Contrasting Effects of *t*10,*c*12- and *c*9,*t*11-Conjugated Linoleic Acid Isomers on the Fatty Acid Profiles of Mouse Liver Lipids

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ABSTRACT: The purpose of this study was to examine the effects of two purified isomers of CLA (*c*9,*t*11-CLA and *t*10,*c*12-CLA) on the weights and FA compositions of hepatic TG, phospholipids, cholesterol esters, and FFA. Eight-week-old female mice (n = 6/group) were fed either a control diet or diets supplemented with 0.5% *c*9,*t*11-CLA or *t*10,*c*12-CLA isomers for 8 wk. Weights of liver total lipids and those of individual lipid fractions did not differ between the control and the *c*9,*t*11-CLA groups. Livers from animals fed the *t*10,*c*12-CLA diet contained four times more lipids than those of the control group; this was mainly due to an increase in the TG fractions (fivefold), but cholesterol (threefold), cholesterol esters (threefold), and FFA (twofold) were also significantly increased. Although *c*9,*t*11-CLA did not significantly alter the weights of liver lipids when compared with the control group, its intake was associated with significant reductions in the weight percentage (wt% of total FAME) of 18:1n-9 and 18:1n-7 in the TG fraction and with significant increases in the weight percentage of 18:2n-6 in the TG, cholesterol ester, and phospholipid fractions. On the other hand, *t*10,*c*12-CLA intake was linked with a significant increase in the weight percentage of 18:1n-9 and a decrease in that of 18:2n-6 in all lipid fractions. These changes may be the result of alterations in the activity of Δ9-desaturase (stearoyl CoA desaturase) and the enzymes involved in the metabolism of 18:2n-6. Thus, the two isomers differed not only in their effects on the weights of total liver lipids and lipid fractions but also on the FA profile of the lipid fractions.

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CLA is a collective term for a group of isomers of linoleic acid that have conjugated double bonds. Depending on the position and geometry of the double bonds, several isomers of CLA have been reported (1). The major dietary sources of *c*9,*t*11-CLA are dairy products and ruminant meat, whereas those of *t*10,*c*12-CLA are partially hydrogenated vegetable oils from margarines and shortenings (2). Most of the published studies have used mixtures of CLA isomers, that were composed of two major forms, *cis*9,*trans*11-CLA (*c*9,*t*11-CLA) and *trans*10, *cis*12-CLA (*t*10,*c*12-CLA), and a number of minor isomers. Feeding a mixture of CLA isomers to animal models has been reported to alter blood lipids, atherogenesis, diabetes, body composition, chemically induced carcinogenesis, and immune cell functions (3). Results from studies in mice (4–7), rats (8–11), chickens (12,13), pigs (14–18), and fish (19) have indicated that supplementing diets with a mixture of CLA isomers causes a reduction in body fat.

Because liver is the major site for FA synthesis, an understanding of the effects of dietary CLA isomers on liver lipids and their FA composition is important in understanding their overall effects on body fat metabolism. Results showing the effects of dietary CLA on liver lipids have been variable. It increased liver lipids in mice (20), increased (12) or decreased (13) them in chickens, decreased them in fish (19), and had no effect in hamsters (21) and rats (22,23). Part of these variations may be due to differences in lipid metabolism among the different species, but they could also be due to differences in the composition of CLA isomers in the mixtures used. Studies conducted with purified isomers have shown that the one responsible for reducing body and adipose tissue weights in mice (5,24–26) and hamsters (21) and for altering mammary lipid metabolism in dairy cows (27) is the *t*10,*c*12-CLA isomer.

Although feeding a mixture of CLA isomers did not alter the concentration of liver lipids in rats (22,23), it altered their FA composition (28). Other studies in rats using purified isomers have shown that the isomer responsible for altering the FA profile of the rat liver lipids was *t*10,*c*12-CLA, whereas the naturally occurring *c*9,*t*11-CLA had no effect (29,30). Thus, the two CLA isomers differ in their effects on the FA profile of rat liver lipids, but the effects of purified isomers on the FA profile of liver lipids in other species have not been studied.

