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Dietary Conjugated Linoleic Acid did not Affect on Body Fatness, Fat Cell Sizes and Leptin Levels in Male Sprague Dawley Rats*

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This study was designed to observe the effect of conjugated linoleic acid (CLA) supplementation on body fatness, fat cell sizes and leptin levels in male Sprague Dawley rats. Following weaning, forty rats were divided into 4 groups beef tallow (BT), fish oil (FO). beef tallow with CLA supplementation (BTC), and fish oil with CLA supplementation (FOC) group. For four weeks, all rats were fed experimental diets containing 12% of total dietary fat (w/w) with or without 1% CLA. After 4 weeks, the animals were sacrificed; the total carcass fat, plasma leptin levels, epididymal fat pad weights and fat cell sizes in adipose tissue were measured. CLA supplementation did not significantly affect the rat's body weights, total body fat, epididymal fat pad weights, and fat cell sizes. CLA also did not have a significant effect on plasma leptin levels.These results suggest that CLA supplement was not an effective way to reduce the body weights of male Sprague Dawley rats.

Key words : conjugated linoleic acid, body fatness, fat cell size, leptin, rats

INTRODUCTION

Obesity has been a nutritional problem in the western world for a long time, and it is emerging as a problem in Korea; this trend is widely attributed to changes in dietary habits occurring with economic development. Increases in obesity in Korea are apparent through the Korean Nutrition Survey; in the 1995 survey,[1] 20.5% of the subjects who were 20 years and over had Body Mass Index (BMI) values in excess of 25, compared to the 17.1% and 19.6% reported in 1991[2] and 1992,[3] respectively. Obesity is an underlying cause as well as a risk factor[4-6] for diabetes mellitus, hypertension, and hyperlipidemia, as well as for cardiovascular diseases. Thus, the prevention of obesity is becoming an important issue, and deserves a lot of attention in the future.

Conjugated linoleic acid (CLA) is an umbrella term for all linoleic acids with double bonds in different locations. as well as all isomers. Numerous studies[7-14] have reported that CLA given to mice and obese persons resulted in an increased body mass and a decreased body fat. However, it has been also observed that CLA given to rats exerted no effects on body weights or fat cell numbers, while the size of fat cells was affected.[17,18] It appears that the results of various studies carried out so far on the role of CLA in reducing body fat are not consistent, and research on CLA's biochemical mechanism is limited.

Leptin is produced and secreted in fat cells as a result of instructions received from the obesity gene. Leptin is produced in amounts which vary according to an individual's nutritional status, and functions as a satiety regulator[19] by stimulating the satiety center in the hypothalamus of the brain. Leptin also increases thermogenesis and physical activities, and decreases food intakes, there by reducing body weights and body fat.[19-21] However, further research is needed on the role of leptin and its relation to internal body fat.

Thus, the present research was designed to study the effects on the body fat, fat cell sizes, and plasma leptin levels of growing rats of CLA supplementation to diets based on two different dietary fats (beef tallow and fish oil).

MATERIALS AND METHODS

1. Experimental design

Forty 3-week old male Sprague Dawley rats, each with a body weight of approximately 47g, were given a laboratory chow diet for 3 days as an adaptation period; the rats were then randomly divided into 4 groups, with the 10 rats in each group following the same experimental diet for a period of four weeks. The BT group was fed a beef tallow diet with no CLA supplementation; the BTC group was fed a beef tallow diet with CLA supplementation; the FO group was fed a fish oil diet with

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no CLA supplementation; and the FOC group was fed a fish oil diet with CLA supplementation.

