatty acids, whereas the main polyunsaturates were 18:2(n-6), 20:4(n-6) and 22:6(n-3) fatty acids (Table 5). There was a 10-fold difference in CFAM intake between the 0.1 and the 1 g/100 g CFAM groups, whereas the CFAM content in the total liver lipids was only 75% lower in the former group than in the latter group. The sum of the monounsaturated fatty acids originating primarily from a \(\Delta^9\)-desaturation was significantly lower in the 1 g/100 g CFAM group than in the 0.1 g/100 g CFAM group or in the control group (Table 5). Although not significant \((P = 0.25)\), the 0.1 g/100 g CFAM group had slightly more monounsaturates than the control group. This was also true for \(\Delta^9\)-desaturase (Table 3); this activity was closest associated with the monounsaturated fatty acid content in liver of rats from either dietary group \((r = 0.714; P \leq 0.05; \text{Fig. 2})\).

A commensurate decrease of some selected polyunsaturated fatty acids (PUFA) such as 22:6(n-3) and 22:4(n-6) occurred along with increased CFAM consumption (Table 5). Additionally, an inverse relationship was found between ACO activity and the same group of selected fatty acids, i.e., 22:6(n-3) \((r = -0.55; P \leq 0.05, \text{Fig. 3A})\) and 22:4n-6 \((r = -0.57; P \leq 0.05; \text{Fig. 3B})\). CFAM intake was also accompanied by increases in the liver content of both 20:5(n-3) and 20:4(n-6) (Fig. 3A). The increases of these two fatty acids were positively correlated with the peroxisomal ACO activity \([r = 0.77; P = 0.05, r = 0.57; P = 0.05 \text{ for } 20:5(n-3) \text{ and } 20:4(n-6); \text{Fig. 4} \text{ and } B, \text{respectively}]\). The decrease in 22:6(n-3) fatty acid level with increased CFAM intake was inversely related to an increase in the 20:5(n-3) content of liver lipids (Table 4) \((r = -0.55; P \leq 0.05; \text{data not shown for the linear regression fit})\). The same trend for the (n-6) fatty acids homologs \([i.e., 22:4(n-6) \text{ and } 20:4(n-6)]\) was observed, but was not significant for the relationship between arachidonic acid [22:4(n-6)] and arachidonic acid [20:4(n-6)] \((r = -0.34; P = 0.18; \text{data not shown})\). Interestingly, the level of 18:3(n-6), the \(\Delta^6\)-desaturated product of linoleic acid, increased steadily with CFAM consumption \((P \leq 0.05, \text{Table 5})\), with no change in the 18:2(n-6) level.

**DISCUSSION**

In this study, we used a purified fraction of CFAM formed mainly from linoleic acid for direct study of their effect on some liver enzyme activities. We examined the biochemical effects of CFAM generated from 18:3(n-3) because they are formed at a higher level in oils (Martin et al. 1998, Sêbédio et al. 1987). 18:3(n-3)-derived CFAM are representative of all CFAM in terms of the ring (contain either a C5- or a C6-
membered ring) and they can be analyzed easily in their nonhydrogenated form. Rats were fed these fatty acids as TG because this is the natural form of dietary fat and also because it has been demonstrated that this is a bioavailable form of CFAM (Martin et al. 1997).

Recently, Lamboni et al. (1998) studied the relationship between intake of CFAM purified from a heated linseed oil and liver enzyme activity in rats. Some differences exist between their results and ours, such as a decrease in CPT-I activity in vitro due to CFAM intake that was not observed in our study, as well as an increase in the total liver lipids and proteins in their study but not in ours (Table 2). It is noteworthy that variations in the feeding conditions could explain the discrepancies between these studies. In the former study, CFAM were administrated as methyl esters (0.15 g/100 g), in their hydrogenated form for 7 wk to rats weighing 50–60 g at the onset of the experiment. In our study, CFAM were ingested as triacylglycerol (0.1 and 1 g/100 g), in nonhydrogenated form for 2 wk to rats weighing 200–220 g. Nonetheless, we observed a decrease in the glycogen content in the liver, which was also noted in the earlier study (Lamboni et al. 1998). This decrease was maximal with as little as 0.1 g/100g CFAM intake.