We recently reported that supplementing the diets of mice with purified *c*9,*t*11-CLA and *t*10,*c*12-CLA had similar effects on immune cell functions, but only the *t*10,*c*12-CLA increased the liver lipids (24,31). Which liver lipid fraction is altered by the CLA, and the effects of purified isomers on the FA composition of liver lipid fractions in mice are not known. The purpose of this study was to examine the effects of two purified isomers of CLA (*c*9,*t*11-CLA and *t*10,*c*12-CLA) on the weights and FA compositions of hepatic TG, phospholipids, cholesterol esters, and FFA in mice.

MATERIALS AND METHODS

CLA isomers and diets. Highly enriched *c*9,*t*11-CLA and *t*10, *c*12-CLA isomers in the form of FFA were a kind gift from Natural ASA (Hovdebygda, Norway). The analytical data for

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Abbreviations: AA, arachidonic acid; MUFA, monounsaturated FA; PPAR, peroxisome proliferator-activated receptor; SREBP-1, sterol regulating element-binding protein-1; SFA, saturated FA.

these isomers was provided by the supplier and confirmed in our laboratory. The preparation enriched in *c*9,*t*11-CLA contained *c*9,*t*11-CLA = 84.6%; *t*10,*c*12-CLA = 7.7%; 18:1n-9 = 3.8%; *t*9,*t*11-CLA + *t*10,*t*12-CLA = 2.0%; and other FA = 1.9%. In the preparation enriched in *t*10,*c*12-CLA, this isomer was 88.1%, with *c*9,*t*11-CLA = 6.6%; *t*9,*t*11-CLA + *t*10,*t*12-CLA = 2.5%; 18:1n-9 = 1.1%; and other FA = 1.7%.

The concentration of CLA used in this study was 0.5 wt% of the diet, comparable to the concentrations used in previous studies with rodent models, which have ranged from 0.1 to 1.5 wt% of a mixture of CLA isomers. AIN-93G, a high-carbohydrate mouse diet, was used as the basal diet. The nutrient and FA composition of this diet has been reported previously (24,31). Briefly, the control diet contained (g/kg): cornstarch 417.5, casein 200, dextrinized cornstarch 132, sucrose 100, corn oil with tocopherol 50 (α-tocopherol 100 mg/kg corn oil), cellulose 50, mineral mixture (AIN-93G) 35, vitamin mixture (AIN-93) 10, L-cysteine 3, and choline bitartrate 2.5. For the two CLA-containing diets, CLA isomer-enriched oils were added by replacing 5 g/kg of corn oil with an equivalent amount of the CLA source. Diets were constantly flushed with nitrogen gas while being gently mixed in a blender. Diets were packaged in 30-g aliquots, flushed with nitrogen gas, and stored at –20°C. Fresh dietary packets were served each day. The animal protocol was approved by the Animal Use Committee at the University of California, Davis.

Animals, feeding, and tissue collection. Eighteen 8-week-old, pathogen-free C57BL/6N female mice were purchased from Charles River (Raleigh, NC). Female mice were chosen because we were also interested in the effects of CLA isomers on immune cell functions, and because male mice fight when caged together, which can affect their immune cell functions. They were maintained in a sterile air curtain isolator at the animal facility of the University of California Medical School, with controlled temperature (25°C) and light and dark cycles (12 h each). They were fed the laboratory chow diet for the first 7 d and experimental diets for the last 56 d. Animals were divided into three groups at the start of the experimental diets (study day 1), with six per group. Details regarding animal handling, sacrifice, tissue collection, and storage have been published previously (24).

Lipid extraction, the isolation of different lipid classes, and FA analysis. Livers were removed, blotted dry with tissue paper, weighed, placed in liquid nitrogen, and stored frozen at –80°C until processed. Lipid extraction and FA analysis were performed according to previously published methods from our laboratory (32).

