

Conjugated Linoleic Acid Supplementation for Twelve Weeks Increases Lean Body Mass in Obese Humans^{1,2}

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Abstract

Conjugated linoleic acid (CLA) alters body composition in animal models, but few studies have examined the effects of CLA supplementation on body composition and clinical safety measures in obese humans. In the present study, we performed a randomized, double-blind, placebo-controlled trial to examine the changes in body composition and clinical laboratory values following CLA (50:50 ratio of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) supplementation for 12 wk in otherwise healthy obese humans. Forty-eight participants (13 males and 35 females) were randomized to receive placebo (8 g safflower oil/d), 3.2 g/d CLA, or 6.4 g/d CLA for 12 wk. Changes in body fat mass and lean body mass were determined by dual-energy X-ray absorptiometry. Resting energy expenditure was assessed by indirect calorimetry. Clinical laboratory values and adverse-event reporting were used to monitor safety. Lean body mass increased by 0.64 kg in the 6.4 g/d CLA group ($P < 0.05$) after 12 wk of intervention. Significant decreases in serum HDL-cholesterol and sodium, hemoglobin, and hematocrit, and significant increases in serum alkaline phosphatase, C-reactive protein, and IL-6, and white blood cells occurred in the 6.4 g/d CLA group, although all values remained within normal limits. The intervention was well tolerated and no severe adverse events were reported, although mild gastrointestinal adverse events were reported in all treatment groups. In conclusion, whereas CLA may increase lean body mass in obese humans, it may also increase markers of inflammation in the short term. J. Nutr. 137: 1188–1193, 2007.

Introduction

Conjugated linoleic acid (CLA)⁸ is a polyunsaturated fatty acid naturally occurring in foods from ruminant sources. Supplements composed of 2 isomers of CLA, the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12, have been well studied in experimental models in relation to immune function, cancer, atherosclerosis, and glucose maintenance. In addition, CLA has been shown to reduce body fat mass and increase lean body mass (LBM) in several animal models, with the largest effects seen in mice (1). The mechanisms of action are thought to be mediated by increased energy expenditure and involve enhancement of fatty acid oxidation, in both adipocytes and skeletal muscle cells, and increased

lipolysis in adipocytes (2,3). Several clinical trials in humans have reported mixed results with a few studies showing that CLA reduces body fat mass or increases LBM in humans (4–7), whereas others showed no effects on body composition (8–12).

In addition to the effects on body composition, CLA has been shown to induce inflammation and hyperinsulinemia in animal models (13) and, in a few clinical trials to date, to impair insulin sensitivity in humans (14–16). These effects appear to be solely related to the *trans*-10, *cis*-12 isomer when given in isolation, which is also the isomer that has the greatest effect on body composition alterations in animal models (17).

The prevalence of obesity is increasing in the United States and throughout the world. Only a few of the clinical trials of CLA supplementation have been conducted in obese humans (BMI between 30 and 35 kg/m²). Thus, the primary goal of the present study was to examine the effect of 2 doses of CLA supplementation for 12 wk on body composition, weight, resting energy expenditure (REE), and safety measures in obese individuals free of chronic disease. We utilized a mixed isomer form of CLA with the goal of inducing the beneficial effects on body composition while minimizing the potential for any adverse alterations in

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⁸ Abbreviations used: CLA: conjugated linoleic acid; LBM: lean body mass; REE: resting energy expenditure; RQ: respiratory quotient.

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inflammation or insulin sensitivity found with the *trans*-10, *cis*-12 isomer when given alone.

Materials and Methods

Participants and protocol. Fifty-five healthy, nonsmoking obese (BMI between 30 and 35 kg/m²) participants between 18 and 50 y of age were recruited from the population living in the Durham, Chapel Hill, Carrboro, and surrounding areas of North Carolina. The procedures followed were in accordance with the ethical standards of and approved by the Institutional Review Board at the University of North Carolina at Chapel Hill, and all subjects provided informed consent prior to enrolling in the study.

