

Short-Term Intake of Conjugated Linoleic Acid Inhibits Lipoprotein Lipase and Glucose Metabolism but Does Not Enhance Lipolysis in Mouse Adipose Tissue¹

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ABSTRACT Feeding diets supplemented with t10c12 conjugated linoleic acid (CLA) to growing mice reduces body fat mass. The effects are evident after 1 wk and maximal by 3 wk and are accompanied by reductions in fat cell size. This may complicate direct comparisons with adipocytes from control mice. Accordingly, we investigated the early biochemical events that occur within adipocytes during the first week of CLA feeding, before changes in the size of adipocytes have occurred. Female ICR mice were fed a control diet or a diet supplemented with 0.5 g/100 g of CLA for 4 d, at which time there were no differences in body weight, fat mass or adipocyte size (except that CLA-fed mice had fewer adipocytes >90 μ m in diameter). Parametrial adipose tissue from the CLA-fed mice had significantly reduced heparin-releasable lipoprotein lipase (LPL) and intracellular LPL activities and significantly reduced glucose incorporation into CO₂, fatty acid and glycerol. There were no differences between adipose tissues from CLA-fed or control mice in the ratios of 16:0 to 16:1 and 18:0 to 18:1 fatty acids or in norepinephrine-stimulated lipolysis. Serum insulin levels in food-deprived mice, measured at 4 d and 7 wk, did not differ between groups nor did the concentration of free fatty acids in serum of food-deprived or fed mice measured at the same time points. In mice, CLA-induced inhibition of heparin-releasable LPL and glucose metabolism may be the most important early steps leading to subsequent body fat reduction. In addition, CLA does not appear to enhance lipolysis in mouse adipose tissue *in vivo*. J. Nutr. 133: 663–667, 2003.

KEY WORDS: • conjugated linoleic acid • lipoprotein lipase • lipolysis • glucose metabolism

The c9t11 and t10c12 isomers of conjugated linoleic acid (CLA)⁵ induce several physiological effects, some through the independent actions of one of these isomers and others through synergistic interactions involving both isomers (1,2). CLA-induced reduction in body fat gain is an example of a single-isomer effect that is due specifically to t10c12 CLA (3,4). This effect appears to result from a reduction in lipid accumulation in adipocytes and is correlated with inhibition of the activities of heparin-releasable lipoprotein lipase (LPL) and stearoyl-CoA desaturase (SCD) (3,5–7).

We have investigated the biochemical mechanism(s) that may underlie these findings, in particular whether t10c12 CLA acts directly or via a metabolite to regulate lipid accumulation in adipocytes, and identified the signaling pathways through

which t10c12 CLA (or its bioactive metabolite) acts to control body fat gain *in vivo* (3,8). We have reported that a 19-carbon CLA cognate, conjugated nonadecadienoic acid (CNA), inhibited LPL activity and lipid accumulation in 3T3-L1 cells similarly to CLA and was even more effective than CLA in inducing body composition changes in growing mice (3,8). Because CLA and CNA differ in length by one carbon atom, they are unlikely to have a common metabolite to account for these observations. A more likely, although as yet unproven explanation, is that t10c12 CLA and its CNA bioactive counterpart (putatively t11c13 CNA) directly affect adipocytes.

We have found that a number of lipoxygenase inhibitors reduced LPL activity in differentiated 3T3-L1 adipocytes and enhanced the CLA-induced inhibition of LPL (9). Dietary nordihydroguaiaretic acid (used as a model lipoxygenase inhibitor) acts synergistically with a suboptimal dietary concentration of CLA to significantly reduce body fat gain in growing mice (9). These findings are of interest in that CLA affects the eicosanoid-regulated immune system (10,11) and regulates the concentrations of inducible prostanoids and leukotrienes (12), actions that may relate to the mechanisms underlying the effects of CLA on adipocytes and body fat gain (9).

The purpose of this study was to further elucidate the CLA-induced changes in adipocyte physiology that underlie

¹ This study was supported in part by gift funds administrated through the Food Research Institute at the University of Wisconsin-Madison.

