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Biochimica et Biophysica Acta 1631 (2003) 265–273
BIOCHIMICA ET BIOPHYSICA ACTA
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Activity and mRNA levels of enzymes involved in hepatic fatty acid synthesis and oxidation in mice fed conjugated linoleic acid

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Received 10 September 2002; received in revised form 31 January 2003; accepted 21 February 2003

Abstract

The effects of dietary conjugated linoleic acid (CLA) on the activity and mRNA levels of hepatic enzymes involved in fatty acid synthesis and oxidation were examined in mice. In the first experiment, male ICR and C57BL/6J mice were fed diets containing either a 1.5% fatty acid preparation rich in CLA or a preparation rich in linoleic acid. In the second experiment, male ICR mice were fed diets containing either 1.5% linoleic acid, palmitic acid or the CLA preparation. After 21 days, CLA relative to linoleic acid greatly decreased white adipose tissue mass but caused hepatomegaly accompanying an approximate 10-fold increase in the tissue triacylglycerol content irrespective of mouse strain. CLA compared to linoleic acid greatly increased the activity and mRNA levels of various lipogenic enzymes in both experiments. Moreover, CLA increased the mRNA expression of Δ^6- and Δ^5-desaturases, and sterol regulatory element binding protein-1 (SREBP-1). The mitochondrial and peroxisomal palmitoyl-CoA oxidation rate was about 2.5-fold higher in mice fed CLA than in those fed linoleic acid in both experiments. The increase was associated with the up-regulation of the activity and mRNA expression of various fatty acid oxidation enzymes. The palmitic acid diet compared to the linoleic acid diet was rather ineffective in modulating the hepatic lipid levels or activity and mRNA levels of enzymes in fatty acid metabolism. It is apparent that dietary CLA concomitantly increases the activity and mRNA levels of enzymes involved in fatty acid synthesis and oxidation, and desaturation of polyunsaturated fatty acid in the mouse liver. Both the activation of peroxisomal proliferator α and up-regulation of SREBP-1 may be responsible for this.

Keywords: Conjugated linoleic acid; Fatty acid synthesis; Fatty acid oxidation; Gene expression; Mouse

1. Introduction

Conjugated linoleic acid (CLA) is a generic name for a group of positional and geometrical isomers of conjugated dienoic derivatives of linoleic acid and occurs naturally in dairy products and beef fat [1]. CLA reportedly causes profound alterations in energy and lipid metabolism in experimental animals. Studies have shown that CLA reduces body fat mass in mice [2–5], rats [6,7], hamsters [8] and pigs [9]. But CLA causes liver enlargement accompanying an increase in tissue lipid content in mice [2–5,10,11].

There is evidence that CLA affects hepatic fatty acid metabolism in mice. Belury et al. [11] demonstrated that CLA causes up-regulation of the gene expression of acyl-CoA oxidase, a rate-limiting enzyme in peroxisomal fatty oxidation, in the liver of female SENCAR mice. They also showed that CLA increases the mRNA levels of liver fatty acid binding protein and cytochrome P450IV A1, known to be induced during peroxisome proliferation following the activation of peroxisome proliferator activated receptor (PPAR)α. In fact, they subsequently demonstrated [12] that CLA is a potent ligand and activator of PPARα. Using PPARα-null mice in a C57BL/6N genetic background, Peters et al. [5] recently found that CLA increased the mRNA levels of acyl-CoA oxidase, carnitine palmitoyltransferase II, liver fatty acid binding protein and cytochrome P450IV A1 in the liver of wild-type but not PPARα-null mice. CLA also increased the gene expression of short-, medium-, long- and very-long-chain-acyl-CoA dehydrogenases in both wild-type and PPARα-null mice. These observations indicate that CLA increases the gene expression of hepatic fatty acid oxidation enzymes through both PPARα-dependent and -independent mechanisms. In the

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doi:10.1016/S1388-1981(03)00038-6

same study, they found that CLA increased the mRNA levels of proteins involved in the regulation of lipogenesis including fatty acid synthase, spot 14 and stearoyl-CoA desaturase 1 in both PPARα-null and wild-type mice. They therefore suggested that dietary CLA increases both hepatic fatty acid oxidation and synthesis in the mouse liver. However, an increase in both the catabolic and anabolic pathways appears illogical. In addition, their results on the effect of CLA on stearoyl-CoA desaturase 1 gene expression were inconsistent with those reported by Lee et al. [13]. They showed that CLA decreased the mRNA level of stearoyl-CoA desaturase 1 in the liver of ICR mice and in H2.35 mouse liver cells. Therefore, the notion that CLA increases hepatic lipogenesis still needs to be confirmed. Previous information on the physiological activity of CLA in affecting hepatic fatty acid metabolism is mainly based on observations of the mRNA levels and not actual metabolic activity. There is still the possibility that alterations in the mRNA levels do not parallel those in enzyme protein expression. In addition, the responses to dietary CLA of hepatic fatty acid metabolism may differ according to the strains of mice employed [5]. Given the above, we examined the effects of dietary CLA on the activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation and synthesis in the liver of C57BL/6J and ICR mice in the present study.

