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Supplementation of Conjugated Linoleic Acid with γ-Oryzanol for 12 Weeks Effectively Reduces Body Fat in Healthy Overweight Korean Women

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Abstract

The enhancement of the human body fat reduction of conjugated linoleic acid (CLA) with the supplementation of γ-oryzanol (OZ) was investigated on overweight Korean women (n=51, BMI> 23). Subjects were divided into 4 groups of control, CLA, glyceride form of CLA (GCLA), and CLA plus OZ (CLA-OZ). The soft-gel capsule (500 mg) was used to deliver control (500 mg olive oil), CLA (500 mg CLA), GCLA (500 mg GCLA) and CLA-OZ (500 mg CLA plus 50 mg OZ). Three capsules were taken twice a day for 12 weeks. The CLA-OZ supplementation reduced 1.35% body fat that was 0.34% enhancement against CLA supplementation. As considered subject variations, CLA-OZ reduced body fat ranged from 7.9% to -2.7%, equivalent to 5.6 kg loss to 0.7 kg gain in body fat mass, against CLA. The CLA-OZ reduced body weight and body mass index (BMI), relative to control, but the reductions by CLA-OZ were not different from those by CLA and GCLA. All biochemical markers analyzed for safty were not significantly different within or between groups and were within the normal range. The CLA-OZ supplementation significantly reduced blood pressure, as compared to the supplementation of CLA, GCLA and control. These results suggest that OZ could be a useful ingredient to mix with CLA for the reduction of human body fat.

Key words: conjugated linoleic acid (CLA), γ-oryzanol, body fat, body mass index (BMI), overweight Korean women

INTRODUCTION

Conjugated linoleic acid (CLA) was first identified from fried ground beef as an anticarcinogenic compound (1). Further research supported that CLA also acts as an anticarcinogenic nutrient (2-7). After anticancer activity of CLA was claimed, many studies focused on the beneficial health effects of CLA such as immune system stimulation (8,9), protection against arteriosclerosis (10,11), normalization of Type 2 Diabetes Mellitus (12), and body fat reduction (13). Among these biological effects of CLA, the body fat reduction activity is the most used function for commercial products. Many studies in experimental animals and human showed that body fat was reduced and lean body mass was increased by CLA supplementation (13-30). The mechanistic actions of CLA for body fat reduction were associated with the increased lipolysis in adipocytes and skeletal muscle cells (17-20), carnitine palmitoyl transferase activity in both fat and skeletal muscle (18,31), and hormone sensitive lipase activity in adipocytes (32). CLA also reduced heparin-releasable lipoprotein lipase activity and intracellular concentration of triacylglycerol and glycerol (15).

Currently, human clinical studies for the body fat reduction have been carried out for 3 months (26) and 12 months (29) towards obese Europeans (22,23,28). There are more concerns on body fat reduction by Korean women not only for the beauty, but for the control of diseases. Previously we examined the effectiveness of CLA supplementation for body fat reduction and safety by clinical assessment on obese Korean women with various levels of CLA (25). The results showed that daily intake of 2.25 g pure CLA was the most effective among tested dosages, which is lower than 3.4 g pure CLA of Europeans when tested for 3 months (22). Similar results in body fat reduction were observed between treatments of 2.25 g pure CLA against obese Korean women (25) and treatments of 3.4 g pure CLA against Europeans (22). However, one of these human studies does not mean that the efficacy of CLA in the body fat reduction is good enough to maintain healthy body condition. Hence, needs to enhance CLA activity

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of body fat reduction and lean body mass increase in obese Korean women prompted to search for substances in natural sources to meet the requirement.

The γ-oryzanol (OZ) is a phytochemical occurring naturally in rice bran having several biological activities such as antioxidant activity, inhibiting platelet aggregation, anti-atherogenic effect, lowering triglycerides, increasing HDL cholesterol levels, reducing cholesterol levels in the liver, inhibiting LDL cholesterol oxidation, maintaining estrogen balance in post-menopausal women, anti-mutagenic and anti-cancer properties, and improving lean body mass (33-39). The data providing evidence of the increase of lean body mass by OZ supplementation (34) have prompted to search for the synergistic effect of OZ on the body fat reduction of CLA.

In the present study, the supplementation effect of OZ with CLA on the body fat reduction and safety was investigated against obese Korean women.

MATERIALS AND METHODS

Materials

CLA and glyceride form of CLA (GCLA) were used as CLA sources manufactured by HK Biotech., Co. Ltd. (Jinju, Korea). Fatty acid composition of CLA and GCLA are in Table 1. The composition of CLA isomers was 37.95% *c*9,*t*11 CLA and 38.84% *t*10,*c*12 CLA in CLA sample, and 37.83% *c*9,*t*11 CLA and 38.55% *t*10,*c*12 CLA in GCLA. Both samples contained less than 3.0% *t,t* CLA isomers, and less than 21% other fatty acids including palmitic, stearic, oleic and linoleic acids.

Table 1. Composition of major fatty acids in the CLA and GCLA samples

Fatty acid[1)]	Treatment	
	CLA	GCLA
Plamitic (C16:0)	4.39[2)]	4.88
Stearic (C18:0)	1.32	1.55
Oleic (C18:1)	14.20	13.56
Linoleic (C18:2)	0.98	0.79
*c*9,*t*11 CLA	37.95	37.83
*t*10,*c*12 CLA	38.84	38.55
*c*9,*c*11 CLA	0.96	0.98
*t*9,*t*11 CLA	1.35	1.86
Total	100	100

[1)]Fatty acid methyl esters were prepared and analyzed by the method in reference (41). The conditions of gas chromatograph (Agilent ALS): column, Supelcowax-10 (60 m × 0.32 mm i.d.); initial oven temperatures, 180°C (0 min); final oven temperature, 200°C (30 min); and oven temperature programmed at rate of 2°C/min from 0 to 200°C.

