Title

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Journal

Lipids, 35(7)

ISSN

0024-4201

Authors

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Publication Date

2000-07-01

DOI

10.1007/s11745-000-0586-y

Peer reviewed

Conjugated Linoleic Acid Supplementation in Humans: Effects on Circulating Leptin Concentrations and Appetite

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ABSTRACT: Conjugated linoleic acid (CLA) has been demonstrated to reduce body fat in animals. However, the mechanism by which this reduction occurs is unknown. Leptin may mediate the effect of CLA to decrease body fat. We assessed the effects of 64 d of CLA supplementation (3 g/d) on circulating leptin, insulin, glucose, and lactate concentrations in healthy women. Appetite was assessed as a physiological correlate of changes in circulating leptin levels. Analysis of plasma leptin concentrations adjusted for adiposity by using fat mass as a covariate showed that CLA supplementation significantly decreased circulating leptin concentrations in the absence of any changes of fat mass. Mean leptin levels decreased over the first 7 wk and then returned to baseline levels over the last 2 wk of the study in the CLA-treated group. Appetite parameters measured at around the time when the greatest decreases in leptin levels were observed showed no significant differences between supplementation and baseline determinations in the CLA-supplemented group or between the CLA and placebo-supplemented groups. There was a nonsignificant trend for mean insulin levels to increase toward the end of the supplementation period in CLA-treated subjects. CLA did not affect plasma glucose and lactate over the treatment period. Thus, 64 d of CLA supplementation in women produced a transient decrease in leptin levels but did not alter appetite. CLA did not affect these parameters in a manner that promoted decreases of adiposity.

Paper no. L8438 in *Lipids 35,* 783–788 (July 2000).

Conjugated linoleic acid (CLA) is the generic name for a group of positional and geometric conjugated dienoic isomers of linoleic acid. CLA has received considerable attention for its anticarcinogenic (1) and antiatherogenic activities (2,3). More recently CLA has been demonstrated to reduce body fat in mice (4–6) and lower glucose and insulin levels in genetically obese Zucker fatty (fa/fa) rats (7). However, CLA has also been shown to increase plasma insulin levels in mice (4). Because CLA supplementation is being considered for the treatment and prevention of obesity and diabetes, it would be useful to determine the effects of CLA on plasma insulin and glucose levels in humans.

The mechanism by which CLA exerts its effects on body composition is unknown. *In vitro* studies suggest that CLA reduces body fat by acting directly on adipocytes to enhance lipolysis and decrease lipoprotein lipase activity (5). However, it is also possible that CLA mediates reductions of body fat through leptin, the *ob* gene product that regulates adiposity through decreases of food intake and increases in metabolic rate (8–11). Leptin has also been shown to directly stimulate lipolysis in adipose tissue explants (12) and cultured adipocytes (13,14). CLA has been shown to decrease food intake and increase metabolic rate to varying degrees in mice (5,6,15). Thus, increases of plasma leptin concentrations could indeed mediate the effect of CLA to reduce body fat.

In this study, we sought to assess the effects of 9 wk of CLA supplementation on circulating leptin levels in healthy women. Because a number of studies indicate that insulin (16) and insulin-mediated glucose metabolism (17) regulate leptin production by adipose tissue as well as changes of circulating leptin concentrations in response to energy intake (18–20) and energy restriction (21,22), plasma insulin and glucose concentrations were measured throughout the study. Appetite was assessed as physiological correlate of changes in leptin production.

MATERIALS AND METHODS

Subjects and study design. Twenty-four women were recruited for this study, and 17 women completed it. Potential subjects completed a medical and physical examination, standard blood test, diet history, assessment of eating behavior to rule out eating disorders, and a urine test for pregnancy. Subjects selected for inclusion were all healthy, nonsmoking women between the ages of 20 and 41 yr of age and with normal menstrual cycles. Subjects lived in the metabolic suite at the Western Human Nutrition Research Center, University of California (Davis, CA) 24 hr/d, 7 d/wk for the entire study, which consisted of a 30-d stabilization period followed by a 64-d intervention period. At the end of the baseline period,

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Abbreviations: AUC, area under the curve; BMI, body mass index; CLA, conjugated linoleic acid; PPAR-γ, peroxisome proliferator activated receptor-γ.