2. Materials and methods

2.1. Animals and diets

Male C57BL/6J and ICR mice were purchased from CLEA Japan (Tokyo, Japan) at 5 weeks of age. After an acclimatization period of 7 days in our housing conditions, the animals were randomly divided into groups and fed purified experimental diets for 21 days. In the first experiment (Exp. 1), two groups of ICR and C57BL/6J mice were fed either a diet containing 1.5% of a fatty acid preparation rich in CLA or a preparation devoid of CLA and mainly consisting of 9c,12c-linoleic acid (70.8%). In the second experiment (Exp. 2), three groups of ICR mice were fed a diet containing either 1.5% linoleic acid, palmitic acid or the CLA preparation. The fatty acid preparations rich in linoleic acid and CLA were generously donated by Rinoru Oil Mills, Aichi, Japan. The palmitic acid with a purity greater than 98% used as a dietary fatty acid source in Exp. 2 was purchased from Wako Pure Chemicals, Osaka, Japan. In addition to these fatty acid preparations, all the experimental diets contained 13.5% palm oil as a dietary fat. The contents of major fatty acids in the experimental diets are shown in Table 1. All the experimental diets contained 12.8 g of fatty acids as triacylglycerol and 1.5 g of free fatty acid in 100 g. The linoleic acid diet and CLA diet contained equal amounts of palmitic, stearic and oleic acids. Total amounts of ocatadecadienoic acid were also the same between the two diets. The former contained ocatadecadienoic acid exclusively as linoleic acid, while the latter contained this fatty acid as both linoleic acid and various CLA isomers. Therefore, the linoleic acid content was about two times higher in the linoleic acid diet than in the CLA diet. The amount of linoleic acid was the same between the palmitic acid diet and CLA diet. But these diets differed in the contents of palmitic acid and total octadecadienoic acids. Although the CLA content of the fatty acid preparation employed in the present study (70.5%) was considerably less than those employed by others (86–98%), the 1.5% dietary level employed in the present study may provide a sufficiently high amount of CLA (about 1% in diet, Table 1) to exert its physiological activity [2–11]. The CLA preparation employed to date in studies to examine its physiological activities in experimental animals consisted principally of 9c,11t/9t,11c- and 10t,12c-CLA in similar amounts. These isomers were also the main components of the CLA preparation employed in the present study and the composition was similar to those used by other investigators [2–11]. The composition of the experimental diet was (in wt.%): casein, 20; corn starch, 15; palm oil, 13.5; the fatty acid preparation, 1.5; cellulose, 2; mineral mixture [14], 3.5; vitamin mixture [14], 1.0; DL-methionine, 0.3; choline bitartrate, 0.2; and sucrose, 43. The body weight at the start of the feeding period was, in Exp. 1, 25–34 g for ICR and 18–22 g for C57BL/6J mice and, in Exp. 2, 31–36 g for ICR mice. We followed our institute's guidelines in the care and use of laboratory animals.

Table 1
Fatty acid contents of experimental diets

Fatty acid contents (g/100 g diet)	Experimental diets		
	Linoleic	Palmitic	CLA
16:0	5.92	7.31	5.91
18:0	0.60	0.56	0.60
9c-18:1	5.27	5.01	5.27
9c,12c-18:2	2.27	1.21	1.22
CLA			
9c,11t-/9t,11c-18:2	0.00	0.00	0.49
10t,12c-18:2	0.00	0.00	0.51
9c,11c-/10c,12c-18:2	0.00	0.00	0.03
9t,11t-/10t,12t-18:2	0.00	0.00	0.02
Total CLA	0.00	0.00	1.06

2.2. Enzyme assays

At the end of the experimental period, the animals were sacrificed by bleeding from the inferior vena cava under diethyl ether anesthesia. The livers were then quickly excised. About 0.6 g of each liver was homogenized with 6 ml of 0.25 M sucrose containing 1 mM EDTA and 3 mM Tris–HCl (pH 7.2) and centrifuged at 200,000 × *g* for 30 min. The activity of hepatic fatty acid oxidation enzymes was analyzed using whole liver homogenate as an enzyme source as detailed previously [15–17]. The mitochondrial and peroxisomal fatty acid oxidation rates were analyzed

using [1-^{14}C]palmitoyl-CoA as a substrate. Carnitine palmitoyltransferase and acyl-CoA oxidase activities were measured using palmitoyl-CoA as a substrate. Enoyl-CoA hydratase activity was measured using both crotonyl-CoA (C4) and *t*-2-octenoyl-CoA (C8) as substrates. 3-Hydroxyacyl-CoA dehydrogenase activity was measured in reverse reaction in Exp.1 and in both reverse and forward reactions in Exp. 2. Acetoacetyl-CoA and 3-hydroxybutyryl-CoA were used as substrates for the reverse and forward reactions, respectively. 3-Ketoacyl-CoA thiolase activity was measured using acetoacetyl-CoA (C4) as a substrate in Exp. 1 and using both acetoacetyl-CoA and 3-ketooctanoyl-CoA (C8) substrates in Exp. 2. The activity of enzymes involved in fatty acid synthesis was measured using the 200,000 × *g* supernatant fraction [17–19].

2.3. RNA analysis

Hepatic RNA was extracted and the mRNA levels of enzymes involved in fatty acid metabolism were analyzed by slot-blot hybridization as reported previously [17–19]. Briefly, RNA samples (30 μg) were denatured and applied to a nylon membrane using a slot-blot apparatus (Bio-Rad Laboratories, Hercules, CA) and fixed with UV irradiation. RNAs on nylon membranes were hybridized with specific cDNA probes labeled with [α-^{32}P]-dCTP and quantified by an imaging analyzer (Bio-Rad Laboratories). The values were corrected for those obtained with a cDNA probe specific to 18S rRNA. cDNA probes to detect the respective mRNAs for enzymes involved in fatty acid synthesis and oxidation were all described previously [17–19]. We also carried out Northern blot hybridization for typical samples to confirm the specificity of the probes.

2.4. Statistical analysis

Data for Exp. 1 were analyzed by two-way ANOVA to establish the significance of the effects of dietary fatty acid types and mouse strains. Data for Exp. 2 were analyzed by one-way ANOVA to establish the significance of the effect of dietary fatty acid types and significant differences of the means were evaluated at the level of $P<0.05$ using the Tukey's test [20].