[2)]Composition (%) was calculated by area ratios of fatty acids in CLA and GCLA samples. The CLA percent of total fatty acids is 79.11% in CLA and 79.22% GCLA samples.

OZ (98%) was purchased from Oryza oil & Fat Chemical. Co., Ltd. (Tokyo, Japan).

Soft gel capsule preparation

Four different soft gel capsules were prepared to provide for subjects. One capsule for control placebo group contained 500 mg olive oil, for CLA group contained 500 mg CLA, for GCLA group contained 500 mg GCLA, and for CLA-OZ group contained 500 mg CLA plus 50 mg OZ.

Human subjects

Healthy overweight Korean women volunteers (n=117, age 19～50 y) with the body mass index (BMI) of 23 or above were recruited by announcement on the web page of Seoul Women's University and Korea National Open University. All subjects were informed about this study by written consent. Subjects were excluded if they were pregnant, lactating, participating in a special dietary program, receiving drug therapy, or taking other dietary supplements for weight loss. Subjects were also excluded if they are in any abnormal clinical condition such as renal, liver, pancreatic, infectious diseases, hypertension, cardiac failure, malignant tumors, and active thyroid disease. The study was approved by the Institutional Review Board (IRB) of Seoul Women's University. The subjects (n=117) were randomly assigned to four groups of placebo control, CLA, GCLA, and CLA-OZ with 29～30 subjects per group. A total of 66 subjects was dropped over the course of the study, and at 12 weeks the dropped subject numbers were 18, 14, 19, and 15 in placebo control, CLA, GCLA, and CLA-OZ, respectively. Thus, data from 51 subjects were used for the analysis of this study. Large numbers of dropped subjects were not because of any adverse effects, but because of the fail to attend on the designated check up day.

Study design

The study was designed as a randomized, double-blinded, and placebo-controlled study. The subjects were randomly assigned to four groups of control (3.0 g olive oil/day), CLA (3.0 g CLA/day), GCLA (3.0 g GCLA/day), and CLA-OZ (3.0 g CLA plus 0.3 g OZ/day). The daily dosage (6 capsules/day) was divided into two servings (3 capsules each) taken at 30 minutes after meals. One hour running on the running machine for three days weekly was included in the study. The treatment period was 12 weeks.

Diet and exercise

The individual diet and exercise were assessed by questionnaire at 0, 3, 6, 9, and 12 weeks. Calorie and

nutrient intake of all participants were reported to researchers with a questionnaire providing information on the quantity and types of food consumed. The results were analyzed by Can-pro (Computer Aided Nutritional Analysis Program ver. 3.0); a specially designed software program that uses the basal metabolic equation to convert the food intake to caloric intake. The researchers gave subjects dietary advice of a general nature and guideline of exercise running 1 hr for 3 days a week at a health center.

Measurement of body composition by DEXA and anthropometric characteristics

Body fat mass (BFM) and lean body mass (LBM), were determined using Direct Digital Bone Densitometer (DEXA; Lunar Radiation Corp, Madison, WI, USA) with Lunar Prodigy software (version 5.6; Lunar Radiation Corp). Body weight was measured by Bio-electrical impedance fat analyzer (Inbody 3.0, Bio Space Co., Ltd., Seoul, Korea). Height and waist size were measured by a trained person for the consistent results. The measurements were performed at 0, 3, 6, 9, and 12 weeks.

Clinical assessment

Blood pressure, heart rate, electrocardiogram, and pulse were measured at Inje University Paik Hospital (Seoul, Korea) before the start of the program for screening the subjects. Urinalysis was performed for glucose, protein, bilirubin, and pH by Aution mini-4290 (ARKRAY, Japan). Blood samples were obtained from fasting subjects, and analyzed for biochemical clinical assessment of hemoglobin (Hb), hematocrit (Hct), white blood cell (WBC), and red blood cell (RBC) by auto blood analyzer (Advia 2120, Bayer, USA), Ca, P, Na, K, and Cl by Adiva (Adiva 1650, Bayer, Japan), insulin, leptin and thyroid-stimulating hormone (TSH) by γ-counter (COBRA 5010 Quantum, Packard, USA), HbAlc by Variant (Variant Ⅱ turbo bio-rad, Germany), and lipoprotein (a) [LP(a)] by Hitachi 7180 (Hitachi, Japan).

Energy expenditure calculation

Total energy expenditure (TEE) was calculated by the following equation at 0, 6, and 12 weeks: TEE=354-6.91 × age + PA [9.36 × body weight (kg) + 726 × hight (m)], where PA=1.0 (very low active), 1.12 (low active), 1.27 (active), and 1.45 (very active) (26).

Statistical analysis

Data were analyzed by Statistical Analysis System (SAS ver 9.1). Average values were used for the estimation of the value for distributed variables, and are given with standard deviation (SD). Differences among within and between treatment groups were analyzed with repeated measure ANOVA. Significance of differences within and between groups was obtained by ANOVA multiple range test ($p<0.05$ or 0.01).

RESULTS

Effects of CLA-OZ supplementation on body composition and anthropometric characteristics

Base line characteristics of the subjects participated ated in up to 12 weeks on weight and body composition are in Table 2. There were no significant differences between treatment groups for age, height, weight, BMI, BFM, LBM, and waist size at 0 week. The change in body weight and BMI of subjects after 12 weeks of treatment are in Table 3. Body weight and BMI were reduced most effectively in CLA-OZ group, which was assigned to receive 3.0 g/day of CLA and 0.3 g/day of OZ, whereas they were rather elevated in control and GCLA treatments. No significance in body weight and BMI was observed between CLA-OZ and CLA treatments.

