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Acute reduction of serum leptin level by dietary conjugated linoleic acid in Sprague-Dawley rats

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The purpose of this study was to clarify the effect of conjugated linoleic acid on lipid accumulation in adipose tissue. Sprague-Dawley rats were fed a diet containing 2% conjugated linoleic acid for 1, 3, 6, and 12 weeks. In rats fed 2% conjugated linoleic acid, the weight of perirenal white adipose tissue was comparable with that of rats fed a conjugated linoleic acid-free diet. For fatty acid composition of perirenal white adipose tissue, both 16:1/16:0 and 18:1/18:0 ratios were significantly lower in the conjugated linoleic acid-fed group than the control group. Although there was no remarkable difference in serum triglyceride, total cholesterol, and phospholipid levels between dietary groups, serum leptin level was significantly lower than the control group, and lipid content in the perirenal white adipose tissue exerted a tendency toward low compared to the control value at 1-week feeding. On the other hand, leptin level in perirenal white adipose tissue was significantly lower in the conjugated linoleic acid-fed group than the control group at 12-week feeding. In conclusion, these observations suggest dietary conjugated linoleic acid is an acute reducer of serum leptin level. This may afford an explanation of the mechanism of anti-obesity effect in conjugated linoleic acid. (J. Nutr. Biochem. 11:467–471, 2000)

Keywords: conjugated linoleic acid; leptin; white adipose tissue; fatty acid composition

Introduction

Conjugated linoleic acid (CLA) is a generic term of positional and structural isomers of octadecadienoic acid. Recently, several results on anti-obesity activity of CLA have been reported. For example, dietary CLA reduces body fat,[1–4] weight, and triglyceride level in white adipose tissue.[5,6] Among CLA isomers, Park et al. reported that 10*t*, 12*c* isomer exerted a body fat-reducing effect stronger than 9*c*, 11*t*.[4] It has also been reported that CLA inhibits differentiation and proliferation of 3T3-L1 preadipocytes.[7] On the other hand, Satory and Smith reported that CLA promoted de novo lipogenesis in 3T3-L1 adipocytes.[8] So far, there are various putative candidates as a mechanism of the action of CLA to alter body composition.[9]

Leptin is the product of *ob* gene and is released from mature adipocytes. It inhibits food intake and accelerates energy expenditure. Serum leptin is correlated to body fat level, and *ob* gene expression in adipose tissue is accelerated at obesity state.[10–12] It has been reported that a high-fat diet enhances *ob* gene expression in white adipose tissue.[13–15] The purpose of this study was to examine the effect of dietary CLA on lipid metabolism including leptin expression serum leptin level.

Methods and materials

Experimental animals and diet

Male 4-week-old Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka, Japan) were fed nonpurified diet and water ad libitum for a week after arrival. After acclimation, rats were separated into two groups, with 16 rats each. Experimental diets

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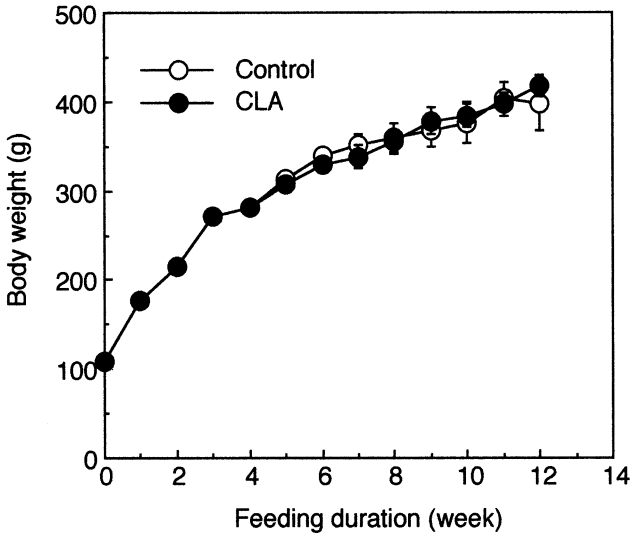

