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Short-term Administration of Conjugated Linoleic Acid Reduces Liver Triglyceride Concentration and Phosphatidate Phosphohydrolase Activity in OLETF Rats

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The present study explored the short-term effects of dietary conjugated-linoleic acid (CLA) on liver lipid metabolism in starved/refed Otsuka Long Evans Tokushima Fatty (OLETF) rats. Male OLETF rats (12 weeks old) were starved for 24 hours, then refed for 48 hours with either a CLA diet [7.5% CLA and 7.5% Safflower oil (SAF)] or a SAF control diet (15% SAF). The results demonstrated a 30% reduction of hepatic triglyceride (TG) concentration in the CLA group when compared to the control group. Liver cholesterol concentration was also 26% lower in the CLA fed rats. The activity of mitochondrial carnitine palmitoyltransferase, the rate-limiting enzyme of fatty acid oxidation, was moderately elevated by 1.2-fold in the livers of the CLA group when compared to the control. In contrast, phosphatidate phosphohydrolase, the rate-limiting enzyme for TG synthesis, was found to be 20% lower in the livers of the CLA-fed rats. Therefore, dietary CLA evidently lowers liver lipid concentrations through a reduced TG synthesis and enhanced fatty acid oxidation in starved/refed OLETF rats.

Keywords: Carnitine palmitoyltransferase, Conjugated-linoleic acid, Phosphatidate phosphohydrolase

Introduction

Conjugated linoleic acid (CLA) refers to a group of positional (9/11 or 10/12 double bonds) and geometric (various *cis*/*trans* combinations) isomers that are derived from linoleic acid (*cis-9, cis12*-octadecadienoic acid). They are found in edible foods, such as ruminants meats, pasteurized dairy products, and processed cheeses (Ha *et al.*, 1989; Chin *et al.*, 1992). CLA exhibits several beneficial effects, such as a protective effect against cancer and heart disease (Ip *et al.*, 1991; Ip *et al.*, 1994; Lee *et al.*, 1994), and reduced body fat mass in experimental animals (Park *et al.*, 1997; West *et al.*, 1997; Park *et al.*, 1999). Furthermore, Houseknecht *et al.* showed that CLA is capable of improving glucose tolerance in hyperglycemic Zucker rats (Houseknecht *et al.*, 1998).

Re-feeding after starvation leads to a significant increase in lipogenesis in the liver and white adipose tissue in experimental animals (Owens *et al.*, 1979; Kochan *et al.*, 1997). This physiological alteration may have some advantages for survival by directing the newly-synthesized fatty acids to form triglycerides as a high-energy source; this increases weight and lipid accumulation in the liver and adipose tissues (Nace *et al.*, 1976; Baltzell *et al.*, 1985). Belury *et al.* observed that CLA can modulate hepatic lipid composition in rats. However, it is unclear whether or not the short-term feeding of CLA influences the hepatic lipid metabolism in the starved/re-fed animal model.

In the present study, we employed Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which develop obesity, non-insulin dependent diabetes mellitus with early hypertriglyceridemia, and hyperinsulinemia in adulthood (Kawano *et al.*, 1992; Kawano *et al.*, 1994). We, therefore, evaluated the dietary influences of CLA on hepatic lipid metabolism in this animal model.

Materials and Methods

Animals and diets Male OLETF rats were a generous gift from the Tokushima Research Institute (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan). The rats were individually housed in metal cages in a temperature-controlled (24ºC) room under a 12-hour light/dark cycle. All of the animals were fed chow powder *ad libitum* until they were given the experimental diet. At 12 weeks of age (body eight 440-450 g), the rats were divided into control and

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CLA groups. Each group contained five rats. Both of the rat groups were starved for 24 hours, then re-fed for 48 hours with one of the following diets. The CLA group was fed with powdered chow that was supplemented with 7.5% safflower oil (SAF) and 7.5% CLA. The control group was fed with powdered chow that was supplemented with 15% SAF. CLEA, Inc. (Tokyo, Japan) provided the powdered chow. The ingredient composition was as follows: crude protein 25.4%, crude fat 4.2%, non-nitrogen compound 50.3%, and moisture 8.9%. CLA and SAF oil were obtained from Rinoru Oil Mills Co. Ltd. (Nagoya, Japan). The CLA mixture was composed of 33.2% of 9-*cis*, 11 *trans*/9 *trans*, 11 *cis* 18:2; 34.2% of 10 *trans*, 12 *cis* - 18:2; 2.4% of 9 *cis*, 11 *cis*/10 *cis*, 12 *cis* - 18:2; and 1.8% of 9 *trans*. 11 *trans*/10 *trans*, 12 *trans*-18:2 fatty acids. At the end of the experimental period, the rats were sacrificed under diethyl ether anesthesia. Blood samples were taken from the vena cava, and the tissues were excised and kept frozen at −80°C until they were analyzed.