Briefly, a portion of the frozen liver was weighed and freeze-dried, and a portion of the freeze-dried sample was then weighed and transferred into a 7-mL glass hand homogenizer. The sample was homogenized with 5 mL chloroform/methanol 2:1 (vol/vol) containing 0.005% each BHT and hydroquinone. The homogenate was filtered through prewashed sharkskin paper; the homogenizer and residue on the paper were then washed twice with 1 mL additional solvent, and the combined filtrates were dried under nitrogen. The residue was dissolved in 1 mL chloroform and filtered through prewashed cotton into a 2-mL tared vial. The solvent was removed under dry nitrogen, freeze-dried, and weighed to yield the total lipids extracted (*ca.* 0.01–0.04 g). This was dissolved in a small amount of chloroform and applied as a band to a 20 cm × 20 cm × 250 μm activated silica gel plate. The plate was developed using hexane/ether/acetic acid (85:15:2, by vol), in a chamber flushed with nitrogen and equilibrated with the developing solvent. The plate was dried under a stream of nitrogen at room temperature, then sprayed with 2′,7′-dichlorofluorescein reagent; the bands were then visualized under UV light and scraped into test tubes. Each lipid fraction was extracted with chloroform/methanol (2:1), filtered, and the solvent removed and weighed. Each lipid fraction was transferred into small screw-capped test tubes, to which were added 1 mL dry methanol and one drop toluene. This was followed by the addition of 0.5 mL 0.5 M methanolic sodium methoxide, after which the tubes were flushed with nitrogen and capped. The tubes were then heated at 53–55°C for 10 min and cooled to room temperature. Methanolic HCl, 0.5 mL 3 N, was added to each tube, which was then capped, heated at 53–55°C for 15 min, and cooled to room temperature. Water (4.5 mL) and hexane (2 mL) were then added and mixed. The layers were allowed to separate, and the top hexane layer was transferred to another test tube. The bottom layer was extracted with an additional 2 × 1 mL hexane. The combined hexane extracts were dried over sodium sulfate/sodium bicarbonate (4:1) and filtered through prewashed cotton; the solvent was then removed under a stream of dry nitrogen to yield the transmethylated product. This was dissolved in 5–100 μL isooctane and analyzed on an Agilent 6890 gas–liquid chromatograph equipped with an FID and using a Supelco 2380 column (100 m × 0.25 mm × 0.2 μm film thickness). Oven conditions were as follows: hold at 75°C for 4 min, heat at 13°C/min to 175°C, hold for 27 min, heat at 4°C/min to 215°C, and hold for 20 min. A second 6890 gas–liquid chromatograph equipped with an Agilent 5973 mass selective detector was used to verify the identity of the GLC peaks as necessary.

Statistical analysis. The data were first checked for homogeneity of variance using Levene's test. When heterogeneity was found, either a transformation was used to stabilize the variances among diets or the SAS PROC MIXED was used to incorporate the heterogeneity into the model. The model was a one-way ANOVA, and Dunnett's test was used for diet comparisons with the control group (33).

RESULTS

Effect of CLA isomers on the weights of different classes of liver lipids. Table 1 contains data regarding the total liver lipids and the lipid classes for mice fed the control and CLA-containing diets. Livers from the animals fed the control diet contained an average of 156 mg lipids per liver, which represented 11.3% of the liver weights. Liver lipid contents of the animals fed the *c*9,*t*11-CLA diet did not differ significantly from those in the control group. However, the livers of animals fed diets containing

TABLE 1
Effect of CLA Isomers on Liver Lipid Classes (wt% of total liver lipids or mg/liver)[a]

Lipid fraction	Control	*c*9,*t*11-CLA	*t*10,*c*12-CLA
Lipids (wt% of liver)	11.30 ± 1.30	11.80 ± 0.50	30.30 ± 4.70*
Total lipids (mg/liver)	156.1 ± 18.1	172.2 ± 13.0	641.1 ± 119.1*
TG			
wt% of lipids	63.2 ± 3.40	68.0 ± 2.7	81.9 ± 1.0*
mg/liver	98.7 ± 24.0	117.1 ± 18.0	524.8 ± 63.8*
Phospholipids			
wt%	17.1 ± 2.6	15.9 ± 1.7	4.7 ± 0.4*
mg/liver	26.7 ± 3.1	27.4 ± 4.9	29.5 ± 2.8
Cholesterol			
wt%	6.2 ± 0.7	5.8 ± 0.4	4.8 ± 0.5
mg/liver	9.7 ± 1.2	9.9 ± 2.1	31.4 ± 3.6*
Cholesterol esters			
wt%	9.5 ± 0.9	7.1 ± 0.5*	7.0 ± 0.4*
mg/liver	14.8 ± 2.9	12.3 ± 2.7	44.6 ± 5.5*
FFA			
wt%	4.0 ± 0.6	3.1 ± 0.5	1.7 ± 0.3*
mg/liver	6.2 ± 0.5	5.4 ± 1.6	11.2 ± 2.3

[a]Data are mean ± SEM (n = 6). Numbers marked with an asterisk are significantly ($P < 0.05$) different from corresponding values in the control group.

