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Selectively hydrogenated soybean oil with conjugated linoleic acid modifies body composition and plasma lipids in rats

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Abstract

The present study examined effects of a selectively hydrogenated soybean oil (SHSO) containing about 21% CLA on body composition, adipose depots and organ weights, and plasma lipid profiles in rats. Male Sprague Dawley rats were fed for 6 weeks a purified diet containing 0%, 1%, 3%, and 5% of SHSO. Different levels of SHSO supplementation did not significantly affect growth performance, although there was a trend toward decreased body weight gain with increasing dietary SHSO levels. The weights of inguinal, epididymal, and retroperitoneal adipose depot, but not mesenteric, were significantly influenced by dietary SHSO supplementation ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively). Although the absolute weight of body protein in the control rats was higher in SHSO-fed rats, the effect on absolute weight of body protein is diluted and eliminated when the data are adjusted for eviscerated carcass weight as a percentage base. Therefore, as dietary SHSO level increased, body protein as a percentage of carcass weight increased ($P < 0.05$), although as dietary SHSO level increased, body fat proportion in carcass decreased ($P < 0.01$). Plasma triglycerides (TG) and total cholesterol (TC) concentrations were beneficially decreased, and HDL-cholesterol (HDL-C) to TC ratio was also beneficially increased by SHSO supplementation ($P < 0.05$, $P < 0.001$, and $P < 0.01$, respectively). However, plasma HDL-C concentration undesirably decreased with dietary SHSO supplementation ($P < 0.05$). The present study observed that body composition and plasma lipids were beneficially modulated by SHSO supplementation at least 3% levels (0.6% of CLA), and suggested that SHSO is a useful fat source because of the high level of CLA.

Keywords: Selectively hydrogenated soybean oil; Conjugated linoleic acid (CLA); Body composition; Antiobesity; Plasma lipid

1. Introduction

Conjugated linoleic acid (CLA) is a collective name for the mixture of positional and geometric isomers of linoleic acid (c-9, c-12-octadienoic acid) and has been reported to exert various beneficial effects. CLA has been reported to reduce body fat in humans [1], pigs [2], mice [3,4], rats [5] and broilers [6], and it was suggested that CLA has an antiobesity effect in various animal models and in humans. In addition, CLA decreased plasma lipid levels and reduced the development of atherosclerosis in rabbits [7] and hamsters [8].

Therefore, there is increasing interest in producing CLA as a food ingredient and health supplement because of the possible health benefits associated with its consumption. Dietary CLA predominately originates from dairy products via biohydrogenation of polyunsaturated fatty acids by rumen bacteria [9,10]. Chin et al. [10] reported that dairy products such as milk, butter, cheese, and yogurt as well as beef contained 3–8 mg of total CLA per gram of fat. The commercially available CLA are manufactured by alkali isomerization of linoleic acid or linoleic acid rich oils such as safflower oil and sunflower oil. Jung and Ha [11] originally reported that a large quantity of CLA (98 mg /g oil) was formed during a selective hydrogenation of soybean oil [11]. Jung et al [12,13] reported that catalyst type and amounts, temperature, hydrogen pressure, and agitation rate greatly affected the quantity of total CLA and individual isomers as well as the time to reach the maximal quantity of CLA in the hydrogenated soybean oil. Although it has been reported that SHSO contained high level of CLA, its health benefits have never been studied.

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Table 1
Composition of experimental diets

	Control	SHSO Diet, %		
		1.5	3	5
Casein	200	200	200	200
DL-methionine	30	30	30	30
Corn starch	150	150	150	150
Sucrose	500	500	500	500
Cellulose	50	50	50	50
Soybean oil	50	35	20	—
SHSO (CLA)	—	15	30	50
Mineral mix, AIN-76A	35	35	35	35
Vitamin mix, AIN-76A	10	10	10	10
Choline bitartrate	2	2	2	2

Data are given as g/kg diet.

The objectives of the present study were to estimate effects of feeding SHSO containing CLA to rats on their growth performance, weights of organs, and abdominal adipose depots. Also, potentially hypocholesterolemic effects of CLA on plasma lipid profiles were determined.