Body composition of treated subjects was analyzed by DEXA and the data is shown in Table 4. CLA-OZ treatment most effectively reduced body fat, relative to CLA, GCLA, and control treatment groups. CLA-OZ treatment

Table 2. Baseline anthropometric characteristics for subjects in treatment groups

Parameter	Treatment[1)]			
	Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)
Age	29.50±2.89[2)]	26.33±2.35	29.50±2.70	27.64±2.41
Height (cm)	162.42±1.10	161.52±1.08	159.13±1.51	162.52±1.70
Weight (kg)	70.01±1.99	65.89±1.73	64.34±2.32	66.64±2.60
Waist (cm)	83.88±1.86	83.75±1.70	83.38±2.86	79.75±1.27
BMI (kg/m^2)	26.47±0.57	25.23±0.54	25.42±0.83	25.15±0.66
Fat (%)	34.14±0.98	34.93±1.02	34.53±1.11	34.30±1.06
SLM[3)] (kg)	43.51±1.31	40.30±0.84	39.58±1.16	41.15±1.37
BFM (kg)	23.92±1.02	23.16±1.13	22.35±1.42	23.01±1.43
LBM (kg)	46.07±1.37	42.73±0.88	41.99±1.22	43.63±1.44

[1)]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day) + OZ (0.3 g/day).
[2)]Mean±SE; no significance was observed between treatment groups for given parameters.

Table 3. Change in anthropometric parameters of overweight and obese women with treatments

Parameter	Week	Treatment[1)]			
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)
Weight (kg)	0	70.01±1.99[2)]	65.89±1.73	64.34±2.32	66.64±2.60
	6	70.66±2.02	65.79±1.83	64.06±2.68	65.86±2.41
	12	70.35±1.98	64.02±1.62	64.48±2.49	65.74±2.47
	Δ(12-0)[3)]	0.34±0.37	-1.87±0.38	0.14±0.78	-0.90±0.73
BMI (kg/m^2)	0	26.47±0.57	25.23±0.54	25.42±0.83	25.15±0.66
	6	26.54±0.63	25.18±0.60	25.54±1.02	24.84±0.61
	12	26.66±0.57	24.76±0.60	25.50±1.00	24.81±0.69
	Δ(12-0)	0.19±0.14	-0.47±0.15	0.08±0.29	-0.34±0.28

[1)]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day)+OZ (0.3 g/day).
[2)]Mean±SE. No significance was observed within and between treatment groups for given parameters.
[3)]Value at 12 week−Value at 0 week.

Table 4. Changes in body composition determined by DEXA in overweight and obese women with treatments

Parameter		Treatment[1)]			
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)
Fat (%)	Ave	-0.38±0.56[2)]	-1.01±0.40	-1.05±0.69	-1.35±1.03
	Range	-3.3～2.5	-3.7～0.6	-5.9～0.5	-11.6～3.3
BFM (Kg)	Ave	0.02±0.38	-0.36±0.32	-0.71±0.60	-1.14±0.75
	Range	-2.4～1.8	-2.4～1.6	-5.0～1.1	-8.0～2.3
LBM (Kg)	Ave	0.68±0.36	0.93±0.23	0.43±0.30	0.16±0.33
	Range	-0.13～2.7	-0.6～3.0	-1.5～1.7	-2.7～1.4

[1)]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day)+OZ (0.3 g/day).
[2)]Mean±SE of values taken by subtraction from 12 week to 0 week. No significance was observed between treatment groups for given parameters.

reduced 1.14 kg BFM, whereas CLA and GCLA treatments reduced only 0.36 and 0.71 kg BFM, respectively. Control treatment rather elevated 0.02 kg BFM. Subjects participated in CLA-OZ treatment showed body fat reduction up to 11.6% corresponding BFM 8.0 kg, while other subjects in the same treatment group rather gained body fat up to 3.3% corresponding BFM 2.3 kg. Although this indicates that there exists a large variation on individual response by CLA-OZ treatment, one can expect for the highest level of body fat reduction by CAL-OZ treatment. As compared to the other three groups, CLA-OZ was the most effective for body fat reduction. No difference was seen in LBM from all treatments. These results suggest that OZ enhances the body fat reduction in overweight women induced by CLA.

Effects of CLA-OZ treatment on safety

Clinical assessments, including blood pressure, urinalysis and blood analysis were carried out to determine safety of CLA supplementation with and without OZ. Table 5 shows the changes in systolic and diastolic blood pressures. The blood pressure of all treated subjects at 6 and 12 weeks were in the normal systolic and diastolic blood pressures, ranged from 110 to 116 and from 67 to 77, respectively. The changes in both systolic and diastolic blood pressures by CLA-OZ treatment were significantly, $p<0.05$, different from those by other treatments. Meanwhile, both systolic and diastolic blood pressures were reduced, $p<0.01$, in the subjects supple-

Table 5. Changes in blood pressure of overweight and obese women with treatments

Parameter	Treatment[1)]			
	Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)
Systolic BP (mmHg)	2.58±4.03[2)a]	2.21±6.45[a]	4.62±3.02[a]	-3.15±2.37[**b]
Diastolic BP (mmHg)	-1.53±1.94[a]	-1.61±2.14[a]	3.92±2.47[a]	-5.71±1.84[**b]

[1)]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day)+OZ (0.3 g/day).
[2)]Mean±SE of values taken by subtraction from 12 week to 0 week. [**]Significantly different from corresponding 0 week at $p<0.01$ within group. Means with different small superscript letters between treatments represent significant difference at $p<0.05$.

