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Short-Term Feeding of Conjugated Linoleic Acid Does Not Induce Hepatic Steatosis in C57BL/6J Mice

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Summary We investigated the effect of short-term feeding of conjugated linoleic acid (CLA) on adipose tissue weights, liver weight, hepatic lipid metabolism, and serum lipoprotein profiles in C57BL/6J mice. Mice were fed semi-synthetic diets containing either 6% high-linoleic safflower oil (HL-SAF) or 4% HL-SAF+2% CLA for 1 wk. Short-term feeding of CLA showed an anti-obesity effect without inducing hepatomegaly in mice. In addition to the decline of hepatic triglyceride concentration, significant inhibition of $\Delta 9$ desaturation of fatty acid in the total liver lipids was found in CLA-fed mice. The CLA diet significantly increased the activities of peroxisomal β-oxidation and decreased the activities of diacylglycerol acyltransferase, a triglyceride synthesis-related enzyme, in the liver. Moreover, serum lipoprotein profiles of CLA-fed mice showed preferable changes in the atherogenic indices. However, serum leptin and adiponectin were drastically decreased by CLA feeding, suggesting that prolonged administration of CLA would induce further decrease of serum adipocytokine levels, which may be a cause of lipodystrophy in mice. These results show that short-term feeding of CLA does not induce adverse effect in C57BL/6J mice.

Key Words C57BL/6J mice, conjugated linoleic acid, diacylglycerol acyltransferase, hepatic steatosis

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. It is found in meat and dairy products, such as beef, milk, and processed cheese (*1*, *2*). CLA has attracted considerable attention because of its potentially beneficial effects in inhibiting carcinogenesis, attenuating atherosclerosis, alleviating diabetes, and reducing body fat in animal models and humans (*3–7*). Recently, we reported that CLA and its isomer (10*trans*,12*cis*-CLA) prevent the development of both obesity-related and essential hypertension in model animals (*8–10*). Feeding a CLA mixture and the 10*trans*,12*cis*-CLA isomer with a low-fat diet for more than 1 mo, however, induced lipodystrophy, which is characterized by an increase in hepatic lipid contents concomitant with a decrease in body fat mass, in mice (*11*, *12*). CLA-induced hepatic steatosis has been found only in mice and has not been reported in other species (*13–16*). Increasing the amount of fat in a CLA-supplemented diet substantially reduces the lipodystrophy in mice (*17*). Lipodystrophy may occur in mice because they are sensitive to CLA-induced body fat reduction. Therefore, short-term feeding should be sufficient to reveal the physiological effects of CLA in mice.

In the present study, we investigated the effect of short-term feeding of CLA on adipose tissue weights, liver weight, hepatic lipid metabolism, and serum lipoprotein profiles in C57BL/6J mice.

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MATERIALS AND METHODS

Animals and diets. Male C57BL/6J mice aged 7 wk were purchased from CLEA Japan, Inc. (Osaka, Japan) and housed individually in an air-conditioned room (24°C) with a 12-h light/dark cycle. After a 1-wk adaptation period, the mice were assigned to two groups (six rats each).

The basal diets were prepared according to the recommendation of the AIN-93G and contained (in weight %): casein, 20; α-cornstarch, 13.2; sucrose, 10; cellulose, 5; vitamin mixture (AIN-93^{TM}), 1; mineral mixture (AIN-93G^{TM}), 3.5; L-cystein, 0.3; choline bitartrate, 0.25; fat, 6; *tert*-butylhydroquinone, 0.0014; and β-cornstarch, 40.7486. Dietary fats were composed of 6% high linoleic safflower oil (HL-SAF) in the control diet and a mixture of 4% HL-SAF+2% CLA (triglyceride form) in the CLA diet. The composition of the semi-synthetic diets and their fatty acid contents are listed in Table 1. The animals received the diets ad libitum using Rodent CAFE (KBT Oriental Co. Ltd., Saga, Japan) for 1 wk. At the end of the feeding period, the mice were sacrificed by exsanguinations from the heart after a 9-h starvation. White adipose tissues and liver were excised immediately, and serum was separated from the blood. All aspects of the experiment were conducted according to the guidelines provided by the Ethical Committee for Experimental Animal Care at Saga University.