2. Methods and materials

2.1. Animals and diets

A total of 48 Sprague-Dawley rats were obtained from Jung-Ang Laboratory (Seoul, Korea) at 6 weeks of age and habituated to individual housing in hanging stainless steel metabolic cages in a room maintained at an ambient temperature of 22° ± 2°C with 12:12-h light–dark cycle. Initially, the rats were maintained *ad libitum* on purified AIN-76A diet for 7 days. After the adaptation period, the rats were placed on the experimental diets with 0%, 1.5%, 3%, and 5% of SHSO. The supplementation of SHSO levels were 0%, 1.5%, 3%, and 5%, and soybean oil was added to make 5% of total fat level in the diet. The composition of experimental diets is shown in Table 1. The SHSO was obtained by 20 minutes hydrogenation of soybean oil with 5% selective type catalyst (Pricat 9908, Unichema) under the condition of reaction temperature of 230°C, hydrogen pressure of 0.25 kg/cm^2, and agitation rate of 300 rpm [12,13]. The total CLA contents and their isomeric distribution, and fatty acid profiles in SHSO were analyzed by gas chromatography with a 100-m highly polar fused silica capillary column (cyanopripyl siloxane phase, SP2380, 100 m × 0.25 mm, 0.25 μm thickness (Supelco Inc., Bellefonte, PA, USA) [12–14]. Total CLA content in SHSO was 208 mg/g oil. The distribution of CLA isomers in SHSO is shown in Table 2, and the actual supplementations of CLA to the experimental diets were 0%, 0.31%, 0.62%, and 1.04 %, respectively. The fatty acids composition of SHSO is shown in Table 3. The defined powdered diets were placed on the bottom of the cages and replaced with fresh diet on every 2 days. The rats were weighed three times each week and food intake (corrected for spoilage and measured to 0.3 g) was also measured at these times. To ensure a homogenous mixture, SHSO was mixed with soybean oil before adding to the basal diet, keeping the total fat at 5 g/100 g. Diets were mixed weekly and stored at 4°C.

Table 2
Distribution of CLA isomers in SHSO

CLA Isomer	Oil (mg/g)
trans-7, *cis*-9/*cis*-9, *trans*-11/*trans*-8, *cis*-10	42.07
cis-10, *trans*-12/*trans*-9, *cis*-11/*cis*-11, *cis*-13	16.08
cis-12, *trans*-14/*trans*-10, *cis*-12	8.45
trans-11, *cis*-13/*cis*-9, *cis*-11	27.44
trans-12, *cis*-14/*cis*-10, *cis*-12/*cis*-11, *cis*-13	12.08
trans-12, *trans*-14	8.45
trans-11, *trans*-13	5.0
trans-10, *trans*-12/*trans*-9, *trans*-11/*trans*-8, *trans*-10/*trans*-7, *trans*-9	89.04

2.2. Adipose depots, organs, muscle, and blood sampling

After completing 6 weeks of the experimental period, rats were fasted overnight (12 h) and anesthetized with diethyl ether, and selected adipose depots and organs were removed and weighed. The liver, left and right kidney, left and right testis, heart, and spleen were removed and weighed to 0.0001 g. Similarly, the left and right inguinal, epididymal, retroperitoneal adipose depots, and mesenteric adipose depot were removed and weighed. For body composition analyses, gut contents were removed to obtain empty carcass weight, and the carcasses were frozen at −20°C until analysis.

2.3. Analytical methods

Frozen carcasses were chopped, ground, and freeze-dried to determine water content. Total nitrogen was analyzed by

Table 3
Fatty acid composition of selectively hydrogenated soybean oil (SHSO)

Fatty Acid	Proportion (%)
Palmitic acid	11.53
Stearic acid	6.13
trans Isomer of oleic acid	5.53
Oleic acid	19.28
cis Isomers of oleic acid	2.30
Unconjugated isomers of linoleic acid	7.48
Linoleic acid	20.70
Arachidic acid	0.42
Isomers of linolenic acid	0.65
Linolenic acid	1.07
Behenic acid	0.77
Conjugated linoleic acids	22.97
Unknown	1.06