Determination of serum lipids Serum was separated by centrifuging the blood at 3000 rpm for 15 min. Triglyceride, total cholesterol, HDL-cholesterol, non-esterified fatty acid (NEFA), and phospholipids in serum were measured with commercial kits that were supplied by Roche Diagnostics GmbH, Germany.

Determination of liver lipids A portion of the fresh liver from each rat was homogenized in an ice-cold 10 mM Tris-HCl buffer (pH 7.4) that contained 0.25 M sucrose and 1 mM EDTA (buffer A). Then the homogenate was centrifuged at $20,000 \times g$ for 20 min at 4°C. The supernatant of each homogenate was filtered through a nylon mesh, then centrifuged again at $105,000 \times g$ for 45 min at 4°C. The resulting supernatant was collected as a cytosolic fraction. The pellet was gently homogenized with a small volume of buffer A, and used as a microsomal fraction. Protein levels were measured by the method of Lowry *et al*. with bovine serum albumin as the standard (Lowry *et al*., 1951). Hepatic lipid concentrations were measured according to established protocols (Cha *et al*., 1998; Ikeda *et al.* 1998).

Enzyme assay Phosphatidate phosphohydrolase (PAP; EC 3.13.4), malic enzyme (EC 1.1.1.40), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), and carnitine palmitoyltransferase (CPT; EC 2.3.1.23) were measured as described previously (Cha *et al*., 1998; Ikeda *et al*., 1998; Martin *et al*., 2000).

Stastistical analyses All of the values are expressed as means ± SE. Data were analyzed by a one-way analysis of variance (ANOVA), followed by an inspection of all of the differences by a Students *t*-test. The differences were considered significant at $p < 0.05$.

Results and Discussion

The present study investigated the effect of the short-term feeding of the dietary CLA on hepatic lipid metabolism in starved/refed OLETF rats. The results demonstrated a 31% reduction of hepatic triglyceride content in the CLA-fed OLETF rats when compared to the control rats (Table 1). Similarly, the liver cholesterol concentration was 26% lower in the CLA fed rats.

The investigation then researched what leads to the reduction of hepatic lipid after the CLA administration. We measured the activities of two key enzymes; CPT for fatty acid oxidation and PAP for TG synthesis in liver. The present study found that there was a tendency to increase the CPT activity in the livers of the CLA-fed rats when compared to the control rats (Table 2). Short-term CLA feeding also enhanced the CPT activity in the brown and white adipose tissues of CLA-fed OLETF rats. Regarding the CPT activity increase in the CLA-fed OLETF rats, it may be assumed that CLA might alter the cellular expression of CPT mRNA. Another possibility is the involvement of peroxisome proliferator-activated receptors (PPARs). It was reported that CLA has structural and physiological characteristics that are similar to PPARs (Moya-Camarena *et al*., 1999). It is known that liver fatty acid catabolism is regulated by PPAR-alpha

Table 1. Effect of CLA on hepatic lipid concentration in starved/re-fed OLETF rats

	Control (mg/g liver)	CLA (mg/g liver)
Triglycerides	49.0 ± 2.8	34.3 ± 3.1*
Total cholesterol	4.05 ± 0.25	3.01 ± 0.26*
Phospholoipid	23.1 ± 0.7	23.8 ± 0.6

Data are means ± SE of five rats. Rats were starved for 24 h and then re-fed for 48 h with one of the following diets: CLA group was fed powdered chow supplemented with 7.5% safflower oil and 7.5% CLA, and the control group was fed powdered chow supplemented with 15% safflower oil. *(p<0.05 vs. control).

Table 2. Effect of CLA on enzyme activities in liver of starved/re-fed OLETF rats

	Enzyme activity expressed as nmol/min/mg protein	
	Control	CLA
CPT	11.1 ± 1.6	14.8 ± 2.1
PAP	22.2 ± 0.5	17.8 ± 0.5***
Malic enzyme	53.5 ± 2.0	59.1 ± 4.4
G6PDH	47.0 ± 2.3	47.3 ± 5.0

Data are mean ± SE of five rats. Rats were starved for 24 h and then re-fed for 48 h with one of the following diets: CLA group was fed powdered chow supplemented with 7.5% safflower oil and 7.5% CLA, and the control group was fed powdered chow supplemented with 15% safflower oil. PAP activity was measured using liver microsomal fraction, malic enzyme and G6PDH were measured in liver cytosolic fraction, while CPT activity was measured in liver homogenate fraction. CPT; Carnitine palmitoyl transferase, PAP; Phosphatidate phosphohydrolase, G6PDH; Glucose-6-phosphate dehydrogenase. ***(p<0.0001 vs. control)