3. Results

3.1. Animal growth, tissue weights and liver lipid levels

Significant effects of the CLA preparation compared to the fatty acid preparation rich in linoleic acid on decreasing growth and food intake were detected by two-way ANOVA in Exp. 1 (Table 2). These parameters were significantly higher in ICR mice fed the palmitic acid diet than in those fed the CLA and linoleic acid diets (Exp. 2, Table 3). However, significant differences in these parameters between mice fed the linoleic acid diet and CLA diet were not confirmed in Exp. 2. The CLA diet compared to the linoleic acid diet greatly reduced the weight of epididymal and perirenal white adipose tissue in both Exps. 1 and 2. The values in ICR mice fed the palmitic acid diet were the same as those in the animals fed the linoleic acid diet (Exp. 2). In contrast, the CLA diet compared to the linoleic acid diet approximately doubled the liver weight of animals accompanying large increases in the triacylglycerol (8- to 13-fold) and cholesterol (3- to 5-fold) levels. CLA also caused a significant increase (40–70%) in the content of hepatic phospholipids. The palmitic acid diet relative to the linoleic acid diet, however, did not affect these parameters (Exp. 2). In Exp. 1, the serum triacylglycerol, cholesterol, phospholipid and free fatty acid concentrations were significantly higher in ICR mice than in C57BL/6J mice (data not shown). However, no significant effects of CLA on these parameters were found. Also, no significant differences in the serum lipid levels were detected among groups of ICR mice fed the linoleic acid, palmitic acid and CLA

Table 2
Effects of dietary CLA on growth parameters, tissue weights and liver lipid levels in ICR and C57BL/6J mice (Exp. 1)

	Mouse strains and dietary fatty acids				Significance of the effect of		
	ICR		C57BL/6J		CLA	Strain	CLA × Strain
	Linoleic	CLA	Linoleic	CLA			
Body weight (g)	40.6 ± 1.1	37.6 ± 0.4	26.5 ± 0.4	23.6 ± 0.3	$P<0.01$	$P<0.01$	NS
Body weight gain (g/21 days)	9.67 ± 1.51	6.76 ± 0.94	6.60 ± 0.29	3.65 ± 0.21	$P<0.01$	$P<0.01$	NS
Food intake (g/day)	4.56 ± 0.19	4.12 ± 0.22	3.04 ± 0.03	2.65 ± 0.02	$P<0.01$	$P<0.01$	NS
Epididymal white adipose tissue (g/100 g body weight)	3.09 ± 0.30	1.41 ± 0.16	2.10 ± 0.11	0.17 ± 0.07	$P<0.01$	$P<0.01$	NS
Perirenal white adipose tissue (g/100 g body weight)	1.29 ± 0.16	0.18 ± 0.04	0.55 ± 0.05	0.01 ± 0.01	$P<0.01$	$P<0.01$	$P<0.01$
Liver (g/100 g body weight)	5.32 ± 0.11	11.1 ± 1.2	4.61 ± 0.07	7.91 ± 0.33	$P<0.01$	$P<0.01$	NS
Liver lipids (μmol/100 g body weight)							
Triacylglycerol	416 ± 45	3397 ± 575	141 ± 13	1852 ± 259	$P<0.01$	$P<0.01$	NS
Cholesterol	33.3 ± 3.3	174 ± 21	25.8 ± 1.5	85.3 ± 5.8	$P<0.01$	$P<0.01$	$P<0.01$
Phospholipid	194 ± 8	330 ± 17	165 ± 3	257 ± 8	$P<0.01$	$P<0.01$	$P<0.05$

Male ICR and C57BL/6J mice were fed either a diet containing 1.5% linoleic acid or the CLA preparation for 21 days.
Values represent means ± S.E. of eight mice.

Table 3
Effect of dietary CLA on growth parameters, tissue weights and liver lipid levels in ICR mice (Exp. 2)

	Dietary fatty acids		
	Linoleic	Palmitic	CLA
Body weight (g)	40.9 ± 1.0[ab]	43.9 ± 1.5[b]	38.1 ± 0.5[a]
Body weight gain (g/21 days)	6.5 ± 0.6[a]	9.5 ± 1.3[b]	4.6 ± 0.4[a]
Food intake (g/day)	4.3 ± 0.1[a]	5.0 ± 0.1[b]	4.0 ± 0.1[a]
Epididymal white adipose tissue (g/100 g body weight)	2.94 ± 0.56[b]	3.00 ± 0.43[b]	1.05 ± 0.12[a]
Perirenal white adipose tissue (g/100 g body weight)	1.07 ± 0.25[b]	1.00 ± 0.14[b]	0.14 ± 0.02[a]
Liver (g/100 g body weight)	5.73 ± 0.13[a]	5.47 ± 0.15[a]	10.7 ± 0.6[b]
Liver lipids (μmol/100 g body weight)			
Triacylglycerol	314 ± 78[a]	381 ± 108[a]	2902 ± 389[b]
Cholesterol	50.9 ± 5.3[a]	53.0 ± 4.9[a]	156 ± 17[b]
Phospholipid	223 ± 8[a]	210 ± 7[a]	323 ± 12[b]

Male ICR mice were fed a diet containing either 1.5% linoleic acid, palmitic acid or the CLA preparation for 21 days. Values represent means ± S.E. of eight to nine mice.
[ab]Values with different superscripts differ significantly at $P<0.05$.

Table 5
Effect of dietary CLA on the activity and mRNA levels of enzymes involved in fatty acid synthesis and mRNA levels of Δ^6- and Δ^5-desaturases, and sterol regulatory element binding protein-1 in the liver of ICR mice (Exp. 2)

	Dietary fatty acids		
	Linoleic	Palmitic	CLA
Enzyme activity (μmol/min/liver/100 g body weight)			
Acetyl-CoA carboxylase	1.74 ± 0.13[a]	2.84 ± 0.17[a]	6.24 ± 0.60[b]
Fatty acid synthase	9.67 ± 0.44[a]	14.4 ± 0.7[a]	37.4 ± 2.2[b]
ATP-citrate lyase	7.83 ± 0.80[a]	14.6 ± 1.1[a]	40.7 ± 3.4[b]
Glucose 6-phosphate dehydrogenase	4.17 ± 0.24[a]	6.90 ± 0.33[b]	10.3 ± 0.7[c]
Malic enzyme	87.6 ± 9.6[a]	104 ± 8[a]	328 ± 24[b]
Pyruvate kinase	95.8 ± 6.7[a]	138 ± 6[a]	197 ± 21[b]
mRNA level (%)			
Acetyl-CoA carboxylase	100 ± 9[a]	128 ± 9[a]	285 ± 16[b]
Fatty acid synthase	100 ± 10[a]	115 ± 27[a]	447 ± 40[b]
ATP-citrate lyase	100 ± 4[a]	111 ± 4[a]	193 ± 11[b]
Malic enzyme	100 ± 5[a]	111 ± 10[a]	216 ± 13[b]
Pyruvate kinase	100 ± 11[a]	127 ± 20[a]	172 ± 13[b]
Δ^6-Desaturase	100 ± 14[a]	116 ± 10[a]	307 ± 38[b]
Δ^5-Desaturase	100 ± 6[a]	103 ± 7[a]	212 ± 18[b]
SREBP-1	100 ± 6[a]	97.0 ± 4.8[a]	161 ± 11[b]