Table 4
Effect of dietary selectively hydrogenated soybean oil (SHSO) on growth performance of rats

	Control	SHSO, %		
		1.5	3	5
Initial body weight (g)	215.6 ± 4.8	214.2 ± 4.2	213.0 ± 2.9	214.9 ± 3.3
Final body weight (g)	405.4 ± 12.8	409.0 ± 9.4	394.7 ± 8.2	395.8 ± 8.2
Body weight gain (g/d)	5.43 ± 0.26	5.58 ± 0.17	5.21 ± 0.19	5.20 ± 0.18
Food intake (g/d)	24.86 ± 0.78	25.67 ± 0.53	23.65 ± 0.68	24.02 ± 0.52
Food conversion efficiency	0.22 ± 0.017	0.22 ± 0.014	0.22 ± 0.013	0.22 ± 0.018

Data are presented as mean ± SE.

the Kjedahl method [15]. Carcass fat content was measured by extraction with diethyl ether overnight using a Soxhlet apparatus. Total ash content was determined by incineration (500−600°C, overnight). Water content of the carcass was calculated by subtracting the dried carcass weight from the original weight of the eviscerated carcass. An adiposity index was calculated by dividing the summed weight of the seven excised adipose depots by the weight of the eviscerated carcass that includes all organs without the gastrointestinal tract and the seven adipose depots [16]. Blood was collected by cardiac puncture, and plasma samples were separated by low-speed centrifugation (1500 × *g* for 15 minutes). Total plasma cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C) were determined by enzymatic assays using kits (ASAN Pharmacy, Co., Korea).

2.4. Statistical analysis

Statistical differences were determined by an analysis of variance, with mean separations performed by the Duncan multiple range test using the general linear model procedure of the SAS statistical software [17]. The results are expressed as mean ± SE.

3. Results

3.1. Animal performance and body composition

Effect of dietary SHSO on animal performance of rats is shown in Table 4. There were no significant effects of dietary SHSO on initial and final body weights, daily body weight gain, food intake, and food conversion efficiency between treatments. Although there was a trend to decrease the final body weight, daily body weight gain, and food intake in 3% and 5% SHSO treatments (0.6% and 1.0% of CLA, respectively). Effects of dietary SHSO supplementation on body composition of rats are shown in Table 5. The eviscerated carcass weights of rats was decreased by 3% and 5% SHSO supplementations, but was increased by 1.5% SHSO supplementation ($P < 0.001$). The proportion of body fat in 5% SHSO-fed rats was significantly lower than with other treatments ($P < 0.01$), but the proportion of body protein increased with the increased level of dietary SHSO supplementation ($P < 0.05$). Body protein content was more effectively modified by relatively lower level of SHSO (1.5%, 3%, and 5%) than body fat changes. It should be clarified that the absolute weight of body protein in the control rats was higher in CLA-fed rats. In addition, final

Table 5
Effect of dietary selectively hydrogenated soybean oil (SHSO) on body composition of rats

	Control	SHSO, %		
		1.5	3	5
Body composition				
Eviscerated carcass (g)*	323.13 ± 3.60^{ab}	329.95 ± 4.30^{a}	308.25 ± 2.43^{b}	288.58 ± 6.95^{c}
Fat (%)†	14.31 ± 0.44^{a}	14.14 ± 0.24^{a}	13.30 ± 0.15^{a}	10.97 ± 0.04^{b}
Protein (%)‡	21.73 ± 0.18^{b}	21.85 ± 0.04^{ab}	21.90 ± 0.17^{ab}	22.45 ± 0.23^{a}
Water (%)‡	61.32 ± 0.39^{ab}	60.53 ± 0.37^{b}	62.10 ± 0.28^{a}	62.33 ± 0.49^{a}
Ash (%)	3.52 ± 0.02	3.43 ± 0.09	3.59 ± 0.04	3.49 ± 0.04
Adiposity index§	0.08 ± 0.01^{a}	0.08 ± 0.00^{a}	0.07 ± 0.00^{ab}	0.06 ± 0.00^{b}

Data are given as mean ± SE. Mean values in a row with different superscript letters differ significantly ($P < 0.05$)
* $P < 0.001$.
† $P < 0.01$.
‡ $P < 0.05$.
§ Adiposity index is calculated by dividing the sum of the adipose depots weights by the weight of the eviscerated carcass minus the weight of the adipose depots.