Table 6. Changes in serum lipid and uric acid levels of overweight and obese women with treatments

Parameter	Week	Treatment[1]				Normal range[2]
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)	
Total cholesterol (mg/dL)	0	194.85±8.52[3]	194.40±9.62	202.57±14.51	188.06±8.51	<220
	6	172.39±10.05	178.09±9.71	205.77±13.42	190.14±12.23	
	12	189.78±7.02	192.79±8.31	194.01±8.47	197.29±9.59	
	Δ(12-0)[4]	-5.08±4.88	-2.45±7.04	-11.51±17.95	13.17±6.16	
Triglyceride (mg/dL)	0	91.30±9.11	123.53±19.31	92.27±15.38	87.91±12.34	<200
	6	96.89±20.26	90.31±8.59	97.58±10.12	99.63±22.74	
	12	88.24±14.02	104.58±14.91	97.03±20.21	103.18±21.08	
	Δ(12-0)	-3.06±12.19	-21.34±17.34	-2.00±15.95	12.04±13.45	
LDL-cholesterol (mg/dL)	0	108.98±9.02	105.89±7.60	118.13±10.90	101.29±6.96	<160
	6	99.88±7.97	105.58±7.90	120.26±8.89	109.25±10.70	
	12	108.07±6.86	112.91±8.32	110.05±5.86	115.72±8.35	
	Δ(12-0)	-0.91±6.14	8.08±8.03	-8.19±12.97	18.18±6.39	
HDL-cholesterol (mg/dL)	0	67.61±3.94	63.81±3.33	65.99±3.27	69.19±3.20	>50
	6	53.13±3.27	54.45±2.60	56.29±3.65	60.96±4.26	
	12	64.06±3.08	58.97±3.07	64.56±5.27	60.94±2.64	
	Δ(12-0)	-3.55±3.36	-6.26±4.51	-2.93±6.33	-7.42±3.69	
Uric acid (mg/dL)	0	4.71±0.28	4.91±0.24	5.05±0.35	4.77±0.29	2.4～5.7
	6	4.67±0.39	4.10±0.26	4.34±0.44	4.68±0.27	
	12	4.70±0.28	4.31±0.25	4.32±0.43	4.47±0.24	
	Δ(12-0)	-0.01±0.29	-0.45±0.21	-0.92±0.24	-0.46±0.21	

[1]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day)+OZ (0.3 g/day).
[2]Korean Guidelines of Hyperlipidemia Treatment.
[3]Mean±SE. No significance was observed within and between treatment groups for given parameters.
[4]Value taken by subtraction from 12 week to 0 week.

mented with CLA-OZ, relative to the subjects with others, at 12 weeks, which could be a beneficial effect for people bearing high blood pressure problems. Further investigation on the effects of CLA-OZ on the lowering of blood pressure is in progress through continued research in our laboratory.

Changes in the serum lipid and uric acid levels after 12 weeks treatment are in Table 6. The levels of total cholesterol, triglyceride, and LDL-cholesterol were slightly higher in subjects supplemented with CLA-OZ as compared changes in subjects with other treatments, whereas the level of HDL-cholesterol was slightly lower. These values were in the normal range. The changes in these biochemical markers were not significantly different from treatment groups. The effects of CLA, GCLA, and CLA-OZ supplementations on the glucose metabolism and leptin concentration in blood serum are shown in Table 7. Fasting insulin level was reduced, $p<0.05$, in the subjects at 6 weeks after supplementation with CLA-OZ, relative to that in the subjects with CLA, GCLA and control, but it was back to 0 week level at 12 weeks supplementation. Glucose level was slightly increased, but was not significantly different. HbAlc was not affected by all treatments, and leptin concentration was slightly reduced by all treatments without significance. All of these biochemical markers were in the normal range. Subjects who participated in all treatment groups had no symptoms of abnormal glucose metabolism with the treatments for 12 weeks. No significant change in the serum mineral concentration was observed between treatments during course of the study (Table 8).

Changes in biochemical parameters related to the liver function by CLA-OZ supplementation for 12 weeks are shown in Table 9. There were no significant changes in those biochemical parameters of total protein, albumin, GOT, GPT, alkaline phosphatase, and bilirubin by CLA supplementation with and without OZ. They were in the normal range. Therefore, we conclude that CLA supplementation with or without OZ has no significant effect on liver function of those whose liver function normally. Comparison between groups on blood urea nitrogen (BUN) and creatinine concentrations, which are biochemical markers of kidney function, 12 weeks after treatment are shown in Table 10. BUN and creatinine levels were in the normal range, indicating that no adverse effect on kidney function of normal healthy person by supplementation of CLA with and without OZ.

Influence of CLA supplement with and without OZ on blood cell counts, Hct and Hb levels are shown in Table 11. There were no significant differences between the treatment groups in RBC count, WBC count, Hb and Hct levels. All subjects in groups were in normal range.

