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Evidence That the *trans*-10,*cis*-12 Isomer of Conjugated Linoleic Acid Induces Body Composition Changes in Mice

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ABSTRACT: We investigated the effects of conjugated linoleic acid (CLA) preparations, which were enriched for the *cis*-9,*trans*-11 CLA isomer or the *trans*-10,*cis*-12 CLA isomer, on body composition in mice. Body composition changes (reduced body fat, enhanced body water, enhanced body protein, and enhanced body ash) were associated with feeding the *trans*-10,*cis*-12 CLA isomer. In cultured 3T3-L1 adipocytes, the *trans*-10,*cis*-12 isomer reduced lipoprotein lipase activity, intracellular triacylglycerol and glycerol, and enhanced glycerol release into the medium. By contrast, the *cis*-9,*trans*-11 and *trans*-9,*trans*-11 CLA isomers did not affect these biochemical activities. We conclude that CLA-associated body composition change results from feeding the *trans*-10,*cis*-12 isomer.

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CLA is the acronym for a class of positional and geometric conjugated dienoic isomers of linoleic acid. The term originated in 1987 when we reported biological activity (i.e., anticarcinogenic activity) associated with CLA isolated from grilled ground beef or produced from linoleic acid by base-catalyzed isomerization (1). Since then substantial interest has developed in the biochemical actions of CLA and its potential application to foods, feeds, and pharmaceuticals (2; for a current listing of the scientific literature on CLA since 1987, see http://www.wisc.edu/fri/clarefs.htm).

Dietary CLA has been shown to affect body composition (reduction in body fat, enhancement of fat-free mass) in mice (3,4), rats (5,6), and pigs (7–10). Previously we (3) reported that CLA exerts direct effects on adipocytes, which are the principal sites of fat storage, and skeletal muscle cells, which are the principal sites of fat combustion. We found that adding CLA to the culture medium of mouse 3T3-L1 adipocytes produced a dose-dependent reduction in lipoprotein lipase (LPL) activity and apparently induced lipolysis as well in this cell line. Additionally, skeletal muscle from mice fed CLA exhibited elevated carnitine palmitoyltransferase (CPT) activity. Evidence was also presented indicating that CLA enhanced whole body protein accretion. Based on these findings we proposed that the physiological mechanism of body fat reduction in mice by CLA involved inhibition of fat storage in adipocytes coupled with both elevated β-oxidation in skeletal muscle and an increase in skeletal muscle mass (3).

A central question concerns the biochemical mechanism(s) whereby CLA induces these physiological effects on adipocytes and skeletal muscle. This complex matter logically begins with the issue of CLA metabolism. The CLA preparations used to date in this research consisted principally of two isomers present in similar amounts: *cis*-9,*trans*-11 CLA, and *trans*-10,*cis*-12 CLA (1,3,11–13). Hence it is possible that either, or both, of these isomers could be involved in inducing body composition change. Previously (14) we provided evidence indicating that the *trans*-10,*cis*-12 CLA isomer decreased the expression of hepatic stearoyl-CoA desaturase mRNA in mice.

We now report on the effects, on body composition in mice, of feeding CLA preparations that contained both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA in approximately equal amounts, and preparations that were enriched for one or the other of these isomers. Body composition change was associated with the *trans*-10,*cis*-12 CLA isomer content of the preparations. A commercial preparation that was highly enriched for the *trans*-10,*cis*-12 isomer was also active in reducing LPL activity in cultured 3T3-L1 adipocytes and enhancing triacylglycerol release from these cells. By contrast, commercial preparations highly enriched for either the *cis*-9,*trans*-11 or *trans*-9,*trans*-11 CLA isomers did not affect these biochemical activities.

MATERIALS AND METHODS

Materials. Linoleic acid was purchased from Nu-Chek-Prep Corporation (Elysian, MN); triolein, [9,10-^{3}H(N)], (specific activity 12 Ci/mmol) from American Radiolabeled Chemicals (St. Louis, MO); and [1-^{14}C]linoleic acid (specific activity 55 mCi/mmol) from Amersham Life Science (Arlington

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Abbreviations: CLA, conjugated linoleic acid; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; LPL, lipoprotein lipase;.