Values represent means ± S.E. of eight to nine mice.
[abc]Values with different superscripts differ significantly at $P<0.05$.

diets (Exp. 2). No strain- or CLA-dependent alteration in the serum glucose concentration was seen in Exp. 1 (1170–1593 μmol/dl). However, in Exp. 2, the parameter was significantly higher in ICR mice fed the CLA diet (1656 ± 128 μmol/dl) than in those fed the linoleic acid diet (1041 ± 54 μmol/dl) and the palmitic acid diet (1196 ± 137 μmol/dl).

3.2. Activity and mRNA levels of enzymes in fatty acid synthesis

As dietary CLA increased the liver weight of mice in both Exps. 1 and 2, the activity of enzymes was expressed as total activity (μmol/min per liver/100 g body weight). Dietary CLA compared to linoleic acid increased the activity of various lipogenic enzymes in both ICR and C57BL/6J mice (Table 4, Exp. 1). The extent of the increase was least in pyruvate kinase (1.6- and 1.4-fold increases in ICR and C57BL/6J mice, respectively). The activity of other enzymes was more than two times higher in mice fed CLA than in those fed linoleic acid. The activity of lipogenic enzymes except for glucose 6-phosphate dehydrogenase was somewhat lower in C57BL/6J mice than in ICR mice.

The mRNA levels of lipogenic enzymes were expressed as percentages assigning the value in ICR mice fed the

Table 4
Effect of dietary CLA on the activity and mRNA levels of enzymes involved in fatty acid synthesis in the liver of ICR and C57BL/6J mice (Exp. 1)

	Mouse strains and dietary fatty acids				Significance of the effect of		
	ICR		C57BL/6J		CLA	Strain	CLA × Strain
	Linoleic	CLA	Linoleic	CLA			
Enzyme activity (μmol/min/liver/100 g body weight)							
Acetyl-CoA carboxylase	1.74 ± 0.11	4.94 ± 0.48	1.40 ± 0.10	3.96 ± 0.33	$P<0.01$	$P<0.05$	NS
Fatty acid synthase	8.97 ± 0.48	30.8 ± 4.0	6.30 ± 0.63	25.2 ± 2.4	$P<0.01$	NS	NS
ATP-citrate lyase	8.24 ± 0.57	38.2 ± 5.3	8.09 ± 1.36	24.7 ± 2.6	$P<0.01$	$P<0.05$	$P<0.05$
Glucose 6-phosphate dehydrogenase	3.76 ± 0.33	9.14 ± 1.76	3.96 ± 0.43	12.0 ± 1.2	$P<0.01$	NS	NS
Malic enzyme	73.6 ± 5.4	262 ± 44	59.2 ± 11.0	190 ± 22	$P<0.01$	NS	NS
Pyruvate kinase	101 ± 7	157 ± 19	94.0 ± 5.4	130 ± 15	$P<0.01$	NS	NS
mRNA level (%)							
Acetyl-CoA carboxylase	100 ± 7	241 ± 25	96.4 ± 3.6	167 ± 10	$P<0.01$	$P<0.01$	$P<0.05$
Fatty acid synthase	100 ± 10	371 ± 58	87.6 ± 15.8	207 ± 20	$P<0.01$	$P<0.01$	$P<0.05$
ATP-citrate lyase	100 ± 5	189 ± 17	102 ± 7	134 ± 6	$P<0.01$	$P<0.01$	$P<0.05$
Malic enzyme	100 ± 6	218 ± 26	85.6 ± 5.7	180 ± 7	$P<0.01$	NS	NS

Values represent means ± S.E. of eight mice.

linoleic acid preparation as 100. Consistent with the results obtained with enzyme activity, CLA-dependent increases in the mRNA levels of lipogenic enzymes were seen in both strains. The extent of the increase was generally lower for the gene expression than the enzyme activity. However, CLA still caused more than a twofold increase in gene expression of acetyl-CoA carboxylase, fatty acid synthase and malic enzyme in both strains with one exception (1.7-fold increase in acetyl-CoA carboxylase in C57BL6/J mice). CLA caused more than a threefold increase in ATP-citrate lyase activity in both strains. But the extent of the increase in the level of mRNA for this enzyme was rather moderate (1.9- and 1.3-fold in ICR and C57BL/6J mice, respectively).

Our results comparing the effect of diets containing fatty acid preparations rich in CLA and linoleic acid indicate that dietary CLA increases hepatic fatty acid synthesis. Dietary polyunsaturated fatty acids including linoleic acid reportedly decrease hepatic fatty acid synthesis [21–23]. The linoleic acid content of the diet containing the CLA preparation was half that of the diet containing the preparation rich in linoleic acid (Table 1). Therefore, there is the possibility that differences in the activity and mRNA levels of hepatic lipogenic enzymes between mice fed CLA and the linoleic acid preparations are ascribable to dietary linoleic acid levels, but not to the specific physiological activity of CLA. In this context, we compared the physiological activity of diets containing fatty acid preparations rich in linoleic acid, palmitic acid and CLA in Exp. 2 using ICR mice. The dietary level of linoleic acid was indistinguishable between the palmitic acid diet and CLA diet (Table 1).