2. Experimental diets

The experimental diets were composed of 20.9% protein, 53.5% carbohydrate, and 25.6% fat (12% w/w) in terms of their contribution to total calories, and other nutrients were identical (Table 1). The two sources of dietary fat were beef tallow (BT), containing saturated fatty acids, and fish oil (FO) containing docosahexaenoic acid (DHA), an n-3 fatty acid. CLA (prepared by Kyung-Sang University), chemically synthesized from safflower oil, was added to the diets of the CLA-supplementation groups at 1% (w/w). The purity of the CLA was 80%, and the CLA contained 50% cis-9, trans-11 CLA and 50% of trans-10, cis-12 CLA. As both beef tallow and fish oil are lacking in essential fatty acids, corn oil which is rich in n-6 linoleic acid was also added. In order to prevent oxidation of the fish oil, dl-α-tocopherol (145.6 mg/100g oil) was added to the fish oil diets. The animals were kept under 12 hour dark and light cycle conditions, and food and water were given *ad libitum*. Body weights were measured at the same designated time once a week.

Table 1. Diet composition of experimental groups (g/100g diet)

Ingredients	Dietary groups[1]			
	BT	BTC	FO	FOC
Corn Starch	56.50	56.50	56.50	56.50
Casein	22.00	22.00	22.00	22.00
L-methionine	0.30	0.30	0.30	0.30
Cellulose	4.00	4.00	4.00	4.00
Fat or Oil				
Beef tallow	9.64	8.39	0.00	0.00
Corn oil	2.36	2.36	2.61	2.61
Fish oil	0.00	0.00	9.39	8.14
CLA[2]-rich oil	0.00	1.25	0.00	1.25
Mineral mix[3]	4.00	4.00	4.00	4.00
Vitamin mix[4]	1.00	1.00	1.00	1.00
Choline bitartrate	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00

1) BT : Beef tallow, BTC : Beef tallow with CLA supplementation, FO : Fish oil, FOC : Fish oil with CLS supplementation
2) CLA rich oil contained 80% of total conjugated linoleic acid (50% c9t11, 50% t10c12)
3) AIN 76 mineral mixture
4) AIN 76 Vitamin mixture

3. Sample collections

After 4 weeks on their respective experimental diets, animals were fasted for 12 hours. The animals were anaesthetized with ethyl ether, and blood was collected from the inferior vena cava into test tubes pre-treated with heparin. The blood was centrifuged at 800×g for 15min at 4℃; the plasma was then collected from the supernatant and was stored at -70℃ for further analysis.

Epididymal fat pads were removed, and washed in saline solution (0.9% NaCl). After the solution water was blotted dry, the pads were weighed. One gram of fat was kept in 4% formalin solution in order to determine the sizes of fat cells. The carcass was kept at -40℃ for body fat determination.

4. Biochemical analysis

(1) Triglyceride and leptin levels in the plasma

The measurement of triglycerides was done by using a kit provided by Young-Dong pharmaceutical company. Leptin was measured by using the RIA kit (Linco Research, Inc).

(2) Size and distribution of fat cells in the epididymal fat pads

The epididymal fat pads were dyed with hematocyrine eosin(HE), and an image analyzer (Bioquant, RND, Nashville, TN) was used to determine the size and distribution of fat cells.

(3) Body fat in carcass

For the determination of body fat, the carcass was prepared by using the modified method of Mickelsen & Anderson.[22] The frozen carcass was autoclaved at 15lb pressure for 60 minutes. Then, distilled water (2.5 times the weight of the carcass) was added to the hot carcass, and the mixture was blended at low speed. The mixture was filtered by using a strainer (425㎛ mesh size), and was washed by distilled water. Rubber spatulas were used for thorough collection of the filtrate, and the volume of the total filtrate was measured. Lipids were extracted from the filtrate by using the method of Brian and Dyer.[23] Five ml of filtrate was placed in a separate flask. A solution of 26ml Methanol : Chloroform (1 : 1) was added to the filtrate, and was shaken. After standing for an hour, 7 ml of distilled water was added and well shaken, and was left for 24 hours. After the lower chloroform layer was removed into a cylinder, 13ml of chloroform was added to the supernatant for the second lipid extraction. After the resulting lower layer was added to the cylinder containing the first chloroform extract, the combined volume of the extracts were measured, and 10 ml was dried by N_2 gas for determination of lipids.