10 subjects were randomly assigned to receive a CLA supplement and 7 were assigned to receive a sunflower oil placebo. The subjects and technical/support staff were blinded as to the treatment assignments.

At the beginning of the intervention period, 3 g/d (approximately 1% of daily energy intake) of CLA was given in the form of a treatment capsule from Pharmanutrients, Inc. (Lake Bluff, IL) until the study was completed. Of the fatty acids in the capsules 65% were CLA isomers, and gas chromatography showed that the isomer composition was 22.6% *trans*-10, *cis*-12; 23.6% *cis*-11,*trans*-13; 17.6% *cis*-9,*trans*-11; 16.6% *trans*-8,*cis*-10; 7.7% *trans*-9,*trans*-11 and *trans*-10,*trans*-12; and 11.9% other isomers. Placebo capsules made from sunflower oil contained 72.6% linoleic acid and no detectable CLA; they were taken during the baseline period by all subjects and during the intervention period by the control group. The CLA and placebo capsules were identical in appearance.

Both baseline and intervention diets met the Recommended Daily Allowance for all known nutrients and were matched with respect to energy as a percentage of calories from carbohydrate, protein, and fat (55, 15, and 30%, respectively). The caloric intake of each subject was estimated with the Harris-Benedict equation and intake adjusted during the baseline period to maintain body weight. The diet was provided as a rotating 5-d menu. Four meals were served daily. Meal times were set at 8:30–9:00 A.M. for breakfast, 12:00–12:30 P.M. for lunch, 5:00–5:30 P.M. for dinner, and 7:00–7:30 P.M. for evening snack. Subjects walked 2 mi twice daily; this and other activities were controlled carefully throughout the study. For all subjects, blood was collected between 7:00–8:00 A.M. by antecubital venipuncture after an overnight fast. Weight was assessed daily before breakfast. All subjects gave their informed consent. The study protocol was approved by the Human Subjects Committees of the U.S. Department of Agriculture and the University of California, Davis.

Appetite assessment. Appetite was assessed with the use of visual analog scales. Subjects marked their answers on a line displayed on the screen of a handheld computer (Palm Pilot©). Feelings of hunger, fullness, and prospective consumption (an assessment of the amount of food that could be eaten) were reported hourly from 7:00 A.M. to 10:00 P.M.; the area under the curve (AUC) was then calculated from the responses. Baseline appetite was assessed during the third week of the baseline period, and treatment effects were measured after 6 wk of intervention. Appetite measurements were performed every other day for three replicates. The same menu days of the baseline test were chosen as the measurement days for the treatment period.

Assays. Leptin concentrations in the plasma were determined with radioimmunoassay kits (Linco Research, St. Louis, MO) as previously described (23). For human leptin, the intra- and interassay coefficients were <8%. Plasma insulin concentrations were measured with a specific radioimmunoassay for human insulin (ICN Diagnostic Div., ICN, Costa Mesa, CA) according to the method of Yalow and Berson with minor modifications (24). Plasma glucose and lactate concentrations were measured with a YSI 2300 StatPlus Glucometer (Yellow Springs Instruments, Yellow Springs, OH).

Data analysis. A linear model with time taken as a repeated measure was used to determine the effects of CLA on appetite, plasma leptin, glucose, and lactate levels. The leptin data were also analyzed after adjustment for body fat mass by including body fat mass as a covariate in the model. The fat mass values utilized were obtained as previously described (25). These analyses were performed using the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Owing to the wide variation in fasting insulin levels between subjects, the insulin data were analyzed as the percentage change in insulin from baseline levels for each subject. To determine the effect of CLA on plasma insulin levels, the AUC (trapezium rule) for the percentage change in insulin levels was calculated for each subject, and the mean AUC for the CLA and placebo-supplemented groups were compared by a two sample *t*-test. Data are expressed as the mean ± SEM. The probability level for significance was set at $P < 0.05$, and a Bonferroni adjustment applied to multiple comparisons where appropriate.