Heights, IL). The isomer contents of the CLA preparations used in this study are presented in Table 1. UW-CLA was prepared as described (12). UW-CLA-1 and UW-CLA-2 were prepared by low-temperature crystallization of UW-CLA as described below. Natural Lipids (Hovdebygda, Norway) kindly provided CLA-SF (prepared by base isomerization of safflower seed oil), CLA-DC (prepared from dehydrated castor oil), and *trans*-10,*cis*-12 CLA. The *cis*-9,*trans*-11 CLA and *trans*-9,*trans*-11 CLA preparations were purchased from Matreya Inc. (Pleasant Gap, PA). 3T3-L1 preadipocytes were purchased from American Type Culture Collection (Rockville, MD).

Low temperature crystallization. Low-temperature crystallization of UW-CLA was performed as described (15) with slight modification. Methyl esters of UW-CLA were prepared with 4% HCl in methanol (12). The CLA methyl esters were dissolved in acetone, and the solution was cooled to a range of −60 to −70°C. The solvent was removed by evaporation from the supernatant fluid. The supernatant fluid was redissolved in methanol and subjected again to recrystallization; the resulting supernatant fluid, which was enriched for *cis*-9,*trans*-11 CLA, was designated UW-CLA-1. The crystalline fraction from the first crystallization was redissolved in acetone and recrystallized at the same temperature; this preparation, which was enriched for the *trans*-10,*cis*-12 CLA isomer, was designated UW-CLA-2. UW-CLA-1 and UW-CLA-2 were hydrolyzed with 1 M KOH in methanol and extracted with hexane after acidification. Fractions were analyzed with gas chromatography (GC); the extent of methylation or hydrolysis was determined with thin-layer chromatography. GC was conducted with a Hewlett-Packard 5890 series II (Wilmington, DE) fitted with a flame-ionization detector and 3396A integrator. A Supelcowax-10 fused-silica capillary column (60 m × 0.32 mm i.d., 0.25 μm film thickness) (Bellefonte, PA) was used, and oven temperature was programmed from 50 to 200°C with an increase of 20°C per min, held for 50 min, increased 10°C per min to 225°C, and held for 20 min.

TABLE 1
Isomer Distribution of the Conjugated Linoleic Acid (CLA) Preparations Used in this Study[a]

	Fatty acid (%)			
	c-9,*t*-11[b]	*t*-10,*c*-12	*t*-9,*t*-11/*t*-10,*t*-12	Other CLA isomers[c]
UW-CLA[d]				
(used in Exp. 1)	41.1	43.5	10.0	3.04
(used in culture)	45.7	47.6	1.71	2.79
UW-CLA-1[d]	72.4	13.0	3.51	9.22
UW-CLA-2[d]	16.2	79.2	3.06	1.56
cis-9,*trans*-11 CLA[e]	96.3	N.D.[g]	2.60	1.08
trans-10,*cis*-12 CLA[f]	1.61	92.8	1.16	1.64
trans-9,*trans*-11 CLA[e]	N.D.	N.D.	100[h]	N.D.
CLA-SF[f]	41.9	43.5	1.78	1.47
CLA-DC[f]	29.1	2.63	8.44	20.1[i]

[a]Methylated with 4% HCl/methanol at room temperature for 30 min to minimize artifact formation.
[b]*c*-9,*t*-11, *cis*-9,*trans*-11; *t*-10,*c*-12, *trans*-10,*cis*-12; *t*-9,*t*-11, t*rans*-9,*trans*-11; *t*-10,*t*-12, *trans*-10,*trans*-12.
[c]Mostly *cis*-9,*cis*-11 or *cis*-10,*cis*-12 isomers.
[d]CLA, prepared as described in Ref. 12; for preparation of UW-CLA-1 and UW-CLA-2 see the Materials and Methods section.
[e]Purchased from Matreya, Inc. (Pleasant Gap, PA).
[f]Materials and analysis provided by Natural Lipids (Hovdebygda, Norway). CLA-SF and CLA- DC were made from safflower seed oil and dehydrated castor oil, respectively.
[g]N.D., not detected.
[h]*trans*-9,*trans*-11 isomer only.
[i]Additionally includes 8,10 and 11,13 isomers.

