

Conjugated linoleic acid supplementation alters the 6-mo change in fat oxidation during sleep^{1–3}

Rachel N Close, Dale A Schoeller, Abigail C Watras, and Elizabeth H Nora

ABSTRACT

Background: Conjugated linoleic acid (CLA) is a family of positional and geometric isomers with 2 conjugated double bonds formed from linoleic acid and linolenic acid. CLA has a wide range of biological effects, including body fat reduction.

Objective: The aim of our study was to determine CLA's effects on energy expenditure, macronutrient utilization, and dietary fat oxidation in overweight adults after 6 mo of supplementation.

Design: We recruited 23 subjects from our main CLA efficacy study who were receiving either 4 g/d of 78% active CLA isomers (3.2 g/d: 39.2% *cis*-9,*trans*-11 and 38.5% *trans*-10,*cis*-12) or 4 g/d of safflower oil. Energy expenditure and substrate utilization were measured before and after 6 mo of CLA supplementation by using whole-room indirect calorimetry. Dietary fat oxidation was measured by using stable isotope-labeled oleate and palmitate.

Results: Our substudy detected a difference in the change in fat utilization between the CLA (4 ± 8 g) and placebo (-7 ± 11 g) groups during sleep after 6 mo of supplementation. In addition, the percentage of energy from protein was reduced during sleep in the CLA group (CLA: $-3.3 \pm 2.6\%$; placebo: $0.3 \pm 5.7\%$). We also detected a difference in the change in energy expenditure during sleep (CLA: 0 ± 38 kcal; placebo: -43 ± 90 kcal). We did not detect a change in labeled dietary fat oxidation after 6 mo of CLA supplementation given with a breakfast meal.

Conclusion: Mixed isomer CLA supplementation, but not placebo, positively altered fat oxidation and energy expenditure during sleep. *Am J Clin Nutr* 2007;86:797–804.

KEY WORDS Energy expenditure, fat oxidation, substrate utilization, overweight, obesity treatment

INTRODUCTION

Conjugated linoleic acid (CLA) is a family of positional and geometric isomers with 2 conjugated double bonds formed from linoleic acid, which displays a wide range of biological effects. CLA-related health benefits first received notice in 1985 when Pariza and Hargraves (1) isolated a compound in grilled ground beef that inhibited epidermal carcinogenesis in mice. In 1997, CLA's effects on reducing body fat were first reported (2). CLA is being further evaluated for its potential role in atherosclerosis (3–5), the immune response (6–9), body composition (2, 10, 11), lipid metabolism (12, 13), and glucose and insulin tolerance (14–16).

CLA's role in altering body composition is fairly well established in animal models, but studies in humans have shown

mixed results. Some short-term human studies detected a decrease in body fat (17–21), whereas other studies did not detect an effect (11, 22). Gaullier et al (10, 23) conducted a 2-y study, the longest human study to date, and clearly showed a decrease of ≈ 2 kg in body weight and body fat mass in an overweight mixed-sex population with no serious adverse events.

The mechanism through which CLA attenuates adiposity is thought to involve either adipocyte apoptosis (24–26) or decreased adipocyte cell size secondary to a decrease in triacylglycerol uptake (27–29). Others proposed that CLA decreases percentage body fat by increasing energy expenditure (30), fat oxidation, or both (27); yet, when an increase in fat oxidation was detected, it was unknown whether it was from increased dietary fat oxidation or endogenous fat oxidation. Numerous studies investigated the effect of CLA on the fatty acid composition of agricultural production animals and found an increased ratio of saturated fatty acids to monounsaturated fatty acids (31–33); however, little is known about the ability of CLA to alter the type of fatty acids being oxidized in the body.

The primary aim of the present study was to investigate whether CLA increases total fat oxidation under eucaloric conditions. Secondary aims were to test for any alterations in dietary fat oxidation by using stable isotopes, endogenous fat, or potentially both and to test for any changes in energy expenditure by using a whole-room indirect calorimeter. Our study was a substudy performed as part of a larger study for which the primary aim was to determine whether 3.2 g/d of CLA for 6 mo would alter body fat mass in an overweight adult population (34).

SUBJECTS AND METHODS

Protocol

The present study was done as part of a double-blind, randomized, 6-mo intervention trial. The first participants began the trial in August 2004, and the last subjects ended the trial in March

¹ From the Departments of Nutritional Sciences (RNC, DAS, and ACW) and Internal Medicine (EHN), University of Wisconsin-Madison, Madison, WI.

² Supported by Cognis Nutrition & Health (LaGrange, IL) and by NIH grant RR03186.

³ Reprints not available. Address correspondence to DA Schoeller, 1415 Linden Drive, Madison, WI 53706. E-mail: dschoell@nutrisci.wisc.edu.

Received July 27, 2006.

Accepted for publication May 14, 2007.

2005. Subjects were screened and then underwent baseline evaluation. The substudy evaluation measured 24-h energy expenditure and substrate utilization by using a whole-room indirect calorimeter. Dietary fat oxidation was measured by mixing [$1-^{13}\text{C}$]oleate and D_{31} -palmitate into a breakfast meal and then collecting breath carbon dioxide and urine to measure the end products of oxidation. Subjects were then provided either 4 g/d of 78% active CLA isomers (3.2 g/d: 39.2% *cis*-9,*trans*-11 and 38.5% *trans*-10,*cis*-12) or 4 g/d of safflower oil placebo as 1-g gel capsule supplements. The baseline evaluations were repeated 6 mo later.

Subjects

The aim of the main study was to compare the effects of 4 g/d of 78% active CLA isomers (3.2 g/d: 39.2% *cis*-9,*trans*-11 and 38.5% *trans*-10,*cis*-12) and 4 g/d of safflower oil placebo supplement for 6 mo on body composition by using the 4-compartment model (34).