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⁵ Abbreviations used: CLA, conjugated linoleic acid; CNA, conjugated nonadecadienoic acid; LPL, lipoprotein lipase; NDGA, nordihydroguaiaretic acid; SCD, stearoyl-CoA desaturase.

the reduction in body fat gain. Accordingly, we investigated the early biochemical events in the adipocytes of mice fed CLA-supplemented diet, before the occurrence of changes in body composition, accompanied by reduced adipocyte size, which may complicate direct comparisons of adipocytes from control and CLA-fed mice.

MATERIALS AND METHODS

Materials. Adenosine deaminase, BSA, ascorbic acid, collagenase (type II), HEPES and a triglyceride kit (catalog No. 337-40A) were purchased from Sigma Chemical (St. Louis, MO). Triolein, [9,10-³H(N)] (specific activity, 12 Ci/mmol), was purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO). [1-¹⁴C]linoleic acid (specific activity, 55 mCi/mmol) was purchased from Amersham Life Science (Arlington Heights, IL). D-[¹⁴C(U)]glucose (specific activity, 245 mCi/mmol) was obtained from Moravsek Biochemicals (Brea, CA). A Coat-A-Count Insulin kit was obtained from Diagnostic Products Corporation (Los Angeles, CA). NEFA C free fatty acid kit purchased from Waco (Neuss, Germany). CLA (90% purity) was purchased from Natural Lipids Ltd (Hovdebygd, Norway). CLA was checked by gas chromatography, and its isomer content was (in g/100 g) c9t11, 40.1; t10c12, 41.6; c9c11/c10c12, 2.3; and t9t11/t10t12, 5.3. Semipurified rodent diet (TD 94060) was purchased from Harlan Teklad (Madison, WI).

Animals and diets. We obtained 8-wk-old female ICR mice and 3-wk-old male CD rats from Harlan Sprague-Dawley (Madison, WI). The semipurified diet contained (in g/kg) sucrose, 481; casein, vitamin-free diet, 210; corn starch, 150; DL-methionine, 3; corn oil, 50; cellulose, 50; mineral mix, AIN-76 (13), 35; vitamin mix, AIN-76A, 10; calcium carbonate, 4; choline bitartrate, 2; and ethoxyquin, 0.1. The fatty acid profile (in g/100 g of diet) of the control diet was 14:0, 0.1; 16:0, 11.5; 16:1, 0.2; 18:0, 2.1; 18:1, 28.0; 18:2, 55.7; α -18:3, 0.8; and 20:0, 0.4. The fatty acid profile (in g/100 g of diet) of the CLA diet was 14:0, 0.1; 16:0, 10.1; 16:1, 0.2; 18:0, 1.9; 18:1, 25.7; 18:2, 54.8; α -18:3, 0.7; 20:0, 0.4; c9,t11 CLA, 5.2; t10,c12 CLA, 5.5; c,c CLA isomers, 0.3; and t,t CLA isomers, 0.5.

The mice and rats were housed individually in a windowless room on a 12-h light/dark cycle, in strict accordance with guidelines established by the Research Animal Resources Center of the University of Wisconsin-Madison, and fed a semipurified diet for 1 wk before experiments commenced. They were then randomly assigned to groups and fed either CLA-containing diet (semipurified diet with 0.5 g of CLA/100 g) or control diet (semipurified diet with additional 0.5 g of corn oil/100 g) for the period(s) indicated in the tables and figures. After the feeding period, the mice were killed by CO₂ asphyxiation. Mouse parametrial adipose tissue was isolated and minced as described (14). Sera from food-deprived rats were analyzed for insulin concentration.

Metabolic studies: glucose metabolism and lipolysis. We investigated the effects of CLA on glucose metabolism and lipolysis using minced fat pads rather than isolated fat cells. Based on our experience, minced fat pads are technically easier to manipulate and yield more consistent results.