Animal studies and body composition analyses. Weanling ICR mice (females for experiment 1, males for experiment 2) and semipurified diet (TD94060, 99% basal mix) were purchased from Harlan Sprague-Dawley (Madison, WI). The diet was composed as follows (ingredient, g/kg): sucrose, 476; casein, "vitamin-free" test, 210; corn starch, 150; DL-methionine, 3; corn oil, 60; cellulose, 50; mineral mix, AIN-76, 35; vitamin mix, AIN-76A, 10; calcium carbonate, 4; choline bitartrate, 2; and ethoxyquin, 0.1. For the treatment diets, 5 g UW-CLA, 3 g UW-CLA-1, or 2.5 g UW-CLA-2 (for Experiment 1), 5 g CLA-SF, 5 g or 9 g CLA-DC (for Experiment 2) were added at the expense of corn oil to maintain 6% fat. Diet was stored at −20°C until use. Mice were housed individually in a windowless room with a 12-h light-dark cycle in strict accordance to guidelines established by the Research Animal Resources Center of University of Wisconsin-Madison. Diet and water, available *ad libitum*, were freshly provided three times per week. After a 5-d adaptation period mice were randomly separated into groups and fed control diet, or diet supplemented with 0.5% UW-CLA, 0.3% UW-CLA-1, or 0.25% UW-CLA-2 (Experiment 1); or 0.5% CLA-SF, 0.5% CLA-DC, or 0.9% CLA-DC (Experiment 2).

For body composition analyses, animals were sacrificed, gut contents were removed (to obtain empty carcass weight), and the carcasses frozen at −20°C. Frozen carcasses were chopped and then freeze-dried to determine water content. Each dried carcass was ground to give a homogeneous sample before further analysis. Total nitrogen was analyzed by the Kjeldahl method (16). Carcass fat content was measured by extraction with diethyl ether overnight using a Soxhlet apparatus. Total ash content was determined by incineration (500–600°C, overnight).

Adipocyte cell culture. 3T3-L1 preadipocytes were cultured as described (17). Briefly, 3T3-L1 preadipocytes were grown to confluence at 37°C in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS). At 2-d postconfluence (designated "day 0") cell differentiation was induced with a mixture of methylisobutylxanthine (0.5 mM), dexamethasone (0.25 μM), and insulin (1 μg/mL) in DMEM containing 10% FBS. On day 2 this medium was replaced with DMEM medium containing 10% FBS and insulin only. On day 4 and thereafter the medium consisted of DMEM plus 10 %FBS only; this medium was subsequently replaced with fresh medium at 2-d intervals. Fatty acid–albumin complexes were prepared as described (3). All culture media including control had a final concentration of 100 μM albumin.

Free and esterified glycerol were determined using a Sigma Diagnostic Kit (Sigma Chemical Co., St. Louis, MO).

Heparin-releasable LPL activity (EC 3.1.1.34, 10 U heparin/mL media for 1 h at 37°C) was measured as described (18). Recovery of free fatty acid was estimated at 71% by using [^{14}C]linoleic acid. Protein was determined using Bio-Rad DC Protein assay kit (Hercules, CA).

Statistical analyses. Data were subjected to analysis using the Statistics Analysis System (SAS Users Guide: Statistics, SAS Institute Inc., Cary, NC). Data in Tables 2–4, and Figures 1 and 2 were analyzed with one-way analysis of variance (ANOVA). Two-way ANOVA (treatments and experiments) were performed on data as log values for Figures 3 and 4. If the interaction between treatment and experiment was significant, this interaction was then used as the error term in the Least Square Means analysis. For Figure 4, standard errors were computed using a nested model including variability between experiments and variability among dishes with an experiment as described (19).

RESULTS

Table 1 shows the relative isomer content of the CLA preparations employed in this study. UW-CLA was characterized primarily by two peaks with retention times corresponding to the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers. UW-CLA-1 was enriched for the *cis*-9,*trans*-11 isomer, whereas UW-CLA-2 was enriched for the *trans*-10,*cis*-12 CLA isomer. Preparations designated *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, and *trans*-9,*trans*-11 CLA, respectively, contained more than 90% of the indicated isomer. CLA-SF was similar to UW-CLA. CLA-DC contained a number of CLA isomers including the *cis*-9,*trans*-11 isomer, but very little of the *trans*-10,*cis*-12 isomer.