Table 6
Effects of dietary selectively hydrogenated soybean oil (SHSO) on weight of organs and adipose depots of rats

	Control	SHSO, %		
		1.5	3	5
Organ Weights (g)				
Kidney*	2.40 ± 0.13[b]	2.59 ± 0.09[ab]	2.66 ± 0.07[ab]	2.78 ± 0.08[a]
Heart	1.13 ± 0.05	1.13 ± 0.03	1.10 ± 0.02	1.14 ± 0.05
Testis[†]	2.85 ± 0.18[b]	3.47 ± 0.06[a]	3.40 ± 0.07[a]	3.41 ± 0.04[a]
Liver	12.41 ± 0.55	13.16 ± 0.40	12.39 ± 0.46	13.59 ± 0.45
Spleen	0.68 ± 0.03	0.75 ± 0.04	0.68 ± 0.03	0.74 ± 0.10
Adipose Depot Weights (g)				
Inguinal[‡]	8.02 ± 0.81[a]	8.39 ± 0.59[a]	6.91 ± 0.41[ab]	5.36 ± 0.42[b]
Epididymal[‡]	8.90 ± 0.74[a]	8.99 ± 0.46[a]	7.49 ± 0.30[ab]	6.23 ± 0.64[b]
Retroperitoneal[†]	3.59 ± 0.39[a]	3.17 ± 0.24[a]	2.81 ± 0.16[a]	1.90 ± 0.09[b]
Mesenteric	6.11 ± 0.81	5.94 ± 0.39	6.53 ± 0.39	5.27 ± 0.41
Total[†§]	26.62 ± 2.22[a]	26.49 ± 1.46[a]	23.73 ± 1.11[a]	18.76 ± 1.21[b]

Data are given as mean ± SE. Mean values in a row with different superscript letters differ significantly ($P < 0.05$).
* $P < 0.05$, and
† $P < 0.001$.
‡ $P < 0.01$.
§ Total adipose depot was sum of inguinal, epididymal, retroperitoneal and mesenteric fat depot.

body and eviscerated carcass weight were higher in the control rats in comparison to the CLA-fed rats. This may indicate that the effect on absolute weight of body protein is diluted and eliminated when the data are adjusted for eviscerated carcass weight as a percentage base. Therefore, from this point of view, the change of body protein can be better expressed by the percentage, rather than weight itself. The water proportion in the body was decreased in 1.5% SHSO treatment, but increased in 3% and 5% SHSO treatments compared with control ($P < 0.05$). The proportion of body ash was not significantly different between treatments. The adiposity index in 1.5% SHSO treatment group was similar to the control, but those in 3% and 5% SHSO treatment groups were significantly lower than the control ($P < 0.05$).

3.3. Adipose depots and organ weights

Weights of inguinal and epididymal adipose depots were significantly lower in 3% and 5% SHSO treatments than in control ($P < 0.01$ and $P < 0.01$, respectively) (Table 6). However, weights of inguinal and epididymal adipose depots in 1.5% SHSO supplementation group were similar to those in control. The weights of retroperitoneal and total adipose depots were decreased by 5% SHSO supplementation, as compared with other treatments ($P < 0.001$ and $P < 0.01$, respectively). The weights of kidney and testis were significantly increased by dietary SHSO supplementation, as compared with control ($P < 0.05$ and $P < 0.001$, respectively). However, the SHSO supplementations did not affect the weight of heart, liver, and spleen.