Exclusion criteria. Individuals were ineligible if they reported any of the following: chronic illness, including history of cancer, cardiovascular disease, diabetes, or gastrointestinal disorder; anemia; HIV positive; significant abnormal clinical laboratory (including unacceptable hematopoietic, hepatic, and renal function); food allergies or intolerances; current drug therapy for a diagnosed disease, including medications known to alter lipid metabolism; current use of weight-lowering medications or diets; use of tobacco products; use of CLA-containing dietary supplements during the previous 3 mo; consuming a medically prescribed diet that may interfere with the intervention; current or planned pregnancy; or no interest in participating in a clinical trial. The screening for eligibility included a complete physical exam by the study physician and a review of clinical laboratory analyses.

Study protocol. The study was a single-center, randomized, double-blind, placebo-controlled clinical trial. Fifty-five participants were randomized to receive 1 of 3 treatments: placebo (8 g safflower oil), 3.2 g/d CLA, or 6.4 g/d CLA for 12 wk. Participants were given 2 bottles of pills each (placebo group was given 2 bottles of placebo pills, 3.2 g/d CLA group was given 1 bottle of placebo and 1 bottle of CLA pills, and the 6.4 g/d CLA group was given 2 bottles of CLA pills) and instructed to take 1 pill from each bottle 4 times/d (once at each of 3 mealtimes and once before bedtime). The CLA pills (Tonalin, Cognis) were composed of the C18:2 *cis*-9, *trans*-11 and the C18:2 *trans*-10, *cis*-12 isomers in a 50:50 ratio. The placebo pills and CLA pills were identical in appearance. The randomization list was kept secure by the General Clinical Research Center (GCRC) Pharmacy and was opened only after participants had completed the study protocol. Participants were instructed to maintain their normal diet and exercise routine throughout the study protocol.

Body composition and EE measurement. Dual-energy X-ray absorptiometry was used to determine body composition at baseline and the final 12-wk visit (Hologic Delphi W model). Indirect calorimetry was used to estimate REE and respiratory quotient (RQ) at baseline and 12 wk. The Medgraphics Cardiorespiratory Diagnostic Systems CPX D Series Indirect Calorimeter was used for indirect calorimetry. At the start of each day, the machine underwent both a syringe volume and manual gas analyzer calibration. Each participant fasted overnight and then rested in the supine position for 10–30 min prior to being measured to allow time for breathing to become regular and to avoid the effects of previous voluntary activity on REE. Each participant was fitted with a noseclip and a flexible disposable mouthpiece that contained the pneumotach and gas detection line. Measurements were taken for 20 min in a dark, quiet, thermoneutral environment. All data used to derive REE and RQ are taken from a period of equilibrium or steady state that has been identified according to statistically defined guidelines and study protocol. The mean REE and RQ values over this accepted period were reported for each participant.

Clinical assessment. Participants reported to the GCRC 3 times throughout the 12-wk intervention, i.e., at baseline, at wk 6, and a final visit at wk 12. Because 24 h were needed for complete laboratory results to be obtained after the baseline visit, participants were randomized into the study on the day of their baseline visit and were instructed not to begin taking the study pills until the next day, following confirmation of

eligibility. Heart rate and blood pressure were monitored at each visit. Clinical laboratory analyses were conducted on fasting blood draws at each visit using validated methods at the McClendon Clinical Laboratories at the University of North Carolina Hospital, which is accredited by the Clinical Laboratory Improvement Act and the College of American Pathologists. The analyses included the following assessments: lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerols), alanine transaminase, aspartate transaminase, alkaline phosphatase, hemoglobin, hematocrit, bilirubin, calcium, chloride, creatinine, erythrocytes, white blood cells, platelets, γ -glutamyltransferase, lactate dehydrogenase, leukocytes, potassium, sodium, glucose, C-reactive protein, and IL-6. In addition, weight, height, and waist-to-hip ratio were measured at each visit.