Figure 1 Effect of dietary conjugated linoleic acid (CLA) on the body weight of rats. Data are means ± SE for 4 rats.

that contained 0% CLA + 8% safflower oil (control) or 2% CLA + 6% safflower oil (CLA-fed), were prepared according to AIN-93G diets. The composition of dietary CLA was 9*c*, 11*t* 34.7%, 10*t*, 12*c* 35.6%, 9*c*, 11*c* and 10*c*, 12*c* 2.3%, 9*t*, 11*t* and 10*t*, 12*t* 1.6%. Body weight and food intake were measured every other day. Four animals of each group were killed after 1-, 3-, 6-, and 12-week feeding by withdrawing blood from abdominal aorta under light anesthesia by diethylether, and perirenal white adipose tissue (PWAT) was excised and weighed. Serum was prepared immediately after the blood collecting, and then PWAT and serum were kept at −30°C before analysis. This experiment was carried out under the guidelines for Animal Experiment in the Faculty of Agriculture and Graduate Course of Kyushu University No. 6 of the Japanese government.

Analysis of lipid content in PWAT and serum lipid parameter

Lipid extraction was performed by the method of Folch et al.[16] The measurements of triglyceride (TG), phospholipid (PL), and total cholesterol were performed by using commercial kits (Wako Pure Chemicals, Osaka, Japan).

Determination of leptin levels in serum and PWAT

To extract leptin from PWAT, the tissue was homogenized with 1 μM acetic acid, then the homogenate was centrifuged at 1,000 × *g* for 10 min and hexane was added to the supernatant to remove lipid components. Protein content of the residue was measured by using BCA Protein Assay Kit (Pierce, Rockford, IL USA). Leptin levels of serum and PWAT were measured using a commercial kit (Yanaihara Institute Inc., Fujinomiya, Japan).

Measurement of fatty acid composition of PWAT

The procedure of lipid extraction from PWAT utilized the same method as mentioned above. The total fatty acid fraction isolated from PWAT was mixed with 2 mL of 0.87% sulfuric acid in methanol and 1 mL of dimethylsulfoxide for methylation. The analysis of fatty acid composition was performed by gas–liquid chromatography (Shimadzu GC-17A) using Supelcowax 10 column (0.32 mm × 60 m, film thickness 0.25 μm, Supelco Inc., Bellefonte, PA USA). Column temperature was raised from 150°C to 220°C at the rate of 4°C/min, and the detector and injector temperature was 250°C. Identification of the CLA peaks was performed by equivalent chain length[17] using gas chromatography–mass spectrometry (JEOL Auto MS 50).

Results

Growth parameter

As shown in *Figure 1*, there was no significant difference in body weight between dietary groups throughout the feeding period. In addition, there was no significant difference between dietary groups in food intake or in weights of kidney, spleen, lung, and heart (data not shown). The weight of PWAT tended to be lower in the CLA-fed group at 1-, 3-, and 6-week feeding, but not significantly (*Table 1*); the value was comparable between dietary groups at 12-week feeding.

Serum lipid profiles

Serum lipid contents are shown in *Table 2*. There were no significant differences in serum TG and PL levels between the control and CLA-fed groups at any feeding duration. Total cholesterol level of CLA-fed group was significantly lower than that of control at 3-week feeding, but there was no significant difference at other feeding durations.

Lipid content in PWAT

At 1-week feeding, lipid content in PWAT of CLA-fed group exerted a lower tendency than that of control group, (76% of control; *Table 3*). The difference in the PWAT lipid content between the two groups gradually disappeared with elongation of feeding period. The relative value to the control was 94%, 100%, and 98% at 3-, 6-, and 12-week feeding, respectively. Lipid content tended to be low with feeding duration in control rats, and the level was constant at 6- and 12-week feeding.

Table 1 Effect of dietary conjugated linoleic acid (CLA) on the weight of renal white adipose tissue (g/100 g body weight) of Sprague-Dawley rats

	Feeding duration (week)			
Dietary group	1	3	6	12
Control	0.76 ± 0.16	1.36 ± 0.07	2.90 ± 0.76	1.71 ± 0.30
CLA	0.62 ± 0.11	1.17 ± 0.17	2.44 ± 0.60	1.90 ± 0.32

Data are means ± SE (n = 4).

