Trans-10,*cis*-12 CLA Increases Liver and Decreases Adipose Tissue Lipids in Mice: Possible Roles of Specific Lipid Metabolism Genes

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ABSTRACT: Although consumption of CLA mixtures has been associated with several health effects, less is known about the actions of specific CLA isomers. There is evidence that the *t*10,*c*12-CLA isomer is associated with alterations in body and organ weights in animals fed CLA, but the mechanisms leading to these changes are unclear. The purpose of this study was to determine the effects of two commonly occurring isomers of CLA on body composition and the transcription of genes associated with lipid metabolism. Eight-week-old female mice (n = 11 or 12/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 or 0.2% of the peroxisome proliferator-activated receptor α (PPARα) agonist fenofibrate for 8 wk. Body and retroperitoneal adipose tissue weights were significantly lower (6–10 and 50%, respectively), and liver weights were significantly greater (100%) in the *t*10,*c*12-CLA and the fenofibrate groups compared with those in the control group; body and tissue weights in the *c*9,*t*11-CLA group did not differ from those in the control group. Livers from animals in the *t*10,*c*12-CLA group contained five times more lipids than in the control group, whereas the lipid content of the fenofibrate group did not differ from that in the control group. Although fenofibrate increased the mRNA for PPARα, *t*10,*c*12-CLA decreased it. These results suggest that PPARα did not mediate the effects of *t*10,*c*12-CLA on body composition. The CLA isomers and fenofibrate altered mRNA levels for several proteins involved in lipid metabolism, but the most striking difference was the reduction of mRNA for leptin and adiponectin in the *t*10,*c*12-CLA group. These initial results suggest that changes associated with energy homeostasis and insulin action may mediate the effects of *t*10,*c*12-CLA on lipid metabolism.

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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). Most of the published studies have used a mixture of CLA isomers, which comprised 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. 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).

Feeding a mixture of CLA isomers to animal models has been reported to alter chemically induced carcinogenesis, atherogenesis, diabetes, body composition, and immune cell functions (3). Since a mixture of CLA isomers has been used in most of the studies, it is not known which of the CLA isomers is responsible for its effects listed above. Studies conducted with purified isomers show that *c*9,*t*11-CLA and *t*10,*c*12-CLA did not differ in their effects on immune cell functions in mice (4), but other studies have reported that the isomer responsible for reducing body and adipose tissue weights in mice (5) and hamsters (6) and for altering mammary lipid metabolism in dairy cows (7) is the *t*10,*c*12-CLA isomer. Supplementing diets of mice with a mixture of CLA isomers also causes an increase in liver and spleen masses and a several-fold increase in the amount of hepatic lipids (8,9). Which isomer causes the increase in liver lipids is not known. The liver lipids of mice fed purified isomers of CLA were not examined (5), but in hamsters fed similarly liver lipids did not change (6). Furthermore, the mechanisms by which individual CLA isomers may alter liver and adipose tissue fat contents are poorly understood.

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 body weight, weight of liver and its lipid content, adipose tissue weight, and possible means by which the CLA isomers may alter body composition in mice. One approach was to compare CLA effects to those of fenofibrate, a known peroxisome proliferator-activated receptor α (PPARα) agonist; the other approach was to examine the mRNA for a number of proteins that can be involved in lipid metabolism. The mRNA levels examined included acyl CoA oxidase (ACO), a rate-limiting enzyme for β oxidation; lipoprotein lipase (LPL), an enzyme that removes FFA from TG-rich lipoproteins; apolipoprotein C-3 (apoC-3), an inhibitor of LPL; microsomal cytochrome P450A1 (CYP450A1), a catalyst of ω hydroxylation of FA; mitochondrial uncoupling protein-2

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Abbreviations: ACO, acyl-CoA oxidase; Acrp30, complement-related protein 30, (Adiponectin); apoA-I, apolipoprotein A-I; apoC-3, apolipoprotein C-3; CYP4A1, microsomal cytochrome P450 4A1; PPAR, peroxisome proliferator-activated receptor; UCP, mitochondrial uncoupling proteins.

(UCP2), a protein that uncouples ATP synthesis from oxygen consumption and results in the production of heat; apolipoprotein A-1 (apoA-1), the primary protein constituent of HDL; PPARα, a transcription factor that controls synthesis and catabolism of TG-rich lipoproteins; and two adipose tissue hormones (leptin and adiponectin) that play an important role in regulation of lipid metabolism. Our results show that the two isomers differed in their effects on liver and retroperitoneal adipose tissue lipids, and in their effects on the adipocyte-associated hormones, leptin and adiponectin.

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 these isomers were provided by the supplier and confirmed in our laboratory. The preparation enriched in *c*9,*t*11-CLA contained 84.6% *c*9,*t*11-CLA; 7.7% *t*10,*c*12-CLA; 3.8% 18:1 *c*9; 2.0% *t*9,*t*11-CLA + *t*10,*t*12-CLA; and 1.9% other FA. The preparation enriched in *t*10,*c*12-CLA contained 88.1% *t*10,*c*12-CLA; 6.6% *c*9,*t*11-CLA; 2.5% *t*9,*t*11-CLA + *t*10,*t*12-CLA; 1.1% 18:1 *c*9; and 1.7% other FA. Fenofibrate was purchased from Sigma Chemical Co. (St. Louis, MO).