Glucose metabolism was investigated as described (15,16) with modifications. Fat pads were rinsed with warm phosphate-buffered saline, finely minced and preincubated twice (2 × 15 min) in culture buffer (5 mmol of glucose, 10 mmol of HEPES, 120 mmol of NaCl, 5 mmol of KCl, 1.2 mmol of MgSO₄ · 7H₂O, 1.2 mmol of KH₂PO₄, 1.3 mmol of CaCl and 25 mmol of NaHCO₃ per L, pH 7.4). Experiments were commenced by adding ~100 mg of minced fat to flasks containing 0.5 μ Ci of D-[¹⁴C(U)]glucose in culture buffer. Flasks were gassed with 95% O₂ and 5% CO₂ and sealed with rubber stoppers holding center wells that contained filter paper. After a 2-h incubation, the reaction was stopped by injecting 0.25 mL of 0.5 mol of H₂SO₄/L into each flask. The CO₂ was then trapped in 0.4 mL ethanolamine that was injected into the filter paper, and after 30 min the contents of the center well were transferred to vials containing 10 mL of scintillation fluid. Total lipids in each flask were extracted with chloroform/methanol (2:1). Radioactivity in the chloroform phase was analyzed to determine radiolabeled glucose incorporation into total lipids. Then 1 mL of the lower phase was dried under nitrogen

and methylated with 1 mol HCl/L at 60°C for 20 min. Methyl esters of fatty acids were extracted in hexane and counted. The difference in radioactivity between total lipids and fatty acids represented glucose incorporation into glycerol.

Lipolysis was determined as described (16) with modifications. Fat pads were washed with warm saline, blotted, minced and then preincubated twice in wash media (5 mmol of glucose, 10 mmol of HEPES and 20 g of BSA per L in Krebs' bicarbonate buffer, pH 7.4) for 15 min. About 100 mg of minced fat pad was incubated for 2 h at 37°C in an atmosphere of 95% O₂ and 5% CO₂ with varying concentrations of norepinephrine (0–10,000 nmol/L) in incubation medium (wash medium with 100 mU of adenosine deaminase and 0.1 mmol of ascorbic acid per L). The reaction was stopped by the injection of 0.25 mL of 0.5 mol of H₂SO₄/L. The medium was collected and used for glycerol determination. Lipolysis was defined as the nmol of glycerol released per hour per milligram of adipose tissue.

Lipoprotein lipase. Heparin-releasable and intracellular LPL activities were determined as described (17,18) with modifications. For heparin-releasable LPL, samples were prepared by incubating minced fat tissue fragments (~40 mg) in 0.3 mL of elution buffer (10 mmol of Tris-HCl, 10,000 U of heparin and 100 mL of serum per L, pH 7.4) at 37°C for 1 h, after which 0.2-mL samples were assayed for LPL activity. For intracellular LPL, homogenized samples were prepared by putting tissue fragments (~0.4 g) in ice-cold buffer (0.223 mol of Tris-HCl, 0.25 mol of sucrose, 2 g of deoxycholate, 0.08 g of Nonidet P-40, 10,000 U of heparin and 10 g of BSA per L, pH 8.3) and homogenizing with a sonicator. Homogenates were centrifuged at 12,000 × g for 15 min at 4°C. Aliquots of the supernatant (below the fat cake) were diluted 1:20 in detergent-free buffer and used in the LPL assay. A triolein emulsion was prepared by mixing 600 mg of unlabeled triolein, 200 μ Ci of triolein [9,10-³H(N)] and 36 mg of lecithin in 10 mL of glycerol. The assay substrate was prepared immediately before use by mixing the triolein emulsion with 0.2 mol of Tris-HCl/L (pH 8.0) containing 30 g of BSA/L and heat-inactivated fasted rat serum (1:4:1, v/v/v). The assay was initiated by the addition of 100 μ L of assay substrate to 100- μ L sample. The reaction mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 3.25 mL of fatty acid extraction mixture (methanol/chloroform/heptane 1.41:1.25:1), followed by 1.05 mL of 0.1 mol of potassium carbonate-borate buffer/L (pH 10.5). After extraction, the reaction mixtures were centrifuged at 1,400 × g for 30 min. A 1-mL aliquot of the methanol water upper phase was used for counting. The recovery of the free fatty acid was estimated to be 71%, using [¹⁴C]linoleic acid.