Analysis of lipids. Liver lipids were extracted according to the method of Folch et al. (*18*), and the concentrations of triglyceride and cholesterol were measured

Table 1. Fatty acid composition of experimental oils.

	HL-SAF	CLA-TG
	(Weight%)	
16:0	6.6	6.6
18:0	2.4	2.4
18:1	16.1	16.4
18:2	73.0	1.7
CLA (9c,11t)	—	32.2
(10t,12c)	—	33.1
(9c,11c)	—	1.2
(10c,12c)	—	1.1
(t,t)	—	2.8
18:3	0.5	—
20:0	0.3	—
20:1	0.2	—
Others	0.9	2.6

HL-SAF, high linoleic safflower oil; CLA-TG, triglyceride-form conjugated linoleic acid.

by the methods of Fletcher (*19*) and Sperry and Webb (*20*), respectively. Fatty acid composition of total liver lipids was analyzed by gas-liquid chromatography as described previously (*21*). Serum lipoproteins were analyzed by an on-line dual enzymatic method for simultaneous quantification of triglyceride and cholesterol by high-performance liquid chromatography at Skylight Biotech Inc. (Akita, Japan) according to the methods of Usui et al. (*22*). The serum glucose level was measured using a commercial enzyme assay kit (Glucose CII-test from Wako Pure Chemical Industries, Ltd., Tokyo, Japan). Serum insulin, adiponectin, and leptin levels were measured using commercial mouse ELISA kits (Shibayagi Co. Ltd., Shibukawa, Japan; Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan; and Morinaga Co. Ltd., Yokohama, Japan, respectively).

Preparation of liver subcellular fractions. A piece of liver from each mouse was homogenized in six volumes of a 0.25 M sucrose solution containing 1 mM EDTA in a 10 mM Tris-HCl buffer (pH 7.4). After precipitating the nuclei fraction, the supernatant was centrifuged at 10,000×*g* for 10 min at 4°C to obtain mitochondria. The resulting supernatant was recentrifuged at 125,000×*g* for 60 min to precipitate microsomes, and the remaining supernatant was used as the cytosol fraction. The microsomal pellet was resuspended in a 0.25 M sucrose solution containing 1 mM EDTA in a 10 mM Tris-HCl buffer (pH 7.4). Protein concentration was determined by the method of Lowry et al. (*23*), with bovine serum albumin used as the standard.

Assays of hepatic enzyme activity. The enzyme activities of carnitine palmitoyltransferase (CPT) (*24*), peroxisomal β-oxidation (*25*), fatty acid synthase (FAS) (*26*), and diacylglycerol acyltransferase (DGAT) (*27*) were determined as described in the respective references.

Statistical analyses. All values are expressed as mean±SE. Data were analyzed with Student's *t*-test, and differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Lifestyle-related diseases such as hyperlipidemia, arteriosclerosis, diabetes mellitus, and hypertension are widespread and increasingly prevalent in industrialized countries and have contributed to the increase in cardiovascular morbidity and mortality (*28*, *29*). Although the pathogenesis of lifestyle-related diseases is complicated and the precise mechanisms have not been elucidated, based on epidemiologic studies obesity has emerged as one of the major cardiovascular risk factors (*30–32*). Recently, CLA has attracted considerable attention because of its potentially beneficial effects in alleviating obesity. CLA reduced body fat and enhanced lean body mass in animal models (*33*, *34*), and dietary CLA supplementation reduced the percentage of body fat compared with control groups in humans (*7*, *35*). Studies using prolonged CLA feeding, however, reported that a drastic decrease of adipose tissue induced lipodystrophy, such as hepatic steatosis and hyperinsulinemia, in mice (*11*, *12*). Therefore, we investigated the effects of short-term feeding of CLA in this hyper-responsive animal.

The effects of dietary CLA on the body weight, food intake, and relative liver weight in mice are shown in Table 2. There was no significant difference in those growth parameters between the groups. As shown in the Fig. 1, perirenal and west subcutaneous white adipose tissue weights were significantly decreased by CLA feeding. These results showed that short-term feeding of CLA is enough to reveal the anti-obese effect without inducing hepatomegaly in mice.