3.4. Plasma lipid profiles

Plasma TG, TC, and HDL-C of rats are shown in Table 7. Plasma TG concentration was increased in 1.5% SHSO treatment, but decreased in 3% SHSO treatment compared with control. However, plasma TG was not significantly different between rats receiving 5% of dietary SHSO and control. Plasma TC concentration was decreased with in-

Table 7
Effects of dietary selectively hydrogenated soybean oil (SHSO) on plasma lipids of rats

	Control	SHSO, %		
		1.5	3	5
TG*	222.80 ± 19.55[ab]	233.61 ± 23.99[a]	158.98 ± 10.92[b]	206.97 ± 26.20[ab]
TC[†]	114.85 ± 10.02[a]	102.28 ± 5.42[ab]	87.44 ± 6.35[b]	63.96 ± 5.06[c]
HDL-C*	52.17 ± 3.78[a]	41.85 ± 1.95[b]	49.08 ± 3.65[ab]	40.80 ± 2.24[b]
HDL-C/TC[‡]	0.47 ± 0.03[b]	0.42 ± 0.02[b]	0.57 ± 0.02[ab]	0.70 ± 0.09[a]

Data are given as mean ± SE (mg/dL). Mean values in a row with different superscript letters differ significantly ($P < 0.05$).
* $P < 0.05$.
† $P < 0.001$.
‡ $P < 0.01$.
TG = triglyceride; TC = total cholesterol; HDL-C = HDL-Cholesterol; HDL-C/TC = HDL-Cholesterol to total cholesterol ratio.

creasing level of dietary SHSO ($P < 0.001$). Plasma HDL-C concentration was unfavorably decreased with dietary SHSO supplementation ($P < 0.05$). HDL-C to TC ratio was beneficially significantly increased with dietary SHSO level in 3% and 5% SHSO treatments, but was not affected in 1.5% SHSO treatment compared with control ($P < 0.01$).

4. Discussion

The main measurements in the present study involved effects of SHSO supplementation with 0%, 1.5%, 3%, and 5% levels (0%, 0.3%, 0.6%, and 1.0% of CLA, respectively) on changes in animal performance, body composition, adipose depot and organ weight, and plasma lipid profiles in rats.

The observation that SHSO fed as a useful fat source containing CLA did not significantly affect growth performance of rat is consistent with previous reports that body weight and daily body weight gain was not significantly influenced by 0.5% [3] and 1.0% CLA [18] of diet to mice. In addition, this study observed that body fat content was decreased, but body protein content was increased without change in growth performance by SHSO feeding, and these results were consistent with the observation of DeLany et al. [16]. The changed body composition therefore caused reduction of adiposity index. West et al. [18] suggested no change in body weight by dietary CLA supplementation, indicating an increase in lean mass as well as a reduction in fat mass in the CLA-supplemented mice. Similarly, no significant changes in body weight reflected that body fat content and adipose depot weights were significantly decreased, but increased body protein content by SHSO feeding in this study. Previous studies reported that body fat and protein were significantly changed by CLA, particularly at the supplementation level higher than 0.5% of diet to mice [3,4,16]. In our present study, we also observed that body protein was significantly affected by a relatively low dose of SHSO (1.5–5.0% SHSO, which correspond to 0.3–1.0% CLA). On the other hand, the present result was contrasted with the previous findings, which showed the reduction of body protein in mice [4], and which reported no significant changes in body protein in mice by CLA feeding [16,19]. This discrepancy may be due to different experimental conditions such as animal model, diet composition, and level and isomer of CLA.

There are several possible mechanisms for the reduced body fat accumulation in response to CLA feeding. Two *in vitro* studies suggest that CLA might impact body composition in part by increasing lipolysis and β-oxidation of fatty acids, and reducing the deposition of fatty acids in adipose tissue [3], and different isomers of CLA might have different effects on body composition [20]. Park et al. [21] observed that the trans-10, cis-12 CLA isomer stimulated lipolysis, whereas, the cis-9, trans-11 and trans-9, trans-11 CLA isomers were ineffective in cultured adipocytes from mice. In addition, some *in vivo* studies have reported that effects could involve a reduction *de novo* lipogenesis, a reduction in use of preformed fatty acids for lipid synthesis, an increase in rates of lipolysis or some combination of these in pigs [19,22], and/or an increase in fat oxidation in mice [4]. Two studies demonstrated that adipose tissue mass decrease by CLA was mainly due to apoptosis of adipose tissue cells in mice [23], and that the reduction in adipose tissue mass in response to dietary CLA was accounted for a decrease in cell size rather than a change in cell number in rats [24]. In addition, it is implying that CLA inhibits lipid filling of adipocytes, and other studies have demonstrated an inhibitory effect of CLA *in vitro* on proliferation of 3T3-L1 preadipocytes [25,26].