Table 7. Changes in serum glucose, insulin, HbA1c and leptin levels of overweight and obese women with treatments

Parameter	Week	Treatment[1)]				Normal range[2)]
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)	
Glucose (mg/dL)	0	87.88±1.98[3)]	84.51±1.68	81.34±1.65	82.24±1.55	<126
	6	84.89±3.17	82.67±1.61	83.77±1.50	88.56±3.13	
	12	91.23±2.73	88.62±2.37	89.44±3.97	84.68±7.54	
	Δ(12-0)[4)]	4.35±1.49	5.42±1.87	9.00±3.89	2.12±7.40	
Fasting insulin (μIU/mL)	0	5.85±0.70	7.44±1.45	4.97±1.07	4.92±0.82	<22
	6	3.58±1.08	4.35±1.61	3.88±0.59	2.99±0.64*	
	12	5.03±1.07	8.64±3.38	4.34±0.94	6.61±2.25	
	Δ(12-0)	-0.82±1.42	1.05±3.15	-1.00±1.77	1.62±2.15	
HbA1c (%)	0	5.38±0.15	5.62±0.44	5.30±0.12	5.31±0.06	3.5～6.5
	6	5.45±0.08	5.78±0.54	5.38±0.12	5.39±0.05	
	12	5.31±0.10	5.23±0.08	5.27±0.15	5.20±0.07	
	Δ(12-0)	-0.07±0.13	-0.03±0.05	-0.07±0.04	-0.10±0.05	
Leptin (ng/mL)	0	13.41±1.75	12.83±0.79	13.24±3.31	11.61±1.30	3.7～11.1
	6	13.31±1.71	10.81±1.30	11.27±2.98	10.38±1.61	
	12	13.29±1.54	11.37±1.22	9.61±0.92	10.47±1.78	
	Δ(12-0)	-0.12±1.52	-1.88±1.21	-3.92±3.04	-0.92±1.27	

[1)]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day)+OZ (0.3 g/day).
[2)]Korean Guidelines of Hyperlipidemia Treatment.
[3)]Mean±SE. *Significantly different from corresponding 0 week at $p<0.01$ within treatment group. No significance was observed within and between treatment groups for given parameters, otherwise mentioned.
[4)]Value taken by subtraction from 12 week to 0 week.

Table 8. Changes in serum mineral levels of overweight and obese women with treatments

Parameter	Week	Treatment[1)]				Normal range[2)]
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)	
Ca (mg/dL)	0	9.35±0.11[3)]	9.36±0.09	9.23±0.10	9.29±0.08	8.1～10.5
	6	9.25±0.11	9.44±0.07	9.42±0.08	9.37±0.07	
	12	9.29±0.07	9.29±0.06	9.18±0.11	9.36±0.09	
	Δ(12-0)[4)]	-0.06±0.08	-0.03±0.08	-0.04±0.14	0.05±0.08	
Na (mmol/L)	0	141.42±0.60	141.07±0.67	141.20±0.70	142.43±0.58	135～145
	6	143.73±0.85	142.73±0.71	141.80±1.24	142.23±0.82	
	12	138.00±0.58	139.00±0.58	138.44±0.87	138.62±0.54	
	Δ(12-0)	-3.42±0.76	-2.58±0.97	-2.89±1.33	-3.69±0.52	
K (mmol/L)	0	4.28±0.16	4.31±0.10	4.19±0.13	4.24±0.10	3.5～5.3
	6	4.36±0.21	4.29±0.12	4.58±0.13	4.35±0.11	
	12	4.36±0.13	4.52±0.12	4.36±0.17	4.53±0.13	
	Δ(12-0)	0.08±0.20	0.23±0.14	0.12±0.23	0.26±0.17	
Cl (mmol/L)	0	102.83±0.56	103.27±0.64	102.40±1.18	103.71±0.37	98～110
	6	105.64±0.31	104.53±0.52	103.30±1.33	103.77±0.57	
	12	103.08±0.34	103.33±0.84	103.22±0.74	103.08±0.46	
	Δ(12-0)	0.25±0.69	0.00±0.84	0.67±1.48	-0.69±0.66	
P (mg/dL)	0	3.64±0.16	3.99±0.13	3.94±0.12	3.63±0.18	2.5～5.0
	6	3.74±0.19	3.93±0.13	3.79±0.13	3.86±0.11	
	12	3.81±0.19	3.86±0.14	3.74±0.11	3.83±0.13	
	Δ(12-0)	0.17±0.18	-0.03±0.16	-0.22±0.09	0.14±0.14	

[1)]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day)+OZ (0.3 g/day).
[2)]Korean Guidelines of Hyperlipidemia Treatment.
[3)]Mean±SE. No significance was observed within and between treatment groups for given parameters, otherwise mentioned.
[4)]Value taken by subtraction from 12 week to 0 week.

Changes in concentration of TSH, which is a biomarker of thyroid function, and LP(a) of subjects at 12 weeks are in Table 12. The average values of TSH and LP(a) were in the range of normal and changes in values between treatment groups had no significant difference and all values were in the normal range. From the results

Table 9. Changes in biochemical parameters related to liver function of overweight and obese women with treatments