In Experiment 1, mice were fed control diet, or diet containing 0.5% UW-CLA, 0.3% UW-CLA-1, or 0.25% UW-CLA-2. Body weight gains during the course of this experiment are shown in Figure 1; total feed intake is shown in Table 2. Mice fed diets supplemented with UW-CLA-2 gained significantly less body weight than controls. Mice fed diets supplemented with UW-CLA or UW-CLA-1 appeared to gain less weight than controls, but the differences were not statistically significant (however, the mean empty carcass weight of mice fed diet supplemented with UW-CLA was significantly different from controls, see Table 3). Feed intake was significantly reduced for mice fed UW-CLA and UW-CLA-2, but there was no difference in feed intake between controls and mice fed diet supplemented with UW-CLA-1.

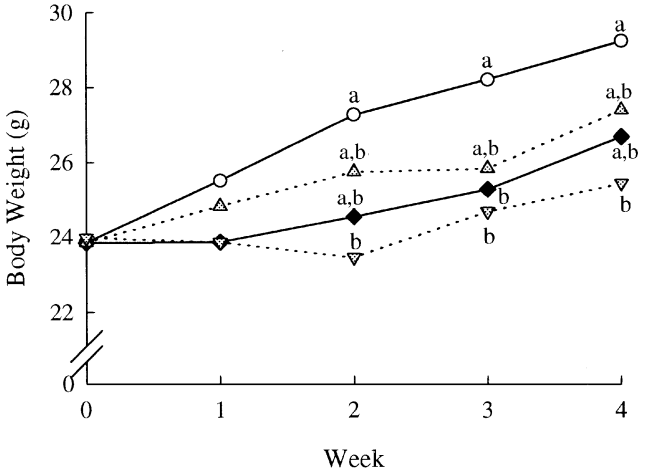

FIG. 1. Body weights, Experiment 1. Animals were fed one of the following diets for 4 wk: control (circle); UW-CLA, 0.5% (diamond); UW-CLA-1, 0.3% (triangle); UW-CLA-2, 0.25% (inverted triangle). Reported values are means ± SE for 5 or 6 animals. At each time point, means with different roman letters are significantly different at $P < 0.05$. UW-CLA, diet devised at University of Wisconsin containing conjugated linoleic acid (Ref. 12). For preparation of UW-CLA-1 and UW-CLA-2, see the Materials and Methods section.

TABLE 2
Food Intake[a]

	Food Iintake (g/mouse/4 wk)
Experiment 1	
Control	$129.2^{a} \pm 2.5$
UW-CLA	$110.6^{b} \pm 4.5$
UW-CLA-1	$131.7^{a} \pm 1.5$
UW-CLA-2	$113.9^{b} \pm 6.8$
Experiment 2	
Control	$129.5^{a} \pm 2.0$
CLA-SF	$112.9^{c} \pm 1.7$
CLA-DC 0.5%	$117.9^{b,c} \pm 5.2$
CLA-DC 0.9%	$122.8^{a,b} \pm 1.8$

[a]Female (Experiment 1) or male (Experiment 2) mice were fed treatment diet for 4 wk. Reported values are mean ± SE ($n = 5$ or 6 for Experiment 1, and 7 or 8 for Experiment 2). For each experiment, means with different superscript roman letters are significantly different ($P < 0.05$).

Body composition results from Experiment 1 are shown in Table 3. The percentage of body fat of the mice fed diet supplemented with UW-CLA was reduced by 70% relative to controls, whereas the percentages of whole body water, protein, and ash were significantly increased. This is consistent with our previous observations (3). A similar reduction in percentage of whole body fat, accompanied by increased percentages of whole body water, protein, and ash, was observed for mice fed diet containing UW-CLA-2. By contrast, mice fed diet containing UW-CLA-1 exhibited a smaller reduction in percentage of body fat, and smaller increases in percentages of whole body water, protein, and ash relative to controls.

In Experiment 2, mice were fed control diet or diet containing 0.5% CLA-SF, 0.5% CLA-DC, or 0.9% CLA-DC. Feeding CLA-DC at 0.9% of the diet provided an amount of the *cis*-9,*trans*-11 isomer that was equivalent to that in diet supplemented with 0.5% CLA-SF; however, the diet supplemented with 0.5% CLA-SF contained about 10 times more *trans*-10,*cis*-12 CLA than diet supplemented with 0.9% CLA-DC (Table 1).