The effects of dietary CLA on the hepatic lipids are

Table 2. Growth parameters of C57BL/6J mice after 1 wk of feeding.

	Control	CLA
Final body weight (g)	17.6±0.5	17.7±0.4
Total food intake (g)	15.4±0.3	14.9±0.7
Liver weight (g/100 g BW)	4.33±0.11	4.41±0.14

Values are expressed as mean±SE of six mice.

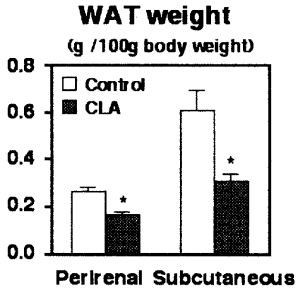

Fig. 1. Effect of dietary fatty acids on white adipose tissue (WAT) weights. Mice were fed semi-synthetic diets containing either 6% HL-SAF or 4% HL-SAF+2% CLA for 1 wk. Values are expressed as mean±SE of six mice. See Table 1 for composition of diets. Asterisks show significant difference at $p<0.05$.

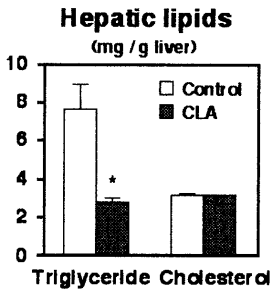

Fig. 2. Effect of dietary fatty acids on the concentrations of hepatic lipids. Mice were fed semi-synthetic diets containing either 6% HL-SAF or 4% HL-SAF+2% CLA for 1 wk. Values are expressed as mean±SE of six mice. See Table 1 for composition of diets. Asterisk shows significant difference at $p<0.05$.

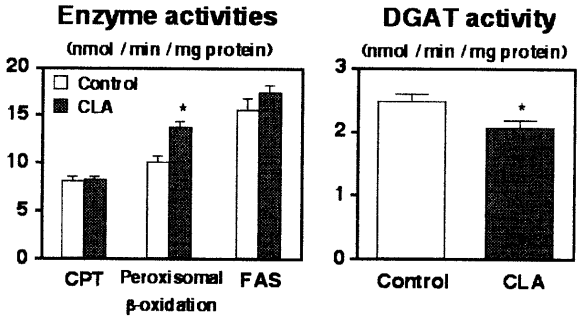

Fig. 3. Effect of dietary fatty acids on the activities of mitochondrial carnitine palmitoyltransferase (CPT), peroxisomal β-oxidation, cytosolic fatty acid synthase (FAS) and microsomal diacylglycerol acyltransferase (DGAT) in the liver of C57BL/6J mice. Mice were fed semi-synthetic diets containing either 6% HL-SAF or 4% HL-SAF+2% CLA for 1 wk. Values are expressed as mean±SE of six mice. See Table 1 for composition of diets. Asterisks show significant difference at $p<0.05$.

shown in Fig. 2. In contrast to previous reports showing that feeding CLA to mice induced hepatic steatosis (*11*, *12*), short-term feeding of CLA decreased the triglyceride content in the liver of mice. These findings reveal that CLA also has a lipid-lowering effect in mice, as has been shown in other animal models (*36–38*). In this study, cholesterol content in the liver was not influenced by dietary CLA.

We measured the activities of CPT, peroxisomal β-oxidation, FAS, and DGAT in the liver (Fig. 3). Although the activity of mitochondrial CPT was not changed, the activity of peroxisomal β-oxidation was significantly increased by CLA feeding. Thus, CLA feeding may activate peroxisomal proliferation in the liver of mice. Because fibrate agents, artificial peroxisome proliferators, have been reported to reduce hepatic triglyceride content (*39–41*), the activation of peroxisomal β-oxidation by CLA may contribute to the reduction of hepatic triglyceride content. The activity of cytosolic FAS, a late-limiting enzyme of fatty acid synthesis, was not altered by CLA feeding. However, the activity of microsomal DGAT, a triglyceride synthesis-related enzyme, was significantly reduced in the liver of CLA-fed mice. The reduction of hepatic triglyceride content may be attributable to the inhibition of triglyceride synthesis through the decreased activity of DGAT.