5. Statistical analysis

All the results were analyzed by using the general linear model (GLM) of the Statistic Analysis System (SAS) program. Duncan's multiple range test was used to determine the statistical differences at $p<0.05$. Two-way ANOVA-unbalanced design was used to determine the effects of CLA and of different dietary fats. All results were expressed by using mean±standard deviation.

RESULTS

1. Food intakes and body weight gains

Table 2 shows the average daily food intakes, food efficiency ratios and body weight gains of the rats after 4 weeks on their respective diets. Weight gains were similar in the groups with CLA supplementation (BTC and FOC) and without supplementation (BT and FO). However, the groups fed beef tallow (BT and BTC) showed significantly lower weight gains compared to the groups given fish oil (FO and FOC).

2. Plasma triglyceride and leptin concentrations

As shown in Table 3, no differences were observed in plasma triglyceride and leptin levels among the groups after 4 weeks, regardless of CLA supplementation and of the different dietary fats.

3. Size and distribution of fat cells

There were no differences in fat cell sizes among the four groups after 4 weeks of respective experimental diets (Table 3). The distribution of fat cell sizes is illustrated in Fig 1. When the fat cells were divided into 3 groups according to their sizes, 63 - 78% of fat cells in all the animals were small (26-55μm in diameter), 22 - 36% were medium (56 - 95 μm in diameter), and almost none were large (96-115μm in diameter).

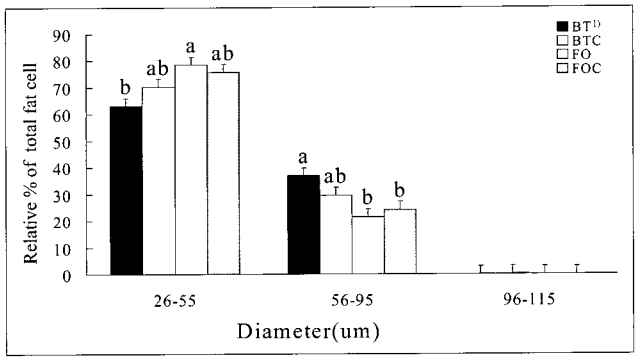

Fig 1. Comparison of the size distribution of fat cell in rats fed experimental diet for 4 weeks

1) BT : Beef tallow, BTC : Beef talloow with CLA supplemmentation, FO : Fish oil, FOC : Fish oil with CLA supplementation
Bars sharing common letters are not significantly different at $p<0.05$

Table 2. Mean food intakes, body weight gains and food efficiency in 4 week feeding experiment

Dietary groups[1)]	Food intakes	Body weight changes			Food efficiency
		Initial	Final	Weight gain	
	g/day	g	g	g/day	
BT	23.89±0.46	45.56±0.96	241.11±5.66^{b}	6.98±0.17^{b}	3.80±0.06
BTC	24.05±0.19	47.64±0.97	243.64±2.14^{b}	7.00±0.06^{b}	3.85±0.05
FO	24.58±0.38	46.78±0.64	255.22±4.19^{a}	7.44±0.15^{a}	3.75±0.08
FOC	24.18±0.28	47.00±0.74	251.64±2.80^{ab}	7.31±0.11^{ab}	4.07±0.48
P-value					
Oil	NS	NS	0.0053	0.0043	NS
CLA	NS	NS	NS	NS	NS
Oil * CLA	NS	NS	NS	NS	NS

1) BT : Beef tallow, BTC : Beef talloow with CLA supplemmentation, FO : Fish oil, FOC : Fish oil with CLA supplementation
Values sharing common superscripts in the same column are not significantly different at $p<0.05$

Table 3. Effects of CLA supplementation on plasma triglycerides, plasma leptin, fat cell sizes, weights of epididymal fat pads, and total body fat in 4 weeks feeding experiment