Compliance was measured at 6 wk and 12 wk by comparing the number of unused pills with the number expected to be taken as well as by measuring plasma CLA values from baseline to wk 12 in each group, as follows. Lipids were extracted using the method of Bligh and Dyer (18). The lower (chloroform) phase was transferred to a clean tube and completely evaporated under nitrogen. The residual lipids were saponified and the fatty acids were trans-methylated by the sequential addition of 1 mL of 4.25% NaOH in CHCl₃:MeOH (2:1, v:v) and 1N HCl in saline (19). Fatty acid methyl esters were then resuspended in 50 μ L undecane, and analyzed using capillary GC on a Perkin Elmer Auto-System XL Gas Chromatograph, split injection, with helium as the carrier gas. The methyl esters were separated on a capillary column coated with 70% cyanopropyl polysilphenylene-siloxane (10 m \times 0.1 mm ID-BPX70 0.2 μ m; SGE); injector 240°C and detector 280°C. Data were analyzed with the Perkin Elmer Totalchrom Chromatography software, version 6.2. Heptadecanoic acid (17:0) was added to the samples as an internal standard to correct for recovery and quantitation. The identity of the CLA mixed isomers was confirmed by comparing retention times of individual isomers in the mixture against pure isomers of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA (Matreya).

Diet and exercise. Participants were instructed to maintain their current diet and exercise routines throughout the study period. To assess compliance with these instructions and examine potential changes in diet or exercise that may affect the outcome parameters, five 24-h dietary recalls were administered over the telephone to each participant over the 12 wk period. The timing of these varied from subject to subject, but were planned to occur once every 15 to 18 d. The recalls were telephone-administered by a trained interviewer using a multiple-pass, 24-h recall method using Nutrition Data System for Research (NDSR) software, version 5.0.35 developed by the Nutrition Coordinating Center at the University of Minnesota. Foods, beverages, preparation methods, amounts, and recipes reported by the subject were entered by an interviewer into the NDSR software package to obtain an estimate of nutrient intake. Following completion of all quality checks, the data were then rerun using the latest NDSR database version at the time (2005). A brief physical activity questionnaire was administered at baseline, 6 wk, and 12 wk to assess moderate to vigorous leisure-time activity (20) in the 3 mo prior to study enrollment and during the 12-wk intervention period. Activities were assigned a MET value, and the mean number of MET-h/wk was calculated for each participant.

Statistical analyses. Assuming a difference of 2.8 kg between the change in body fat mass of the placebo group and the 6.4 g/d CLA group with a SD of 2.4 kg, based on results from a previous study (4), an estimated 16 participants were required in each group for 90% statistical test power and 5% significance level. Means \pm SD or SEM for continuous variables and frequencies for categorical variables were calculated for each variable by treatment group. One-way ANOVA on continuous variables and chi-square test on categorical variables were used to determine whether baseline demographic characteristics and adverse event reporting rates differed between intervention groups. Log-transformation was performed on variables not normally distributed. Pre- and postgroup means were compared by *t* test. One-way ANOVA was used to examine whether changes from wk 0 to wk 12 were different between treatment groups. If ANOVA revealed that the overall test for treatment was significant, *t* tests were used for post-hoc comparisons. All

statistical tests were assessed at a level of significance $\alpha = 0.05$; each test was 2-sided (specified a null hypothesis of equality). For example, the *t* test comparing the pre- and postobservations was a one-sample *t* test of the difference (equivalent to a paired *t* test), which specified $H_0 \text{ diff} = 0$ vs. $H_a \text{ diff} \neq 0$. All data analyses were conducted using SAS statistical software, version 9.1 (SAS Institute). Subjects with only a baseline visit (wk 0) were not included in the analyses. Because there was a limited number of subject visits, the last-value-carried-forward method was not used here.

