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Effect of Structured Lipids Containing CLA on Hepatic Antioxidant Enzyme Activity in Rats Fed a Normal Diet*

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Conjugated linoleic acid (CLA) has been shown to have a range of biological activities, including anti-carcinogenic, anti-atherosclerotic, anti-adipogenic and anti-diabetogenic effects. Recent reports also showed that CLA has free radical scavenging capacity, which may have health benefits for human beings. The current study was performed to investigate the effect of structured lipid (SL)-containing CLA on plasma lipids and hepatic antioxidant enzyme activity. Sprague-Dawley rats were fed 5% and 10% SL-containing normal diet for 6 wks and these groups were compared to rats fed 5% and 10% corn oil. In plasma lipids, total-cholesterol was not affected by fat source or dietary fat level while triglyceride level decreased significantly in groups fed 10% fat diet compared to the other groups. Plasma thiobarbituric acid reactive substances (TBARS) level decreased significantly in the S5 and S10 groups compared to the C5 and C10 groups, although hepatic TBARS level was not altered by fat source. On the other hand, in terms of hepatic antioxidant enzyme activity, superoxide dismutase activity increased in the S10 group, whereas catalase activity decreased in the S10 group. Glutathione peroxidase activity decreased significantly in the SL groups compared to the C5 group. Glutathione reductase activity increased and glucose-6-phosphate dehydrogenase activity decreased in the C10 group compared to the C5 and S5 groups. In conclusion, the free radical scavenging activity of CLA seemed to suppress oxidative stress, which reduced lipid peroxidation resulting in lower hepatic antioxidant enzyme activity.

Keywords: Structured lipid, Conjugated linoleic acid, Lipid peroxidation, Antioxidant enzyme activity

INTRODUCTION

The concept of structured lipids (SL) for nutritional and medical use was introduced in 1987 by Babayan.[1)] SL are triacylglycerols (TG) modified to alter the fatty acid composition and/or their location in the glycerol backbone via chemical or enzymatic means. The triacylglycerols in SL consist of glycerol moieties esterified to a mixture of short-, medium- and long-chain fatty acids for functional purposes.[2)] Lipids can be restructured to meet essential fatty acid requirements or to incorporate specific fatty acids of interest. SL may offer the most efficient means of delivering target fatty acids for nutritive or therapeutic purposes as well as to alleviate specific disease and metabolic conditions. In particular, SL containing medium- and long-chain fatty acids that are esterified at the *sn*-1, 2 or 3 positions of the glycerol molecule can be used for distinct nutritional and medical purposes by utilizing their unique metabolism. Likewise, through the structural configuration of fatty acid, diaclyglyceride (DG) could be produced. Nagao et al.[3)] reported that dietary DG, in contrast to TG, decreased both body weight and visceral fat mass in healthy men, as determined by semi-quantitative analysis using computed tomography.

Conjugated linoleic acid (CLA) is a group of octadecadienoic acids containing conjugated double bonds that are separated by one single bond. Much attention has been given to CLA because it is a natural component of foods and has interesting health benefits.[4)] CLA has been investigated in terms of its physiological and pharmacological activities, appearance in foods, separation, characterization methods and the procedures used to prepare it. The observed physiological and pharmacological activities of CLA include anticarcinogenic, antiatherosclerotic, antioxidant and immunomodulatory antibacterial effects, alteration of tissue fatty acid composition and metabolism,[4-10)] influence on signal transduction and effects on body composition and metabolism. However, the mechanism of its biological actions is still poorly understood.

Recently, we incorporated CLA into unsaturated fatty

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acid-rich corn oil via lipase-catalyzed acidolysis. Thus, the present study was carried out to compare the effect of corn oil and a corn oil-based SL-containing CLA on antioxidative activity in AIN-76 diet-fed rats.

METHODS

1. Fat Sources

Two types of dietary fat used in the animal diet were corn oil triglyceride (TG) and its structured lipids (SL). SL was prepared by immobilized lipase-catalyzed esterifying glycerol with fatty acids from corn oil using the method of Lee and Akoh.[11] The fatty acid composition of the test oil is shown in Table 1. The composition of TG, DG and monoacylglyciride (MG) of SL was 51%, 35%, 14%, respectively.