The main CLA efficacy study involved 48 overweight subjects, from which 23 subjects (4 men and 19 women) were selected at random for participation in the substudy. The substudy required 2 additional overnight visits to the University of Wisconsin-Madison Hospital's General Clinical Research Center (GCRC). During recruitment, subjects were informed about the substudy and were excluded from both studies if they chose to opt out of the substudy at the time of random assignment.

The main study and this substudy were approved by the Health Sciences Internal Review Board of the University of Wisconsin-Madison. The subjects provided written informed consent for both studies before the beginning of the study.

Inclusion criteria were the following: 18–44 y of age, overweight but not obese [body mass index (BMI; in kg/m^2) ≥ 25 to < 30], stable weight within 3 kg for at least the previous 6 mo, and no current or planned pregnancies. Exclusion criteria were the following: pregnancy or lactation, smoking, a physical limitation causing difficulty to participate in a prescribed walking program, recent use of a weight-loss program, metabolic disease history, psychiatric or eating disorders, abnormal electrocardiogram, fasting plasma cholesterol concentrations ≥ 300 mg/dL, and triacylglycerol concentrations ≥ 500 mg/dL.

Because of scheduling limitations, it was not possible to fully control for menstrual cycle. Of the 16 women, at baseline, 4 in the CLA group and 5 in the placebo group were in the first half of their menstrual cycle, whereas the remaining 5 and 2 women in the CLA and placebo groups, respectively, were in the second half of their menstrual cycle. For the final overnight visit, 2 women in the CLA group and 5 women in the placebo group were in the first half of their menstrual cycle, whereas the remaining 7 and 2 women in the CLA and placebo groups, respectively, were in the second half of their menstrual cycle.

Respiratory chamber

During the overnight visits in the GCRC, subjects stayed in a 12-m^3 shell volume whole-room respiratory chamber for measurement of macronutrient utilization, energy expenditure based on carbon dioxide production ($\dot{V}\text{CO}_2$), oxygen consumption ($\dot{V}\text{O}_2$), and urinary nitrogen production. This chamber was previously described (35). Analyzers measured the concentration of carbon dioxide and oxygen in the chamber effluent on a minute-to-minute basis in combination with measuring carbon dioxide

and oxygen concentrations of 3 calibration gases for 1 min every 15 min. Protein oxidation was determined by analysis of urinary nitrogen concentrations. This information was then used to calculate $\dot{V}\text{CO}_2$, $\dot{V}\text{O}_2$, energy expenditure, respiratory quotient (RQ), and macronutrient utilization by using the equations of Jequier et al (36). First, protein oxidation was calculated by using urinary nitrogen concentrations and total void volumes. Then, nonprotein $\dot{V}\text{CO}_2$ and $\dot{V}\text{O}_2$ were calculated. Finally, fat utilization and carbohydrate utilization were calculated by using the nonprotein $\dot{V}\text{CO}_2$ and $\dot{V}\text{O}_2$ data from the chamber.

To ensure the calibration of the unit, routine methanol burns were conducted. This involves burning a known amount of anhydrous methanol (99.8% pure; Acros Organics, Geel, Belgium) for a known amount of time, ≥ 10 h, while collecting $\dot{V}\text{O}_2$, $\dot{V}\text{CO}_2$, and oxygen production data. Subjects' chamber data were adjusted if the methanol burns deviated $> 2\%$ from the theoretical recoveries for either oxygen or carbon dioxide.

Energy and substrate metabolism

Subjects arrived at the GCRC for admission at 2000; they had been asked to refrain from exercise for the 24 h before admission. Female subjects provided a urine sample at this time to test for pregnancy. Before entering the respiratory chamber at 2100, all subjects emptied their bladders. They were instructed to provide urine samples at 2300 on the night of admission and on the next day at the following time points: 0645, 0800, 1200, 1600, 2000, and 2200. Subjects were instructed to be in bed at 2300 and to lie quietly in the dark with minimal movements if unable to sleep. Subjects were awakened at 0645 and exited the chamber at 0700 for showering and other grooming. At 0745 they reentered the chamber, and at 0800 they provided a baseline breath sample. Once samples were collected, breakfast was given. This meal included a heated drink into which the tracer fats had been mixed. The 2 tracers were [$1-^{13}\text{C}$]oleate (10 mg/kg body wt) and D_{31} -palmitate (15 mg/kg body wt). Subjects then provided hourly breath samples and the remaining scheduled urine samples. If they had to void between urine collection time points, that urine was also collected. Meals were provided at 1200 and 1900, with a snack at 1600. Subjects were released from the respiratory chamber at 2100; however, they remained at the GCRC until their final urine sample was collected at 2200. This overnight stay was conducted before the start of CLA supplementation and then again at the end of the 6-mo supplementation period. Data from 3 time periods were analyzed: a 24-h time period; a waking time period, which was from 2100 to 2300 and from 0645 to 2100; and a sleeping time period, which was from 2300 to 0645.

