

Conjugated linoleic acid supplementation for 1 y does not prevent weight or body fat regain¹⁻³

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ABSTRACT

Background: Conjugated linoleic acid (CLA) is marketed as a safe, simple, and effective dietary supplement to promote the loss of body fat and weight. However, most previous studies have been of short duration and inconclusive, and some recent studies have questioned the safety of long-term supplementation with CLA.

Objective: Our aim was to assess the effect of 1-y supplementation with CLA (3.4 g/d) on body weight and body fat regain in moderately obese people.

Design: One hundred twenty-two obese healthy subjects with a body mass index (in kg/m²) > 28 underwent an 8-wk dietary run-in with energy restriction (3300–4200 kJ/d). One hundred one subjects who lost >8% of their initial body weight were subsequently randomly assigned to a 1-y double-blind CLA (3.4 g/d; *n* = 51) or placebo (olive oil; *n* = 50) supplementation regime in combination with a modest hypocaloric diet of –1250 kJ/d. The effects of treatment on body composition and safety were assessed with the use of dual-energy X-ray absorptiometry and with blood samples and the incidence of adverse events, respectively.

Results: After 1 y, no significant difference in body weight or body fat regain was observed between the treatments. The CLA group (*n* = 40) regained a mean (±SD) 4.0 ± 5.6 kg body weight and 2.1 ± 5.0 kg fat mass compared with a regain of 4.0 ± 5.0 kg body weight and 2.7 ± 4.9 kg fat mass in the placebo group (*n* = 43). No significant differences in reported adverse effects or indexes of insulin resistance were observed, but a significant increase in the number of leukocytes was observed with CLA supplementation.

Conclusion: A 3.4-g daily CLA supplementation for 1 y does not prevent weight or fat mass regain in a healthy obese population. *Am J Clin Nutr* 2006;83:606–12.

KEY WORDS Conjugated linoleic acid, dietary supplement, obesity, body fat, safety

INTRODUCTION

The long-term effects of conventional weight-management programs are unsatisfactory, and alternative therapies, including dietary supplements, are repeatedly called for by obese persons and society. Although the use of dietary supplements is widespread, the documentation on their efficacy and safety is not convincing (1). Conjugated linoleic acid (CLA) is a mixture of linoleic acid isomers with conjugated double bonds that has been studied intensively (2). CLA is sold commercially as dietary supplements for weight and fat loss. The products often have a 40%:40% content of *cis*-9, *trans*-11 (*c9,t11*) and *trans*-10, *cis*-12

(*t10,c12*) fatty acids, and the remaining 20% is usually composed of ≈1–4% other conjugated fatty acids and 15–19% other non-conjugated fatty acids (3). The CLA *c9,t11* isomer is a natural constituent in the human diet, and the average daily intake of the *c9,t11* isomer in Western societies is between 150 and 200 mg/d (4), whereas the intake of the *t10,c12* isomer is negligible.

In humans, a dose-response study of CLA supplementation (a mixture of *c9,t11* and *t10,c12*) over 3 mo reported a decrease in body fat, which was assessed by dual X-ray absorptiometry (DXA) scanning, at CLA doses ≤3.4 g/d without additional effects at doses >3.4 g/d (5). The only published long-term (>6 mo) placebo-controlled human study provided subjects with CLA (a mixture of *c9,t11* and *t10,c12*, as either free fatty acids or triacylglycerols) for 12 mo and induced significant losses of 2.0 kg body weight (in the CLA triacylglycerol group only) and 2.2 kg body fat mass (in both CLA supplementation regimens) compared with placebo (6). An open label 1-y extension of that study showed not only that the placebo group lost body fat mass when given CLA, but also that a 2-y treatment did not add more loss of fat mass than did the 1-y treatment (7).

To our knowledge, only 2 studies have investigated the effect of CLA during weight gain in humans, and both found no effect on body weight or body fat regain after a 3- or ≈6-mo treatment with CLA (as a mixture of *c9,t11* and *t10,c12*) after an initial loss of body weight (8, 9). However, the 3-mo study found that CLA did increase the amount of lean body mass compared with placebo (8).

In the present study, we investigated whether 1-y supplementation with CLA (3.4 g/d of a mixture of *c9,t11* and *c10,t12* as triacylglycerols) could decrease body weight and body fat mass regain in moderately obese persons after a low calorie diet (LCD)-induced weight loss (primary endpoint). In addition, we assessed the safety of the treatment [by monitoring adverse events, vital signs, electrocardiograms (ECGs), and blood variables, including indexes of insulin resistance] and the influence

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of CLA on hormones that may influence growth or body fat metabolism, such as insulin-like growth factor I (IGF-I), growth hormone (GH), thyroid-stimulating hormone (TSH), and testosterone (secondary endpoints).