Table 1. Fatty acid composition of test oil(unit : %)

Fatty acid	Control (Corn oil)	SL
10:00	-	9.4
16:00	11.4	9.1
16:01	0.2	0.1
18:00	1.3	1.3
18:01	32.3	25.6
18:02	53.4	46.6
18:03	1.4	1.1
CLA 1	-	3.0
CLA 2	-	3.3
CLA 3	-	0.3
CLA 4	-	0.3
Total SAT	12.7	19.8
Total MUFA	32.5	25.7
Total PUFA	54.8	54.6
PUFA/SAT	4.3	2.8

CLA 1 : c9 t11
CLA 2 : t10 c12
CLA 3 : c9 c11 t11 t13
CLA 4 : t8 t10 t9 t11 t10 t12

2. Animal and Diets

Forty-four male Sprague Dawley rats, weighing between 90 and 100 g, were purchased from Bio Genomics Inc. (Seoul, Korea). The animals were individually housed in stainless steel cages in a room with controlled temperature (20-23 ℃) and lighting (alternating 12 h periods of light and dark) and fed a pelletized commercial non-purified diet for 1 week after arrival. They were randomly divided into 4 groups (n=11) and fed AIN-76 basal diets containing corn oil and structured lipid at 5% and 10% for 6 wks (Table 2). The composition of the mineral and vitamin mixture was previously described.[12,13] Free access was given to food and water. Food consumption and body weight were measured daily and weekly, respectively. At the end of the experimental period, the rats were anesthetized with Ketamin-HCl after food had been withheld for 12 h. Blood samples were taken from the inferior vena cava for the determination of plasma lipids. All samples were stored at -70 ℃ until analyzed.

Table 2. Composition of Diets (%)

Component	Control		SL	
	C5	C10	S5	S10
Casein	20.0	20.0	20.0	20.0
D,L-methionine	0.3	0.3	0.3	0.3
Corn starch	15.0	15.0	15.0	15.0
Sucrose	50.0	45.0	50.0	45.0
Cellulose powder	5.0	5.0	5.0	5.0
Test oil[1]	5.0	10.0	5.0	10.0
Cholinebitartrate	0.2	0.2	0.2	0.2
AIN-Mineral[2]	3.5	3.5	3.5	3.5
AIN-Vitamin[3]	1.0	1.0	1.0	1.0
Total	100	100	100	100

1) Control is a commercial corn oil and SL is its structured lipids containing CLA
2) AIN-76 mineral mixture (Harlan Teklad Co. USA)
3) AIN-76A vitamin mixture (Harlan Teklad Co. USA)

3. Plasma Lipids

Plasma cholesterol was determined using a commercial kit (Sigma) based on a modification of the cholesterol oxidase method of Allain et al..[14] Plasma triglyceride concentrations were measured enzymatically using a kit from Sigma Chemical Co., a modification of the lipase-glycerol phosphate oxidase method.[15]

4. Preparation of Hepatic Antioxidant Enzyme Source

The preparation of the enzyme source fraction in the hepatic tissue was as follows. The hepatic tissue was homogenized in a five-fold vol. of a 0.25 M sucrose buffer, centrifuged at 600 ×g for 10 min to remove any cell debris and then the supernatant was centrifuged at 10,000 ×g for 20 min to remove the mitochondria pellet. Finally, the supernatant was further ultracentrifuged at 105,000 ×g for 60 min to obtain the cytosol supernatant. The amount of protein in the mitochondrial and cytosolic fractions was measured according to the method of Bradford[16] using bovine serum albumin as the standard.

5. Antioxidant Enzyme Activities

Superoxide dismutase (SOD) activity was measured using Marklund and Marklund's method[17] with a slight modification. One hundred microliters of the cytosol supernatant was mixed with 1.5 mL of a Tris-EDTA-HCl buffer (pH 8.5), then 100 μL of 7.2 mmol/L pyrogallol was added and the reaction mixture incubated at 25 ℃ for 10 min. The reaction was terminated by the addition

of 50 μL of 1 mol/L HCl and measured at 420 nm. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed in U/mg protein. Catalase (CAT) activity was measured using Aebi's[18] method with a slight modification, in which the disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to determine CAT activity. Activity was defined as the amount of enzyme that oxidized H_2O_2 μmol/min/mg protein. Glutathione peroxidase (GSH-Px) activity was measured using Paglia and Valentine's[19] method with a slight modification. The reaction mixture contained 2.6 mL of a 0.1 mol/L of Tris-HCl (pH 7.2) buffer, 100 μL of 30 mmol/L glutathione and 100 μL of 6 mmol/L NADPH. One hundred microliters of the cytosolic supernatant was added to 2.9 mL of the reaction mixture and incubated at 25 ℃ for 5 min. The reaction was initiated by the addition of 100 μL of 7.5 mmol/L H_2O_2 and the absorbance was measured at 340 nm for 5 min. A molar extinction coefficient of 6.22×10^3 $(mmol/L)^{-1}\cdot cm^{-1}$ was used to determine the activity. One unit of GSH-Px was defined as the amount of enzyme that oxidized 1 μmol per min per mg protein. Glutathione reductase (GR) activity was determined using the method of Pinto and Bartley[20] by monitoring the oxidation of NADPH at 340 nm. The reaction mixture contained 1 mM EDTA and 1 mM GSSG in 0.1 M potassium phosphate buffer (pH 7.4). Activity was expressed as oxidized NADPH nmol/min/mg protein. Glucose-6-phosphate dehydrogenase (G6PD) activity was determined using the method of Pitkanen.[21] The reaction mixture contained 55 mM Tris-HCl (pH 7.8), 3.3 mM $MgCl_2$ buffer and 6 mM G-6-P. Activity was expressed as reduced NADPH nmol/min/mg protein.