Results

Study subjects. Fifty-five participants were recruited into the study (Fig. 1). Three participants were determined to be ineligible after randomization (at the end of the baseline visit). Three participants withdrew within the intervention time period for personal reasons, and one withdrew due to pregnancy. Thus, the main analyses were conducted on a total of 48 participants. There were no differences in baseline age, BMI, or body fat mass among participants who withdrew and those who completed the study. LBM was significantly higher in those who withdrew from the study than in those who completed the study, which may be explained by the fact that a higher proportion of males did not complete the study (71% of noncompleters were male) compared with those who completed the study (25% of completers were male). Of the 7 individuals who were randomized but did not complete the study, 1 was in the placebo group, 4 were in the 3.2 g/d CLA group, and 2 were in the 6.4 g/d CLA group.

Treatment groups did not differ in demographic characteristics or in weight, BMI, body fat mass, LBM, or physical activity at baseline (Table 1). Based on pill count, compliance was 88% for the placebo group, 92% for 3.2 g/d CLA group and 92% for the 6.4 g/d CLA group. Supporting this, plasma CLA concentrations increased in both the 3.2 g/d ($+0.02 \pm 0.02$ mol/L, $P = 0.002$; range = -3.4 to $+57.1$ mol/L) and 6.4 g/d ($+0.05 \pm 0.05$ mol/L, $P = 0.002$; range = 0 to $+99.5$ mol/L) groups, but did not change in the placebo group.

Changes in weight, body composition, REE, RQ, estimated energy intake and physical activity. Body fat mass, weight, BMI, and percentage of fat did not change in any of the groups after 12 wk of intervention (Table 2), and there was no effect of the intervention on the waist-to-hip ratio (data not shown). LBM increased by 0.64 kg in the 6.4 g/d CLA group ($P < 0.05$) and tended to increase by 0.65 kg in the 3.2 g/d CLA group ($P = 0.18$). There were no changes in REE or RQ as measured by indirect calorimetry in any of the groups. Physical activity in MET-h/wk reported throughout the 12-wk intervention period

for each group was, for the placebo group, 23.6 ± 12.2 ; for the 3.2 g/d CLA group, 22.4 ± 11.9 ; and for the 6.4 g/d CLA group, 20.5 ± 13.8 , which represented a significant decrease from baseline for the placebo and 6.4g/d CLA groups ($P < 0.05$). Estimated energy intakes throughout the 12-wk intervention period did not differ among the groups and were 7949.2 ± 1852.0 kJ/d for the placebo group, 8167.3 ± 1563.8 kJ/d for the 3.2 g/d CLA group, and 7651.0 ± 2087.0 kJ/d for the 6.4 g/d CLA group.

Effects of CLA on clinical laboratory analyses. HDL-cholesterol decreased in both the placebo (-0.1 mmol/L) and 6.4 g/d CLA groups (-0.1 mmol/L) following the intervention (Table 3). C-reactive protein ($P = 0.03$), IL-6 ($P = 0.04$), WBC ($P = 0.005$), and alkaline phosphatase ($P = 0.002$) increased, and hemoglobin ($P = 0.01$), hematocrit ($P = 0.03$), and sodium ($P = 0.0008$) decreased in the 6.4 g/d CLA group from wk 0 to wk 12, although the absolute mean values remained within normal limits. Of the parameters that changed in the 6.4 g/d CLA group, only the changes in C-reactive protein, IL-6, and WBC differed significantly from those in the placebo group. Other clinical laboratory values did not change across the intervention in any of the groups (data not shown).

Adverse events. Thirteen participants (27%) reported adverse events (4 in the placebo group reported gas or bloating; 2 in the 3.2 g/d CLA group reported gas or itching of the face; and 7 in the 6.4 g/d CLA group reported gas, bloating, indigestion, diarrhea, or heartburn). There were no serious adverse events, and the majority of adverse events were mild gastrointestinal symptoms that may have been related to the study pills. Subjects who withdrew from the study reported no adverse events during the intervention nor did they report an adverse event as the reason for withdrawal.