SUBJECTS AND METHODS

The study was initiated in January 2002, the first subject was included in February 2002, and the clinical part of the study was terminated in June 2003. The subjects were healthy participants of both sexes, aged between 18 and 65 y, with body mass indexes (BMI; in kg/m^2) between 28 and 35. The subjects were recruited by 2 research centers (the Department of Human Nutrition, RVA University, Copenhagen, Denmark and the Department of Clinical Nutrition Hvidovre Hospital, Copenhagen, Denmark). All subjects provided written informed consent before inclusion into the study. Subjects were not included if they did not have a stable weight (± 3 kg in the past 2 mo) or if they were receiving drug therapy, consuming a special diet, or taking dietary supplements for weight loss. In addition, pregnant or lactating women were excluded. Subjects with renal, liver, pancreatic, or cardiac diseases, those with chronic inflammatory or infectious diseases, and those with malignant tumors were excluded. Diet-treated diabetic subjects as well as subjects with treated simple hypertension were included. Subjects who had active thyroid disease or who were receiving thyroid hormone treatment, and subjects taking adrenergic agonists, with known or suspected drug or alcohol problems, or with any clinical condition rendering them unfit to participate were excluded. The study was approved by the regional Ethics Committee for the districts of Copenhagen and Frederiksberg, Denmark (journal no. KF 01-247-01). The study was performed according to the Declaration of Helsinki (Edinburgh Amendment 2000) and the current International Conference on Harmonization guidelines.

The study was a randomized, double-blind, placebo-controlled, parallel-group study with 2 treatment arms. Initially, all subjects followed an LCD (Nutrilett; Collett Pharma, Lysaker, Norway) with an energy content of 3300–4200 kJ/d for 8 wk. All subjects attended 6 group meetings where experienced dietitians guided them through the weight-loss regimen. The subjects that lost $\geq 8\%$ of their initial weight during the LCD were randomly assigned to receive either CLA or placebo. The 2 treatment groups received either 6×750 mg CLA capsules (TONALIN; Natural ASA, Hovdebygd, Norway) or 6 placebo capsules (4.5 g olive oil) per day. The dose was selected based on previous studies (5, 10). The CLA content of the capsules was $\approx 80\%$ of the total lipid content (≈ 3.4 g CLA/d), consisting of 39% *c9,t11* CLA and 41% *t10,c12* CLA as triacylglycerols; the remaining 20% of the lipid content consisted of other triacylglycerols, as analyzed by the manufacturer. The soft gel capsules were opaque and identical in taste and appearance, and the energy content in both types of capsules was matched. The randomization sequence was generated by Scandinavian Clinical Research (Contract Research Organization) by using a simple block randomization procedure without any stratifications. The allocation sequence was provided to each center's study personnel via an internet-based interface. Both centers followed the study's randomization procedure and did not break the code at any time during the study. The randomization list was kept confidential and was opened only after closure of the database. Assuming a difference of 1.7 kg fat mass between treatment groups, which

was based on prior studies of 3.4 g CLA/d compared with placebo supplementation (5), and an estimated SD of 2.2 kg (based on prior experience of within-group detectable differences with the use of DXA methodology), an estimated 37 subjects per group were required (for 90% statistical test power and 5% significance level). To account for a high dropout rate, the required number of subjects needed for initial inclusion was estimated to be 60 per group. After the 8-wk LCD-period (at week 0), the 101 subjects who achieved the weight-loss goal were randomly assigned to either the CLA- or the placebo-supplemented group. For a total of 52 wk, the subjects were provided with CLA or placebo in combination with a modest hypocaloric diet of ≈ -1250 kJ/d (1 kcal = 4.18 kJ). The dietary instruction was given by dietitians in 14 individual consultations throughout the treatment period ($\approx 1/\text{mo}$) and was based on the isocaloric interchangeable and educational diet program "Eat for life" (11). The energy requirement during the 52-wk hypocaloric period was estimated by using appropriate equations according to body weight, sex, and age (12).