Parameter	Week	Treatment[1]				Normal range[2]
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)	
Total protein (g/dL)	0	7.93 ± 0.83[3]	8.12 ± 0.10	7.76 ± 0.26	8.12 ± 0.15	6.6 ~ 8.5
	6	6.06 ± 0.88	7.07 ± 0.43	6.98 ± 0.71	6.86 ± 0.80	
	12	6.28 ± 0.85	7.57 ± 0.57	8.01 ± 0.15	8.00 ± 0.15	
	Δ(12-0)[4]	-1.65 ± 0.80	-0.67 ± 0.62	0.22 ± 0.35	-0.05 ± 0.22	
Albumin (g/dL)	0	4.73 ± 0.07	4.80 ± 0.07	4.56 ± 0.13	4.75 ± 0.07	3.5 ~ 5.0
	6	4.59 ± 0.09	4.65 ± 0.07	4.68 ± 0.11	4.64 ± 0.10	
	12	4.80 ± 0.07	4.88 ± 0.12	4.86 ± 0.12	4.96 ± 0.05	
	Δ(12-0)	0.07 ± 0.06	0.06 ± 0.10	0.30 ± 0.18	0.25 ± 0.08	
GOT (U/L)	0	19.35 ± 0.99	18.60 ± 1.26	21.74 ± 3.61	21.09 ± 1.71	=31
	6	17.77 ± 0.93	16.57 ± 1.36	14.79 ± 1.11	16.99 ± 0.83	
	12	19.64 ± 1.49	18.03 ± 1.51	16.99 ± 1.16	19.96 ± 1.69	
	Δ(12-0)	0.29 ± 1.24	-0.75 ± 1.27	-5.84 ± 4.52	-1.31 ± 1.43	
GPT (U/L)	0	15.91 ± 1.06	15.36 ± 2.20	17.65 ± 2.60	18.94 ± 4.16	=31
	6	14.51 ± 1.38	16.40 ± 3.34	14.51 ± 1.78	12.19 ± 1.26	
	12	19.20 ± 3.32	15.63 ± 3.05	14.34 ± 1.43	14.52 ± 1.37	
	Δ(12-0)	3.29 ± 2.74	0.41 ± 1.53	-4.38 ± 2.51	-4.86 ± 4.24	
Alkaline phosphatase (U/L)	0	62.08 ± 3.44	66.60 ± 4.26	59.25 ± 5.58	60.44 ± 3.03	39 ~ 117
	6	59.62 ± 5.63	62.04 ± 4.16	66.06 ± 7.44	57.64 ± 5.23	
	12	58.42 ± 3.80	60.19 ± 4.52	58.68 ± 6.03	57.92 ± 2.60	
	Δ(12-0)	-3.66 ± 1.96	-4.60 ± 2.54	-1.61 ± 2.91	-1.93 ± 2.29	
Bilirubin (mg/dL)	0	0.83 ± 0.08	0.87 ± 0.11	0.96 ± 0.10	0.88 ± 0.06	0.2 ~ 1.2
	6	0.94 ± 0.10	0.97 ± 0.14	0.78 ± 0.05	0.93 ± 0.12	
	12	1.11 ± 0.15	1.04 ± 0.10	0.97 ± 0.17	1.10 ± 0.12	
	Δ(12-0)	0.26 ± 0.19	0.22 ± 0.18	-0.06 ± 0.19	0.24 ± 0.12	

[1]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day) + OZ (0.3 g/day).
[2]Korean Guidelines of Hyperlipidemia Treatment.
[3]Mean ± SE. No significance was observed within and between treatment groups for given parameters, otherwise mentioned.
[4]Value taken by subtraction from 12 week to 0 week.

Table 10. Changes in biochemical parameters related to renal function of overweight and obese women with treatments

Parameter	Week	Treatment[1]				Normal range[2]
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)	
BUN (mg/dL)	0	14.39 ± 1.22[3]	14.84 ± 1.26	13.90 ± 1.33	12.98 ± 1.47	4.5 ~ 23.5
	6	11.45 ± 1.02	11.33 ± 1.20	12.57 ± 1.51	13.31 ± 0.88	
	12	16.89 ± 1.41	13.68 ± 1.03	13.83 ± 0.89	15.36 ± 1.88	
	Δ(12-0)[4]	2.50 ± 2.07	-1.06 ± 2.02	-0.00 ± 1.93	2.81 ± 2.49	
Creatinine (mg/dL)	0	0.98 ± 0.02	0.97 ± 0.02	0.94 ± 0.03	1.01 ± 0.02	0.7 ~ 1.3
	6	0.93 ± 0.04	0.86 ± 0.02	0.96 ± 0.03	0.94 ± 0.02	
	12	0.88 ± 0.02*	0.91 ± 0.03	0.87 ± 0.02	0.91 ± 0.02*	
	Δ(12-0)	-0.10 ± 0.03	-0.06 ± 0.03	-0.08 ± 0.05	-0.09 ± 0.02	

[1]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day) + OZ (0.3 g/day).
[2]Korean Guidelines of Hyperlipidemia Treatment.
[3]Mean ± SE. *Significantly different from corresponding 0 week at $p<0.05$ within group. No significance was observed within and between treatment groups for given parameters, otherwise mentioned.
[4]Value taken by subtraction from 12 week to 0 week.

of this study, CLA supplementation with and without OZ did not affect on the thyroid function.

Effects of CLA-OZ treatment on daily energy intake and energy expenditure

Daily energy intake and energy expenditure of treatment groups are in Table 13. Daily energy intake of CLA-OZ group was decreased, $p<0.05$, at week 12, but placebo control and other groups also decreased. Food intake of subjects supplemented with control, CLA, GCLA and CLA-OZ was decreased by about 300 kcal, but no significance was seen from all treatment groups. These results might be, in part, attributed to the perform-

Table 11. Changes in blood cell counts, hemoglobin and hematocrit levels of overweight and obese women with treatments