Meals

A 3-d lead-in diet menu and the corresponding foods were provided to subjects before their admission dates. The lead-in diet consisted of frozen meals and shelf-stable items calculated to give 36% of energy as fat, 18% as protein, and 46% as carbohydrate. The diet was tailored to meet the energy needs of each subject on the basis of the Harris Benedict equation with 7% subtracted to account for the equation's tendency to overestimate caloric need for overweight and obese subjects. This was then multiplied by an assumed 1.65 physical activity level. If subjects did not eat all food listed on their menu, they recorded what they did not eat, and their lead-in diet records were adjusted accordingly. During hospital stays, subjects ate a diet of 36% fat, 18%



protein, and 46% carbohydrate, which was formulated by the research dietitian. Breakfast, lunch, dinner, and the afternoon snack provided 25%, 30%, 35%, and 10% of their daily energy intake, respectively. The caloric need for each subject in the chamber was calculated in the same manner as the lead-in diet except that the physical activity level applied was only 1.4 because of the restrictive nature of the respiratory chamber. During the stay, subjects were instructed to eat all food provided. Any amount left on the plate was measured and subtracted from the inpatient diet records. The lead-in diet and inpatient diet did not allow for any caffeine or alcohol.

Sample collection and analysis

Nitrogen

Complete urine samples were collected and analyzed for total nitrogen, from which protein oxidation was calculated. While in the indirect calorimeter, the subjects' total volume of each void, scheduled and unscheduled, was recorded. From each void, 15-mL urine aliquots were immediately put into a 50-mL falcon tube containing citric acid (99% pure; Acros Organics) to prevent loss of ammonia. They were later pooled into a "waking" specimen and a "sleeping" specimen by taking an aliquot that was proportional to the volume of the void, thus creating a volume-weighted sample. The 2 pooled samples for waking and sleeping were diluted 1:100 with nitrogen-free water. The diluted samples were then analyzed for total nitrogen in triplicate by using an Antek 9000N Chemiluminescent Nitrogen analyzer (Antek Instruments Inc, Houston, TX). This instrument was calibrated by using gravimetric urea solutions, which were then used as standards during the sample runs. Pearson's regression coefficients for the calibration curve were >0.999.

Stable isotopes

The oxidation of stable isotope-labeled dietary fats was assessed on the basis of breath carbon dioxide and urinary water. The excess ^{13}C recovery in the breath carbon dioxide was used to calculate dietary monounsaturated fat utilization during the chamber stay as previously described (35). Breath samples were collected by having subjects blow through a straw into a labeled 15-mL red top no-additive Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ), which was capped immediately after being filled. The ratio of $^{13}\text{C}:^{12}\text{C}$ in the sample was analyzed by injecting a small amount into an isotope ratio gas chromatography mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany). Each sample was injected in duplicate, and the mean ^{13}C abundance (in ppm) was used in further calculations. The acceptable average SD $^{13}\text{C}:^{12}\text{C}$ analysis was ≤ 0.3 ppm. If the replicates were more variable, the analysis was repeated. $^{13}\text{CO}_2$ recovery was calculated by subtracting baseline ^{13}C in breath carbon dioxide taken at 0800, with correction for background ^{13}C variations by using control subjects not dosed with labeled oleate, and then multiplying by the measured carbon dioxide production by using the trapezoid rule. An acetate correction, previously attained by testing sedentary subjects dosed with [^{13}C]acetate in the respiratory chamber, was used to adjust for the loss of ^{13}C in intermediary metabolism (35).

Deuterium recovery was calculated as previously described to determine dietary saturated fat utilization (35). At each scheduled time point for urine collection, 5-mL urine aliquots were put into a 5-mL Vacutainer with no additive and stored in a -70°C

TABLE 1

Study subjects' characteristics at baseline for the main study and the substudy¹

	Main study		Substudy	
	CLA (n = 22)	Placebo (n = 18)	CLA (n = 12)	Placebo (n = 7)
Sex (n)				
Male	5	3	3	1
Female	17	15	10	9
Age (y)	34 ± 8 ²	32 ± 7	33 ± 7	33 ± 6
Weight (kg)	80.0 ± 9.1	79.0 ± 10.9	81.4 ± 10.0	74.8 ± 5.0
BMI (kg/m ²)	27.6 ± 1.8	28.0 ± 2.2	27.5 ± 1.8	27.1 ± 1.9

¹ CLA, conjugated linoleic acid. No statistically significant between-group differences were detected.

² $\bar{x} \pm \text{SD}$ (all such values).

freezer until analyzed for deuterium abundance. Each of the urine samples was decolorized by using carbon black and was then filtered through a 0.45- μm pore size acetate filter. The decolorized urine samples were then put into individual glass vials and capped, and the average $^2\text{H}:^1\text{H}$ ratio abundances (in ppm) were assessed in duplicate with the use of a Delta Plus isotope ratio mass spectrometer equipped with an H/D Device (Finnigan MAT). The acceptable average SD for $^2\text{H}:^1\text{H}$ analysis was ≤ 0.08 ppm. If results were more variable, the analysis was repeated. The deuterium abundances were corrected for each individual's baseline abundances in the urine specimen collected before the breakfast dose. The cumulative percentage dose recovery was calculated by multiplying the excess deuterium abundance at each time point by the subject's total body water, as measured by ^{18}O dilution, then dividing by the amount of deuterium from the D_{31} -palmitate provided in the breakfast shake. The resulting dietary fat oxidation value was assigned to the time of the midpoint between successive voids.

Statistical analysis

All statistical analyses were performed with SAS software (version 8.2; SAS Institute Inc, Cary, NC). Means \pm SDs were calculated for the continuous outcomes. Between-group differences for changes from month 0 to month 6 were assessed by *t* tests and also by Wilcoxon's rank tests because normality could not be assured given the small sample size. The results were consistent between the 2 tests. Within-group differences for changes from month 0 to month 6 were assessed by paired *t* tests. All data were also analyzed with an adjustment for sex, which did not alter the findings. All values are presented as means \pm SDs, and statistical significance was set at an α level of 0.05 for all analyses.