Adipocyte isolation and cell size determination. Adipocytes were isolated by the method of Rodbell (16) with modifications. Parametrial fat tissues were cut into small pieces and digested with collagenase (0.5 g/L) in wash media (2 mmol of glucose, 0.2 g of BSA and 30 mmol of HEPES in Krebs' bicarbonate buffer per L, pH 7.4) for 60 min at 37°C. Cells were filtered through a nylon filter (200 μ m) and washed in wash media. Cell sizes were determined using a Coulter Counter (Coulter Electronics, Miami, FL) connected to a Coulter Channelyzer 256. An orifice tube containing a 280- μ m aperture was used, and the Coulter Counter was calibrated with latex particles of known diameter. Cell collection was performed in the manometer mode (2 mL volume).

Fatty acid analysis. Fat was extracted from parametrial adipose tissues with chloroform/methanol (2:1), and fatty acid methyl esters were prepared by reaction with 1 mol HCl/L in methanol at 60°C for 20 min and identified by comparison with standards by gas chromatography. Gas chromatographic analysis was conducted with a Hewlett-Packard 5890 series II gas chromatograph fitted with a flame-ionization detector and 3396A integrator (Avondale, PA). A Supelcowax-10 fused-silica capillary column (60 m × 0.32 mm i.d., 0.25- μ m film thickness) was used, and oven temperature was programmed from 50° to 200°C, increased 20°C/min, held for 50 min, increased 10°C/min to 220°C and held for 60 min.

Serum insulin and free fatty acids. Mice were food-deprived for 12 h before the experiment. They were then anesthetized using isoflurane, and blood was collected via cardiac puncture. Serum insulin and free fatty acid concentrations were determined by using a radioimmunoassay kit and colorimetric assay kit, respectively.

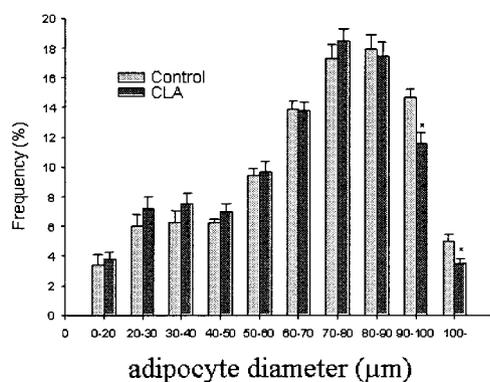


FIGURE 1 Size distribution of adipocytes from parametrial adipose tissue of 8-wk-old female mice fed control or CLA-supplemented diet for 4 d. Values are means \pm SEM, $n = 9$ mice. *Different from control group, $P < 0.05$.

Statistical analysis. Because only control and CLA groups were compared, data in Figures 1 and 2 and the tables were analyzed using an unpaired Student's t test. Data in Figures 3 and 4 were analyzed by analysis of variance using the General Linear Models procedure of the SAS Institute (SAS Users Guide: Statistics; SAS Institute Inc., Cary, NC). Differences were considered significant at a P value of <0.05 .

RESULTS

Body weights for control mice and mice fed CLA-supplemented diet for 4 d were not different (30.8 ± 0.5 g and 30.9 ± 1.0 g, respectively; $n = 9$). Relative parametrial fat mass also did not differ between groups (3.6 ± 0.3 and 3.1 ± 0.4 g/100 g of body, respectively; $n = 9$). The diameters of parametrial adipocytes for control and CLA-fed mice were 68.3 and 65.5 μm , respectively (Fig. 1). There were no differences except in the range of 90 μm diameter and larger, where there was a lower percentage of adipocytes in the CLA-fed group (Fig. 1).