The subjects' characteristics (including smoking and drinking habits) and demographic data were recorded when the subjects entered the study. Body weight, adverse events, and concomitant medication were recorded at each visit with the dietitian, ie, a total of 14 times during the 52-wk treatment. Physical measurements, blood samples, urine samples, ECGs, DXA scans, and measurements of blood pressure, waist circumference, hip circumference, and pulse were taken 4 times in total: before the LCD period (week -8), after the LCD period (week 0), and after ≈ 26 and 52 wk of treatment. The dietary supplements were provided to (and returned from) the study subjects at every bi-monthly visit. Fasting blood samples (taken after the subjects fasted for >10 h) were obtained between 0700 and 1300 at each visit, and the time point of sampling was repeated if possible. Except for the analysis of insulin concentrations, the samples were all analyzed in an accredited laboratory (Capio Diagnostik, Copenhagen, Denmark). Insulin concentrations were analyzed with a commercially available kit (IMMULITE 1000 Insulin; DPC Biermann GmbH, Bad Neuheim, Germany).

Blood was analyzed for the following: hemoglobin, erythrocytes, leukocytes, platelets, alanine aminotransferase, aspartate aminotransferase, γ glutamyltransferase, creatinine, IGF-I, GH, TSH, glucose, insulin, and total testosterone. An index of insulin resistance [homeostasis model of assessment ratio (HOMA-R)] was derived from fasting values of glucose and insulin according to the following formula: $(\text{glucose} \times \text{insulin})/22.5$. Urine was obtained for the analysis of blood, glucose, and protein content and for pregnancy testing. All blood samples were taken and DXA scans and clinical assessments (except body weight) were performed at the same center, ie, at the Royal Veterinary and Agricultural University, Copenhagen, Denmark. Blood samples were obtained at all time points from a total of 75 subjects. Compliance was measured every 2 mo by a comparison between the number of unused capsules and the number of capsules that were given. A subject was considered compliant when he or she completed $\geq 75\%$ of the capsules provided.

DXA scans (Lunar Radiation Corp, Madison, WI) were used to measure body composition with LUNAR PRODIGY software (version 5; Lunar Radiation Corp). All DXA scans were performed by the same experienced technical assistant in the mornings of each visit; the scans were performed within the same 1-h time frame for each subject. The results of the DXA scans were

divided into body fat mass (FM) and body fat-free mass (FFM). FFM was calculated as lean tissue mass + bone mineral content. Percentage FM is calculated as $FM(DXA)/[FM(DXA) + FFM(DXA)] \times 100$. Waist and hip circumferences were measured immediately before DXA scanning. Body weight was assessed at each visit on regularly calibrated electronic weight scales (SCALE; Lindells Inc, Kristianstad, Sweden). In addition, body weight was also assessed as the sum of FM and FFM as assessed by DXA scanning at 4 time points during the study.

Diet records were completed 3 times: before entering the study (week -8) and after ≈ 24 and 52 wk of treatment. The subjects recorded their diets for 3 consecutive days before medical center visits. The method provided information on the quantity and type of food consumed during the 3-d record period. Each subject was given detailed instructions on how to fill out the food diary, and the clinical dietitian monitored all returned dairies. Food intake was converted into energy intake.

Statistics

All analyses were performed with SPSS 12.0 software (SPSS Inc, Chicago, IL). Statistical tests were performed with the use of 5% as the nominal level of significance, and interval estimates were constructed with the use of 95% as the level of confidence. We defined a modified intention-to-treat population (83 subjects comprised of 77 patients who completed the 26-wk treatment + 6 subjects who withdrew or who we were unable to contact during the last 26-wk treatment), which was used for the analysis of changes from baseline to 1 y. A last-observation-carried-forward analysis was used for this modified intention-to-treat population, which completed the 26-wk treatment but did not complete the 1-y period, for the analyses of changes in FM, FFM, percentage fat, and body weight. Last-observation-carried-forward was also applied to missing values in the 8-wk LCD period for body weight. No substitution of missing data was performed on other variables. No stratification was used. Fisher's exact test was used for testing differences in sex distribution and dropout rates between treatment groups. Analysis of covariance was used to compare the changes in FM, FFM, percentage fat, and body weight in the CLA and placebo groups with the use of the week 0 value and body weight change (weight at week 0 minus weight before the LCD period) as a covariate. The treatment center and sex were also inserted as covariates. We also performed repeated analyses of changes in FM, FFM, percentage fat, and body weight. The values for each variable at weeks 0, 25, and 52 were compared between treatment groups with the use of sex, treatment center, and the change in body weight (weight at week 0 minus weight before the LCD period) as covariates. The changes from week 0 to week 52 within treatment groups were tested with the paired *t* test. Between-group analyses of changes in waist and hip circumferences, energy intake, and all blood variables were analyzed with an unpaired *t* test.