RESULTS

Effect of CLA on plasma leptin concentrations. Leptin levels initially decreased and then returned to baseline levels in CLA-treated subjects (Table 1, Fig. 1A). Analysis of plasma leptin concentrations adjusted for adiposity by using fat mass as a covariate showed that CLA supplementation significantly decreased leptin levels ($P = 0.05$). Adiposity-adjusted leptin levels tended to be low at 33 d and were decreased significantly after 49 d in the CLA-supplemented group compared to the placebo-supplemented group and baseline values ($P = 0.02$ and $P = 0.04$, respectively). From the low point at 49 d, leptin concentrations in the CLA-treated group increased until they returned to near-baseline levels by the end of the study. After 57 d of supplementation, mean leptin levels in the CLA-treated group, although lower, were not significantly different from leptin levels in the placebo-treated group or from baseline values ($P = 0.12$ and $P = 0.17$, respectively). Since plasma leptin levels were not assessed between 33 and 49 d, the maximal effect of CLA on plasma leptin levels could have occurred during this time period. Changes of absolute and plasma leptin concentrations normalized as the ratio of leptin to fat mass or percentage fat mass in the CLA-supplemented group (Table 1) were similar to changes of adiposity-adjusted leptin concentrations. For example, mean absolute leptin levels were at their lowest point after 49 d of CLA supplementation. Over the entire supplementation period, CLA tended to decrease absolute and normalized plasma concentrations of leptin ($P = 0.10$). All of the observed changes in leptin concentrations occurred in the absence of detectable changes of fat mass (25) and body mass index (BMI) (Table 1).

Effect of CLA on plasma insulin, glucose, and lactate concentrations. There was a nonsignificant trend for mean plasma insulin levels to increase in CLA-treated subjects

TABLE 1
Mean BMI; Absolute and Normalized Plasma Leptin, Insulin, Glucose, and Lactate Concentrations; and Mean Change During 63 d of Treatment with Placebo or CLA[a]