Body weight changes during the course of this experiment are shown in Figure 2; feed intake data are indicated in Table 2. Animals fed diet containing CLA-SF exhibited reduced body weight gain relative to controls, whereas mice fed diet supplemented with CLA-DC at 0.5% or 0.9% exhibited

TABLE 3
Body Composition, Experiment 1[a]

	ECW (g)[b]	Fat (%)	Water (%)	Protein (%)	Ash (%)
Control	$27.43^{a} \pm 1.21$	$22.27^{a} \pm 1.80$	$54.30^{a} \pm 1.35$	$16.26^{a} \pm 0.49$	$3.29^{a} \pm 0.13$
UW-CLA	$24.28^{b} \pm 0.76$	$6.69^{c} \pm 0.86$	$65.59^{c} \pm 0.68$	$19.04^{b} \pm 0.24$	$3.78^{b} \pm 0.10$
UW-CLA-1	$25.53^{a,b} \pm 0.59$	$13.08^{b} \pm 1.66$	$60.99^{b} \pm 1.14$	$18.09^{b} \pm 0.50$	$3.54^{a,b} \pm 0.13$
UW-CLA-2	$23.44^{b} \pm 0.92$	$6.80^{c} \pm 1.26$	$65.35^{c} \pm 1.13$	$19.33^{b} \pm 0.29$	$3.83^{b} \pm 0.08$

[a]Female mice were fed treatment diet for 4 wk. Reported values are means ± SE (n = 5 or 6). In each column, means with different superscript roman letters are significantly different ($P < 0.05$).
[b]ECW, empty carcass weight.

TABLE 4
Body Composition, Experiment 2[a]

	ECW (g)[b]	Fat (%)	Water (%)	Protein (%)	Ash (%)
Control	$31.4^{a} \pm 0.4$	$15.31^{a} \pm 1.66$	$58.3^{a} \pm 1.2$	$19.57^{a} \pm 0.41$	$3.35^{a} \pm 0.11$
CLA-SF	$27.6^{b} \pm 0.6$	$4.78^{c} \pm 0.57$	$66.1^{c} \pm 0.4$	$22.59^{c} \pm 0.17$	$3.84^{b} \pm 0.11$
CLA-DC 0.5%	$30.7^{a} \pm 1.3$	$12.27^{a,b} \pm 1.84$	$60.5^{a,b} \pm 1.5$	$20.58^{a,b} \pm 0.47$	$3.32^{a} \pm 0.08$
CLA-DC 0.9%	$30.4^{a} \pm 0.9$	$10.06^{b} \pm 0.99$	$61.9^{b} \pm 0.7$	$20.93^{b} \pm 0.24$	$3.31^{a} \pm 0.09$

[a]Male mice were fed treatment diet for 4 wk. Reported values are mean ± SE (n = 7 or 8). In each column, means with different superscript roman letters are significantly different ($P < 0.05$).
[b]ECW, empty carcass weight.

similar weight gain to controls. Mice fed CLA-SF ate significantly less feed than controls. Mice fed a diet supplemented with 0.5% CLA-DC also appeared to eat significantly less feed than controls, but this difference was not evident for mice fed diet supplemented with 0.9% CLA-DC.

The data of Table 4 indicate that the mice fed a diet supplemented with CLA-SF exhibited significantly greater changes in body composition relative to controls than mice fed diet supplemented with CLA-DC at either 0.5 or 0.9%.

We previously reported (3) that CLA inhibited heparin-releasable LPL activity in 3T3-L1 adipocytes. Using this model, we studied the effects of UW-CLA, *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, and *trans*-9,*trans*-11 CLA on LPL activity (Fig. 3). UW-CLA and *trans*-10,*cis*-12 CLA significantly reduced LPL activity, whereas *cis*-9,*trans*-11 CLA and *trans*-9,*trans*-11 CLA were without effect (Fig. 3A). UW-CLA and *trans*-10,*cis*-12 CLA also significantly enhanced glycerol release (Fig. 3B), and significantly reduced intracellular triacylglycerol (Fig. 3C) and glycerol (Fig. 3D). By contrast, *cis*-9,*trans*-11 CLA and *trans*-9,*trans*-11 CLA appeared to produce no effect on intracellular triacylglycerol (Fig. 3C) or glycerol (Fig. 3D). Both *trans*-9,*trans*-11 CLA and *cis*-9,*trans*-11 CLA appeared to induce significant enhancements in glycerol release at the concentrations tested (Fig. 3B).