RESULTS

One hundred thirty-five subjects were screened for potential inclusion into the study, and 122 subjects were enrolled in the 8-wk LCD-period. A total of 101 subjects were randomly assigned to 1 of 2 treatment groups after completing the 8-wk LCD and having lost $\geq 8\%$ of their initial body weight. The 83 subjects who completed the 26-wk treatment (40 subjects in the CLA group and 43 subjects in the placebo group) were included in the

1-y (modified) intention-to-treat analyses. Seventy-seven subjects completed the whole treatment period (**Figure 1**). Mean (\pm SD) compliance was high: $95.7 \pm 8.7\%$ and $96.7 \pm 8.8\%$ in the CLA and placebo groups, respectively; the difference in compliance between the 2 groups was not significant. The dropout rate after 12 mo did not differ significantly between the treatment groups (27.5% for the CLA group compared with 26.0% for the placebo group).

Baseline characteristics

The subjects were all white, and the study groups were well-matched with respect to sex, ethnic origin, smoking habits ($n = 83$), habitual alcohol intake, and body height (data not shown). No significant differences in vital signs (blood pressure and heart rate), medical conditions, or concomitant medications were observed between groups at the start of the study. Significant differences in body weight, BMI, body FM, and lean body mass were observed between the groups at week -8 (before the LCD period) and week 0 (baseline) ($P < 0.05$). The subjects in the placebo group had higher body weights and BMIs than did the subjects in the CLA group. Consequently, body FM and FFM were also higher in the placebo group than in the CLA group. However, the percentage body FM was not significantly different between the groups at weeks -8 or 0 (baseline). Hip circumference measurements were slightly higher in the placebo group at baseline than in the CLA group, whereas waist circumferences and the ratio of waist to hip measurements were not significantly different between treatment groups (**Table 1**).

Changes in body weight, body composition, and dietary records

During the LCD period, both groups of subjects lost ≈ 10 kg body weight, of which $\approx 70\%$ was fat mass; the difference between the groups was not significant (Table 1). According to the food records, energy intake was lower in both groups at week 25 and week 52 than before the LCD, with no significant difference observed between the groups. During the 1-y supplementation period, both groups regained ≈ 3 kg body weight when measured by DXA scan (data not shown) and ≈ 4 kg when assessed on a weight scale, with no significant differences observed between the groups (**Figure 2**). Similarly, the gains in FM ($\bar{x} \pm$ SD: 2.1 ± 5.0 kg for the CLA group compared with 2.7 ± 4.9 kg for the placebo group) and FFM (0.9 ± 1.7 kg for the CLA group compared with 0.5 ± 1.8 kg for the placebo group) were not significantly different between the groups. Repeated analyses provided essentially the same results ($P = 0.56$ for changes in body FM). It should be noted that the last-observation-carried-forward analysis is rather conservative, making it less likely to detect a treatment effect (the dropouts are more likely to be persons who could not maintain the weight loss; therefore, the reported mean weight gain data are probably too low). Changes in hip and waist circumferences did not significantly differ between the groups at any time point.

Adverse events

A total of 563 adverse events (AEs) were reported, but with no significant difference between the 2 groups. Of these AEs, 35 were considered related to the treatment and 528 were not. Of the subjects, 94.1% and 98.0% of the CLA and placebo groups, respectively, experienced AEs during the study (no significant