	Baseline	33-d Treatment	49-d Treatment	57-d Treatment	63-d Treatment
BMI (kg/m^2)					
Placebo	22.2 ± 1.3	22.1 ± 1.2	22.6 ± 1.6	22.1 ± 1.4	22.2 ± 1.4
Δ		−0.05 ± 0.07	0.0 ± 0.1	−0.1 ± 0.2	0.1 ± 0.2
CLA	23.2 ± 0.5	23.3 ± 0.5	23.0 ± 0.5	23.1 ± 0.5	23.1 ± 0.5
Δ		−0.05 ± 0.07	−0.2 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
Leptin (ng/mL)					
Placebo	16.7 ± 4.3	15.5 ± 5.0	16.8 ± 5.9	16.2 ± 5.0	15.7 ± 4.6
Δ		−0.2 ± 0.8	1.0 ± 1.6	−0.6 ± 1.6	−1.0 ± 1.8
CLA	16.0 ± 2.5	13.3 ± 2.4	12.9 ± 2.3[a,b]	13.8 ± 2.2	15.1 ± 3.6
Δ	−2.8 ± 0.7	−3.2 ± 0.6	−2.3 ± 1.1	0.3 ± 1.8	
Leptin/fat mass (ng/mL/kg)					
Placebo	0.79 ± 0.12	0.73 ± 0.11	0.76 ± 0.15	0.76 ± 0.09	0.76 ± 0.11
Δ		0.02 ± 0.02	0.05 ± 0.08	−0.03 ± 0.08	−0.03 ± 0.11
CLA	0.83 ± 0.10	0.67 ± 0.07[a]	0.66 ± 0.08[a]	0.68 ± 0.07	0.78 ± 0.15
Δ		−0.16 ± 0.07	−0.17 ± 0.04	−0.14 ± 0.07	0.02 ± 0.08
Leptin/% fat mass (ng/mL)					
Placebo	0.51 ± 0.10	0.51 ± 0.10	0.51 ± 0.13	0.50 ± 0.10	0.50 ± 0.10
Δ		0.03 ± 0.02	0.03 ± 0.05	−0.01 ± 0.05	−0.01 ± 0.07
CLA	0.52 ± 0.07	0.43 ± 0.06	0.41 ± 0.06[b]	0.43 ± 0.05	0.52 ± 0.10
Δ		−0.09 ± 0.04	−0.10 ± 0.03	−0.09 ± 0.04	0.03 ± 0.05
Insulin (pmoles/L)					
Placebo	64.6 ± 13.2	55.6 ± 8.3	71.5 ± 13.2	54.2 ± 9.0	70.1 ± 13.2
Δ		−14.6 ± 6.9	1.4 ± 4.2	−10.4 ± 6.9	5.6 ± 6.3
CLA	54.9 ± 10.4	50.7 ± 5.6	65.3 ± 13.2	60.4 ± 8.3	65.3 ± 6.3
Δ		−3.5 ± 6.3	10.4 ± 6.3	6.3 ± 8.3	10.4 ± 10.4
Glucose (mmoles/L)					
Placebo	4.11 ± 0.17	4.05 ± 0.21	4.25 ± 0.24	3.92 ± 0.29	4.08 ± 0.22
Δ		−0.10 ± 0.17	0.10 ± 0.19	−0.18 ± 0.23	−0.02 ± 0.17
CLA	4.04 ± 0.09	4.04 ± 0.18	4.01 ± 0.16	4.11 ± 0.19	4.1 ± 0.23
Δ		−0.01 ± 0.11	−0.03 ± 0.11	0.07 ± 0.14	0.06 ± 0.18
Lactate (mmoles/L)					
Placebo	2.25 ± 0.29	2.11 ± 0.40	3.06 ± 0.59	2.36 ± 0.17	2.43 ± 0.21
Δ		−0.14 ± 0.46	0.62 ± 0.54	0.11 ± 0.33	0.18 ± 0.22
CLA	2.39 ± 0.34	2.68 ± 0.34	2.89 ± 0.29	2.78 ± 0.39	2.33 ± 0.23
Δ		0.29 ± 0.37	0.49 ± 0.42	0.39 ± 0.62	−0.06 ± 0.48

[a]Mean ± SEM; $n = 7$ for placebo and $n = 10$ for CLA. Treatment change (Δ) values represent mean change from baseline; BMI, body mass index; CLA, conjugated linoleic acid. [a]$P < 0.05$ vs. baseline. [b]$P < 0.05$ vs. placebo.

(Table 1 and Fig. 1B). The mean percentage change in insulin levels from baseline increased over the last 2 wk of the study in the CLA-supplemented group; this change coincided with the increase in leptin from the low point in leptin concentrations at 49 d (Figs. 1A and 1B). The mean AUC for the percentage change in insulin levels from baseline was 1039 ± 708 and −208 ± 895 for the CLA and placebo-supplemented groups, respectively. Although the difference in the mean AUC between the two groups was not significant ($P = 0.17$), it is possible that significance would have been attained with a larger sample size. CLA did not have any effect on plasma glucose ($P = 0.524$) and lactate concentrations ($P = 0.845$) (Table 1).

Effect of CLA on appetite. The AUC ratings during the baseline and supplementation periods for hunger, fullness, and prospective consumption showed that none of the three appetite measures was significantly affected by CLA treatment (Table 2). In addition, an approximately equal number of subjects had increased or decreased hunger, fullness, or prospective consumption in the CLA and placebo-supplemented groups (data not shown).