*t*10,*c*12-CLA contained four times more lipids than the amount found in the control group, and lipids constituted 30% of the liver weight in this group.

TG, phospholipids, cholesterol, cholesterol esters, and FFA, constituted 63.2, 17.1, 6.2, 9.5, and 4.0 wt% of the total liver lipids in animals fed the control diet (Table 1). The percentage distribution of lipids among the different fractions did not differ between the control and the *c*9,*t*11-CLA groups, except that intake of *c*9,*t*11-CLA was associated with a significant reduction in the weight percentage of cholesterol esters. In the animals fed *t*10,*c*12-CLA, the weight percentage of TG was increased ($P < 0.05$), and the weight percentages of phospholipids, cholesterol esters, and FFA were decreased as compared with the corresponding values in the animals fed the control diet. Because not all lipid fractions changed proportionally, these changes did not represent changes in the absolute weights of the different fractions. Based on the total lipids and their percentage distribution among different classes, we calculated the absolute weight (mg/liver) of each lipid class. On the basis of mg/liver, none of the lipid classes differed between the control and the *c*9,*t*11-CLA groups. On this basis, the weights of TG, cholesterol, cholesterol esters, and FFA in the *t*10,*c*12-CLA group were five, three, three, and two times those of the corresponding values in the control group; the absolute weights of phospholipids per liver did not differ among the three groups. These data emphasize that changes in the weight percentage and absolute weight of different lipid fractions can lead to different interpretations, and both should be determined.

FA composition of liver TG, phospholipids, cholesterol esters, and FFA of mice fed the control or CLA-containing diet. The weight percentage (wt% of total FAME) concentrations of CLA and major FA found in the different classes of lipids in the livers of mice fed the control or experimental diet are shown in Table 2. The highest weight percentage of *c*9,*t*11-CLA was found in cholesterol esters (3.1%), followed by TG (1.3%), FFA (1.2%), and phospholipids (0.1%), respectively. The weight percentage of *t*10,*c*12-CLA ranged from 0.3% in TG to 0.1% in cholesterol esters. In animals fed *t*10,*c*12-CLA, the concentrations of *c*9,*t*11-CLA were below the detection limit; however, in animals fed *c*9,*t*11-CLA, the levels of *t*10,*c*12-CLA were detectable in the cholesterol esters and phospholipids. This may be due to impurities in the isomers added to the diets. These data show that both isomers of CLA were incorporated into in all four of the lipid classes investigated; however, the amounts incorporated differed between the two isomers and among the different lipid classes. The relative proportions of *t*10,*c*12-CLA incorporated into all lipid classes except the phospholipids were much smaller than the corresponding proportion of *c*9,*t*11-CLA.

*c*9,*t*11-CLA had only modest effects on the FA profiles of all the lipid fractions when compared with the corresponding values in animals fed the control diet (Table 2). The weight percentages of 18:1n-9 and 18:1n-7 were significantly decreased in the TG fraction, and that of 18:2n-6 was increased in TG, phospholipids, and cholesterol esters in the group fed the *c*9,*t*11-CLA diet. Changes in the proportions of these three FA in other lipid fractions did not attain statistical significance. The sum of monounsaturated FA (MUFA) was significantly decreased in TG, and that of PUFA was increased in the TG, cholesterol ester, and FFA fractions but was unchanged in the phospholipid fraction by the feeding of *c*9,*t*11-CLA compared with the corresponding values in the control group. The weight percentages of all other

TABLE 2
FA Composition (wt%) of Liver TG, Phospholipids, Cholesterol Esters, and FFA of Mice Fed Control or Experimental Diets[a]