Table 2 Effect of dietary conjugated linoleic acid (CLA) on serum lipid levels of Sprague-Dawley rats

Feeding duration (week)	Triglyceride (mg/dL)		Total cholesterol (mg/dL)		Phospholipid (mg/dL)	
	Control	CLA	Control	CLA	Control	CLA
1	129 ± 16	133 ± 18	120 ± 9	114 ± 14	171 ± 13	148 ± 6
3	134 ± 36	133 ± 27	113 ± 8	73 ± 6*	196 ± 17	183 ± 13
6	153 ± 34	140 ± 14	82 ± 9	84 ± 13	147 ± 7	157 ± 11
12	89 ± 34	116 ± 18	98 ± 14	91 ± 8	137 ± 28	141 ± 19

Data are means ± SE ($n = 4$). *Significantly different from value of control in each feeding duration at $P < 0.05$.

Serum and PWAT leptin levels

As shown in *Table 4*, serum leptin level in CLA-fed group at 1 week was significantly lower than that of the control rats (37% of control). The difference of serum leptin level between dietary groups tended to be reduced with the elongation of feeding period. The relative value to the control was 62%, 78%, and 76% at 3-, 6-, and 12-week feeding, respectively. In PWAT, leptin was not detected at 1-week feeding in both groups, and leptin level increased with the elongation of feeding period in both groups (*Figure 2*). The increase of leptin level in PWAT was significantly slower in the CLA group compared to the control group.

Fatty acid composition of PWAT

Fatty acid composition of PWAT is shown in *Table 5*. CLA was not detected in the control group at any feeding duration, whereas the level increased with the elongation of feeding period in the CLA-fed group. Although linoleic acid (LA) level was comparable between dietary groups at 1-week feeding, the LA level of control rats increased with elongation of feeding period. On the other hand, LA level in the CLA-fed group was not increased with the elongation of feeding period. This led to a significantly lower LA level in CLA-fed rats compared to control rats after 3-, 6-, and 12-week feeding. Palmitic acid (16:0) and stearic acid (18:0) levels were higher, and palmitoleic acid (16:1) level was lower in the CLA-fed group compared to the control group at 3-, 6-, and 12-week feeding, and some significant difference was recognized. *Figure 3* shows the effect of dietary CLA on the indices of Δ9 desaturase activity. In the CLA-fed group, 16:1/16:0 and oleic acid (18:1)/18:0 ratios in the CLA-fed rats were significantly lower than those of control rats after 3-, 6-, and 12-week feeding, although there was no significant difference after 1-week feeding.

Discussion

We previously reported that dietary CLA reduced TG level in white adipose tissue after 3 weeks of feeding.[6] As shown in *Table 3*, the reducing effect of dietary CLA on TG content of PWAT was most remarkable at 1-week feeding, when serum leptin level was significantly lower in the CLA-fed group than the control group, as shown in *Table 4*. As a mechanism to express these effects, it is considered that dietary CLA acts as a PPARγ activator. It has been already reported that CLA could be a ligand of PPARα, has activated PPARγ, and has impaired NIDDM of Zucker fa/fa rats.[18–20] On the other hand, it has been reported that thiazolidinediones (PPARγ activators) repress *ob* gene expression[21–23] and increase the ratio of small adipocytes of *ob* Zucker rats.[24] So, CLA might reduce serum leptin level

Table 3 Effect of dietary conjugated linoleic acid (CLA) on lipid content (mg/g) in perirenal white adipose tissues of Sprague-Dawley rats*

Dietary group	Feeding duration (week)			
	1	3	6	12
Control	781 ± 133	770 ± 141	641 ± 63	671 ± 29
CLA	594 ± 35	725 ± 41	639 ± 7	657 ± 18

Data are means ± SE ($n = 4$).