Discussion

We conducted a randomized, double-blind, placebo-controlled clinical trial to examine the effects of 2 doses of CLA supplementation for 12 wk on body composition and clinical safety measures in healthy obese humans. No substantial changes in body fat mass, weight, BMI, REE, or RQ were observed during the intervention. LBM increased significantly in the 6.4 g/d CLA-supplemented group. Significant increases in C-reactive protein and IL-6 were observed in the 6.4 g/d CLA group, suggesting an increase in inflammation during the short-term supplementation.

Previous clinical trials examining the effects of CLA supplementation on body composition in humans have found either

FIGURE 1 Study participants' randomization and trial completion.

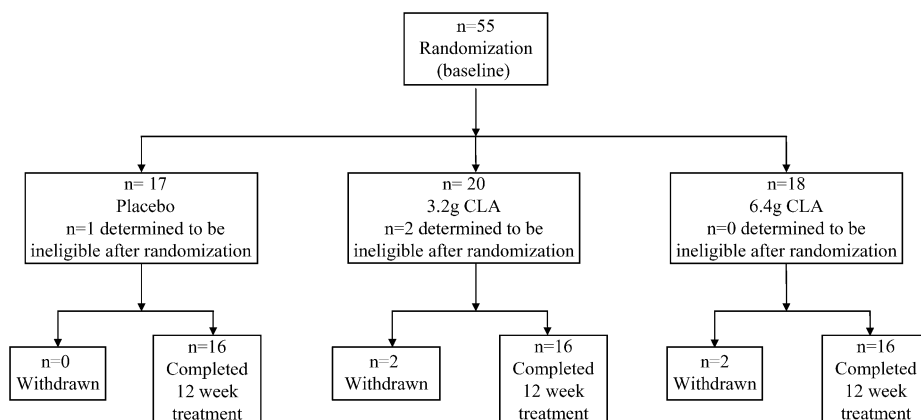


TABLE 1 Baseline characteristics of the healthy obese study population by intervention group¹

	Placebo	3.2 g/d CLA	6.4 g/d CLA
Sex, % (n)			
Male	25 (4)	31 (5)	25 (4)
Female	75 (12)	69 (11)	75 (12)
Race, % (n)			
African American	31 (5)	31 (5)	38 (6)
White	69 (11)	63 (10)	62 (10)
Other	0	6 (1)	0
Age, y	34.9 ± 8.0	36.3 ± 8.9	34.1 ± 8.9
Height, cm	166.7 ± 8.9	169.2 ± 7.1	166.3 ± 8.1
Weight, kg	91.1 ± 10.1	94.0 ± 9.2	90.5 ± 8.3
BMI, kg/m ²	32.7 ± 1.9	32.7 ± 1.8	32.7 ± 1.7
Body fat mass, kg	35.6 ± 4.9	34.8 ± 5.4	34.8 ± 5.3
Lean body mass, kg	50.7 ± 7.9	54.1 ± 9.0	50.9 ± 9.1
Resting energy expenditure, kJ/d	7312.1 ± 1592.6	6944.5 ± 1761.0	7052.8 ± 1085.9
Respiratory quotient	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
Physical activity, MET-h/wk	31.4 ± 19.2	26.9 ± 20.2	24.4 ± 13.1

¹ Values are % (n) or means ± SD, n = 16.

significant changes in a beneficial direction (i.e., decrease in body fat mass or increase in LBM) or null results [6,21,22; see also studies published prior to August 2003 reviewed in (23)]. Our study adds to the evidence that CLA supplementation increases LBM but has no effect on body fat mass, weight, or BMI. Blankson et al. (4) found an increase of 0.88 kg in LBM among overweight and obese individuals supplemented with 6.8g/d CLA daily for 12 wk, although it was unclear whether the effect of LBM was solely related to CLA or to training in this study because the 6.8 g/d CLA group also intensified their physical training during the intervention time period. In comparison, the 6.4 g/d CLA supplemented group in our study increased LBM a mean of 0.64 kg and reported a significant decrease in physical activity during the intervention period, suggesting that the increase in LBM was related to CLA supplementation and not to a concomitant increase in training.