Both heparin-releasable and total LPL activity were reduced in parametrial adipose tissue of the mice fed CLA-supplemented diet for 4 d (Fig. 2). By contrast, lipolysis in parametrial adipose tissue was unaffected in mice fed CLA-supplemented diet for 4 d (Fig. 3). Lipolysis in parametrial adipose tissue from mice fed CLA-supplemented diet for 3 wk was reduced relative to controls (Fig. 4), but by this time adipocyte size was also significantly reduced relative to controls (data not shown). The serum free fatty acid concentra-

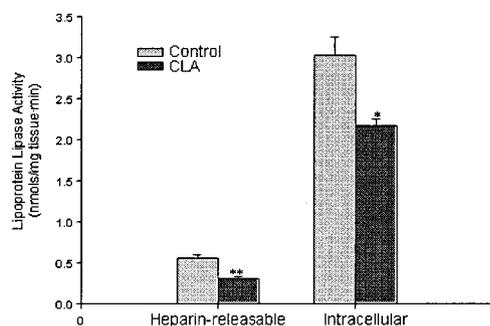


FIGURE 2 Heparin-releasable and intracellular lipoprotein lipase (LPL) activity in parametrial adipose tissue of 8-wk-old female mice fed control or CLA-supplemented diet for 4 d. Values are means \pm SEM, $n = 3$. *Different from control group, $P < 0.05$. **Different from control group, $P < 0.01$.

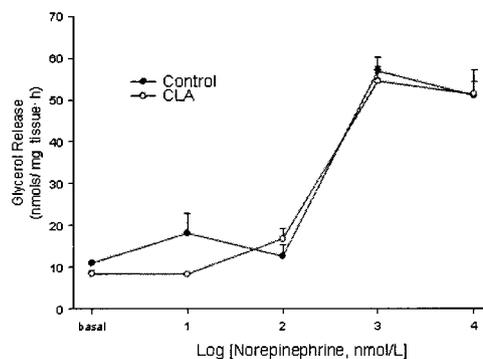


FIGURE 3 Lipolysis in parametrial adipose tissue in 10-wk-old female ICR mice fed control or CLA-supplemented diet for 4 d. Values are means \pm SEM, $n = 3$.

tion did not differ between CLA-fed and control mice at 4 d or 7 wk (Table 1).

The conversion of glucose into fatty acids, glycerol and CO_2 was significantly reduced in parametrial fat tissue from mice fed CLA for 4 d compared with controls (Table 2). Fatty acid profiles for adipose tissue from mice fed control or CLA-supplemented diet for 4 d are shown in Table 3. Notably there were no differences between control and CLA-fed mice in 16:0, 16:1, 18:0 or 18:1. However, there appeared to be more esterified c9t11 than t10c12 CLA in mice fed CLA despite the fact that the CLA diet contained approximately equal amounts of these isomers. In addition, there was significantly less linoleic acid in adipose tissue from the CLA-fed group.

Serum insulin concentrations in food-deprived mice did not differ between groups (50.40 ± 2.77 versus 64.39 ± 10.82 pmol/L for the control and CLA-fed groups after 4 d of feeding, respectively); the findings were similar for mice fed CLA-supplemented diet for 7 wk (73.47 ± 7.94 versus 90.37 ± 23.14 pmol/L for the control and CLA-fed groups, respectively). To provide a species comparison, in a separate study we found that feeding CLA-supplemented diet for 7 wk did not affect the serum insulin concentration of food-deprived male CD rats (400 ± 3.48 and 350 ± 2.96 pmol insulin/L for the control and CLA-fed groups, respectively).

DISCUSSION

A goal of this investigation was to study the early biochemical effects of dietary CLA on adipocytes. Feeding mice diet

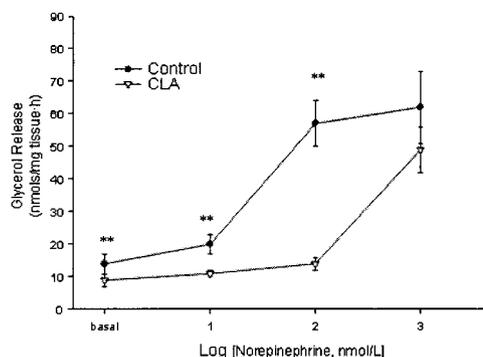


FIGURE 4 Lipolysis in parametrial adipose tissue of 10-wk-old female mice fed control or CLA-supplemented diet for 3 wk. Values are means \pm SEM, $n = 5$. **Different from control group for each concentration of norepinephrine, $P < 0.01$.