Parameter	Week	Treatment[1)]				Normal range[2)]
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)	
RBC (10^6/μL)	0	4.52±0.07[3)]	4.51±0.08	4.52±0.09	4.41±0.08	3.82～5.4
	6	4.37±0.11	4.40±0.08	4.59±0.11	4.36±0.10	
	12	4.39±0.05	4.50±0.09	4.55±0.07	4.38±0.09	
	Δ(12-0)[4)]	-0.13±0.08	0.00±0.08	-0.01±0.09	-0.03±0.06	
WBC (10^3/μL)	0	5.84±0.49	6.73±0.44	7.72±1.09	6.60±0.42	4.2～11.0
	6	6.04±0.98	6.90±0.49	7.08±0.47	6.23±0.38	
	12	5.96±0.42	5.96±0.39	7.29±0.44	6.29±0.62	
	Δ(12-0)	0.11±0.50	-0.46±0.44	-0.69±1.15	-0.32±0.62	
Hct (%)	0	42.26±0.85	42.17±0.62	42.04±1.01	42.75±0.85	36～48
	6	41.40±0.99	40.86±0.50	42.59±1.37	42.16±0.88	
	12	40.58±0.47	41.24±0.65	41.72±1.06	41.46±0.66	
	Δ(12-0)	-1.68±0.65	-1.00±0.48	-0.68±0.92	-1.14±0.56	
Hb (g/dL)	0	13.74±0.32	13.70±0.20	13.70±0.40	13.75±0.26	12～16
	6	13.35±0.31	13.41±0.18	13.87±0.52	13.56±0.27	
	12	13.42±0.23	13.77±0.26	13.72±0.47	13.68±0.28	
	Δ(12-0)	-0.33±0.22	0.13±0.28	-0.07±0.24	-0.03±0.23	

[1)]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day)+OZ (0.3 g/day).
[2)]Korean Guidelines of Hyperlipidemia Treatment.
[3)]Mean±SE. No significance was observed within and between treatment groups for given parameters, otherwise mentioned.
[4)]Value taken by subtraction from 12 week to 0 week.

Table 12. Changes in thyroid function and LP(a) in overweight and obese women with treatments

Parameter	Week	Treatment[1)]				Normal range[2)]
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)	
TSH (μIU/mL)	0	2.80±0.34[3)]	2.52±0.68	2.15±0.24	2.20±0.40	0.46～4.68
	6	1.79±0.18	2.05±0.27	2.67±0.51	1.72±0.30	
	12	2.74±0.47	2.07±0.29	2.21±0.18	1.89±0.21	
	Δ(12-0)[4)]	-0.07±0.39	-0.52±0.73	0.00±0.21	-0.04±0.27	
Lp(a) (mg/dL)	0	13.86±4.91	9.01±4.31	13.00±3.75	14.56±3.87	<30
	6	16.19±5.84	8.18±3.46	14.56±4.60	14.40±4.30	
	12	13.43±4.60	8.58±4.40	12.61±3.92	14.56±4.14	
	Δ(12-0)	-0.43±1.94	-1.68±1.12	-1.26±0.45	-1.02±1.19	

[1)]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day)+OZ (0.3 g/day).
[2)]Korean Guidelines of Hyperlipidemia Treatment.
[3)]Mean±SE. No significance was observed within and between treatment groups for given parameters, otherwise mentioned
[4)]Value taken by subtraction from 12 week to 0 week.

ance of one hour exercise for three days weekly for 12 weeks. Meanwhile, some reduction of energy expenditure in CLA-OZ group was observed, but no significance could be claimed.

DISCUSSION

This study has shown that CLA mixed with OZ reduced the body fat of healthy overweight Korean women with no adverse effect, relative to CLA or GCLA. The efficacy of CLA-OZ in body fat reductions may not be affected by other fatty acids than CLA, considering that no report of other fatty acids (palmitic, stearic, oleic, linoleic acids) contained in CLA-OZ on the body fat reduction has been found like as CLA. The accumulated data from animal experiments and clinical studies proved that CLA is effective on the body fat reduction for both animals and humans (15-30), but no report on the combined effects of CLA and OZ is seen in literature. Previous study on the body fat reduction by CLA supplementation with different daily dosages for healthy overweight Korean women showed 3.0 g of CLA sample, corresponding to 2.25 g pure CLA, was the most effective in terms of body fat reduction and lean body mass increase (25).

Recently, efforts are given for enhancement of CLA activity in body fat reduction by supplementation with other substances. The OZ is one substance that we have found to have the expected activity among natural in-

Table 13. Changes in daily energy intakes and total energy expenditure of overweight and obese women with treatments

Parameter	Week	Treatment[1)]			
		Control (n=12)	CLA (n=15)	GCLA (n=10)	CLA-OZ (n=14)
Energy (kcal)	0	1587.16±94.00[2)]	1553.16±106.85	1520.00±109.13	1685.48±80.51
	6	1444.87±118.84	1200.28±83.02	1516.85±178.85	1222.33±161.13
	12	1253.80±81.23	1239.98±70.58	1215.86±90.40	1326.25±85.34*
	Δ(12-0)[3)]	-333.35±90.32	-273.39±107.34	-304.14±137.35	-310.99±107.82
Protein (g)	0	64.22±5.49	58.01±4.86	61.08±3.93	66.20±3.46
	6	58.35±6.13	46.35±2.71	54.61±5.81	43.41±4.70
	12	48.13±3.24	50.85±3.36	49.53±4.45	54.62±5.27
	Δ(12-0)	-16.09±4.94	-4.62±4.39	-11.55±5.86	-8.96±5.76
Fat (g)	0	47.62±3.88	42.68±3.96	45.56±4.43	48.78±3.06
	6	38.85±5.19	34.96±2.42	42.11±5.52	32.42±5.47*
	12	33.59±2.51	34.00±2.90	35.91±4.75	39.38±3.65
	Δ(12-0)	-14.03±3.65	-6.77±5.24	-9.65±5.47	-7.83±5.24
Carbohydrate (g)	0	225.83±12.65	235.76±14.28	214.35±15.69	229.38±16.64
	6	212.90±13.07	177.18±17.06	214.09±37.36	189.28±25.45
	12	184.51±11.52	180.71±11.82	168.82±9.57	192.03±11.02
	Δ(12-0)	-41.32±14.76	-51.81±15.09	-45.52±18.93	-30.67±22.18
Fiber (g)	0	16.41±1.28	17.61±1.56	15.28±1.21	16.61±1.32
	6	14.45±0.86	13.11±1.35	14.17±1.58	12.84±1.68
	12	13.75±1.48	13.21±1.14	13.54±1.11	15.25±1.69
	Δ(12-0)	-2.66±1.37	-3.82±1.57	-1.74±2.02	-0.27±2.54
Cholesterol (mg)	0	295.40±30.14	271.92±35.26	258.20±41.09	352.92±44.08
	6	280.84±61.97	217.29±32.33	215.37±35.70	235.74±51.95
	12	226.08±22.48	254.06±28.69	246.90±34.52	255.91±35.52
	Δ(12-0)	-69.32±40.04	-9.15±49.66	-11.30±39.48	-76.37±60.19
Total Energy Expenditure (kcal)	0	2455.34±42.52	2427.23±39.76	2382.19±31.17	2453.66±52.57
	6	2469.94±42.66	2425.98±39.55	2367.03±29.66	2444.32±50.62
	12	2459.40±43.41	2389.49±37.48	2383.85±28.45	2442.88±50.42
	Δ(12-0)	4.06±4.44	-1.21±4.43	1.66±9.29	-10.78±8.70