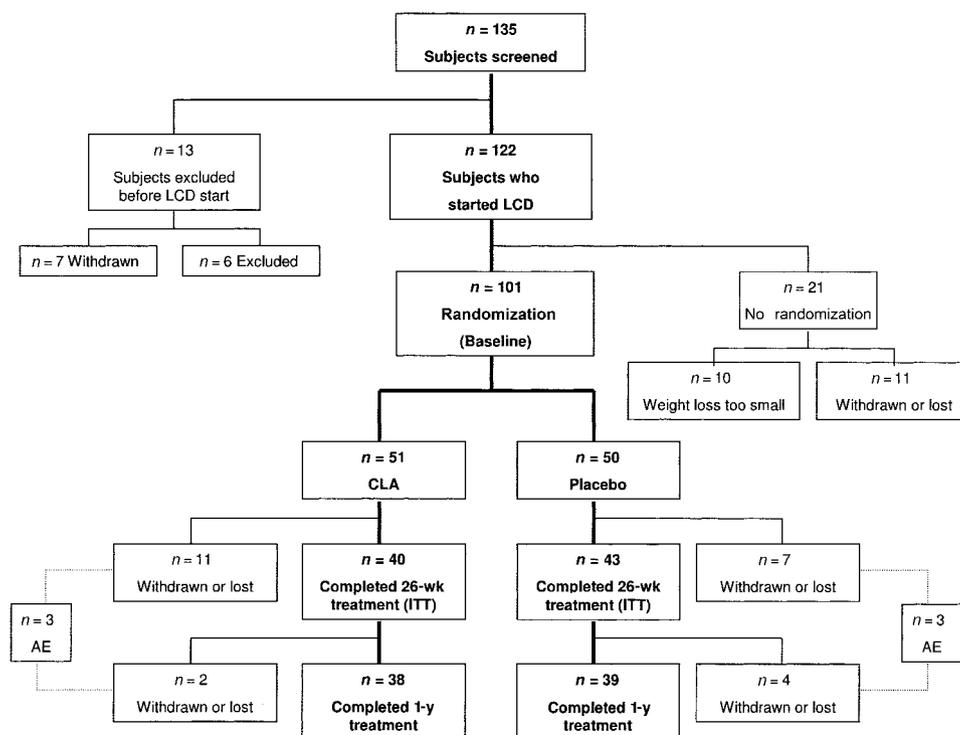


FIGURE 1. Study participant and randomization scheme. LCD, low-calorie-diet period (8 wk before baseline); ITT, intention-to-treat; CLA, conjugated linoleic acid; AE, adverse effect (person withdrawn from study). ITT indicates the study population for which intention-to-treat analyses were performed for changes in body weight, body fat, fat-free mass, and percentage body fat from baseline to 1 y of treatment ($n = 83$ in total). The dropout rate was not significantly different between groups (chi-square test).

difference between the groups). Three subjects from the CLA group and 2 from the placebo group withdrew from the study because of pregnancy or because of AEs (soft stools, depression, air in the stomach, or stomach pain). Five serious AEs were registered, 4 of them in the placebo group; however, none were related to the treatment.

Laboratory analyses

Several of the blood variables measured for the assessment of treatment safety changed during the LCD-induced weight loss, but these variables recovered during the subsequent weight regain period (week 0 to week 52), with no significant difference between the groups (data not shown). Only the change in leukocyte concentration from week 0 to week 52 was significantly different between the groups, with a greater increase in the CLA group ($\bar{x} \pm \text{SD}$: $0.81 \pm 1.21 \times 10^9/\text{L}$) than in the placebo group ($0.19 \pm 1.14 \times 10^9/\text{L}$; $P = 0.03$). This difference between groups appeared only after >25 wk of supplementation. Urine analyses, ECG registrations, systolic and diastolic blood pressures, and heart rates did not uncover any other abnormal observations in any of the study groups (data not shown). CLA did not significantly affect fasting values of plasma glucose and insulin, and insulin resistance was also not affected, as assessed by the HOMA-R index (Table 2). In addition to the safety variables, we assessed a panel of hormones (testosterone, GH, IGF-I, and TSH), but CLA treatment did not significantly affect any of these measures (Table 2).

DISCUSSION

Numerous animal studies have shown that CLA causes repartitioning of body composition; ie, decreased FM and increased

lean body mass (13). In addition, CLA was reported to be an effective inhibitor of atherogenesis in rabbits (14) and of insulin resistance in skeletal muscle in rats (15). Hence, CLA was suggested to be useful in treating diabetes by controlling body fat and weight gain (16), but recent studies in humans have indicated that CLA may actually have negative effects on insulin sensitivity (17). Although the animal studies have been optimistic, and some human studies have also shown positive effects of CLA on body fat, most human studies (duration <6 mo) have shown only marginal effects on body weight and body composition (18). The apparent discrepancies between the animal and short-term human studies have been ascribed to lower doses per kilogram body weight, shorter treatment duration in the human studies, the view that CLA may only be effective during fat accumulation, and the quality and reliability of the measurement methods.