In contrast to the conflicting results from human trials, CLA is effective at reducing body fat mass in animal studies (17,24). Although the biological mechanisms have not been conclusively defined, there is evidence that CLA 1) increases energy expenditure in animals perhaps through changes in gene expression for genes encoding uncoupling proteins, 2) decreases adipocyte size, 3) inhibits preadipocyte differentiation, 4) increases adipocyte

TABLE 2 Changes from wk 0 to wk 12 in weight, BMI, body composition, and REE in the healthy obese adults treated with 0, 3.2, or 6.4 g/d of CLA¹

	Placebo	3.2 g/d CLA	6.4 g/d CLA
		<i>unit/12 wk</i>	
Body weight, kg	+0.43 ± 0.57	+0.40 ± 0.30	+0.39 ± 0.43
BMI, kg/m ²	+0.01 ± 0.27	+0.14 ± 0.14	+0.07 ± 0.19
Body fat mass, kg	+0.14 ± 0.58	-0.09 ± 0.03	-0.17 ± 0.36
Lean body mass, kg	+0.33 ± 0.32	+0.65 ± 0.47	+0.64 ± 0.29*
Fat, %	-0.06 ± 0.44	-0.33 ± 0.39	-0.43 ± 0.30
REE, kJ/d	-169.52 ± 399.66	-143.11 ± 234.99	+28.51 ± 240.31
Respiratory quotient	+0.03 ± 0.04	+0.03 ± 0.02	-0.01 ± 0.02

¹ Values are means ± SEM, n = 16. *Different from zero, P < 0.05.

apoptosis, 5) inhibits lipogenesis, and 6) increases lipid oxidation (25). A review of the human studies published up to August 2003 on the effects of CLA supplementation on body composition, plasma lipids, and insulin resistance finds little evidence for beneficial effects of CLA compared with results in mice (23). One possible reason for the difference in magnitude of effects in mice compared with humans are that the dosages administered to animals are often much higher than those administered to humans. It has also been suggested that an explanation for the disparate results between species may be related to differences in metabolic rates (1). Theoretically, if CLA is working through increasing energy expenditure, the same percentage of increase in energy expenditure would result in larger effects on fat mass in mice than in humans because of the larger REE in mice. We examined whether CLA altered REE and RQ using indirect calorimetry in our population of obese humans. Consistent with a previous clinical study (12), we found no effect of CLA on REE. However, there was a significant (P = 0.02) decrease in exercise reported by the participants during the 12-wk intervention period, and it is possible that decreased exercise could offset modest increases in energy expenditure from CLA supplementation, resulting in no observable effect in this study.

Toxicity of CLA supplementation, such as liver steatosis, has been reported in mice [reviewed in (17)]. To examine the safety of CLA supplementation in healthy obese humans, we assessed the effect of 2 doses of CLA supplementation on liver enzymes, complete blood count, glucose and inflammatory markers. Whereas significant decreases in hemoglobin, hematocrit, and sodium, and significant increases in alkaline phosphatase and white blood cells were observed in the 6.4 g/d CLA-supplemented group, mean values remained within normal limits during the intervention period. It is also important to note that, whereas alkaline phosphatase increased and hematocrit and hemoglobin decreased in the 6.4g/d CLA group, the absolute mean values after the intervention were the same in this group as in the placebo group. Thus, potential toxicities as noted in animal studies were not present in this study at the doses of CLA administered. We should note that our study was not powered specifically to examine changes in clinical laboratory measures. Thus, we cannot exclude the possibility that changes in some of the other clinical laboratory values could have reached significance given a larger sample size. Adverse events of mild gastrointestinal disorders were reported in all intervention groups, with no significant difference in the frequency between groups. Similarly, other studies report no differences in the rate of adverse events between placebo and CLA-supplemented groups (4,6,26).