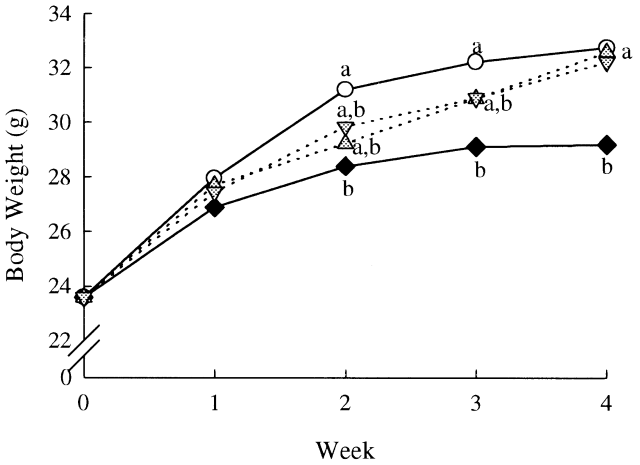

FIG.2. Body weights, Experiment 2. Animals were fed one of the following diets for 4 wk: control (circle); CLA-SF, 0.5% (diamond); CLA-DC, 0.5% (triangle); CLA-DC, 0.9% (inverted triangle). Reported values are means ± SE from 7 or 8 animals. At each time point, means with different roman letters are significantly different at $P < 0.05$. CLA-SF diet (Natural Lipids, Hovdesygda, Norway), prepared by base isomerization of safflower seed oil; CLA-DC (Natural Lipids), prepared from dehydrated castor oil.

We determined the dose-response for *trans*-10,*cis*-12 CLA on LPL activity and intracellular triacylglycerol (Fig. 4). Both of these biochemical markers were reduced in a dose-dependent fashion with addition of *trans*-10,*cis*-12 CLA.

Previously (3) we published data on the inhibition of LPL activity in 3T3-L1 adipocytes by CLA. The CLA preparation employed in that study (3) was identical to UW-CLA in this study; accordingly, it was not possible to determine which isomer was responsible for the inhibitory effect. Figure 5 shows these previously published data (3) plotted as total CLA (top axis) or normalized for the *trans*-10,*cis*-12 or *cis*-9,*trans*-11 isomer content of UW-CLA (bottom axes) (the scales of the bottom axes are similar because the concentrations of the *trans*-10,*cis*-12 and *cis*-9,*trans*-11 isomers in UW-CLA are similar—Table 1). Figure 5 also includes the new data from Figure 4, which were obtained with *trans*-10,*cis*-12 CLA, and additional new data which were obtained with *cis*-9,*trans*-11 CLA. It is clear from this plot of data from independent experiments that the inhibition of LPL activity is effected by the *trans*-10,*cis*-12 isomer, not the *cis*-9,*trans*-11 isomer.