Our main objective was to investigate whether 1-y CLA supplementation in obese adults would safely prevent regain of body FM and body weight after a major initial weight loss compared with placebo. In this respect, we found no effect on either body weight, body FM, or FFM. The absence of a significant effect on FM and body weight corroborates the findings of other recent studies. In a placebo-controlled study, obese subjects were given 6 g CLA/d for 28 wk; the first 12 wk were in conjunction with an LCD (9). CLA affected neither body weight nor body fat content significantly (9). Also, Kamphuis et al (8) performed a study with a CLA preparation similar to that used in our study and found that, after an initial ≈ 6 kg weight loss, 2 CLA supplement doses (1.8 g/d and 3.6 g/d) did not significantly lower FM and body weight after 13 wk. However, CLA did significantly decrease the regain of FFM by $\approx 2.3\%$. Our findings contrast the findings of Gaullier et al (6) who performed a 1-y study in a large sample of

TABLE 1

Body weight, body composition, waist circumference, hip circumference, and energy intake measurements before the low-calorie diet (LCD) and at 0, 25, and 52 wk after the LCD¹

	Before LCD (week -8)	Week 0 (baseline)	Week 25	Week 52	Change (week 52 - week 0)		<i>P</i> between groups ³
					Value	<i>P</i> ²	
Weight (kg)							
CLA	94.5 ± 11.7 ⁴	82.6 ± 9.5 ⁵	84.6 ± 11.3	86.7 ± 13.2	4.0 ± 5.6	< 0.001	0.51
Placebo	100.6 ± 13.2	88.5 ± 12.0	90.7 ± 13.0	92.5 ± 13.0	4.0 ± 5.0		
Fat mass (kg)							
CLA	34.6 ± 6.9	26.9 ± 7.2 ⁵	26.6 ± 7.5	29.0 ± 8.5	2.13 ± 4.99	< 0.001	0.56
Placebo	37.4 ± 7.4	29.9 ± 8.1	29.7 ± 9.5	32.6 ± 9.6	2.73 ± 4.92		
Fat-free mass (kg)							
CLA	59.3 ± 11.5	56.4 ± 11.1 ⁵	57.9 ± 11.5	57.4 ± 11.7	0.94 ± 1.74	< 0.001	0.33
Placebo	62.3 ± 12.8	59.7 ± 12.2	60.7 ± 12.3	60.2 ± 12.3	0.51 ± 1.77		
Percentage fat ⁶							
CLA	37.1 ± 7.3	32.5 ± 9.0 ⁵	31.6 ± 8.5	33.7 ± 8.7	1.1 ± 4.0	0.002	0.47
Placebo	37.9 ± 7.5	33.7 ± 8.7	33.0 ± 9.5	35.2 ± 9.2	1.4 ± 3.9		
Waist circumference (cm)							
CLA	101.9 ± 8.8	93.3 ± 7.8 ⁵	95.0 ± 7.1	97.5 ± 8.95	4.2 ± 5.9	< 0.001	0.88
Placebo	104.9 ± 9.8	95.8 ± 9.6	98.6 ± 10.4	99.7 ± 10.8	4.0 ± 6.7		
Hip circumference (cm)							
CLA	108.3 ± 7.8	101.9 ± 6.5 ^{5,7}	104.2 ± 6.3	106.7 ± 7.3	4.9 ± 6.3	< 0.001	0.37
Placebo	109.6 ± 7.5	104.7 ± 6.5	108.3 ± 8.1	110.7 ± 7.9	6.1 ± 5.9		
Energy intake (kJ/d)							
CLA	10102 ± 3238	—	7167 ± 1999 ⁸	7807 ± 2054	-2295 ± 2690	< 0.001	0.71
Placebo	9889 ± 2378	—	7567 ± 2461 ⁸	7328 ± 1622	-2561 ± 1981		

¹ *n* = 40 for the conjugated linoleic acid (CLA) group (17 men and 23 women) and 43 for the placebo group (19 men and 24 women). The mean (±SD) age was 43.4 ± 8.4 y for the CLA group and 41.7 ± 8.2 y for the placebo group. There were no significant differences in either sex distribution or age between groups. For body weight, fat mass, fat-free mass, and percentage fat, last-observation-carried-forward analysis was applied for the subjects who completed the 26 wk treatment but did not complete the 1-y period.

² Paired *t* test for both groups combined.

³ For analyses of changes in weight, fat mass, fat-free mass, and percentage fat, an analysis of covariance was performed by using the week 0 value, body weight change (week 0 — week -8), treatment center, and sex as covariates. Other variables were analyzed by using unpaired *t* tests. Repeated analyses of all variables provided essentially the same results.

⁴ $\bar{x} \pm$ SD (all such values).

⁵ The change during the 8-wk LCD was not different between the groups.

⁶ Fat mass percentages were calculated as FM (DXA) / [FM(DXA) + FFM(DXA)] × 100.

⁷ Significantly different at baseline (*P* < 0.05).