RESULTS

The subjects were all overweight on the basis of BMI and were mostly female (**Table 1**). As expected with randomization, the participants of the substudy were representative of the main study at baseline. The 2 arms of the study, however, were not balanced, and this was due to 4 dropouts, all from the placebo group. One subject withdrew immediately after the initial inpatient testing

TABLE 2
24-h Substrate utilization at baseline and at month 6¹

	CLA (n = 12)			Placebo (n = 7)		
	Month 0	Month 6	Δ6-0	Month 0	Month 6	Δ6-0
RQ	0.85 ± 0.03	0.84 ± 0.02	-0.01 ± 0.04	0.84 ± 0.03	0.85 ± 0.03	0.01 ± 0.04
Energy expenditure (kcal)	2280 ± 319	2250 ± 305	-30 ± 83	2109 ± 119	2091 ± 148	-18 ± 156
Carbohydrate						
(%)	42.9 ± 7.3	40.7 ± 6.3	-2.2 ± 9.8	39.5 ± 7.2	43.3 ± 9.3	3.8 ± 11.7
(g)	250 ± 58	231 ± 56	-19 ± 48	208 ± 34	226 ± 49	18 ± 59
Fat						
(%)	37.9 ± 7.5	41.6 ± 6.8	3.7 ± 10.7	42.7 ± 7.8	40.2 ± 10.1	-2.5 ± 13.8
(g)	95 ± 19	103 ± 17	8 ± 28	101 ± 22	94 ± 25	-7 ± 34
Protein						
(%)	17.2 ± 1.8	15.4 ± 2.3	-1.8 ± 1.9	15.5 ± 3.3	14.1 ± 3.0	-1.5 ± 3.7
(g)	98 ± 21	87 ± 18	-12 ± 11	81 ± 15	74 ± 16	-8 ± 20

¹ All values are $\bar{x} \pm$ SD. CLA, conjugated linoleic acid; RQ, respiratory quotient. No statistically significant differences were observed between groups at baseline or at 6 mo or for the 0- to 6-mo change (paired *t* tests, *P* < 0.05).

because of time constraints, and another subject withdrew because of time constraints in scheduling the final visit. Two additional subjects were excluded because one started taking a medication known to affect body weight and the other began marathon training despite instructions not to alter exercise patterns. No data from these 4 individuals were used in the analysis. This left 12 subjects in the CLA treatment group and only 7 in the placebo group for data analysis. The decreased sample size may have limited our ability to attain the desired level of significance in the analysis of certain data, specifically the waking respiratory quotient. The only difference between the CLA and placebo groups at baseline was greater energy expenditure in the CLA group during sleep, which remained at month 6.

Energy intake and energy balance

There were no differences between groups at month 0 or month 6 for energy intake for the inpatient diet or the lead-in diet. The macronutrient breakdown for the lead-in diet was 35 ± 0.6%, 18 ± 0.9%, and 48 ± 1.5% for fat, protein, and carbohydrate, respectively, for the CLA group and was 36 ± 1.5%, 18 ± 1.8%, and 48 ± 1.2% for fat, protein, and carbohydrate, respectively, for the placebo group. The inpatient dietary macronutrient

breakdown for both groups was 18 ± 0.1% protein and 46 ± 0.1% carbohydrate, whereas fat was 36 ± 0.05% for the CLA group and 37 ± 0.05% for the placebo group.

24-h Time point

No differences were detected in 24-h energy expenditure or fat oxidation (Table 2). There was no between-group difference in protein utilization. Energy balance was also not different between groups at either month 0 or month 6.

Waking hours

During waking hours (2100–2300 and 0645–2100), we did not detect a difference in energy expenditure or substrate oxidation either within or between groups (Table 3).

Sleeping hours

During sleep (2300–0645), CLA treatment was associated with a number of differences in energy expenditure and substrate utilization (Table 4). The CLA group expended more energy than did the placebo group at baseline (*P* = 0.01) and at month 6 (*P* = 0.04). This appeared to be due to a sex mismatch between

TABLE 3
Waking substrate utilization at baseline and at month 6¹

	CLA (n = 12)			Placebo (n = 7)		
	Month 0	Month 6	Δ6-0	Month 0	Month 6	Δ6-0
RQ	0.86 ± 0.02	0.85 ± 0.02	-0.02 ± 0.03	0.85 ± 0.03	0.85 ± 0.03	0 ± 0.04
Energy expenditure (kcal)	1687 ± 166	1671 ± 246	-16 ± 70	1563 ± 103	1588 ± 84	25 ± 71
Carbohydrate						
(%)	46 ± 6.0	42.7 ± 6.6	-3.3 ± 9.3	42.2 ± 8.0	44.2 ± 9.6	1.9 ± 12.3
(g)	195 ± 45	179 ± 43	-15 ± 40	164 ± 28	175 ± 39	11 ± 45
Fat						
(%)	35.2 ± 6.1	39.5 ± 7.0	4.3 ± 9.8	39.7 ± 7.9	39.5 ± 10.2	-0.2 ± 13.9
(g)	65 ± 13	73 ± 14	8 ± 19	69 ± 17	70 ± 18	0 ± 25
Protein						
(%)	16.8 ± 1.7	15.5 ± 2.4	-1.3 ± 2.7	15.9 ± 3.5	13.4 ± 3.0	-2.0 ± 3.4
(g)	71 ± 17	65 ± 12	-7 ± 12	62 ± 12	55 ± 11	-7 ± 13

¹ All values are $\bar{x} \pm$ SD. CLA, conjugated linoleic acid; RQ, respiratory quotient. No statistically significant differences between groups were observed at baseline or at 6 mo or for the 0- to 6-mo change (paired *t* tests, *P* < 0.05).