Based on previous literature, the effect of CLA supplementation on HDL-cholesterol is unclear. Some studies have shown a decrease in HDL-cholesterol with mixed isomer CLA supplementation (4,6,9,14), one study found an increase in HDL-cholesterol (16), and another reported no effect (27). In another study that examined the effect of supplementation with the individual isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12, no effect on HDL-cholesterol was observed for either isomer after 8 wk supplementation, whereas triacylglycerols significantly increased in the *trans*-10, *cis*-12 CLA supplemented group (28). In the present study, HDL-cholesterol significantly decreased in the 6.4 g/d CLA group and the placebo group, whereas there were no significant changes in triacylglycerols, LDL-cholesterol, or total cholesterol. Thus, the effects of CLA, and in particular, of the individual isomers, on lipid profile need to be further examined to assess the atherogenic potential of CLA supplementation in humans.

TABLE 3 Clinical laboratory values at baseline and after 12 wk of treatment with 0, 3.2, or 6.4 g/d of CLA in healthy obese adults¹

	Placebo			CLA 3.2 g/d			CLA 6.4 g/d		
	Baseline	Wk 12	Change	Baseline	Wk 12	Change	Baseline	Wk 12	Change
Serum lipids, mmol/L									
Triacylglycerols	1.3 ± 0.6	1.4 ± 0.6	+0.1 ± 0.3	1.2 ± 0.8	1.3 ± 0.7	+0.1 ± 0.4	1.4 ± 0.7	1.5 ± 0.2	+0.1 ± 0.7
Total cholesterol	5.3 ± 0.8	5.1 ± 0.7	-0.2 ± 0.5	4.8 ± 0.9	4.9 ± 0.8	+0.1 ± 0.5	4.8 ± 0.9	4.7 ± 1.2	-0.1 ± 0.5
LDL-cholesterol	3.1 ± 0.7	2.9 ± 0.6	-0.2 ± 0.5	2.9 ± 0.8	3.0 ± 0.9	+0.1 ± 0.4	2.7 ± 0.9	2.7 ± 1.0	0 ± 0.4
HDL-cholesterol	1.6 ± 0.3	1.5 ± 0.4	-0.1 ± 0.1*	1.3 ± 0.3	1.3 ± 0.3	0 ± 0.2	1.4 ± 0.4	1.3 ± 0.3	-0.1 ± 0.1*
Serum liver enzymes, U/L									
Alkaline phosphatase	80.2 ± 22.0	82.6 ± 21.3	+2.4 ± 12.6	72.1 ± 15.4	73.9 ± 16.4	+1.8 ± 6.6	74.4 ± 16.1	79.5 ± 16.6	+5.1 ± 5.3*
Alanine transaminase	36.1 ± 25.4	36.1 ± 21.0	0 ± 12.1	33.4 ± 12.1	30.3 ± 10.3	-3.1 ± 10.1	27.4 ± 11.0	34.4 ± 17.6	+7.0 ± 18.2
Aspartate transaminase	28.1 ± 10.2	26.4 ± 9.1	-1.7 ± 6.4	25.9 ± 6.3	24.4 ± 6.7	-1.5 ± 4.7	24.1 ± 5.4	26.2 ± 9.7	+2.1 ± 8.7
γ-Glutamyltransferase	34.3 ± 28.6	36.9 ± 38.7	+2.6 ± 29.5	27.3 ± 11.1	26.9 ± 11.0	-0.4 ± 4.3	23.5 ± 16.3	26.9 ± 20.0	+3.4 ± 6.7
Complete blood count									
Hematocrit	0.39 ± 0.03	0.39 ± 0.03	0 ± 0.02	0.41 ± 0.03	0.41 ± 0.04	0 ± 0.02	0.40 ± 0.03	0.39 ± 0.03	-0.01 ± 0.02*
Hemoglobin, g/L	136.2 ± 13.1	134.6 ± 13.1	-1.6 ± 6.8	140.6 ± 13.3	140.5 ± 15.9	-0.1 ± 6.0	138.6 ± 13.0	134.7 ± 14.3	-3.9 ± 5.4*
Leukocytes, %	2.3 ± 0.9	2.5 ± 1.3	+0.2 ± 1.3	2.6 ± 0.9	2.8 ± 0.7	+0.2 ± 1.0	2.4 ± 0.9	2.2 ± 0.5	-0.2 ± 1.0
White blood cells, n/L (× 10 ⁹)	6.5 ± 1.3	6.6 ± 1.6	+0.1 ± 0.7 ^a	6.2 ± 2.1	5.8 ± 1.6	-0.4 ± 1.7 ^a	6.2 ± 1.1	7.4 ± 1.6	+1.2 ± 1.5 ^{ab}
Other serum analytes									
C-reactive protein, mg/L	11.2 ± 10.7	9.6 ± 7.7	-1.6 ± 3.9 ^a	6.0 ± 2.1	7.5 ± 5.1	+1.5 ± 5.3 ^{ab}	7.2 ± 3.5	10.3 ± 6.7	+3.1 ± 5.1 ^{ab}
IL-6, pg/L	2078.0 ± 1289.3	1713.0 ± 905.4	-365.0 ± 1145.6 ^a	1749.1 ± 1460.3	2071.9 ± 1708.1	+322.8 ± 1248.9 ^{ab}	1632.1 ± 776.1	2071.1 ± 1131.6	+439.0 ± 830.8 ^{ab}
Sodium, mmol/L	142.1 ± 1.8	141.4 ± 3.0	-0.7 ± 2.4	141.5 ± 1.9	141.4 ± 2.3	-0.1 ± 2.0	141.9 ± 1.8	140.6 ± 1.2	-1.3 ± 1.3*
Glucose, mmol/L	5.1 ± 0.3	5.1 ± 0.3	0 ± 0.3	5.1 ± 0.4	5.1 ± 0.5	0 ± 0.4	5.1 ± 0.4	5.2 ± 0.4	+0.1 ± 0.3