⁸ Assessed at week 24, the number of subjects reporting energy intake at all time points was 28 for the CLA group and 20 for the placebo group.

moderately obese subjects given 3.4 g CLA/d. In that study, CLA supplementation induced significant weight and body fat losses of 2.0 and 2.2 kg, respectively. The discrepancy between the studies is not easily explainable, but could be due to the different study designs. Compared with the study by Gaullier et al, our study had a larger sample size and was performed during weight gain. Another explanation may be the absence of diet restrictions during the study by Gaullier et al. A third possible explanation is the significant baseline differences between the groups observed in the present study. However, by using analysis of covariance, this difference is not likely to have influenced the study to any major extent. Finally, given the finding of a numerical difference of ≈0.6 kg FM between the groups and a SD of ≈5 kg, the present study may have been inadequately powered.

The secondary endpoint in the current study was the safety issue. Generally, CLA supplements are considered to be safe in animals (19). However, some studies in mice have shown that CLA, particularly the *t*10,*c*12 isomer, may induce lipotrophy, hyperinsulinemia, and fatty liver (20), and that this may occur at dosing levels that are comparable to the doses used in human

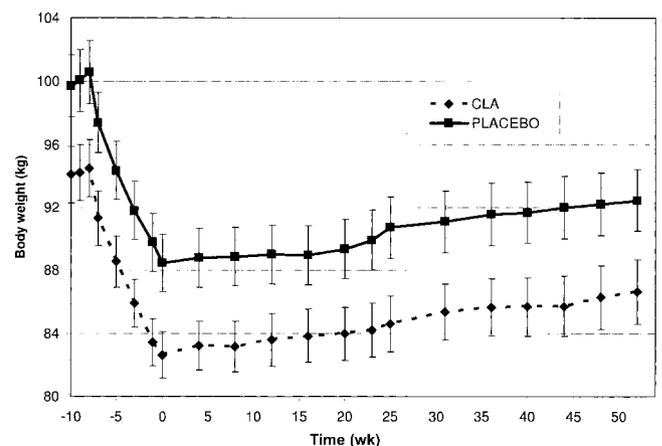


FIGURE 2. Mean (±SEM) body weight measurements for both the conjugated linoleic acid (CLA) and placebo groups during the study. *n* = 40 for the CLA group and 43 for the placebo group. Where relevant, last-observation-carried-forward analysis was conducted to insert body weight measurements taken at week 26 (or later) at week 52. No significant differences in body weight change were observed between the groups throughout the study, *P* > 0.5 (analysis of covariance or repeated-measures analysis).

TABLE 2

Blood variables before the low-calorie diet (LCD) and at weeks 0, 25, 52 after the end of the LCD¹

Blood variable	Before LCD (week -8)	Week 0 (baseline)	Week 25	Week 52	Change (week 52 - week 0)		P between groups ³
					Value	P ²	
Glucose (mmol/L)							
CLA	5.03 ± 0.38 ⁴	4.79 ± 0.44	4.94 ± 0.36	4.94 ± 0.47	0.16 ± 0.73	0.497	0.198
Placebo	5.03 ± 0.50	4.92 ± 0.51	4.85 ± 0.48	4.87 ± 0.47	-0.4 ± 0.61		
Insulin (pmol/L)							
CLA	60.2 ± 19.1	40.6 ± 16.0	44.1 ± 20.3	53.5 ± 21.8	12.9 ± 18.9	< 0.001	0.22
Placebo	58.4 ± 23.0	44.2 ± 23.4	42.9 ± 20.8	50.9 ± 24.4	6.66 ± 24.2		
Growth hormone (μg/L)							
CLA	0.60 ± 0.76	1.20 ± 2.40	1.15 ± 2.34	0.50 ± 0.69	-0.70 ± 2.12	0.036	0.98
Placebo	0.50 ± 0.72	1.59 ± 3.18	0.98 ± 1.61	0.87 ± 1.54	-0.72 ± 3.49		
IGF-I (nmol/L)							
CLA	20.2 ± 5.8	20.0 ± 6.9	24.1 ± 7.4	23.9 ± 5.4	3.71 ± 6.84	< 0.001	0.47
Placebo	22.5 ± 6.9	19.6 ± 6.6	24.5 ± 6.5	24.5 ± 6.9	4.75 ± 4.86		
Testosterone (nmol/L)							
CLA	6.91 ± 6.50	8.60 ± 7.91	9.20 ± 8.19	6.52 ± 6.74	-2.09 ± 2.77	< 0.001	0.15
Placebo	6.78 ± 6.16	8.25 ± 7.05	9.65 ± 8.51	7.14 ± 7.53	-1.10 ± 3.05		
TSH (mIU/L)							
CLA	1.33 ± 0.49	1.20 ± 0.58	1.41 ± 0.59	1.07 ± 0.36	-0.13 ± 0.44	0.016	0.87
Placebo	1.33 ± 0.63	1.24 ± 0.64	1.44 ± 0.90	1.12 ± 0.63	-0.11 ± 0.45		
HOMA-R ⁵							
CLA	13.7 ± 4.7	8.8 ± 4.0	9.9 ± 5.1	11.8 ± 4.7	3.01 ± 4.63	0.001	0.12
Placebo	13.2 ± 5.7	9.9 ± 5.9	9.3 ± 4.6	11.0 ± 5.2	1.10 ± 5.72		
Leukocytes (× 10 ⁹ /L)							
CLA	6.21 ± 1.43	5.56 ± 1.69	6.03 ± 1.39	6.36 ± 1.66	0.81 ± 1.21 ⁶	0.001	0.03
Placebo	5.85 ± 1.53	5.32 ± 1.53	5.89 ± 1.54	5.51 ± 1.30	0.19 ± 1.14		