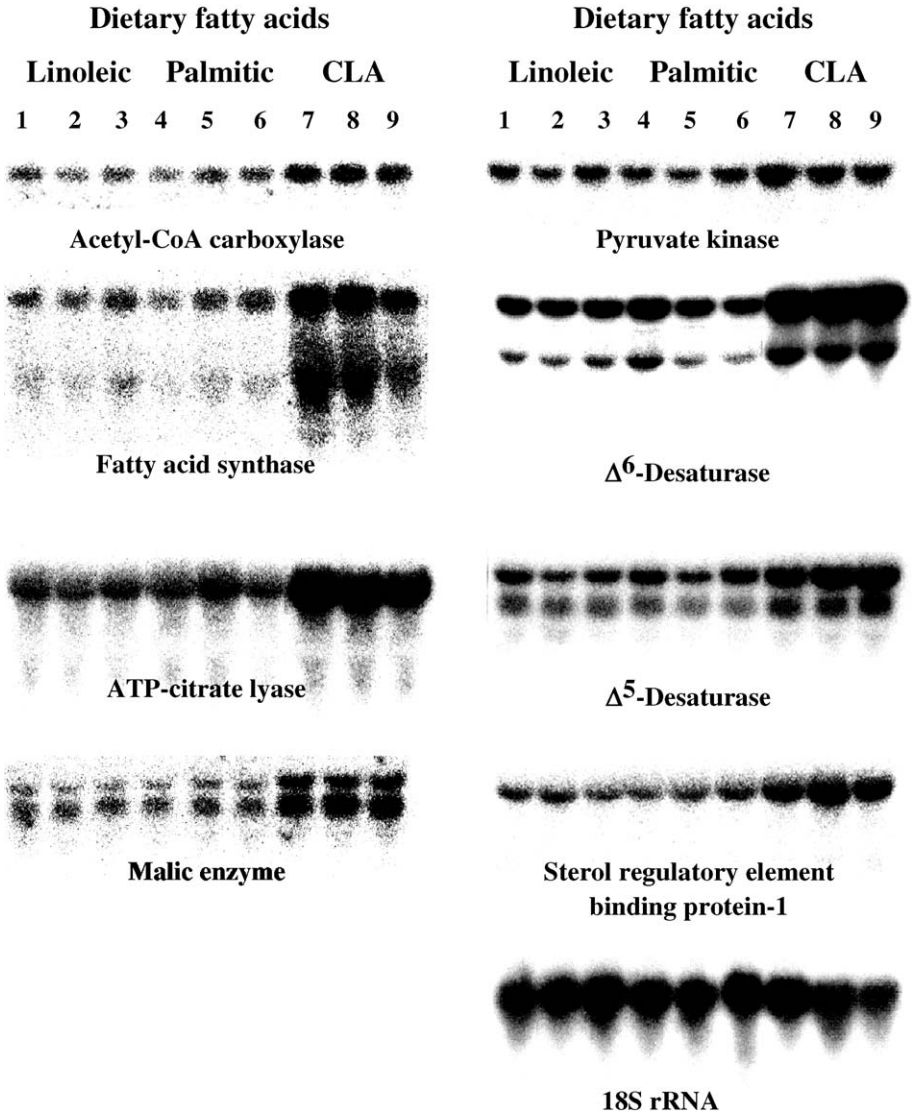

Fig. 1. Northern blot analysis of mRNA of hepatic lipogenic enzymes in ICR mice fed diets containing 1.5% linoleic acid, palmitic acid and CLA preparations (Exp. 2). RNA samples (30 μg) were denatured and subjected to electrophoresis on a 1.1% agarose gel containing 0.66 M formaldehyde, then transferred to a nylon membrane and fixed with UV irradiation. The RNA on nylon membranes was hybridized with radiolabeled cDNA probes specific for lipogenic enzymes and 18S rRNA. Lanes 1–3: mice fed the linoleic acid diet; 4–6: palmitic acid diet; 7–9: CLA diet.

Again, the CLA diet compared to the linoleic acid diet caused more than a twofold increase in the activity of lipogenic enzymes accompanying up-regulation of the gene expression (Table 5). The palmitic acid diet relative to the linoleic acid diet only slightly increased these parameters. As a consequence, the CLA diet even compared to the palmitic acid diet greatly increased the activity and mRNA levels of all the lipogenic enzymes. In addition, the CLA diet relative to both the linoleic and palmitic acid diets significantly increased the mRNA level of sterol regulatory element binding protein (SREBP)-1 that regulates the gene expression of lipogenic enzymes [23,24]. In this experiment, we also analyzed the mRNA level of Δ^6- and Δ^5-desaturases that catalyze the desaturation of polyunsaturated fatty acids. CLA was found to cause large increases in these parameters.

Fig. 1 shows the results of analysis by Northern blot hybridization in Exp. 2 of mRNAs for lipogenic enzymes, Δ^6- and Δ^5-desaturases, and SREBP-1. In addition, the result for 18S rRNA used for the loading control is shown. Dietary CLA-dependent changes in the gene expression of lipogenic enzymes as determined by slot-blot hybridization were further confirmed by this method.

3.3. Activity and mRNA levels of enzymes in fatty acid oxidation

In Exp. 1, the mitochondrial and peroxisomal fatty acid oxidation rates were about 2.5-fold higher in both ICR and C57BL/6J mice fed the CLA diet than in those fed the linoleic acid diet (Table 6). Dietary CLA caused a 2.1- to

Table 7
Effect of dietary CLA on the activity and mRNA levels of enzymes involved in fatty acid oxidation in the liver of ICR mice (Exp. 2)