TABLE 4

Sleeping substrate utilization at baseline and at month 6¹

	CLA (<i>n</i> = 12)			Placebo (<i>n</i> = 7)		
	Month 0	Month 6	Δ6–0	Month 0	Month 6	Δ6–0
RQ	0.84 ± 0.04	0.83 ± 0.02	−0.01 ± 0.03 ²	0.82 ± 0.02	0.84 ± 0.03	0.02 ± 0.04 ²
Energy expenditure (kcal)	580 ± 75 ³	580 ± 67	0 ± 38 ²	546 ± 25 ³	503 ± 85	−43 ± 90 ²
Carbohydrate						
(%)	37.9 ± 11.5	35.1 ± 6.3	−2.8 ± 10.5 ²	31.6 ± 7.7	39.8 ± 10.4	8.2 ± 11.6 ²
(g)	55 ± 20	51 ± 14	−4 ± 14	43 ± 10	51 ± 18	8 ± 17
Fat						
(%)	41.9 ± 12.0	47.5 ± 7.2	5.6 ± 11.7 ²	51.2 ± 9.6	42.9 ± 11.6	−8.3 ± 15.3 ²
(g)	27 ± 8	30 ± 4	4 ± 8 ²	31 ± 6	24 ± 8	−7 ± 11 ²
Protein						
(%)	18.2 ± 2.8 ³	14.9 ± 2.9	−3.3 ± 2.6 ^{2,4}	14.6 ± 3.1 ³	14.9 ± 3.7	0.3 ± 5.7 ²
(g)	26 ± 6 ³	22 ± 6	−5 ± 4	20 ± 4 ¹	19 ± 6	−1 ± 8

¹ All values are $\bar{x} \pm$ SD. CLA, conjugated linoleic acid; RQ, respiratory quotient.² Change from month 0 to month 6 was significant between groups, $P \leq 0.05$.³ Significant difference between groups at month 0, $P < 0.05$ (paired *t* tests).⁴ Change from month 0 to month 6 was significant within group, $P \leq 0.05$.

groups. The 3 male subjects had greater resting metabolic rates than did their female counterparts in the CLA group, whereas there were no male subjects in the placebo group; nonetheless, the change in sleeping energy expenditure in the placebo group (-43 ± 90 kcal/sleep) also differed from that in the CLA group (0 ± 38 kcal/sleep; $P < 0.05$). At baseline, the CLA group had a higher protein oxidative utilization, in terms of percentage of energy ($P = 0.02$) and grams per night ($P = 0.02$), than did the placebo group. After 6 mo, the CLA group utilized less protein for oxidation than at baseline in terms of percentage of energy ($P = 0.001$) and grams per night ($P = 0.004$). We also detected a between-group difference for change in protein utilization as a percentage of energy; the decrease with treatment was greater in the CLA group ($P = 0.03$).

The change in fat oxidation during sleep (0–6 mo) was significantly different in the CLA group (4 ± 8 g/sleep) from that in the placebo group (-7 ± 11 g/sleep; $P < 0.05$). Compared with the placebo group, the CLA group was less dependent on carbohydrates (%) and utilized fewer carbohydrates after 6 mo of supplementation ($P = 0.05$). In turn, this shift in fat and carbohydrate oxidation in the CLA group resulted in a lower RQ after 6 mo than that observed in the placebo group ($P = 0.05$).

Dietary fatty acid oxidation

No difference in the percentage cumulative dose recovery of D₃₁-palmitate ($P = 0.89$) or [1-¹³C]oleate ($P = 0.99$) was detected between the 2 groups 13 h after administration (Figures 1 and 2).

DISCUSSION

We recently reported the results of the main efficacy study of CLA supplementation (34). CLA supplementation for 6 mo was associated with a decrease in weight (-0.6 ± 2.5 kg), whereas a slight weight gain was observed with placebo (1.1 ± 3.2 kg) ($P = 0.04$). These changes were largely due to changes in fat mass after 6 mo, which were also different between the CLA and placebo groups (-1.0 ± 2.2 and 0.7 ± 3 kg, respectively; $P = 0.02$). In our current study, we investigated the physiologic mechanism responsible for the CLA-related loss in body fat. We found that

fat oxidation and energy expenditure increased during sleep in subjects who were randomly assigned to the CLA group; however, our primary outcome of 24-h fat oxidation only trended toward an increase after 6 mo of CLA supplementation.

The effects of CLA on fat and carbohydrate oxidation during sleep were consistent with the nonsignificant decrease in RQ during the 24-h period. The increased fat oxidation was also consistent with results from an animal study in which it was reported that CLA decreased the RQ in mice during sleep with no difference detected during waking hours (37). These results suggest that the mechanism for the efficacy of CLA supplementation for fat loss is at least partially due to increased fat oxidation during sleep.

Additional studies with a larger sample size are required to determine whether the lack of statistical significance for waking hours was a type II error. One possible reason for not finding a significant difference during waking hours was that the SD of fat oxidation in grams was smaller during sleep than during waking hours in our subjects ($P = 0.005$), which reduced the sensitivity of our study with regard to fat oxidation during waking or 24-h periods.

Our findings differ from those of previous studies of energy expenditure and substrate utilization (10, 22, 27). A human study

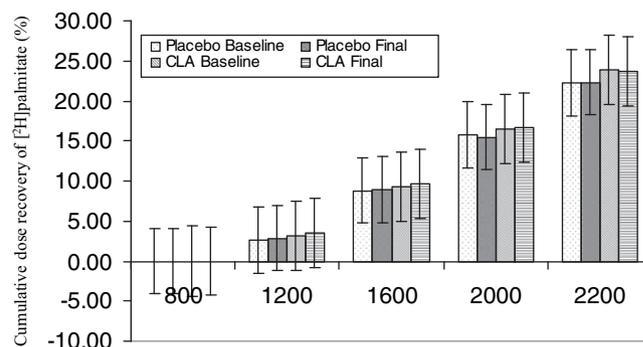


FIGURE 1. Mean (\pm SEM) cumulative percentage dose recovery of dietary [²H]palmitate in the placebo ($n = 7$) and conjugated linoleic acid (CLA; $n = 12$) groups. No statistically significant differences were detected (*t* test).