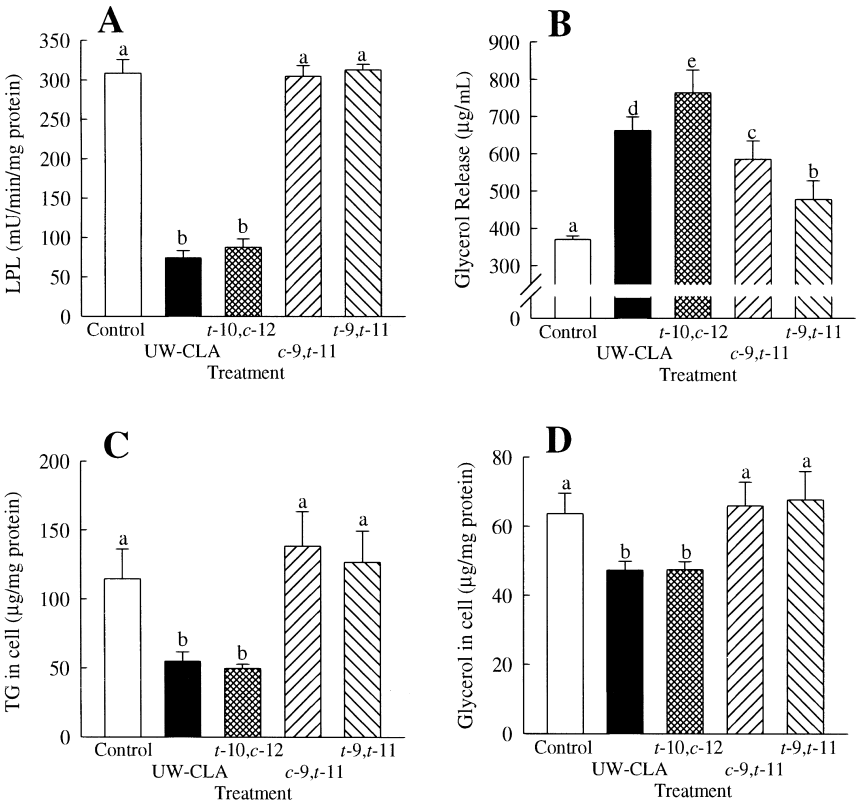

FIG. 3. Effects of CLA on heparin-releasable lipoprotein lipase (LPL) activity (A), glycerol release (B), cellular triacylglycerol (TG) (C), and cellular glycerol (D) in 3T3-L1 adipocytes. Final concentrations are 100 µM for UW-CLA, 43.6 µM for *trans*-10,*cis*-12 isomer (*t*-10,*c*-12), 44.0 µM for *cis*-9,*trans*-11 isomer (*c*-9,*t*-11), and 40.0 µM for *trans*-9,*trans*-11 isomer (*t*-9,*t*-11). Albumin (100 µM) was included in all cases, and incubations were for 48 h. Reported values are mean ± SE (n = 12–16, collected from three independent experiments). Data were analyzed as log value with a two-way analysis of variance using fatty acid (as treatments) and experiment. If the interaction between treatment and experiment was significant, this interaction was used as the error term. Means with different roman letters are significantly different ($P < 0.05$). For other abbreviation see Figure 1.

DISCUSSION

Our present results indicate that the effects of CLA on body composition are associated with the *trans*-10,*cis*-12 CLA isomer. Enrichment of dietary supplements for the *cis*-9,*trans*-11 isomer was not correlated with body composition change. The small amounts of *trans*-10,*cis*-12 CLA in UW-CLA-1 and CLA-DC (Table 1) are most likely responsible for the modest effects of these CLA preparations on body composition (Tables 3,4). In addition, in cultured 3T3-L1 mouse adipocytes, a preparation that was highly enriched for *trans*-10,*cis*-12 CLA significantly reduced LPL activity and enhanced triacylglycerol release. By contrast, preparations highly enriched for either the *cis*-9,*trans*-11 or *trans*-9,*trans*-11 CLA isomers did not affect these biochemical activities (Fig. 3–5). Accordingly, we conclude that CLA-associated body composition change is effected by the *trans*-10,*cis*-12 isomer. It is important now to determine if the 20-carbon metabolites of *trans*-10,*cis*-12 CLA identified by Sebedio *et al*. (20) will induce body composition changes in mice and other species. We have presented preliminary data indicating that conjugated eicosadienoic acid is biologically active in this regard (21).

Our working hypothesis (3) is that the CLA-induced changes in body composition are largely the result of effects of CLA on adipocytes (the major site of fat storage) and skeletal muscle cells (the major site of fat oxidation), although CLA-induced reductions in feed intake (Table 2) may also play an important role. Although the exact relationship between these different factors is unclear, they appear to correlate closely with other CLA-induced effects on lipid metabolism (14,22), indicating that hepatocytes may also be affected by CLA. Indeed, we have already demonstrated that UW-CLA (but not enzymatically synthesized *cis*-9,*trans*-11 CLA) decreased the expression of hepatic stearoyl-CoA desaturase mRNA in mice (14). Accordingly, the conclusion now that body composition changes are effected by the *trans*-10,*cis*-12 CLA isomer indicates a central role for this isomer in mediating many (but not necessarily all) of the biochemical effects attributed to CLA. Given the array of reported