Table 4 Effect of dietary conjugated linoleic acid (CLA) on serum leptin level (ng/mL) of Sprague-Dawley rats

Dietary group	Feeding duration (week)			
	1	3	6	12
Control	662 ± 134	442 ± 80	880 ± 40	724 ± 248
CLA	242 ± 99*	273 ± 80	687 ± 86	549 ± 98

Data are means ± SE ($n = 4$). *Significantly different from the control value in each feeding duration at $P < 0.05$.

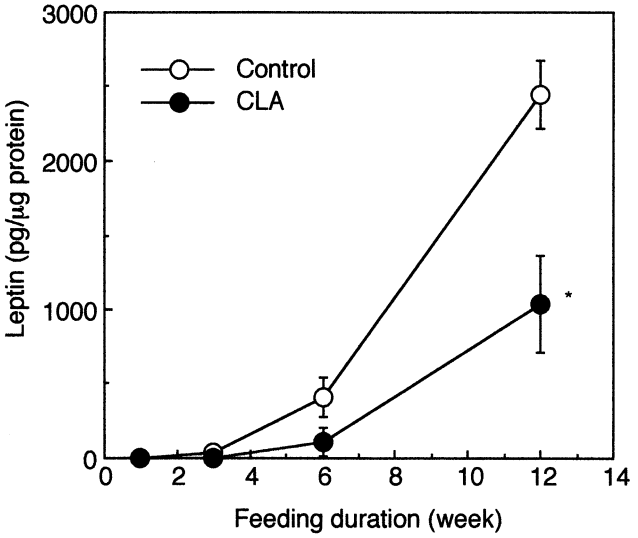

Figure 2 Effect of conjugated linoleic acid (CLA) on leptin level in perirenal white adipose tissue of rats. Data are means ± SE for 4 rats. Significantly different from control value in each feeding duration: $^{*}P < 0.05$.

and lipid content in PWAT as a PPARγ activator. It has been reported that dietary CLA preferentially accumulates in white adipose tissue more than in other tissues.[25] Because adipocytes contain higher concentrations of CLA derived from diet more than other tissues, CLA might act as a potent PPARγ activator in a dietary component.

It has also been reported that a high-fat diet containing safflower oil enhances the expression of leptin mRNA compared to a high-fat diet containing palm oil in rat PWAT, but not in epididymal white adipose tissue.[26] In this experiment, safflower oil was used as basic dietary fat, so CLA might or might not cancel the enhancing effect of safflower oil on the induction of leptin mRNA. Delany et al. reported that dietary CLA reduced serum leptin level of AKR/J mouse in a dose-dependent manner from 0 to 1%, although the difference was not significant.[3] They also reported that dietary CLA at the 1% dose significantly reduced serum leptin level after 6-week feeding compared to control group, but not significantly at 2-, 4-, 8-, and 12-week feeding. As shown in *Table 1*, the weight of PWAT tended to be lower in the CLA-fed group than in the control group after 1-week feeding; a part the lowering

Table 5 Effect of dietary conjugated linoleic acid (CLA) on fatty acid composition of perirenal adipose tissue of Sprague-Dawley rats

Feeding duration (week)	Dietary group	Fatty acids (wt. %)					
		16:0	16:1 (n-9)	18:0	18:1 (n-9)	18:2 (n-6)	CLA
1	Control	24.6 ± 0.5	4.8 ± 0.6	4.2 ± 0.1	27.8 ± 1.0	30.9 ± 1.0	n.d.
	CLA	24.5 ± 0.9	3.7 ± 0.3	3.8 ± 0.1	27.0 ± 0.6	30.2 ± 1.8	1.1 ± 0.3
3	Control	23.3 ± 1.2	5.3 ± 0.5	3.6 ± 0.4	25.9 ± 1.1	36.8 ± 2.5	n.d.
	CLA	26.9 ± 0.4	2.5 ± 0.1*	3.8 ± 0.1	23.1 ± 0.1	27.1 ± 0.4*	2.7 ± 0.2
6	Control	22.1 ± 0.3	4.1 ± 0.4	3.0 ± 0.1	21.4 ± 0.4	41.7 ± 0.9	n.d.
	CLA	31.0 ± 0.5***	2.9 ± 0.1	3.8 ± 0.1**	20.5 ± 0.1	27.6 ± 0.4***	3.2 ± 0.1
12	Control	19.7 ± 0.5	3.1 ± 0.1	3.1 ± 0.0	24.2 ± 0.3	46.3 ± 0.3	n.d.
	CLA	23.8 ± 0.8	2.4 ± 0.2	3.5 ± 0.1*	23.3 ± 0.6	33.2 ± 0.8***	7.2 ± 0.3

Data are mean ± SE for 4 rats.
Significantly different from control value in each feeding duration: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. n.d.–not detected.