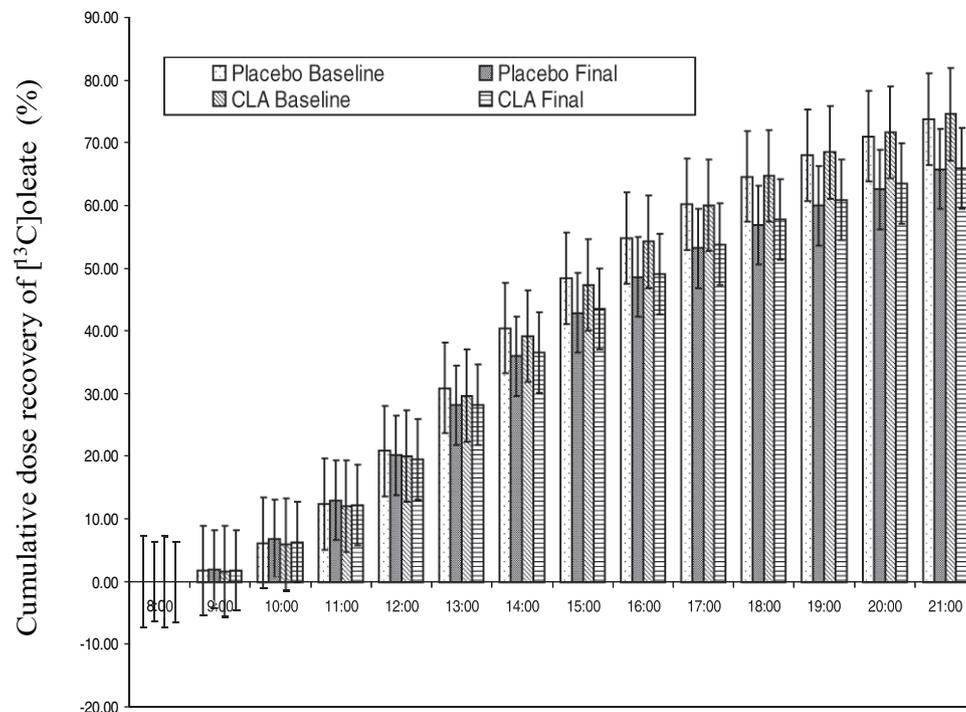


FIGURE 2. Mean (\pm SEM) cumulative percentage dose recovery of dietary [^{13}C]oleate in the placebo ($n = 7$) and conjugated linoleic acid (CLA; $n = 12$) groups. No statistically significant differences were detected (t test).

conducted by Zambell et al (22) found no treatment effect at rest or during a walking protocol in terms of energy expenditure, fat oxidation, and respiratory exchange ratios for 20 min after 30 min of rest at baseline, during week 4 and week 8 of treatment. Their measurement time period was much shorter than our 24-h continuous monitoring; thus, they may not have detected a treatment effect because of short measurement times and the accompanying potential for a type II error. Azain et al (27) found no treatment effect on energy expenditure by indirect calorimetry in rats placed in a respiratory chamber for 48 h at weeks 1, 4, and 7 of treatment. A decrease in RQ was detected at week 4; however, there was no effect at week 7 (27). The irregularity of RQ data from week 4 to week 7 may be a result of the time period length analyzed. Because Azain et al collected data for an entire 48-h time period, the potential sleep time effects of CLA may have been obscured by the greater variation during waking just as they were in our study. Thus, future CLA studies analyzing energy expenditure and macronutrient oxidation would benefit from doing analysis for an entire 24-h period and then further dividing these periods into waking and sleeping hours.

A confounder of our study was that sleeping protein oxidative utilization differed between the CLA and placebo groups at baseline. To ensure that this did not affect our global RQ data, a nonprotein respiratory quotient (NPRQ) was calculated. After 6 mo of supplementation, NPRQ decreased in the CLA group and increased in the placebo group. In addition to the baseline difference, protein utilization also decreased in the CLA group with treatment. This decrease in protein utilization is consistent with the findings from animal model studies (38, 39) that CLA protected against urinary protein losses in an attempt to protect lean body mass stores.

The second aim of our study was to determine whether the alteration in fat oxidation was due to a change in dietary fat,

endogenous fat, or both. We did not detect a change in the oxidation of dietary fat as monitored by labeled oleate and palmitate administered during a breakfast meal. This would suggest that the change in fat oxidation was due to increased endogenous fat. Considering, however, that increased fat oxidation was observed during sleep and that the dietary fat oxidation measures were made during the waking period, further studies of dietary fat oxidation during sleeping hours by using stable-isotope-labeled fats given at a dinner meal are warranted to fully assess CLA's effects on dietary fat oxidation.