Dietary groups[1)]	Triglyceride	Plasma Leptin	Fat cell size	Epididymal fat pad	Total body fat
	mg/dl	ng/ml	Diameter(㎛)	g/100g BW	%
BT	24.22±3.28	0.63±0.09	52.23±1.96	1.58±0.11^{a}	7.64±0.94
BTC	24.35±9.64	0.75±0.19	50.16±3.49	1.67±0.13^{a}	8.08±1.40
FO	25.00±9.62	0.64±0.11	48.11±4.84	1.36±0.19^{b}	8.25±1.37
FOC	25.04±3.87	0.69±0.15	48.36±4.53	1.37±0.08^{b}	6.77±1.28
P-value					
Oil	NS	NS	NS	0.0001	NS
CLA	NS	NS	NS	NS	NS
Oil * CLA	NS	NS	NS	NS	NS

1) BT : Beef tallow, BTC : Beef talloow with CLA supplemmentation, FO : Fish oil, FOC : Fish oil with CLA supplementation
Values sharing common superscripts in the same column are not significantly different at $p<0.05$

4. Weights of epididymal fat pads

The weight of the epididymal fat pads in each animal was expressed as a percentage of body weight (Table 3). The CLA supplementation had no effect on their relative weights. On the other hand, the animals fed fish oil (FO & FOC groups) had significantly lower relative weights of the epididymal fat, compared to those fed beef tallow (BT & BTC groups).

5. Body fat in carcass

Table 3 shows the percentage of fat in the carcasses of the rats. The CLA supplementation did not significantly affect the percentage of body fat in the rats.

DISCUSSION

1. Effects of CLA on food intakes, food efficiency, and body weight gain

Park et al.[11] found almost no differences in body weights in 12-week old mice which had been given 5.0% corn oil supplemented with 0.5% CLA for 6 weeks. On the other hand, West et al.[9] reported that CLA supplementation in mice resulted in less food intakes, lower weight gains and lower fat accumulation. However, Sugano et al.[16] reported that when Sprague Dawley rats were given 0.5 - 1.0% CLA for 3 weeks, their food intakes and weight gains were not different from the control group. This last result is similar to our findings, and this response may be due to the use of rats rather than the mice used in other studies.

2. Effect of CLA on body fat

The study was designed to measure the effects of CLA supplementation in growing rats on the levels of their body fat and on the size of fat cells. When the total body fat % in the carcass was compared, CLA supplementation had no significant effect. Park et al.[11] using mice, reported that, although 0.5% CLA supplementation had no effect on body weight, there were 57 - 60% reductions in body fat. An increased β-oxidation of fatty acids as well as increased energy consumption was proposed to be the mechanism involved in this reduction of body fat. In cell culture, CLA added to bovine serum albumin reduced the activities of LPL, and resulted in the suppression of fat accumulation.[11] According to the research carried out by West et al.[9] in mice, CLA supplementation of 1.0% to low fat diets and of 1.2% to high fat diets for 6 weeks resulted in lower body weights and body fat, compared to the control groups. The reason given for the reduction in body fat was low food intake with CLA supplementation. In the same study, energy consumption increased despite the lower food intake and lower body fat accumulation. The researchers concluded that the increased energy consumption was due to the action of CLA on increased lipolysis and decreased LPL activities; in addition, CLA increases lipolysis during the night, which resulted in increased fatty acid oxidation, and lowered the respiratory quotient (RQ), thus resulting in lowered body fat. Delany et al.[12] reported reduced body fat regardless of food intakes in mice. On the contrary, our study using growing rats did not show any effect of CLA supplementation on body fat. As indicated above, Sugano et al[16] also did not observe any effects of CLA on food intakes and body weight gains in rats. Therefore, it appears that different species of animals show different responses in their lipid metabolism with CLA supplementation. What has been seen in mice was not observed in rats. Park et al.[8] and West et al.[9] proposed that the addition of CLA to the diets influenced the animals' appetite. Further studies on the effect of CLA on appetite, digestion and absorption are needed. In addition, Park et al[11] reported that only the trans-10, cis-12 isomer has an effect on body weight. In our study, 50% of CLA was cis-9, trans-11 CLA and 50% was trans-10, cis-12 CLA, which suggests that only 0.5% "effective" CLA was used in our study instead of 1.0% total CLA. This lower concentration, compared to 1% used in mice, could have been partly responsible for no effects being observed in our study using rats. Compared to the research carried out by Park,[11] where 5.0% corn oil (w/w) rich in linoleic acid and 0.5% CLA were given to mice, our present study used 11% fat and 1% CLA. Also West et a[9] added 1.2% CLA to the high fat diet (45kcal from fat / 100 kcal) and 1.0% CLA to the low fat diet (15kcal %). Our study used 25.6 kcal triglyceride per 100 kcal, which is equivalent to a medium fat diet. According to Nam,[24] lipid synthesis is affected by the kinds and amounts of dietary fat; low fat diets induced active lipid synthesis, while medium and high fat diets did not induce active lipid synthesis. According to the research carried out so far, the effect of CLA on body fat is considered to vary according to fat content in experimental diets. In the study by Park et al.[8] where body fat was reduced with CLA supplementation, it appears that low dietary fat suppressed de novo lipid synthesis processes and controlled fatty acid synthesis in the liver and fatty tissues, resulting in low body fat content. Thus, more research is needed in order to clarify the mechanisms used in these processes .