The reduced body fat content associated with reduced regional adipose depot weights was found in the SHSO-supplemented groups of this study. The reductions in adipose depot weights from different sites, except for mesenteric, in response to supplementation of SHSO containing CLA were consistent with previous observations [4,16,18]. West et al. [4] showed that the reductions in adipose tissue in mice subsequent to CLA feeding were regardless of total fat levels in the diet. The authors also observed that different regional adipose depots responded differentially to the effects of CLA, and the weight of retroperitoneal depot was most sensitive to CLA [4]. In this study, weights of inguinal and epididymal depots were decreased 14% and 16%, 33% and 30% by 3% (equivalent to 0.6% CLA) and 5% (equivalent to 1.0% CLA) SHSO dietary supplementation, respectively. Similarly to the result of West et al. [4], retroperitoneal depot was decreased by dietary SHSO.

Unlike the results of West et al. [4], there was a significant increase in the weight of kidneys of rats fed the 5% SHSO. They only observed that the weight of mice was increased by high-fat diets compared with low-fat diets (1% *vs* 1.2% by weight). However, the present experimental diets were formulated to iso-oil, and then required further research for investigation. The increased testis weight in this study is contrasted to previous studies that found that the testis weight was not changed by dietary CLA supplementation [4,16]. This interesting finding may suggest that CLA is associated with spermatozoa, and further study on this subject is required. Previous works observed that CLA feeding caused increase in liver weight [4,16,23,27] due to liver fat accumulation [4,16]. This could be due to the increased delivery of fatty acids to the liver in response to CLA feeding [16]. In our present study, however, no significant increase in liver weight by dietary SHSO with CLA was seen with all treatments. Therefore, the present study suggested that dietary SHSO is not related to a potential negative effect on enlargement of liver.

Plasma TG, TC, and HDL-C/TC ratio were beneficially changed by dietary SHSO. Similar favorable modifications in plasma lipid profiles by CLA feeding were observed in several studies [7,8,28,29]. Particularly, CLA as 0.5–1.0% of the dietary feeding showed a beneficial effect in retarding

atherosclerosis [7,30]. Park et al. [3] suggested that the reduced serum TG may be related to CLA supplementation, which enhanced fatty acid β-oxidation in skeletal muscle and fat pad. Therefore, reduced plasma TG may be associated with reduced adipose depot weights in this study. However, Griffin [31] reported that serum TG is an independent risk factor for atherosclerosis. Although the HDL-C/TC ratio beneficially increased, plasma HDL-C concentration unfavorably decreased by SHSO supplementation. Reduced HDL-C concentration in this study is consistent with previous studies that showed that HDL-C was decreased by CLA feeding [32]. However, other studies have previously reported that HDL-C was not significantly changed by CLA feeding [7,8,28,33–35].

In conclusion, the findings of this study indicates that dietary SHSO containing about 21% of CLA isomers decreased content of body fat and adipose depots, but increased body protein content without growth performance changes. The reduced body fat is partially explained by the reduced adipose depot weight associated with SHSO treatment. In addition, the liver weight was not negatively increased by SHSO feeding. The present data also suggest that SHSO caused favorable plasma lipid levels reflecting decreased TG, TC, and HDL-C/TC ratios, although it induced an undesirable decrease in HDL-C. Finally, it should be emphasized that beneficial effects on the incidence of obese and atherosclerosis from SHSO with CLA were observed at as low as 3% dietary levels of SHSO (0.6% of CLA). The present data suggest that SHSO containing high CLA could be a useful fat source for the reduction of body fat accumulation and for the prevention of atherosclerosis.

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