DISCUSSION

The present study is the first to examine the effects of CLA supplementation on circulating leptin levels in humans. CLA supplementation significantly decreased leptin concentrations without any detectable changes of body fat mass. After 7 wk of treatment, in the CLA-supplemented group, circulating leptin concentrations were at their lowest point. Thereafter, over the last 2 wk of intervention, leptin concentrations rose to pretreatment levels. These data are consistent with a recent study, which showed that plasma leptin concentrations tended to decrease in CLA-treated mice maintained on a high-fat diet (4). In mice fed a diet of 1% CLA by weight, plasma leptin levels were significantly decreased after 6 wk of treatment but did not differ from controls after 8 wk. However, since the 1% CLA diet decreased several different fat depots by greater

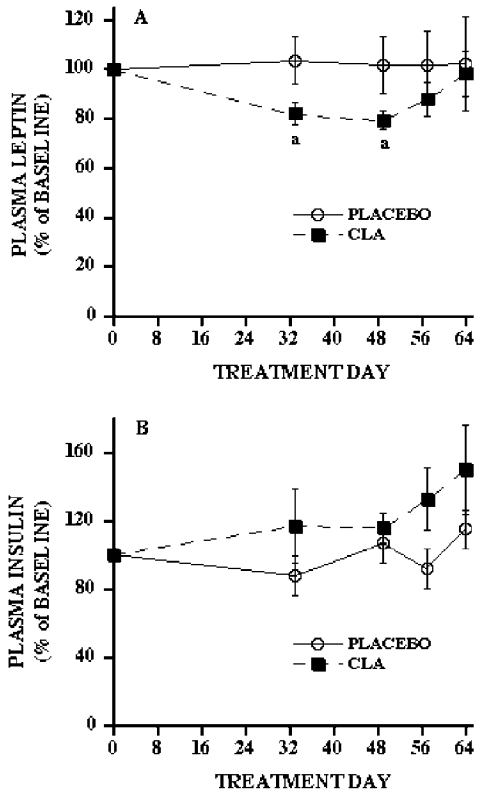

FIG. 1. Percentage change in leptin (A) and insulin (B) concentrations from baseline levels in conjugated linoleic acid (CLA) and placebo-supplemented subjects. Blood samples were collected after an overnight fast at baseline and at days 33, 49, 57, and 63 of intervention. Leptin and insulin concentrations were assessed by radioimmunoassay. Mean ± SEM; $n = 7$ for placebo and $n = 10$ for CLA. $^aP < 0.05$ vs. baseline.

than 50% after 6 wk of treatment, it is possible that the decreased leptin levels resulted from a reduction of body fat in the mice. However, by the end of that study, while overall fat mass decreased nearly 43%, leptin levels no longer differed from control levels. Thus, it is possible, like the present study, that CLA had effects on leptin levels that were independent of changes in fat mass.

To assess whether CLA-mediated changes in leptin levels were related to changes in appetite, self-ratings of hunger, fullness, and prospective consumption were determined. We have previously reported that in energy-restricted human females, subjects with lower leptin concentrations and greater percentage decreases in circulating leptin reported greater feelings of hunger, desire to eat, and prospective consumption than those with higher leptin concentrations and smaller decreases in leptin (26). In the present study, although appetite parameters were measured at around the time when the greatest decreases in leptin levels were observed, there were no significant differences between supplementation and baseline determinations in the CLA-supplemented group or between the CLA and placebo-supplemented groups. It is possible that there were undetected changes in appetite that were related to the decrease in leptin levels; the subjective assessment of appetite may be a gross measure that does not pick

TABLE 2
Mean Ratings of Hunger, Fullness, and Prospective Consumption[a]