We did not directly test any biochemical mechanisms that may have underlain our observation of increased fat oxidation during sleep. Current theories that may explain our results, however, include mechanisms associated with the inhibition of fat deposition within adipocytes, altered lipase activity, and apoptosis of adipocytes. The increase in fat oxidation was likely to have been associated with lipases involved in the storage and mobilization of fat, which are lipoprotein lipase (LPL), hormone-sensitive lipase (HSL) (40), and adipose triacylglycerol lipase (ATGL; 41). CLA supplementation studies have shown decreased adipocyte LPL activity and increased HSL activity (42, 43). No studies to date have researched CLA's effects on ATGL activity. Because rat studies have shown that adipocyte LPL and HSL are linked to circadian rhythms, which lead to a decrease in adipocyte LPL and an increase in HSL during sleeping hours, it is plausible that our sleeping compared with waking results are explained by CLA's effect being further enhanced during the diurnal cycle (44, 45). The increase in nighttime fat oxidation that we observed without an increase in dietary fat oxidation during the day is more consistent with proposed mechanisms involving greater fat mobilization than with those involving reduced storage.

As reported elsewhere, we found that body fat mass decreased by 1 ± 2.2 kg with CLA treatment, whereas it increased by $0.7 \pm$



3.0 kg with placebo after 6 mo (34). These changes corresponded to an average decrease of 5.5 g fat/d compared with an increase of 3.8 g fat/d in the placebo group. These changes are smaller than those that we observed in fat oxidation during the sleep period and are also smaller than the trend in 24-h fat oxidation. As such, these changes in fat oxidation were sufficient to account for the effect of CLA on body fat mass. In contrast, with calculation of the change in energy balance in each group, assuming an energy value of 9.5 kcal/g of endogenous fat, we found that the change in energy balance was twice the change in sleep period energy expenditure and was much larger than the trend for the change in 24-h energy expenditure. This suggests that the changes in fat mass and hence energy balance that we observed in the main study and the changes in fat oxidation that we observed in the present study were related to a decrease in energy intake rather than to an increase in energy expenditure. This, however, can only be suggested because the 95% CIs for energy expenditure are relatively large.

We did not collect data that would have allowed us to determine whether the suggested change in energy intake may have been due to changes in energy absorption on CLA supplementation. Tepstra et al (46) investigated alterations in energy absorption before and after 39 d of CLA supplementation by measuring energy content and composition in feces and excreta. They observed an increase in energy excretion in Balb-c mice without a corresponding increase in energy intake compared with controls. Thus, future CLA research using humans could investigate CLA's ability to alter fecal energy excretion.

In conclusion, CLA altered fat oxidation during sleeping hours and showed a similar trend during waking hours. On the basis of the absence of an alteration in the oxidation of dietary labeled oleate and palmitate in a breakfast meal, CLA does not appear to affect the type of dietary fat oxidized during waking hours. Further studies could be directed at determining whether CLA supplementation alters the type of endogenous fat oxidized on greater utilization of fat by the body, whether dietary fat oxidation is increased during sleeping hours, and whether energy excretion is altered.

We thank the staff of the GCRC for their invaluable efforts and Zhumin Zhang for performing the statistical analysis.

The authors' responsibilities were as follows—RNC: collected and analyzed the data and drafted the manuscript; DAS: designed the study and contributed significantly to the interpretation of the data and to the manuscript revisions; ACW: conducted the associated study and assisted in data collection; and EHN: assisted in monitoring patient safety and health during the length of the study. None of the authors had any conflict of interest.

REFERENCES

1. Pariza M, Hargraves W. A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[a]anthracene. *Carcinogenesis* 1985;6:591–3.
2. Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. *Lipids* 1997;32:853–8.
3. Kritchevsky D, Tepper SA, Wright S, Tso P, Czarnecki SK. Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. *J Am Coll Nutr* 2000;19(suppl):472S–7S.
4. Lee KN, Kritchevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 1994;108:19–25.
5. Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. *Artery* 1997;22:266–77.
6. Cook ME, Miller CC, Park Y, Pariza M. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. *Poult Sci* 1993;72:1301–5.
7. Miller CC, Park Y, Pariza MW, Cook ME. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. *Biochem Biophys Res Commun* 1994;198:1107–12.
8. Nugent AP, Roche HM, Noone EJ, Long A, Kelleher DK, Gibney MJ. The effects of conjugated linoleic acid supplementation on immune function in healthy volunteers. *Eur J Clin Nutr* 2005;59:742–50.
9. Whigham LD, Higbee A, Bjorling DE, Park Y, Pariza MW, Cook ME. Decreased antigen-induced eicosanoid release in conjugated linoleic acid-fed guinea pigs. *Am J Physiol Regul Integr Comp Physiol* 2002;282:R1104–12.
10. Gaullier JM, Halse J, Hoye K, et al. Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. *Am J Clin Nutr* 2004;79:1118–25.
11. Malpuech-Brugere C, Verboeket-van de Venne WP, Mensink RP, et al. Effects of two conjugated linoleic acid isomers on body fat mass in overweight humans. *Obes Res* 2004;12:591–8.
12. Baumgard LH, Matitashvili E, Corl BA, Dwyer DA, Bauman DE. *trans*-10, *cis*-12 Conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. *J Dairy Sci* 2002;85:2155–63.
13. Mashek DG, Grummer RR. Effects of conjugated linoleic acid isomers on lipid metabolism and gluconeogenesis in monolayer cultures of bovine hepatocytes. *J Dairy Sci* 2004;87:67–72.
14. Wargent E, Sennitt M, Stocker C, et al. Prolonged treatment of genetically obese mice with conjugated linoleic acid improves glucose tolerance and lowers plasma insulin concentration: possible involvement of PPAR activation. *Lipids Health Dis* 2005;4:3.
15. Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, et al. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty *fafa* rat. *Biochem Biophys Res Commun* 1998;244:678–82.
16. Ryder JW, Portocarrero CP, Song XM, et al. Isomer-specific antidiabetic properties of conjugated linoleic acid: improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. *Diabetes* 2001;50:1149–57.
17. Blankson H, Stakkestad JA, Fagertun H, Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J Nutr* 2000;130:2943–8.
18. Kamphuis MM, Lejeune MP, Saris WH, Westertep-Plantenga MS. The effect of conjugated linoleic acid supplementation after weight loss on body weight regain, body composition, and resting metabolic rate in overweight subjects. *Int J Obes Relat Metab Disord* 2003;27:840–7.
19. Mougios V, Matsakas A, Petridou A, et al. Effect of supplementation with conjugated linoleic acid on human serum lipids and body fat. *J Nutr Biochem* 2001;12:585–94.
20. Smedman A, Vessby B. Conjugated linoleic acid supplementation in humans—metabolic effects. *Lipids* 2001;36:773–81.
21. Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat in healthy exercising humans. *J Int Med Res* 2001;29:392–6.
22. Zambell KL, Keim NL, Van Loan MD, et al. Conjugated linoleic acid supplementation in humans: effects on body composition and energy expenditure. *Lipids* 2000;35:777–82.
23. Gaullier JM, Halse J, Hoye K, et al. Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans. *J Nutr* 2005;135:778–84.
24. Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, et al. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes* 2000;49:1534–42.
25. Hargrave KM, Meyer BJ, Li C, Azain MJ, Baile CA, Miner JL. Influence of dietary conjugated linoleic acid and fat source on body fat and apoptosis in mice. *Obes Res* 2004;12:1435–44.
26. Miner JL, Cederberg CA, Nielsen MK, Chen X, Baile CA. Conjugated linoleic acid (CLA), body fat, and apoptosis. *Obes Res* 2001;9:129–34.
27. Azain MJ, Hausman DB, Sisk MB, Flatt WP, Jewell DE. Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. *J Nutr* 2000;130:1548–54.
28. Poulos SP, Sisk M, Hausman DB, Azain MJ, Hausman GJ. Pre- and postnatal dietary conjugated linoleic acid alters adipose development, body weight gain and body composition in Sprague-Dawley rats. *J Nutr* 2001;131:2722–31.