	TG			Phospholipids			Cholesterol esters			FFA		
FAME	Control	*c9,t11*	*t10,c12*	Control	*c9,t11*	*t10,c12*	Control	*c9,t11*	*t10,c12*	Control	*c9,t11*	*t10,c12*
16:0	23.3 ± 0.6	24.0 ± 0.5	25.4 ± 0.7*	16.3 ± 0.5	16.3 ± 0.6	16.1 ± 0.5	22.8 ± 2.3	22.9 ± 2.5	16.0 ± 0.6*	33.7 ± 2.2	32.4 ± 1.4	29.1 ± 1.6
16:1n-7	4.9 ± 0.4	4.7 ± 0.5	4.9 ± 0.3	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	8.2 ± 0.4	7.5 ± 0.3	8.7 ± 0.2	1.8 ± 0.2	1.4 ± 0.3	1.9 ± 0.2
18:0	2.3 ± 0.1	2.3 ± 0.2	2.1 ± 0.1	18.3 ± 0.5	18.9 ± 0.5	17.7 ± 0.2	4.4 ± 0.1	5.6 ± 0.6*	3.0 ± 0.1*	16.2 ± 1.6	16.9 ± 2.3	10.7 ± 1.9
18:1n-9	46.6 ± 0.9	40.1 ± 1.8*	52.1 ± 0.7*	9.4 ± 0.4	8.3 ± 0.4	12.5 ± 0.2*	41.0 ± 2.4	39.8 ± 3.0	56.1 ± 1.0*	25.1 ± 1.6	21.6 ± 2.7	37.9 ± 3.3*
18:1n-7	3.9 ± 0.4	2.8 ± 0.3*	4.1 ± 0.4	1.9 ± 0.2	1.8 ± 0.1	2.2 ± 0.1	2.2 ± 0.2	2.0 ± 0.2	2.8 ± 0.0*	2.0 ± 0.3	1.5 ± 0.3	3.0 ± 0.4
18:2n-6	13.2 ± 1.2	17.5 ± 1.9*	6.7 ± 0.2*	12.0 ± 0.1	13.9 ± 0.7*	10.5 ± 0.2*	6.6 ± 0.5	9.2 ± 1.1*	4.5 ± 0.2*	5.4 ± 1.2	7.6 ± 1.8	4.5 ± 0.4
c9,t11-CLA	0.0 ± 0.0	1.3 ± 0.8*	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0*	0.1 ± 0.1*	0.0 ± 0.0	3.1 ± 0.6*	0.2 ± 0.0*	0.0 ± 0.0	1.2 ± 0.3*	0.0 ± 0.0
t10,c12-CLA	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1*
20:4n-6	0.8 ± 0.1	0.9 ± 0.2	0.2 ± 0.0*	25.0 ± 0.3	23.7 ± 0.4*	24.1 ± 0.3	2.9 ± 0.2	3.1 ± 0.5	1.6 ± 0.1	1.0 ± 0.2	2.6 ± 0.4	3.2 ± 1.0*
22:6n-3	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	8.2 ± 0.4	7.8 ± 0.6	5.9 ± 0.1*	0.5 ± 0.0	0.6 ± 0.1	0.3 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.7 ± 0.0
Sum SFA	26.7 ± 0.6	27.6 ± 0.5	28.4 ± 0.7	35.0 ± 05	35.9 ± 0.7	34.2 ± 0.5	28.1 ± 2.3	30.8 ± 2.7	19.7 ± 0.8*	54.3 ± 2.1	55.1 ± 3.8	41.3 ± 3.2*
Sum MUFA	56.1 ± 1.3	48.7 ± 2.3*	62.4 ± 0.9*	12.7 ± 0.6	11.8 ± 0.4	16.0 ± 0.2*	52.5 ± 2.8	50.0 ± 3.1	69.3 ± 2.2*	29.7 ± 1.9	25.1 ± 3.3	44.2 ± 3.9*
Sum PUFA	15.6 ± 0.7	21.2 ± 2.8*	7.6 ± 0.2*	51.2 ± 0.3	50.5 ± 1.0	47.8 ± 0.5*	10.1 ± 0.5	16.1 ± 1.9*	6.8 ± 0.3	6.3 ± 1.1	11.3 ± 1.7*	7.8 ± 0.8

[a]Data are mean ± SEM ($n = 6$). Numbers marked with an asterisk are significantly different ($P < 0.05$) from corresponding numbers in the control group within the same lipid class. SFA, saturated FA; MUFA, monounsaturated FA.

individual FA and the sum of saturated FA (SFA) did not differ significantly between the control and the *c*9,*t*11-CLA groups.