6. Statistical Analysis

All data were presented as mean±SE. Significant differences among the groups were determined by one-way ANOVA using SPSS. Duncan's multiple-range test was performed if differences were identified between groups at p=0.05. Differences of between 5% and 10% were compared by students't test using standard social science statistical packages.

RESULTS

1. Food Intake, Weight Gain and Organ Weight

There were no significant differences in food intake, weight gain or organ (liver, heart and kidney) weight among the groups (Table 3).

Table 3. Effects of structured lipid containing CLA for 6 wks on the food intakes, weight gains and organ weights of rats

	C5	C10	S5	S10
Food Intakes (g/d)	20.74±0.51^{NS}	20.26±0.45	20.43±0.65	20.47±0.43
Weight gains (g/wk)	6.94±0.21^{NS}	7.09±0.31	6.98±0.20	7.43±0.12
Tissue Weight (g)				
Liver	14.44±0.65^{NS}	13.58±0.62	14.41±0.58	14.07±0.39
Heart	1.31±0.04^{NS}	1.28±0.03	1.28±0.04	1.36±0.04
Kidney	2.72±0.09^{NS}	2.73±0.06	2.81±0.08	3.01±0.08

Mean±SE
NS: Not significantly different between groups at $p<0.05$.

2. Plasma Lipids

There was no significant difference in plasma total-cholesterol levels among the groups. Yet, plasma triglyceride levels decreased significantly in the S10 and C10 groups compared to the C5 groups (Table 4).

Table 4. Effects of structured lipid containing CLA for 6 wks on the plasma total-cholesterol and triglycerides concentrations of rats

	C5	C10	S5	S10
Total-Cholesterol (mmol/L)	3.42±0.26^{NS}	3.20±0.21	3.13±0.13	3.02±0.14
Triglyceride (mmol/L)	1.65±0.12^{a}	1.18±0.12^{b}	1.34±0.08^{ab}	1.12±0.11^{b}

Mean±SE
NS: Not significantly different between groups at $p<0.05$.
ab: Means in the same row not sharing a common superscript are significantly different between groups at $p<0.05$.

3. Plasma and Hepatic Lipid Peroxidation

The plasma and hepatic lipid peroxide levels were determined by measuring the TBARS concentration. Hepatic TBARS was not affected by dietary fat source or level. But plasma TBARS level decreased

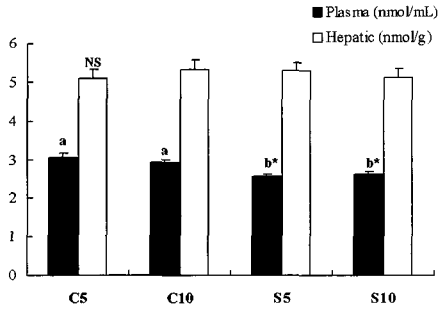

Fig 1. Effects of structured lipid containing CLA for 6 wks on the plasma and hepatic TBARS levels of rats

Mean±SE
NS: Not significantly different between groups at $p<0.05$.
ab: Means not sharing a common superscript are significantly different between groups at $p<0.05$.
* Means significantly different 5% vs 10% within same fat at $p<0.05$

significantly in the SL groups (S5 and S10) compared to the control groups (C5 and C10) (Fig. 1).

4. Hepatic Antioxidant Enzyme Activities

The effects of the structured lipid-containing CLA on the hepatic activity of the antioxidant enzymes in the rats are shown in Table 5. Hepatic glutathione content remained unchanged among the groups. In terms of hepatic antioxidant enzyme activity, SOD activity was significantly higher in the S10 group than in the S5 group, whereas CAT was significantly lower in the S10 group than in the other groups. Dietary fat level did not affect hepatic GSH-Px activity. However, the values in the SL groups seemed to be lower than those in the control groups. Hepatic GSH-Px activity in the S5 and S10 groups was significantly lower than it was in the C5 group. GR activity increased and G6PD activity decreased in the C10 group compared to the C5 and S5 groups. There was no difference between the C5 and S5 groups or between the C10 and S10 groups in terms of GR or G6PD activity.