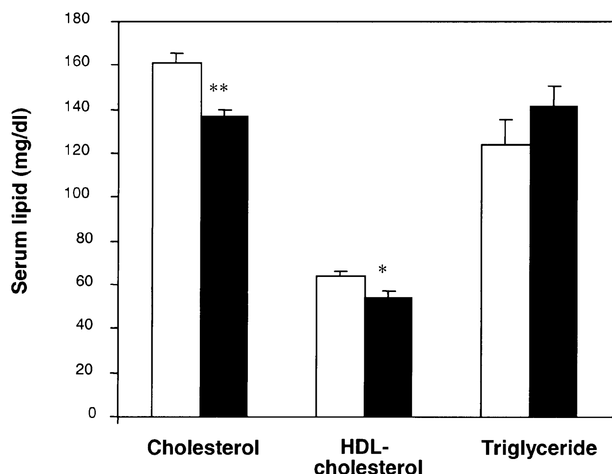

Fig. 1. Effect of CLA on serum lipid concentrations in starved/refed OLETF rats. Data are means ± SE of five rats. Rats were starved for 24 h and then re-fed for 48 h with one of the following diets: CLA group was fed powdered chow supplemented with 7.5% safflower oil and 7.5% CLA (■), and the control group (□) was powdered chow supplemented with 15% safflower oil. (*p<0.05 vs control; **p<0.001).

(Pineda *et al.*, 1999). Hence, it is likely that CLA, by up-regulating PPAR-alpha, may enhance the β-oxidation of fatty acid in liver.

Next, the CLA effect on hepatic TG synthetic enzyme, PAP, was investigated. It was reported that the Mg^{2+}-dependent PAP activity is the rate-limiting enzyme for the TG synthesis (Gomez-Munoz *et al.*, 1992; Jamal *et al.*, 1992). The present study demonstrated a 20% reduction of hepatic microsomal PAP activity in CLA-fed rats ($p < 0.001$) when compared to rats that were fed the control diet (Table 2). This suggests a CLA dependent reduction in liver TG synthesis. Availability of fatty acid is an important factor for TG synthesis (Halminski *et al.*, 1991), because the partition of fatty acids that are supplied from endogenous and exogenous origins into the TG molecule is reciprocally-related to the oxidation process. The decrease in PAP activity, and an increased tendency of CPT activity that is caused by dietary CLA, implies that fatty acids are degraded through β-oxidation rather than utilized for TG synthesis.

The present study also measured the activities of lipogenic enzymes, such as malic enzyme and glucose-6-phosphate dehydrogenase (Table 2). No change in enzyme activities was observed, unlike earlier studies (Nace *et al.*, 1976; Kochan *et al.*, 1997). This could be due to the experimental animal conditions that are employed herein, and those that were reported by others.

In addition to the alteration of hepatic lipids and enzymes that are involved in TG catabolism, dietary CLA reduced the serum total and HDL-cholesterol concentrations (Fig. 1). Our result on the CLA-induced reduction of serum cholesterol concentration confirms earlier reports, where the dietary CLA-induced reduction of serum cholesterol levels in hamsters and rabbits were reported (Lee *et al.*, 1994; Gavino *et al.*, 2000). Moreover, our result indicates that the short-term administration of CLA can reduce the serum cholesterol concentration in obese diabetic OLETF rats.

In this study, we found no changes in the serum TG concentration between the CLA and control groups (Fig. 1), though hepatic TG and PAP activities were reduced. One possibility might be the impaired removal of VLDL-TG after the CLA feeding. There are numerous studies that show the CLA effects on normal rats (Lee *et al.*, 1994; Belury *et al.*, 1997; Park *et al.*, 1997). However, most of these studies have shown the long-term feeding effects (1-4 weeks of feeding). Our data (so far the first of this type) shows that CLA reduces liver TG, as well as blood cholesterol, after 48 hours of feeding. In addition, none of the previous studies showed the lipid-lowering effects of CLA on the liver and blood. From this point-of-view, it is reasonable to say that the effect is unique in this type of rat model. However, no study on the short term CLA effect on normal rats has been undertaken. Therefore, further study will be necessary to clarify this point.

In conclusion, a 48-hr supplementation of CLA to the diet caused a significant reduction of hepatic TG and cholesterol concentration in starved/re-fed OLETF rats. This reduction may be attributed to both the enhanced β-oxidation of fatty acids and the reduced triglyceride synthesis in the livers of starved/re-fed OLETF rats.

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