The addition of CLA to the rats' diets did not significantly influence the weights of the epididymal fat pads relative to total body weight. According to Sisk et al[15] the weights of the epididymal fat of the animals given CLA were significantly reduced, regardless of whether a high fat diet or low fat diet was used. The weight of epididymal fat is known to be directly related to total body fat.[25] Therefore, CLA addition had no effect on

either total body fat or epididymal fat in our study.

3. Effect of CLA on the size of fat cells

Fat cells provide the body with information on energy accumulation. The kind of dietary fat affects the size and number of fat cells according to Lemononnier et al.[26] who reported that the number of fat cells increased drastically when adult rats were fed diets high in saturated fatty acids, and the size of fat cells were increased when a diet high in unsaturated fatty acids were given.[27]

There are reports that when rats were given CLA, the numbers of their fat cells did not change, but the sizes of their fat cells changed.[15,17,18] When 1% CLA was given to mice, their inguinal, epididymal, retroperitoneal, and mesenteric fat decreased; this was attributed to the reduction in LPL activities.[15] Thus, the small amount of fat accumulation in fatty tissue must have led to the small size of fat cells. Our results showed no influence on the size of fat cells in rats after 4 weeks on a CLA supplemented diet. And when the distribution of fat cell size were classified into three groups, small, medium, and large, 63 - 78% of the fat cells were small (26-55 μm in diameter), and large cells (96-115μm) were almost non-existent. Also, CLA supplementation did not show effectiveness on distribution of fat cell size. These may be due to the absence of changes in levels of plasma triglycerides, and also to the lack of change in body weight.

4. Effects of CLA on leptin levels

In our study, the plasma leptin concentrations were not affected by CLA. Delany et al[12] reported that male mice given 1% CLA had reduced weights and body fat, and at the same time the leptin levels were lower. The results of the present study showed no change in body weight, body fat, and plasma triglyceride, levels in rats fed CLA. Therefore, CLA didn't affect the leptin level.

SUMMARY AND CONCLUSION

The effect of CLA (1%, w/w), added to beef tallow and fish oil, on body fattness, fat cell size, triglyceride level, and leptin level were studied in male Sprague Dawley rats, and the results were as follows:

1. CLA given to growing rats for 4 weeks did not affect body weight, body fat, the relative weights of epididymal fat pads and the size of fat cells.

2. CLA did not affect the sizes of fat cells. However, the group given fish oil, compared to those given beef tallow, had significantly lower relative weights of epididymal fat. Plasma leptin concentrations were not significantly affected.

In conclusion, this research found that CLA supplementation in rats did not affect body fat, sizes of fat cells and plasma leptin levels. Thus, further research should use animal species other than rats for in-depth studies on the effects of CLA on body fat.

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