¹ Values are means ± SD, *n* = 16. Means in a row with superscripts without a common letter differ, *P* < 0.05. *Different from wk 0 to wk 12 within treatment group, *P* < 0.05 (paired *t* test).

Previous studies have raised concerns regarding the effects of CLA on oxidative stress, inflammation, and insulin resistance (29), and particularly of the *trans*-10, *cis*-12 isomer when administered in isolation (13,30,31). Increased C-reactive protein was observed in a trial of men with metabolic syndrome in response to supplementation for 3 mo with 3.4 g/d of purified *trans*-10, *cis*-12 CLA isomer (29). A study by Smedman et al. (32) also found increases in C-reactive protein in normal weight humans supplemented with 4.2 g/d CLA as mixed isomers for 12 wk, but did not find changes in other markers of inflammation such as TNF-α, sTNFR1, sTNFR2 or VCAM-1. We found a significant increase in C-reactive protein in the 6.4 g/d CLA group, but not in the 3.2 g/d CLA or placebo groups, with mean concentrations approaching the upper limit of normal for the 6.4 g/d CLA group after the 12 wk intervention, which was not different from the mean concentration of the placebo group at 12 wk. In our study, another marker of inflammation, IL-6, also increased significantly in the 6.4 g/d CLA group. In contrast to our positive findings and those of others, a study by Moloney et al. (16) found no effect on C-reactive protein or IL-6 of 3.0 g/d mixed isomer CLA supplemented for 8 wk among type II diabetics. It is unclear how long-term CLA supplementation may affect inflammatory markers and how transient increases in inflammatory markers may affect adipogenesis or chronic disease risk, and this is an important area of future research.

In conclusion, we found that supplementation with 6.4 g/d CLA for 12 wk increases LBM in healthy obese humans, but has no significant effect on body fat mass, weight, BMI, REE, or RQ. Increases in C-reactive protein and IL-6 in the 6.4 g/d CLA group suggest that the higher dose may increase markers of inflammation in healthy obese humans. CLA is widely used in commercial supplements as well as being present in the diet, thus, these results need to be confirmed by future studies using varying doses of CLA over longer periods of time and in different patient populations.

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