¹ n = 36 for the conjugated linoleic acid (CLA) group and 39 for the placebo group. IGF-I, insulin-like growth factor I; TSH, thyroid-stimulating hormone; HOMA-R, homeostasis model of assessment ratio (index of insulin resistance).

² Paired *t* test for both groups combined.

³ Unpaired *t* test. Statistical analyses using repeated-measures analysis (using the before LCD value as a covariate) gave essentially the same results for all variables.

⁴ $\bar{x} \pm SD$ (all such values).

⁵ Calculated as (glucose × insulin)/22.5.

⁶ Significantly different from placebo group.

studies (21). However, due to species differences, one should always be cautious comparing human and animal studies. In this respect, human studies have also questioned the safety of commercially available supplements of CLA; they have shown that the *t10,c12* isomer (and perhaps the *c9,t11* isomer) produces insulin resistance, even when taken for only 4 mo (22, 23). In contrast, a 1-y study showed that CLA does not seem to impair glucose metabolism or liver function, though it may increase HDL cholesterol, LDL cholesterol, and apolipoprotein B in some circumstances (6). In the present study, CLA supplementation did not significantly affect fasting values of plasma glucose and insulin, and we did not find any effect on HOMA-R. This confirms the previous findings by Riserus et al (22, 23), in which only supplements with purified *trans*-10, *cis*-12 (22) or *cis*-9, *trans*-11 isomers (23) increased insulin resistance in subjects with the metabolic syndrome, whereas a 50:50 mixture, such as the one used in the present study, apparently did not (22).

In the present study, 21% of the reported AEs were severe, with 7% and 5% related to CLA and placebo, respectively. Most AEs were related to effects on the gastrointestinal tract. The number of dropouts (23.7%, with no significant difference between groups) was low for a 1-y study conducted on obese subjects. The compliance was high (96%), which indicated that

taking 6 capsules/d for 1 y was no source of discomfort for the subjects. Some minor changes in the laboratory safety data were observed in both groups in one direction during the LCD period and in the opposite direction during the 1-y supplementation, but with no significant differences between the CLA and placebo groups. Also, urine tests and ECGs were normal until the end of the study. Only leukocyte concentrations were increased by the 1-y CLA treatment. Similar observations have been reported in long-term studies (6, 7, 9). Although the actual increase in leukocyte numbers was generally small, this increase may be of some concern, because previous studies have indicated that leukocytes are an important indicator of inflammation and leukocytes have also been identified as a predictor of coronary heart disease mortality (24). Because the numbers of leukocytes were within reference values (3.0–10.0 × 10⁹/L), and because CLA supplements are used, in most cases, for a limited time period, the clinical relevance of this finding is still unclear. Also, a 12-wk study conducted in humans suggested that CLA may have beneficial effects on immune function (25). Additional studies should be done to clarify this issue. We analyzed whether CLA might affect energy metabolism via effects on testosterone, GH, IGF-I, or TSH. We found no significant changes in any of these



factors, which seems to agree with the apparent lack of effect on body weight and body composition.

CONCLUSIONS

Unfortunately, we did not obtain a perfect group match for body weight at randomization. Despite the reservations that this issue may imply, we conclude that 1-y supplementation with a mixture CLA isomers (3.4 g/d) has no clinically important effect on body weight and body fat regain after an 8-wk LCD-induced weight loss in obese persons. 

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