Table 3. Fatty acid composition of hepatic total lipids in C57BL/6J mice after 1 wk of feeding.

	Control	CLA
	(% of total)	
14:0	0.380±0.024	0.303±0.036
16:0	25.9±0.2	25.4±0.2
16:1	1.72±0.13	0.863±0.055*
18:0	13.0±0.8	18.2±0.3*
18:1*n*-7	1.82±0.10	1.73±0.06
18:1*n*-9	11.4±0.7	8.67±0.42*
18:2*n*-6	23.2±0.9	15.8±0.2*
9c,11t-CLA	n.d.	0.623±0.036
10t,12c-CLA	n.d.	0.384±0.020
18:3*n*-6	0.415±0.024	0.197±0.006*
20:3*n*-6	0.779±0.094	0.650±0.033*
20:4*n*-6	13.3±0.6	14.7±0.1
22:4*n*-6	0.445±0.042	0.961±0.040*
22:5*n*-3	n.d.	0.257±0.008
22:5*n*-6	0.587±0.042	3.57±0.19*
22:6*n*-3	7.07±0.37	7.87±0.33

Values are expressed as mean±SE of six mice.
Asterisks show significant difference at $p<0.05$.

Table 4. Serum parameters of C57BL/6J mice after 1 wk of feeding.

	Control	CLA
VLDL-TG (mg/dL)	23.3±2.9	5.92±0.89*
LDL-TG (mg/dL)	18.6±0.6	7.16±0.89*
HDL-TG (mg/dL)	0.755±0.068	0.617±0.037
VLDL-cholesterol (mg/dL)	4.86±0.56	2.78±0.41*
LDL-cholesterol (mg/dL)	16.3±1.1	10.7±0.9*
HDL-cholesterol (mg/dL)	83.4±1.5	89.9±4.9
Glucose (mg/dL)	210±24	208±25
Insulin (pg/mL)	150±31	94.7±51.7
Adiponectin (μg/mL)	22.4±1.2	6.70±0.27*
Leptin (pg/mL)	427±228	32.7±12.1*

Values are expressed as mean±SE of six mice.
Asterisks show significant difference at $p<0.05$.

Fatty acid composition of liver total lipids are shown in Table 3. Many reports have shown that CLA has an inhibitory effect on the fatty acid $\Delta 9$ desaturation in vitro and in vivo (*42–44*). In this experiment, dietary CLA decreased the proportion of monounsaturated fatty acid contents and increased the contents of CLA isomers in the liver total lipids. Thus, short-term feeding is enough to reveal major physiological effects of CLA, which may be induced directly by CLA isomers incorporated into the liver.

Serum lipoprotein profiles are shown in Table 4. CLA feeding markedly lowered the concentrations of triglyceride and cholesterol in the fractions of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). Cholesterol levels in VLDL and LDL fractions

were decreased and HDL-cholesterol level was not changed in CLA-fed mice. As a result, the calculated anti-atherogenic index (cholesterol ratio in HDL/(VLDL+LDL)) was significantly higher in CLA-fed mice (47.1±6.1) compared with control mice (34.5±1.3). Serum glucose and insulin levels were not significantly changed by CLA feeding. However, serum levels of adiponectin and leptin were drastically decreased in CLA-fed mice. Adiponectin and leptin are both secreted abundantly from adipose tissue and are adipocytokines (*45–47*). Both adipocytokines regulate insulin sensitivity in humans and animals. Therefore, a deficiency of adipocytokine secretion due to a paucity of adipose tissue would cause lipodystrophy, which is characterized by a severe insulin resistance, leading to hyperinsulinemia and hepatic steatosis (*48–50*). In a previous study, a drastic decrease of adipose tissue by CLA feeding caused lipodystropy in mice, but continuous leptin infusion reversed the hyperinsulinemia (*11*). Thus, administering too much CLA may cause lipodystrophy in hyper-responders.

In conclusion, our findings revealed that short-term feeding of CLA does not induce adverse effects in C57BL/6J mice.

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