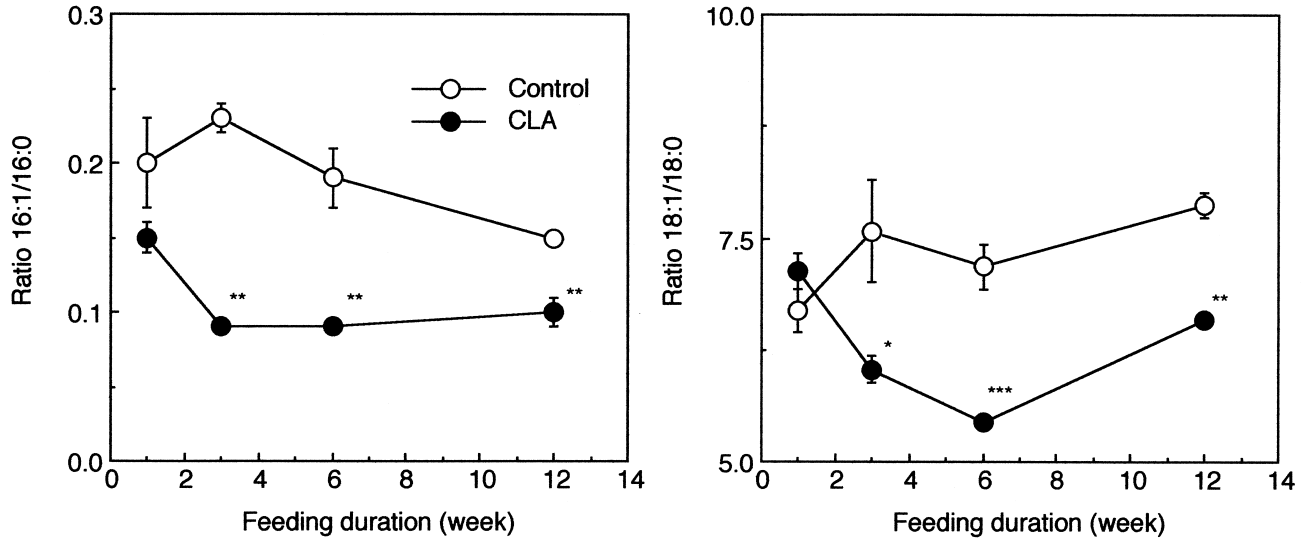

Figure 3 Effect of dietary conjugated linoleic acid (CLA) on the Δ9 desaturation index of rats. Data are means ± SE for 4 rats. Significantly different from control value in each feeding duration: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

effect of dietary CLA on serum leptin was due to the reduction of body fat. The subject to be clarified in future studies is the difference in the response to dietary CLA between the leptin level in serum and PWAT.

Stearoyl-CoA desaturase (SCD) catalyzes the Δ9-*cis* desaturation of saturated fatty acids, and 16:1/16:0 and 18:1/18:0 ratios are indices of SCD activity. As shown in *Figure 3*, these ratios in the CLA-fed rats were significantly lower than those of the control. It has been reported that CLA suppresses SCD1 mRNA expression when 3T3-L1 preadipocytes are differentiated to adipocytes.[28] It has also been reported that SCD mRNA is enhanced accompanied with 3T3-L1 adipocyte differentiation,[29] so dietary CLA might regulate adipocytes differentiation over 3-week feeding. Furthermore, dietary CLA may regulate adipocytes function by the change of membrane fluidity caused by the change of fatty acid composition.

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