TABLE 1

Serum free fatty acid concentration in female mice that were fed a control diet or a diet supplemented with conjugated linoleic acid (CLA) measured at 4 d and 7 wk¹

Time/group	Food-deprived group	Fed group
	<i>mmol/L</i>	
4 d		
Control	0.99 ± 0.03	1.63 ± 0.04
CLA	0.86 ± 0.03	1.65 ± 0.18
7 wk		
Control	0.97 ± 0.03	1.25 ± 0.13
CLA	1.03 ± 0.06	1.13 ± 0.13

¹ Values are means ± SEM, *n* = 4 or 5.

² Mice were deprived of food for 12 h before the experiment.

supplemented with 0.5 g of CLA/100 g for 4 d did not affect parametrial adipocyte size (Fig. 1). Hence, this experimental design is appropriate for the study of early effects of CLA that occur before changes in adipocyte size are apparent.

Park et al. (19) have found that a mixture of CLA isomers inhibited heparin-releasable LPL activity in cultured mouse 3T3-L1 adipocytes and later (3) that this effect was due to the t10c12 CLA isomer. Lin et al. (20) have reported that t10c12 CLA (and, to a lesser extent, c9t11 CLA) inhibited heparin-releasable but not intracellular LPL activity in 3T3-L1 adipocytes and concluded that the CLA isomers acted at the post-translation stage when inactive intracellular LPL protein is activated and translocated to the outer surface of the plasma membrane. However, in mouse parametrial adipocytes *in vivo*, short-term CLA feeding reduced the activities of both intracellular and heparin-releasable LPL (Fig. 2). These findings (Fig. 2) do not preclude a posttranslational effect of CLA as proposed by Lin et al. (20), but they do suggest the possibility that CLA may inhibit LPL gene expression in normal adipocytes *in vivo*.

Our results showed there was no change in glycerol release (indicative of lipolysis) in parametrial adipose tissue after 4 d but a significant reduction after 3 wk of CLA supplementation (Figs. 3 and 4). However, by this time there was substantially less lipid in the adipose tissue of CLA-fed compared with control mice, so one might expect reduced glycerol release even if the rate of lipolysis is unchanged. This is supported by the fact that the free fatty acid concentrations in serum were not affected by feeding CLA-supplemented diet for 4 d or 7 wk (Table 1).

Previously it has been reported that in cultured 3T3-L1 adipocytes, CLA increases glycerol release (3,9,19), but the

TABLE 2

Effect of conjugated linoleic acid (CLA) on glucose metabolism in parametrial fat tissues from mice fed for 4 d¹

Metabolic product	Control group	CLA group
CO ₂ , $\mu\text{mol}/(\text{g of fat} \cdot \text{h})$	1.12 ± 0.17	0.54 ± 0.09 ^a
Fatty acid, $\mu\text{mol}/(\text{g of fat} \cdot \text{h})$	1.35 ± 0.16	0.42 ± 0.08 ^a
Glyceride-glycerol, $\mu\text{mol}/(\text{g of fat} \cdot \text{h})$	1.18 ± 0.12	0.74 ± 0.08 ^a

¹ Values are means ± SEM, *n* = 4.

^a Different from control, *P* < 0.05.

TABLE 3

Fatty acid profile of parametrial adipose tissue in female ICR mice fed a control or conjugated linoleic acid (CLA)-supplemented diet for 4 d¹

Fatty acid	Control group	CLA group
	<i>g/100 g of total fatty acids</i>	
12:0	0.06 ± 0.00	0.07 ± 0.00
14:0	1.37 ± 0.05	1.44 ± 0.01
16:0	24.04 ± 0.24	24.58 ± 0.22
16:1	7.31 ± 0.23	7.62 ± 0.14
18:0	2.24 ± 0.07	2.20 ± 0.03
18:1	34.15 ± 0.17	34.45 ± 0.18
18:2	28.02 ± 0.20	26.35 ± 0.26 ^a
18:3 (n-3)	0.74 ± 0.02	0.67 ± 0.04
20:4	0.22 ± 0.00	0.22 ± 0.02
<i>cis</i> -9, <i>trans</i> -11-CLA	ND ²	0.30 ± 0.01
<i>trans</i> -10, <i>cis</i> -12-CLA	ND	0.19 ± 0.01

¹ Values are means ± SEM, *n* = 9.