Feeding the diet containing *t*10,*c*12-CLA was linked with significant increases in the relative proportions of 18:1n-9 and decreases in the proportions of 18:2n-6 in all lipid fractions when compared with the corresponding values in the control group (Table 2). It was associated with significant reductions in the weight percentages of 16:0 and 18:0 in the cholesterol ester fractions and a significant increase in the proportion of 16:0 in the TG fraction. It was also associated with significant reductions in the weight percentage of DHA in phospholipids, arachidonic acid (AA) in TG, and an increase in the proportion of AA in FFA. The proportion of total MUFA increased and that of PUFA decreased in all lipid fractions except for FFA, where total PUFA did not change; the proportion of total SFA decreased in the FFA and cholesterol ester fractions. Thus, the two CLA isomers differed not only in their incorporation into different lipid fractions but also in the changes they caused in the composition of other FA.

DISCUSSION

We compared the effects of two purified isomers of dietary CLA (*c*9,*t*11-CLA and *t*10,*c*12-CLA) on the weights and FA profiles of murine liver TG, phospholipids, cholesterol esters, and FFA with the corresponding fractions in animals fed diets without CLA; we also compared the incorporation of the two CLA isomers into different lipid fractions. Total liver lipids and their distribution among different fractions did not differ between the control and *c*9,*t*11-CLA groups (Table 1). The livers of animals fed diets supplemented with *t*10,*c*12-CLA contained four times more total lipids than the amount found in the livers of animals fed the control diet. Our results showing an increase in liver lipids by feeding *t*10,*c*12-CLA are consistent with those previously reported with a mixture of CLA isomers (0.5–1.5% fed for 6 wk, Ref. 20) or with *t*10, *c*12-CLA (0.4 or 1.0% fed for 4 wk; 25,26) in mice; the increase in liver lipids with the mixture of CLA isomers ranged from 75 to 150% (20), whereas the increase with *t*10,*c*12-CLA (25) was similar to that found in our study. Feeding a mixture of CLA isomers (0.5% for 6 wk) caused a twofold increase in liver lipids in chickens (12), but it did not increase liver lipids in rats fed at 0.5 to 1.5% for 4–6 wk (22,23). Similarly, feeding a mixture of CLA isomers or the purified *c*9,*t*11-CLA or *t*10,*c*12-CLA (0.66% for 8 wk) did not increase liver lipids in hamsters (21). Still others reported a reduction in liver lipids with the feeding of a CLA mixture in fish (19) and chickens (13). These differences may be due to a number factors, including the species, age of the animal, amount and type of CLA, the duration of its feeding, and the composition of the basal diet.

The increase in liver lipids caused by *t*10,*c*12-CLA in our study was largely due to an increase in the weights of liver TG, although cholesterol, cholesterol esters, and FFA also were significantly elevated. These changes in liver lipids most likely resulted from an altered secretion of leptin and insulin. Published reports have indicated that feeding a mixture of CLA isomers

reduces the concentration of circulating leptin and increases that of insulin in mice, rats, and humans (25,34–37). Other reports have indicated that *t*10,*c*12-CLA reduces the mRNA for leptin and adiponectin in the adipose tissue (24) and increases that of sterol regulatory element-binding protein-1 (SREBP-1) in liver (25). Furthermore, leptin infusion was found to reverse the CLA-caused hyperinsulinemia and fat deposition in the liver (34). Together, these results suggest that *t*10,*c*12-CLA reduced the production of leptin by the adipose tissue, which led to increased production of insulin by the pancreas and insulin-activated SREBP-1 in the liver. An increase in SREBP-1 increases both cholesterol and FA synthesis in the liver (25). Thus, the increase in the liver lipid contents of animals fed diets containing *t*10, *c*12-CLA was most likely due to increased lipid synthesis in the liver; however, a reduction in hepatic lipid secretion also may have contributed to this result. The proposed mechanisms are based on the interaction between several organs in the body, and the response of the liver to CLA may be secondary to the response from other organs. In *in vitro* transactivation assays, both CLA isomers were equally effective in activating peroxisome proliferator-activated receptors (PPAR) α, β, and γ (25; Kelley, D.S., and Lee, J.Y., unpublished results). Thus, PPAR activation by *t*10,*c*12-CLA does not seem to be the mechanism by which liver lipids were increased.

The two CLA isomers differed in the proportion of their incorporation into the different lipid fractions and their effects on the FA profiles of the lipid fractions. Overall, incorporation of *c*9,*t*11-CLA was much higher than that of *t*10,*c*12-CLA, and the highest weight percentage of *c*9,*t*11-CLA was found in the cholesterol esters. These results are consistent with those reported in rats (29).