	Dietary fatty acids		
	Linoleic	Palmitic	CLA
Enzyme activity (μmol/min/liver/100 g body weight)			
Palmitoyl-CoA oxidation			
Mitochondrial	1.80 ± 0.14^a	1.61 ± 0.18^a	4.23 ± 0.32^b
Peroxisomal	11.8 ± 0.7^a	8.81 ± 0.41^a	29.6 ± 2.3^b
Carnitine palmitoyltransferase	7.57 ± 0.31^a	6.52 ± 0.38^a	14.5 ± 0.9^b
Acyl-CoA oxidase	5.57 ± 0.33^a	4.62 ± 0.33^a	13.4 ± 0.9^b
Enoyl-CoA hydratase			
Crotonyl-CoA substrate	1597 ± 65^a	1553 ± 57^a	2901 ± 116^b
t-2-Octenoyl-CoA substrate	1046 ± 33^a	1042 ± 34^a	1954 ± 79^b
3-Hydroxyacyl-CoA dehydrogenase			
Acetoacetyl-CoA substrate	382 ± 15^a	353 ± 13^a	746 ± 32^b
3-Hydroxybutyryl-CoA substrate	262 ± 8^a	238 ± 12^a	511 ± 21^b
3-Ketoacyl-CoA thiolase			
Acetoacetyl-CoA substrate	219 ± 7^a	202 ± 8^a	432 ± 18^b
3-Ketooctanoyl-CoA substrate	448 ± 16^a	399 ± 15^a	842 ± 57^b
mRNA level (%)			
Mitochondrial			
Carnitine palmitoyltransferase I	100 ± 6^a	91.8 ± 4.8^a	234 ± 16^b
Carnitine palmitoyltransferase II	100 ± 4^a	92.2 ± 5.0^a	151 ± 4^b
Long chain acyl-CoA dehydrogenase	100 ± 5^a	87.3 ± 3.3^a	153 ± 3^b
Trifunctional enzyme α subunit	100 ± 5^a	90.7 ± 4.1^a	186 ± 13^b
Trifunctional enzyme β subunit	100 ± 3^a	93.7 ± 3.7^a	173 ± 5^b
Δ^3,Δ^2-Enoyl-CoA isomerase	100 ± 5^a	84.9 ± 2.7^a	143 ± 4^b
2,4-Dienoyl-CoA reductase	100 ± 3^a	104 ± 3^a	156 ± 3^b
Peroxisomal			
Acyl-CoA oxidase	100 ± 7^a	81.8 ± 11.5^a	203 ± 12^b
Bifunctional enzyme	100 ± 7^a	80.4 ± 6.9^a	268 ± 12^b
3-Ketoacyl-CoA thiolase	100 ± 4^a	98.5 ± 6.2^a	244 ± 11^b

Values represent means ± S.E. of eight to nine mice.
[ab]Values with different superscripts differ significantly at $P<0.05$.

Table 6
Effect of dietary CLA on the activity and mRNA levels of enzymes involved in fatty acid oxidation in the liver of ICR and C57BL/6J mice (Exp. 1)

	Mouse strains and dietary fatty acids				Significance of the effect of		
	ICR		C57BL/6J		CLA	Strain	CLA × Strain
	Linoleic	CLA	Linoleic	CLA			
Enzyme activity (μmol/min/liver/100 g body weight)							
Palmitoyl-CoA oxidation							
Mitochondrial	2.08 ± 0.09	4.97 ± 0.44	1.11 ± 0.07	2.72 ± 0.34	$P<0.01$	$P<0.01$	$P<0.05$
Peroxisomal	6.53 ± 0.30	16.7 ± 2.2	4.63 ± 0.18	11.6 ± 0.36	$P<0.01$	$P<0.01$	NS
Carnitine palmitoyltransferase	4.62 ± 0.11	12.4 ± 2.0	4.57 ± 0.21	7.62 ± 0.55	$P<0.01$	$P<0.01$	$P<0.05$
Acyl-CoA oxidase	3.28 ± 0.10	6.79 ± 0.65	2.70 ± 0.02	4.89 ± 0.29	$P<0.01$	$P<0.01$	NS
3-Hydroxyacyl-CoA dehydrogenase	498 ± 11	1040 ± 112	323 ± 3	676 ± 33	$P<0.01$	$P<0.01$	NS
3-Ketoacyl-CoA thiolase	192 ± 4	436 ± 36	166 ± 3	294 ± 30	$P<0.01$	$P<0.01$	$P<0.05$
mRNA level (%)							
Mitochondrial							
Carnitine palmitoyltransferase I	100 ± 14	181 ± 12	95.3 ± 10.4	153 ± 10	$P<0.01$	NS	NS
Carnitine palmitoyltransferase II	100 ± 8	145 ± 8	84.0 ± 3.5	121 ± 7	$P<0.01$	$P<0.01$	NS
Trifunctional enzyme subunit α	100 ± 5	142 ± 8	84.3 ± 4.8	111 ± 8	$P<0.01$	$P<0.01$	NS
Trifunctional enzyme subunit β	100 ± 2	176 ± 18	78.8 ± 3.2	129 ± 6	$P<0.01$	$P<0.01$	NS
Peroxisomal							
Acyl-CoA Oxidase	100 ± 14	152 ± 13	77.7 ± 6.6	125 ± 7	$P<0.01$	$P<0.05$	NS
Bifunctional enzyme	100 ± 9	337 ± 59	90.3 ± 3.7	235 ± 23	$P<0.01$	NS	NS

Values represent means ± S.E. of eight mice.

2.7-fold increase in the activity of these enzymes in ICR mice, and a 1.7- to 2.1-fold increase in C57BL/6J mice. The enzyme activity in fatty acid oxidation was generally higher in ICR mice than in C57BL/6J mice. Two-way ANOVA detected significant CLA- and strain-dependent effects on all these parameters.

Significant CLA effects on the mRNA levels of all the fatty acid oxidation enzymes were detected by two-way ANOVA. CLA caused 30–80% increases in the mRNA levels of various mitochondrial fatty acid oxidation enzymes and peroxisomal acyl-CoA oxidase. The gene expression of peroxisomal bifunctional enzyme was affected most by dietary CLA. The CLA diet compared to the linoleic acid diet caused 3.4- and 2.6-fold increases in the mRNA level in ICR and C57BL/6J mice, respectively. The mRNA levels of various fatty acid oxidation enzymes were somewhat lower in C57BL/6J mice than in ICR mice.