In this study, indirect evidence of a peroxisome proliferation effect was observed in rats consuming CFAM, such as hepatomegaly (increase in the relative liver weight) (Bentley et al. 1993, Hawkins et al. 1987), and an increase of the liver microsomal CYP4A1 (Bentley et al. 1993, Intrasuksri et al. 1998) and peroxisomal ACO activities (Bardot et al. 1995, Bentley et al. 1993) (Tables 3 and 4). It is noteworthy that CFAM seemed to be as potent as the classical peroxisome proliferators because they induced their effect in the same dose range (1–10 mg/g) (Bentley et al. 1993). Nonetheless, this apparent peroxisome proliferation due to CFAM demonstrated differences from the common peroxisome proliferator agents such as fibrates. For example, we did not measure smooth endoplasmic reticulum membrane proliferation (Bentley et al. 1993), which was observed as shown by the absence of microsomal protein increase with CFAM consumption (Table 2). Additionally, in our study, dietary CFAM were strong enough to induce CYP4A1 (ω-laurate hydroxylation) and CYP2E1 ([ω-1]-laurate hydroxylation) activities equally (Table 3). In addition, no induction of CPT-I and Δ9-desaturase activities was observed after CFAM consumption (Table 3), as is usually the case with common peroxisome proliferator drugs such as fibrates (Schoonjans et al. 1996). On the contrary, the Δ9-

**FIGURE 2** Relationship between Δ9-desaturase activity and the sum of monounsaturated fatty acids in rats fed diets containing 0, 0.1 or 1.0 g/100 g cyclic fatty acid monomers (CFAM). For each dietary group (n = 5 or 6), 16:1(n-7), 18:1(n-7), 18:1(n-9), 20:1(n-7) and 20: 1(n-9) were included in the sum of the monounsaturated fatty acids.

**FIGURE 3** Relationship between the peroxisomal acyl-CoA oxidase (ACO) activity and DHA (22:6n-3) (panel A) or adrenic acid (22:4n-6) (panel B) in rats fed diets containing 0, 0.1 or 1.0 g/100 g cyclic fatty acid monomers (CFAM); (n = 5 or 6 per dietary group).
decrease the surprising that CFAM, which are also PUFA, would also be in the liver of mice. It should not be well as conjugated linoleic acids (Belury et al. 1997), can also dose (Table 3). Some PUFA such as arachidonic acid and desaturase activity was even decreased at the 1 g/100 g CFAM dose (Table 3). Some PUFA such as arachidonic acid and eicosapentaenoic acid (EPA) (Miller and Ntambi 1996), as well as conjugated linoleic acids (Belury et al. 1997), can also acutely induce both ACO and CYP4A1 mRNA, and decrease Δ9-desaturase mRNA levels with no effect on CPT-I activity (Park et al. 1997) in the liver of mice. It should not be surprising that CFAM, which are also PUFA, would also be able to induce both the liver ACO and CYP4A1 activities and decrease the Δ9-desaturase activity without affecting CPT-I. Also, the decrease in Δ9-desaturase activity observed in our study may be the result of a direct inhibition by CFAM, as occurs with other cyclic fatty acids such as sterculic acid (a cyclic fatty acid monomers (CFAM); (n = 5 or 6 per dietary group).

FIGURE 4 Relationship between the peroxisomal acyl-CoA oxidase (ACO) activity and eicosapentaenoic acid (EPA) (panel A) or arachidonic acid (panel B) in rats fed diets containing 0, 0.1 or 1.0 g/100 g cyclic fatty acid monomers (CFAM); (n = 5 or 6 per dietary group).

desaturase activity was even decreased at the 1 g/100 g CFAM dose (Table 3). Some PUFA such as arachidonic acid and eicosapentaenoic acid (EPA) (Miller and Ntambi 1996), as well as conjugated linoleic acids (Belury et al. 1997), can also acutely induce both ACO and CYP4A1 mRNA, and decrease Δ9-desaturase mRNA levels with no effect on CPT-I activity (Park et al. 1997) in the liver of mice. It should not be surprising that CFAM, which are also PUFA, would also be able to induce both the liver ACO and CYP4A1 activities and decrease the Δ9-desaturase activity without affecting CPT-I. Also, the decrease in Δ9-desaturase activity observed in our study may be the result of a direct inhibition by CFAM, as occurs with other cyclic fatty acids such as sterculic acid (a cycloprenenopanoic acid) (Jeffcoat, 1977, Legrand et al. 1997). This effect on the desaturase is important because liver Δ9-desaturase activity varies in parallel with hepatic TG secretion, as assessed in vitro in chickens (Legrand et al. 1997) or in vivo in mice (de Antueno et al. 1993). Additionally, the decrease of the Δ9-desaturase activity was associated with a similar lowering of the microsomal-bound PAP activity (Table 4). In rats, this microsome-bound enzyme is rate limiting for the synthesis of TG in the liver (Cha et al. 1998) and in turn, modulates the plasma TG concentration (Cha et al. 1998, Marsh et al. 1987). It is therefore highly conceivable that dietary CFAM, which activate peroxisomal oxidation and see to be potent as peroxisome proliferators, as Δ9-desaturase activity inhibitors and as microsome-bound PAP regulators, may also impair VLDL-TG secretion by the liver. It is interesting to note also that in lymph-canalulated rats (Martin et al. 1997), the same CFAM generated from heated linseed oil are also efficient modulators of lipoprotein secretion rate by the intestine, which is the other important organ for lipoprotein production.