[1)]Control, olive oil (3 g/day); CLA, CLA (3 g/day); GCLA, GCLA (3 g/day); and CLA-OZ, CLA (3 g/day)+OZ (0.3 g/day).
[2)]Mean±SE *Significantly different from corresponding 0 week at $p<0.05$ within group. No significance was observed within and between treatment groups for given parameters, otherwise mentioned.
[3)]Value taken by subtraction from 12 week to 0 week.

gredients tested in a preliminary experiment (25). In the present study, CLA-OZ was the most effective treatment for body fat reduction when compared with placebo control, CLA and GCLA treatments. Although CLA-OZ supplementation did not significantly reduce the body fat in average due to a large variation of the efficacy among individual subjects, one can expect at most 11.6% body fat reduction 12 weeks after treatment of CLA-OZ, while 5.9% and 3.7% from CLA and GCLA, respectively. Mice experiments were carried out with similar treatments, and the results were in accordance with this study (25). The enhanced body fat reducing activity of CLA by mixing with OZ might be, in part, attributed to biological activities of OZ related to lipid metabolism (36,38) and improving lean body mass (34), but further detailed studies for the mechanistic action should be done.

Efficacy of CLA on body fat reduction in healthy overweight Koreans is not much different from previous clinical studies performed on Europeans and Americans (22,23,28). In the 12 week clinical study (22) with various amounts of CLA (3.0 g, 4.5 g, 6 g, and 9 g CLA sample/day, equal to 1.7 g, 3.4 g, 5.1 g, and 6.8 g, respectively, pure CLA/day), performed on European subjects, body fat mass was most effectively reduced by a treatment of 3.4 g pure CLA/day exhibiting 5.7% body fat reduction. Meanwhile, in the present study, the supplementation of a mixture of 3.0 g CLA (2.25 g pure CLA) and 0.3 g OZ to obese Korean women subjects for 12 weeks resulted in the reduction of 2.03% body fat in average, which is 0.34% enhancement in body fat reduction against the supplementation of CLA. As considered the efficacy in subject variations, OZ reduced body fat ranged from 7.9% to -2.7%, equivalent to 5.6 kg loss to 0.7 kg gain in body fat mass, against CLA. This different results could be explained by the differences in body fat mass and body weight between Europeans (30.8～34.7 kg and 79.8～90.1 kg, respectively) and Korean women (average of 22.4～23.9 kg and 64.3～70.0 kg, respectively), and by differences in basal metabolism and life style, who participated in

studies.

Biochemical parameters analyzed for the safety assessment of CLA-OZ were insignificantly elevated or decreased within treatment groups, and their values were in normal range. Fasting insulin levels in CLA-OZ treatment group 6 weeks after treatment was significantly decreased, but elevated to the level of 0 week at 12 weeks . The significant correlation between fasting blood glucose level and body weight change by CLA supplementation has been documented (23), but CLA supplementation showed different responses on glucose metabolism with diabetics and non diabetics. Hence, interpretation on the effects of CLA supplementation in glucose metabolism is more complicated. It is interesting to note that OZ treatment with CLA significantly lowered systolic and diastolic blood pressures, relative to control and CLA treatments without OZ. This finding is very significant because in most cases, an increase in blood pressure occurs coincidently with obesity. CLA treatment with and without OZ lowered uric acid in blood serum at 12 weeks after treatment with the highest reduction in GCLA group. The reason for this can not be explained at this time, and no reports are found at this point. Lowering uric acid by CLA supplementation could be a beneficial factor. The present data from subjects treated with CLA with and without OZ agreed with previous studies for the safety of CLA supplementation on human subjects for 1 year report that adverse events were not differ significantly between the treatment group and placebo control group (23).

Since CLA and OZ are naturally occurring compounds in food sources and human body fat, both compounds are considered to be safe for use as dietary supplements. The *c*9,*t*11CLA in Korean women's breast milk was quantitatively determined, and the typical data showed it contained 4.32～10.12 mg/100 mL, which is much higher than the amount determined in infant formulas (40). Considering that CLA and OZ are naturally occurring compounds and overall results from the present clinical assessments indicate CLA supplementation with OZ against healthy overweight Korean women is effective in body fat reduction, relative to CLA alone, and safe.

In conclusion, the body fat reducing activity of CLA on healthy overweight Korean women was enhanced by 10% (w/w) OZ supplementation in 3 g CLA per day for 12 weeks with no adverse effects. The mechanistic action for these results could be clarified.

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