	Baseline	After 6 wk of treatment
Hunger (AUC as mm·h)		
Placebo	486 ± 59	467 ± 50
CLA	508 ± 77	518 ± 64
Fullness (AUC as mm·h)		
Placebo	798 ± 75	
CLA	716 ± 56	727 ± 32
Prospective consumption (AUC as mm·h)		
Placebo	514 ± 97	500 ± 72
CLA	531 ± 83	568 ± 61

[a]Summarized mean of area under the curve (AUC) ± SEM for 16 hourly inquiries throughout the day; $n = 7$ for placebo and $n = 10$ for CLA.

up subtle changes in appetite. However, in the present study, in contrast to the previous one, the subjects were on a maintenance rather than an energy-restricted diet. Therefore, the decreases of circulating leptin levels were much more modest (~20%) in the present study than during energy restriction (~50–70%). It is also possible that other physiological changes, in addition to decreases in leptin concentration, are required to mediate changes of appetite. Although changes in leptin levels have been shown to modulate energy expenditure (8–11), it was also not affected in the present study by CLA treatment despite the changes in circulating leptin concentrations (25). Thus, the physiological significance of the changes that were observed in circulating leptin levels are not clear.

Plasma insulin levels tended to increase in CLA-treated subjects over the last 2 wk of the supplementation period. This change coincided with the increase in leptin concentrations from their low point at 49 d. A recent study showed that changes in plasma leptin correlate with changes of fasting plasma insulin independent of changes of BMI or percentage body fat (27). It was also demonstrated that insulin administration, at doses producing increments of insulin within the physiological range, increases circulating leptin concentrations in humans (28, 29). Thus, it is possible that the increase in insulin levels mediated the return of leptin concentrations to baseline levels. A trend for circulating insulin levels to increase with CLA supplementation was recently demonstrated in rodents by DeLany and colleagues (4). They reported that plasma insulin levels of CLA-fed mice tended to increase over the treatment period but did not reach significance until after 8 wk. Similar to the present study, this change in insulin levels occurred about 1 wk following the greatest difference in leptin levels between CLA-treated and control mice, and it also coincided with the increase in leptin to control levels. Thus, for both studies, it is possible that increases in insulin levels influenced circulating leptin concentrations.

The increase in insulin levels may have been part of a homeostatic response to counter the effect of CLA to decrease leptin. There are other possible explanations for the transient effect of CLA on circulating leptin concentrations. Initially, as suggested by a recent *in vitro* study (7), CLA could have acted as a ligand that activated peroxisome proliferator-acti-

vated receptor-γ (PPAR-γ). In turn, activation of PPAR-γ has been demonstrated to decrease leptin gene expression in rodents (30) and an adipocyte cell line (31). An event that could have occurred later during the intervention is demonstrated by a study in which rats fed a 1% CLA diet incorporated the *cis*-9,*trans*-11-isomer into membrane phospholipids of mammary epithelial cells (1). The investigators suggested that the incorporation of CLA into phospholipids could have various effects on signal transducing pathways. It is conceivable that altered signaling affected leptin production as CLA incorporation into adipocyte membranes increased.

In summary, 64 d of CLA supplementation in women produced a transient decrease in leptin levels but did not alter appetite or energy expenditure. These results are counter to what would be expected if CLA were able to reduce body fat in humans as it does in animals.

ACKNOWLEDGMENTS

We thank Kimber Stanhope, Peter Taylor and Wing Tung Chau for technical assistance, John Beckmeyer for computer programming assistance, nursing staff and clinical chemistry support staff for assistance with blood drawing and processing, Ginny Gildengorin and Bruce Mackey for statistical consultation. This work was supported by the United States Department of Agriculture, NIH grants DK 50129, DK 35747, the Juvenile Diabetes Association, the American Diabetes Association, and the California Breast Cancer Research Program of the University of California, 1RB-0404. E.A.M. was supported by a Medical Scholars Award from the American Diabetes Association and NIH grant DK09950-01.

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[Received January 14, 2000, and in revised form May 30, 2000; revision accepted June 7, 2000]