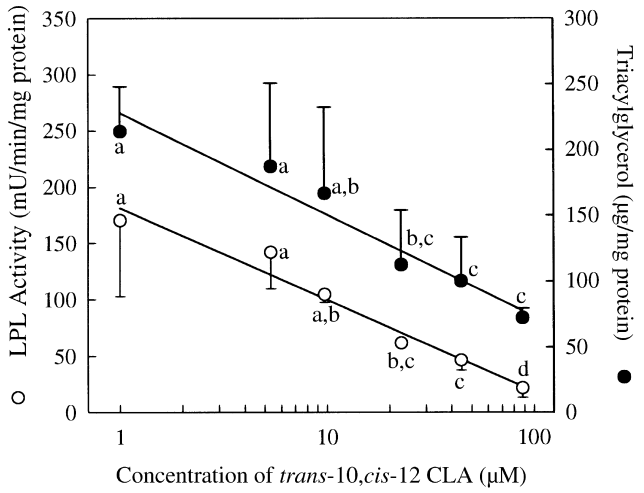

FIG. 4. Dose-response of *trans*-10,*cis*-12 CLA on heparin-releasable LPL (open circle) and amount of cellular TG (closed circle) in 3T3-L1 adipocytes. Albumin (100 μM) was included in all cases, and incubations were for 48 h. Reported values are mean ± SE (n = 4–8, collected from two independent experiments). Data were analyzed as log value with a two-way analysis of variance using fatty acid (as treatments) and experiment. If the interaction between treatment and experiment was significant, this interaction was used as the error term. Means with different roman letters are significantly different ($P < 0.05$). (To avoid zero values of log, the value of 1 was added to all data.) For abbreviations see Figures 1 and 3.

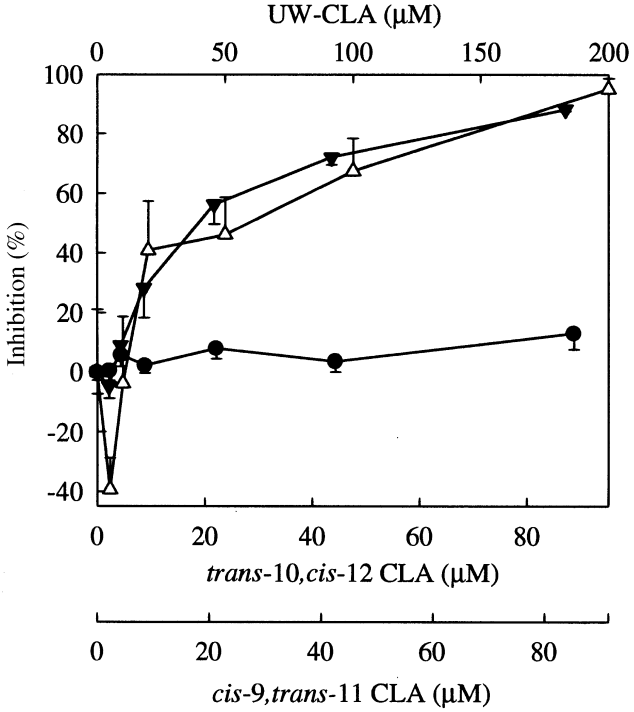

FIG. 5. Effect of UW-CLA, *trans*-10,*cis*-12 CLA, and *cis*-9,*trans*-11 CLA on heparin-releasable LPL activity in 3T3-L1 adipocytes. The top axis indicates the concentration of total CLA and refers to previously published data (3) using UW-CLA (open triangle). The bottom axis indicates the *trans*-10,*cis*-12 isomer concentration, or the *cis*-9,*trans*-11 isomer concentration and refers to data obtained with *trans*-10,*cis*-12 CLA (closed inverted triangle, data from Fig. 4), *cis*-9,*trans*-11 CLA (closed circle), or UW-CLA normalized for *trans*-10,*cis*-12 or *cis*-9,*trans*-11 isomer content. Albumin (100 μM) was included in all cases, and incubations were for 48 h. Reported values are means ± SE of 3–16 culture dishes, normalized against control.

physiological and biochemical effects of CLA, it is likely that the *cis*-9,*trans*-11 CLA isomer (and possibly other CLA isomers as well) will also be found to exert significant biological effects.

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