The effect of dietary CLA on the activity and mRNA levels of fatty acid oxidation enzymes was evaluated in more detail in Exp. 2 (Table 7). Consistent with the results obtained in Exp. 1, the CLA diet compared to the linoleic acid diet caused about a 2.5-fold increase in mitochondrial and peroxisomal palmitoyl-CoA oxidation rates in the liver of ICR mice. Dietary CLA compared to the linoleic acid caused a 2.4-fold increase in acyl-CoA oxidase activity and approximately doubled the level of activity of the other enzymes irrespective of the substrate used.

The CLA-dependent increases in the mRNA levels of enzymes involved in fatty acid oxidation were confirmed in Exp. 2. The CLA diet relative to the linoleic acid diet caused 1.4- to 2.3-fold increases in the mRNA levels of mitochondrial, and 2.0- to 2.7-fold increases in peroxisomal fatty acid oxidation enzymes, respectively. The activity and mRNA levels of fatty acid oxidation enzymes in mice fed the palmitic acid diet were approximately the same as those in the animals fed the linoleic acid diet.

The dietary CLA-dependent increases in the mRNA levels of various mitochondrial and peroxisomal enzymes as determined by slot-blot hybridization were confirmed by Northern blot hybridization (Fig. 2).

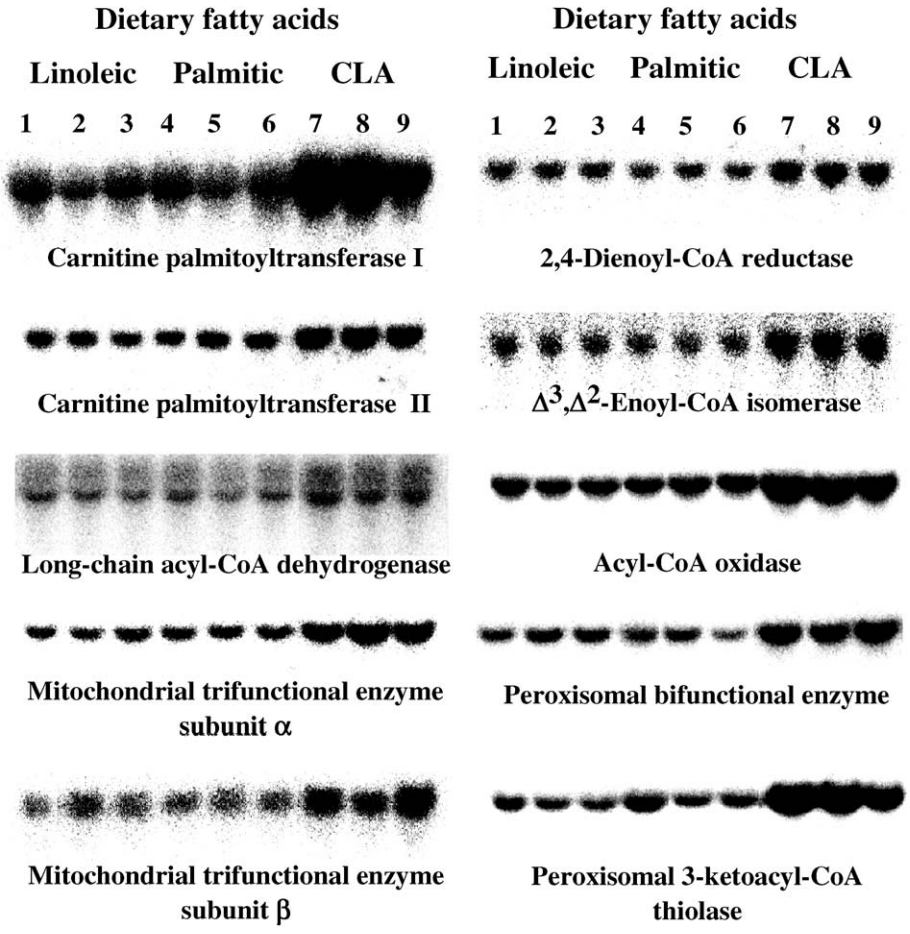

Fig. 2. Northern blot analysis of mRNA of hepatic fatty acid oxidation enzymes in ICR mice fed diets containing 1.5% linoleic acid, palmitic acid and CLA preparations (Exp. 2). RNA samples (30 μg) were denatured, electrophoresed and transferred to a nylon membrane as described in the legend to Fig. 1. The RNA on nylon membranes was hybridized with radiolabeled cDNA probes specific for fatty acid oxidation enzymes. Lanes 1–3: mice fed the linoleic acid diet; 4–6: palmitic acid diet; 7–9: CLA diet.

4. Discussion

Peters et al. [5] unexpectedly found that dietary CLA increased the mRNA levels of proteins relating to lipogenesis in the liver of wild-type as well as PPARα-null mice of the C57BL/6N genetic background. However, the effect of CLA on the activity of hepatic lipogenic enzymes remained to be studied. We demonstrated, in the present study, that CLA compared both to linoleic and palmitic acids up-regulated not only the mRNA expression but also the activity of various enzymes involved in lipogenesis. The extent of the increase in enzyme activity was generally higher than to be expected from the mRNA levels. This implies that CLA not only increased transcription of the lipogenic enzyme gene and (or) mRNA stability but also affected the step of translation and (or) the turnover of the enzyme protein.

SREBPs are membrane-bound transcriptional factors that regulate the gene expression of enzymes in fatty acid and cholesterol biosynthesis. Three types of SREBPs (1a, 1c and 2) have been identified. Studies indicated that SREBP-1 is mainly involved in the regulation of the gene expression of enzymes in fatty acid synthesis while SREBP-2 activates the genes of enzymes involved in cholesterol biosynthesis and the low density lipoprotein receptor. The mature nuclear form of SREBP is generated from the membrane-bound precursor through a sequential two-step proteolytic cleavage process, and its nuclear content appears to be regulated at the steps of both gene expression and proteolytic cleavage of the precursor [19,23,24]. Our observation that CLA increased the mRNA level of SREBP-1 indicates that this fatty acid up-regulates this transcriptional factor at least at the step of gene expression. Therefore, it is possible that the SREBP-1 signaling pathway is involved in the CLA-mediated increase in hepatic lipogenesis.