However, in spite of the above effects on both the peroxisomal β-oxidation and the lipogenic activities measured in this study, there was no modification of the overall lipid content of liver (Table 2). One explanation is that only the TG would be lowered, whereas the phospholipids would be increased, as was already demonstrated in rat liver with tetra-decythioacetic acid, a sulfur-substituted peroxisome proliferator fatty acid (Skorve et al. 1990). From that viewpoint, a detailed analysis of the lipid composition could have been informative.

In addition to the effect of CFAM on liver Δ9-desaturase and downstream on the monounsaturated fatty acid level (Tables 3 and 5, Fig. 2), other modifications of the fatty acid profile due to the CFAM consumption were also found. In particular, the content of the C-22 PUFA decreased in a dose-dependent manner with increasing CFAM intake (Table 4). This decrease was particularly pronounced with 22:6(n-3) and 22:4(n-6), the two main C-22 PUFA, and was accompanied by a similar rise of their biosynthetic precursors, 20:5(n-3) and 20:4(n-6), respectively. Because the decrease of both 22:6(n-3) and 22:4(n-6) and the rise of 20:5(n-3) and 20:4(n-6) were closely associated with peroxisomal ACO activities (Figs. 3 and 4), it is plausible that the levels of both EPA and arachidonic acid in our experimental conditions were dictated by the peroxisomal retroconversion activity of their longer-chain homologs, i.e., docosahexaenoic acid (DHA) and arachidonic acid, respectively (Hagve and Christophersen 1986, Sprecher et al. 1995). However, a precursor-product relationship between adrenic acid [22:4(n-6)] and arachidonic acid [20:4(n-6)] was not significant (r = -0.34; P = 0.18). The relationship between DHA [22:6(n-3)] and EPA [20:5(n-3)] was significant (r = -0.55; P ≤ 0.05). A possible explanation could be that the retroconversion effect of the C-22 metabolites to 20:4n-6 would be somewhat counterbalanced by the contribution of the chain elongation and desaturation system from the abundant precursors [i.e.,18:2(n-6) and 20:3(n-6)]. In addition to the effect of CFAM on liver Δ9-desaturase activity (Table 2), other modifications of the fatty acid profile due to the CFAM consumption were also found. In particular, the content of the C-22 PUFA decreased in a dose-dependent manner with increasing CFAM intake (Table 4). This decrease was particularly pronounced with 22:6(n-3) and 22:4(n-6), the two main C-22 PUFA, and was accompanied by a similar rise of their biosynthetic precursors, 20:5(n-3) and 20:4(n-6), respectively. Because the decrease of both 22:6(n-3) and 22:4(n-6) and the rise of 20:5(n-3) and 20:4(n-6) were closely associated with peroxisomal ACO activities (Figs. 3 and 4), it is plausible that the levels of both EPA and arachidonic acid in our experimental conditions were dictated by the peroxisomal retroconversion activity of their longer-chain homologs, i.e., docosahexaenoic acid (DHA) and arachidonic acid, respectively (Hagve and Christophersen 1986, Sprecher et al. 1995). However, a precursor-product relationship between adrenic acid [22:4(n-6)] and arachidonic acid [20:4(n-6)] was not significant (r = -0.34; P = 0.18). The relationship between DHA [22:6(n-3)] and EPA [20:5(n-3)] was significant (r = -0.55; P ≤ 0.05). A possible explanation could be that the retroconversion effect of the C-22 metabolites to 20:4n-6 would be somewhat counterbalanced by the contribution of the chain elongation and desaturation system from the abundant precursors [i.e.,18:2(n-6) and 20:3(n-6)].

In conclusion, purified CFAM generated from heated linseed oil and given as TG elicited a phenotypic peroxisome proliferator response in rats in dose ranges used commonly for drug-induced peroxisome proliferation. CFAM exerted specific effects on the metabolism of a complex carbohydrate (glycogen) and on the metabolism of unsaturated fatty acids. They regulated some aspects of both lipogenesis and peroxisomal β-oxidation in a coordinated manner, and neither microsome-bound PAP nor mitochondrial β-oxidation was unaffected. The effect on the unsaturated fatty acids would be due to a modulation of the desaturase (Δ9 and possibly Δ6) and of the peroxisomal retroconversion ac-
tivities. On the basis of the effects of CFAM on apparent peroxisomal proliferation, Δ9-desaturase activity and PAP activity, the possibility that CFAM would also reduce the secretion of VLDL-TG by the liver is likely and merits further investigation. It would be interesting to address whether the phenotypic effects observed are supported by a genotypic response, such as a peroxisome proliferator-activated receptor (PPAR)-mediated induction.

LITERATURE CITED


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