29. Sisk MB, Hausman DB, Martin RJ, Azain MJ. Dietary conjugated linoleic acid reduces adiposity in lean but not obese Zucker rats. *J Nutr* 2001;131:1668–74.
30. West DB, Blohm FY, Truett AA, DeLany JP. Conjugated linoleic acid persistently increases total energy expenditure in AKR/J mice without increasing uncoupling protein gene expression. *J Nutr* 2000;130:2471–7.
31. Corino C, Magni S, Pastorelli G, Rossi R, Mourot J. Effect of conjugated linoleic acid on meat quality, lipid metabolism, and sensory characteristics of dry-cured hams from heavy pigs. *J Anim Sci* 2003;81:2219–29.
32. Eggert JM, Belury MA, Kempa-Steczko A, Mills SE, Schinckel AP. Effects of conjugated linoleic acid on the belly firmness and fatty acid composition of genetically lean pigs. *J Anim Sci* 2001;79:2866–72.
33. O'Quinn PR, Nelssen JL, Goodband RD, et al Effects of modified tall oil versus a commercial source of conjugated linoleic acid and increasing levels of modified tall oil on growth performance and carcass characteristics of growing-finishing pigs. *J Anim Sci* 2000;78:2359–68.
34. Watras AC, Buchholz AC, Close RN, Zhang Z, Schoeller DA. The role of conjugated linoleic acid in reducing body fat and preventing holiday weight gain. *Int J Obes (Lond)* 2007;31:481–7.
35. Votruba SB, Atkinson RL, Hirvonen MD, Schoeller DA. Prior exercise increases subsequent utilization of dietary fat. *Med Sci Sports Exerc* 2002;34:1757–65.
36. Jequier E, Acheson K, Schutz Y. Assessment of energy expenditure and fuel utilization in man. *Annu Rev Nutr* 1987;7:187–208.
37. West DB, Delany JP, Camet PM, Blohm F, Truett AA, Scimeca J. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am J Physiol Regul Integr Comp Physiol* 1998;275:R667–72.
38. Yang M, Cook ME. Dietary CLA decreased weight loss and extended survival following the onset of kidney failure in NZB/W F1 mice. *Lipids* 2003;38:21–4.
39. Park Y, Albright KJ, Pariza MW. Effects of conjugated linoleic acid on long term feeding in Fischer 344 rats. *Food Chem Toxicol* 2005;43:1273–9.
40. Fielding BA, Frayn KN. Lipoprotein lipase and the disposition of dietary fatty acids. *Br J Nutr* 1998;80:495–502.
41. Zimmermann R, Strauss JG, Haemmerle G, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* 2004;306:1383–6.
42. Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* 1999;34:235–41.
43. Park Y, Storkson JM, Ntambi JM, Cook ME, Sih CJ, Pariza MW. Inhibition of hepatic stearoyl-CoA desaturase activity by *trans*-10,*cis*-12 conjugated linoleic acid and its derivatives. *Biochim Biophys Acta* 2000;1486:285–92.
44. Benavides A, Siches M, Llobera M. Circadian rhythms of lipoprotein lipase and hepatic lipase activities in intermediate metabolism of adult rat. *Am J Physiol* 1998;275:R811–7.
45. Tsutsumi K, Inoue Y, Kondo Y. The relationship between lipoprotein lipase activity and respiratory quotient of rats in circadian rhythms. *Biol Pharm Bull* 2002;25:1360–3.
46. Terpstra AHM, Beynen AC, Everts H, Kocsis S, Katan MB, Zock PL. The decrease in body fat in mice fed conjugated linoleic acid is due to increases in energy expenditure and energy loss in the excreta. *J Nutr* 2002;132:940–5.