² ND, not detected.

^a Different from control, *P* < 0.01.

effect is induced by both t10c12 and c9t11 CLA, although to a lesser extent by c9t11 CLA (3). Because there is no evidence that c9t11 CLA will reduce body fat gain *in vivo* (2), it is possible that the CLA-induced enhancement of glycerol release in 3T3-L1 adipocytes (3,9,20) may be specific to that model system and not necessarily predictive of what may occur in normal adipocytes *in vivo*. That CLA did not affect glycerol release from cultured human abdominal or thigh adipose tissue was supported by Brown et al. (21). Mechanistic studies to determine possible differences in the regulation of lipolysis in cultured 3T3-L1 adipocytes compared with normal adipocytes *in vivo* are also indicated.

The results showed that CLA did not increase lipolysis *in vivo*. This suggests that there is an early effect of CLA on fat metabolism that involves a reduction of fat deposition and less likely an enhancement of fat release. However, in mice, the t10c12 CLA isomer was able to reduce existing body fat after 12 d feeding, which suggests there may be an additional mechanism such as increased fatty acid oxidation in muscle (2,19).

Glucose metabolism and lipogenesis were reduced by short-term CLA feeding (Table 2). Brown et al. (21) have reported similar findings with human adipocytes cultured in media containing CLA. However, West et al. (22) have reported that CLA feeding did not affect whole body fatty acid synthesis in mice, and Park (23) has found that feeding CLA for 1 wk did not affect the activities of fatty acid synthetase (173.96 ± 32.25 versus 206.23 ± 19.53 mU · min⁻¹ · mg of protein⁻¹ for the control and CLA-fed groups, respectively) or acetyl CoA carboxylase (0.717 ± 0.146 versus 0.716 ± 0.075 mU · min⁻¹ · mg of protein⁻¹, respectively) in mouse fat pads. Additional investigation is warranted in light of our finding that serum insulin concentrations were not significantly affected in mice fed CLA-supplemented diet for 4 d or 7 wk. Rats and mice are reported to respond differently in this regard (2), so it is also noteworthy that in our hands, feeding CLA-supplemented diet to normal rats also did not affect serum insulin concentrations.

It has recently been reported that SCD1-null mice, like CLA-fed mice, have reduced body fat compared with controls (24,25). Interestingly, it has been previously reported that the t10c12 CLA isomer, but not the c9t11 CLA isomer, directly

inhibits SCD activity (6,7). This may contribute to the CLA-induced reduction in lipid accumulation by adipocytes (2). However, in our studies, after 4 d of feeding, CLA modified lipid uptake measured as LPL activity, whereas there was no difference in fatty acid profile (monounsaturated fatty acids vs saturated fatty acids). This indicated that the effect of CLA, especially the t10c12 isomer, on SCD activity was either not involved in the early events of fat metabolism or the experimental period (4 d) was not long enough for the rate of fatty acid turnover in the cells to cause an observable effect on fatty acid profiles. Elsewhere, we discuss the possibility that effects of the biologically active CLA isomers on SCD protein structure may be involved in body fat regulation (26). The effect of CLA on body fat may also be the result of other mechanisms, such as a reduction in arachidonic acid (23,27) and production of its derivatives (23,28) and/or a possible connection to transcription factors, such as peroxisomal proliferator activated receptors (29).

In summary, CLA-induced inhibition of heparin-releasable LPL and glucose metabolism may be the most important early steps leading to subsequent body fat reduction in mice. Also, CLA does not appear to enhance lipolysis in mouse adipose tissue *in vivo*.

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