Table 5. Effects of structured lipid containing CLA for 6 wks on the hepatic antioxidant enzyme activities of rats

	C5	C10	S5	S10
SOD (unit/mg)	16.80±0.48^{ab}	17.82±0.75^{ab}	16.16 ± 0.14^{a}	18.45± 0.79^{b}
CAT (nmol/mg/min)	0.79±0.05^{a}	0.80±0.02^{a}	0.76±0.03^{a}	0.59±0.4^{b*}
GSH-Px (nmol/mg/min)	7.71±0.21^{a}	7.63±0.23^{ab}	6.21±0.23^{c*}	6.89±0.32^{bc}
GR (nmol/mg/min)	36.78±0.93^{a}	41.40±1.49^{b}	37.15±1.12^{a}	40.45±1.41^{ab}
G6PD (nmol/mg/min)	64.21±7.16^{a}	39.92±5.02^{b}	61.77±7.71^{a}	58.99±8.91^{ab}
GSH (μmol/g tissue)	3.65±0.28^{NS}	4.07±0.20	3.49±0.25	4.14±0.16

Mean±SE
NS: Not significantly different between groups at $p<0.05$.
abc: Means in the same row not sharing a common superscript are significantly different between groups at $p<0.05$.
*Means significantly different 5% vs 10% within same fat at $p<0.05$

DISCUSSION

This study was performed to examine the effects of structured lipids, which contain CLA and are composed mainly of TG and DG. The DG oil appears to be metabolized in a manner that favorably impacts dietary fat metabolism when substituted for conventional edible oils. Rats consuming DG oil were observed to have lower triglycerides and decreased levels of post-meal fat-rich chylomicron particles in their blood. The first studies in rats demonstrated that dietary DG compared with TG exerts a potent triacylglycerol-lowering effect in the blood.[22] In this study, SL groups exhibited a tendency toward lower plasma TG compared to the control group, although the tendency was not significant. Interestingly, plasma TG concentration was significantly lower in rats fed 10% corn oil than in those fed 5% corn oil. This seems to be due to the benefits of polyunsaturated fatty acids, levels of which are higher when used at the 10% level in a normal diet. This tendency was also exhibited in the SL groups. High-fat diet (10%), in general, induced increases in lipoprotein lipase (LPL) which resulted in reduced plasma TG levels accompanied by TG accumulation in the adipose tissue and muscle.[23] The dietary fat content used in the study was at normal or moderate levels since we intended to approach the general use of SL in a normal diet.

The susceptibility of an organism to oxidative damage is influenced by the antioxidant defense system's ability to cope with the stress, which in turn can be influenced by nutrition intervention with antioxidants.[24] Inherent antioxidant defense systems consisting of enzymes such as GSH-Px, CAT and SOD and antioxidant nutrients may participate in coping with oxidative stress.[25-27] As antioxidant enzymes have an important role in protecting against free radical damage, a decrease in the activities or expression of these enzymes may predispose tissues to free radical damage.[28,29]

It has been accepted that free radicals and radical-mediated oxidation play roles in many pathological processes, such as carcinogenesis and atherosclerosis.[30] CLA was shown to prevent cancer and atherosclerosis in both animal and cell models.[4,30,31] And the antioxidant activity of CLA has been investigated by several research groups since it was considered as a possible explanation for some of their biological actions,[6,7,10,30] although conflicting results were obtained from previous studies of the antioxidant properties of CLA.[10,30,33-37]

CLA is a functional fatty acid that has diverse physiological effects and antioxidant properties. To better understand the beneficial actions of CLA, it is critical to clarify whether CLA can act as an antioxidant, perhaps by directly quenching free radicals to terminate the radical chain reaction, chelating transition metals to suppress the initiation of radical formation or stimulating antioxidative defense enzyme activities. Ip and co-investigators[33] measured thiobarbituric acid-reactive substances (TBARS) as the indicator of lipid peroxidation in the mammary gland. Radical scavenging activity of CLA could well explain the reduced TBARS observed by Ip and others in their rat-feeding study.

It has been reported that CLA reduces CAT, SOD and GSH-Px but does not induce lipid peroxidation in rat hepatocytes.[35] In addition, dietary CLA reduces glutathione-S-transferase activity in Sprague-Dawley rats, but not significantly. Moreover, liver TBARS levels

tended to be lower in the CLA-fed group.[33] In the present study, hepatic CAT and GSH-Px activity decreased in the SL groups compared to the control group. Although SL treatment did not seem to affect the concentration of hepatic TBARS in rats, the plasma TBARS level, which can reflect the immediate antioxidant response in the body, decreased significantly compared to that of the corn oil-fed control groups. The reduced lipid peroxidation might lead to a decrease in antioxidant enzyme activity.

Overall, the free radical scavenging activity of CLA seemed to suppress oxidative stress, which reduced lipid peroxidation leading to a decrease in hepatic antioxidant enzyme activity. These results suggest the possible role of CLA or DG in maintaining antioxidant status among those on a normal diet. The detailed function of CLA or DG on the antioxidant defense system needs to be elucidated along with its beneficial role in preventing some chronic degenerative diseases.

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