The Δ^6- and Δ^5-desaturation of linoleic acid are the regulatory steps in the biosynthesis of arachidonic acid conversion into eicosanoids. As some studies indicated that dietary CLA interferes with desaturation of linoleic acid [10] and eicosanoid production [25,26], we measured the mRNA levels of Δ^6- and Δ^5-desaturases in the mouse liver in the present study. Unexpectedly, however, we found that CLA greatly increased these parameters. In this context, Matsuzaka et al. [27] recently demonstrated that SREBPs up-regulate the gene expression of the desaturases in mice. In addition, there are strong evidences to indicate that PPARα up-regulates the gene expression of Δ^6- [27,28] and Δ^5-desaturases [27]. Therefore, the CLA-dependent increase in the mRNA levels of Δ^6- and Δ^5-desaturases may be a consequence of both the activation of PPARα [5,12] and up-regulation of SREBP-1.

Previous studies [5,11] indicated that CLA increases the mRNA levels of hepatic fatty acid oxidation enzyme. However, little is known about the effect of CLA on the activity of hepatic fatty acid oxidation enzymes. In the present study, we showed that CLA increased the activity of various fatty acid oxidation enzymes accompanying the up-regulation of mRNA expression of mitochondrial and peroxisomal fatty acid oxidation enzymes in the mice. Therefore, it is apparent that the high levels of mRNA for fatty acid oxidation enzyme in mice fed CLA are associated with increased levels of enzyme activity. CLA appears to be a potent ligand and activator of PPARα [5,12] and hence may induce hepatic fatty acid oxidation [5]. The present finding that dietary CLA increased the gene expression of Δ^6- and Δ^5-desaturases raises the interesting possibility that the eicosanoid signaling pathway is also involved in CLA-dependent increase in hepatic fatty acid oxidation. It is well demonstrated that various eicosanoids are potent activators of PPARs [29,30].

Some studies indicated that CLA is effective in decreasing the serum triacylglycerol but not cholesterol concentration in mice [5,31]. In the current study, no alteration by CLA of the serum lipid levels was observed in both C57BL/6 and ICR mice. An increase in hepatic fatty acid oxidation and synthesis is associated with a reduced and increased very low density lipoprotein secretion rate [32,33], respectively, hence modifying the serum lipid levels. Therefore, it is reasonable to consider that concomitant increases in hepatic fatty acid oxidation and synthesis by CLA may not necessarily cause changes in the serum lipid levels. It is possible that the physiological activity of CLA in affecting the serum lipid level depends on the nature of dietary fat. We used a saturated fat (palm oil) while the other investigators used polyunsaturated fats (corn oil [5] and soybean oil [31]) in experimental diets. As hepatic fatty acid synthesis is strongly down-regulated by polyunsaturated fats [18,21–23], the basal rate of hepatic lipogenesis may be mutually different among these experiments and this may account for the divergent results observed.

Previous studies showed that dietary CLA causes hepatomegaly accompanying the accumulation of lipids in the liver of mice [2–5,10,11]. In the present study, CLA caused about a 10-fold increase in the hepatic triacylglycerol content despite this fatty acid increasing the activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation. It is therefore plausible that enhanced lipogenesis is primarily responsible for the increase in hepatic triacylglycerol. CLA also increased the hepatic cholesterol content, but did not affect the mRNA levels of hepatic cholesterogenic enzymes (T. Ide et al., unpublished data).

The present study demonstrated that dietary CLA increased the activity and mRNA levels of enzymes involved in both fatty acid oxidation and synthesis in the mouse liver. However, it would be reasonable to consider that hepatic fatty acid oxidation and synthesis are regulated reciprocally under various nutritional and physiological conditions. All available information [34–37] supports this notion, and the dietary or physiological factor that causes the concomitant up-regulation of hepatic fatty acid oxidation and synthesis remains unknown. Therefore, CLA is a dietary factor with a unique propensity to affect hepatic fatty acid metabolism.

The physiological basis of the concomitant increase in hepatic fatty acid oxidation and synthesis is not clear at present. It is well demonstrated that CLA feeding greatly decreases white pad mass [2–5] in mice as confirmed in the present study. The result of a recent study [38] indicates that 10t,12c-CLA compared to 9c,11t/9t,11c-isomer is more competent in reducing body fat content. There is the possibility that the enhanced activity and mRNA levels of lipogenic enzymes are a consequence of the large reduction in white pad mass. Moitra et al. [39] have generated a transgenic mouse expressing a dominant-negative protein, termed A-ZIP/F, under the control of the adipose-specific aP2 enhancer/promoter showing a total depletion of white fat tissue and large reduction in brown adipose tissue. This transgenic mouse showed hepatomegaly accompanying an increase in tissue lipid content. Quantitative immunoblot analysis showed a three- to fourfold increase in the levels of lipogenic enzymes in the liver indicating enhanced lipogenesis is a mechanism for the fatty liver. Therefore, the situation in this transgenic mouse resembled that in the animals fed CLA. As white adipose tissue plays a crucial role in metabolizing and converting glucose to fatty acid for storage purposes, the large decrease caused by dietary CLA of white pad mass together with the down-regulation of glucose transporter 4 in this tissue [3] may result in a compensatory increase in hepatic lipogenesis to metabolize glucose.

The current study confirmed the previous observations [5,11,12] that CLA increases the gene expression of enzymes involved in hepatic fatty acid synthesis and oxidation in mice. Measurement of the enzyme activity confirmed that this fatty acid concomitantly up-regulates these metabolic pathways. Moreover, we firstly showed that CLA increases the mRNA levels of Δ^6- and Δ^5-desaturases that catalyze the desaturation of polyunsaturated fatty acids. Inasmuch as no other dietary or physiological factor causing a concomitant increase in both hepatic fatty acid synthesis and oxidation is known, CLA may offer a unique tool to elucidate the mechanism underlying the regulation of hepatic fatty acid metabolism.

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