Feeding *c*9,*t*11-CLA in our study was associated with a significant reduction in the weight percentage of 18:1n-9 and an increase in 18:2n-6 (Table 2). A reduction in the proportion of 18:1n-9 may be due to a decrease in the activity of Δ9-desaturase; an increase in the proportion of 18:2n-6 may be due to a reduction in the activity of Δ6-desaturase or other enzymes involved in the metabolism of this FA. Our results in mice showing changes in the proportions of 18:1n-9 and 18:2n-6 contrast with those showing no change in the FA composition of liver lipids isolated from rats fed diets containing *c*9,*t*11-CLA (29,30). These differences may be due to variance in the lipid metabolism between rats and mice.

In contrast to *c*9,*t*11-CLA, feeding the *c*10,*t*12-CLA isomer was associated with an increase in the weight percentage of 18:1n-9, possibly due to an increased activity of Δ9-desaturase (stearoyl CoA desaturase). Pariza's group has published several papers regarding the effects of CLA on the expression and/or activity of this enzyme. They reported a reduction in the mRNA for stearoyl CoA desaturase-1 in the livers of mice fed a diet containing a mixture of CLA isomers (0.5% for 2 wk) (38), and in 3T3-L1 adipocytes *in vitro* by feeding *t*10, *c*12-CLA (25 to 100 μM) but not by feeding *c*9,*t*11-CLA (39). Subsequently, they reported a reduction in the expression or activity of this enzyme by both CLA isomers (45 μM) in two human breast tumor cell lines (40). Their results suggest that, depending on the model used, either *t*10,*c*12-CLA or both the CLA isomers may inhibit the expression or activity of this enzyme. Other reports have indicated that stearoyl CoA desaturase activity is positively associated with hypertriglyceridemia in humans and mice (41), and that disruption of the gene for this enzyme impairs the biosynthesis of cholesterol esters and TG in mice liver (42). Our results concur with the above-mentioned findings that CLA isomers may alter the activity of stearoyl CoA desaturase. However, our results contrast with those from Pariza's laboratory: Our results suggest a stimulation of this enzyme by *t*10,*c*12-CLA and not inhibition, as reported by this group. Since they used a mixture of CLA isomers in their study with mice, it is possible that a reduction in the liver mRNA for this enzyme was caused by *c*9,*t*11-CLA; this would be consistent with our results regarding the effects of this isomer on the liver FA composition. Furthermore, our interpretation of the activity of the enzyme is based on the FA composition, whereas they measured the mRNA for this enzyme; neither measured enzyme activity.

Feeding *t*10,*c*12-CLA was associated with reductions in the weight percentage of 18:2n-6 in all lipid fractions and that of 22:6n-3 in only the phospholipids. A reduction in the proportion of 18:2n-6 may be due to increased activity of Δ6-desaturase or other enzymes involved in the metabolism of this FA. A reduction in 22:6n-3 may be due to decreased activity of Δ5-desaturase or elongases, or to increased activity of enzymes involved in the metabolism of DHA. The reduction in the proportion of 18:2n-6 in the *t*10,*c*12-CLA group in our study is consistent with that reported after feeding a mixture of CLA isomers to mice (20) or rats (23). Our study extends these findings by showing that *t*10,*c*12-CLA is the isomer that caused a reduction in the weight percentage of 18:2n-6; *c*9,*t*11-CLA actually caused an increase in the weight percentage of this FA. Thus, feeding a mixture of the two isomers may increase, decrease, or have no effect on the proportion of 18:2n-6, depending on the ratio between the isomers. A reduction in the weight percentage of DHA in the phospholipids of animals fed *t*10,*c*12-CLA in our study differs from the increase in this FA in rat liver phospholipids caused by the same isomer (29). We did not measure the activities of lipid-metabolizing enzymes. That the enzymes change is one logical explanation for the changes in FA concentrations. It is also possible that changes in the activities of other FA-metabolizing enzymes may have contributed to the altered concentrations of the FA seen in our study.

In summary, our results show that only the *t*10,*c*12-CLA altered the weights of liver lipids, whereas both isomers altered the FA profiles of the lipid fractions. The two isomers differed in the amounts incorporated into liver lipids and the changes they caused in the FA profiles of the lipid fractions. It is important to note that *c*9,*t*11-CLA did not alter the total lipids but altered the FA composition, whereas the *t*10,*c*12-CLA altered both the total lipids and the FA profile.

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