The concentration of CLA used in this study was 0.5 weight % (wt%) of the diet, which is comparable to the concentrations used in previous studies in rodent models, which ranged from 0.1 to 1.5 wt% of a mixture of CLA isomers. AIN-93G, the high-carbohydrate mouse diet, was used as the basal diet. The nutrient and FA composition of this diet has been previously reported (4). 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. For the fenofibrate diet 2.0 g/kg of this compound replaced an equivalent amount of cellulose. Fenofibrate and the CLA isomers were first mixed with the corn oil and then mixed with the remainder of the dietary components. 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. Extraction of dietary lipids and FA analysis were performed according to previously published methods (10).

Animals, feeding, and tissue collection. Forty-six 8-wk-old, pathogen free C57BL/6N female mice were purchased from Charles River (Raleigh, NC). Female mice were chosen because of their docility for housing in groups. 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 a light and dark cycle (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 four groups at the start of experimental diets (study day 1), 11/group for the control and fenofibrate diets, and 12/group for the two CLA diets. They were housed 5 or 6/cage and were offered fresh diets every day (5 g/animal/d). This is based on our previous experience regarding the amount of food consumed by mice in the age range used in this study (11). All of the food was consumed within 4–6 h after it was offered, in all groups. This regime avoided the need for paired feeding and oxidation of the CLA if left for too long at room temperature. Animals were weighed on study days 10, 25, 35, 42, 49, and 56. All conditions and handling of the animals were approved by the Animal Care and Use Committee at the University of California, Davis.

At the end of the study, mice were anesthetized by interperitoneal injection of xylazine (0.7 mg/mL), ketamine (9.4 mg/mL), and acepromazine (0.2 mg/mL). Blood was collected into EDTA-containing syringes by heart puncture. The mice were then killed by cervical dislocation; spleens, livers, hearts, soleus muscles, and retroperitoneal fat pads were removed, weighed, frozen in liquid nitrogen, and stored at –70°C. Animals were without food for 16 h prior to sacrifice.

Blood lipid analysis. Total cholesterol, HDL, LDL, and TG were analyzed by commercially available diagnostic kits (catalog nos. 1554506, 1930672, 1985604, and 450032, respectively; Roche Diagnostics, Indianapolis, IN) and performed in duplicate on a Roche/Hitachi 902 analyzer. Between-day percent CV for all these kits ranged from 1 to 3%.

cRNA probe generation and ribonuclease protection assay. To generate cRNA probes for most of the target genes, cDNA fragments were amplified from commercially available mouse heart or liver cDNA (Ambion, Austin, TX) using sense/antisense primers designed to generate T7 promoter sites at the 5′ end of the fragment. Three fragments (ACO, apoA-I, and apoC-3) were subcloned into a vector containing a T7 or T3 promoter site and linearized for probe production. All primers used to generate the cDNA target templates were designed using Lasergene PRIMERSELECT software (DNASTAR Inc., Madison, WI) based on previously sequenced fragments of target genes. The primers used are listed from 5′ to 3′, where the nucleotide numbers indicate the primer location in the corresponding sequences of *Mus musculus* origin obtained from the GenBank/EMBL database (Table 1).

The identity of each fragment was confirmed by ribonuclease digestion at a predicted restriction site. All of the PCR templates that were not cloned were transcribed from the integrated T7 promoter site with the exception of the 18S loading control template fragment (Ambion) and apoA-I fragments, which were transcribed from the T3 promoter sites. All ^{32}P-labeled cRNA probes were synthesized using the Maxiscript *in vitro* transcription system according to the manufacturer's instructions (Ambion) and purified by PAGE.

To determine the steady-state transcript levels of the target genes, RNase protection assays were performed using a commercially available RNase protection assay kit (RPA III; Ambion). The cRNA probes were hybridized to target total RNA

TABLE 1
Primers[a] Used to Generate cDNA Target Templates

mRNA examined (accession #)	5′ primer[b]	3′ primer[b]
Adiponectin (NM_009605)	GTACCGGTACCTCCTGCCC AGTCATGCCGAAGAT	TAATACGACTCACTATAGGAA GCTTGCCCCACACTGAACGCTGAG
Apolipoprotein A-I (NM_009692)	GATGAAAACAAGCTTGCT GGCCGTGGCTCTGGTC	TGTAAGAAAGGTACCGCGG GGGTGGGGAGTGGAAGC
Apolipoprotein C-3 (L04150)	GATGTATAGGGATCCTTG CTGCTGGGCTCTGTG	TATCTGGAGAAGCTTGG TCCTCAGGGTTAGAATCC
Cytochrome P450 (NM_007822)	GAAATATACTCGGGCCAC CCACCCTGAGCACCAA	TAATACGACTCACTATAGGAAAGCTT GCAGAAAGATGAGATGACAGGA
Leptin (NM_008493)	TACACGGTACCTATCCGC CAAGCAGAGGGTCACT	TAATACGACTCACTATAGGAAGCT TATCTGCAGCACATTTTGGGAAGG
Lipoprotein lipase (NM_008509)	GGGGGGTACCGGATTGTT GCCGCTGTTTTGTTTTAC	TAATACGACTCACTATAGGAAGC TTGCATGTGGGTTGGTGTTCAGA
Peroxisomal acyl-CoA oxidase (AB034914)	CACAGTGACTGAATTCAACGCT GTGGCTTTGGTGGATG	CTTCCCGAGGTCTAGAGTCGG CCTGGGCTACTACTGC
PPAR-α (X57638)	CTGGCCAACTTGGTACCGAA GAGGGCTGAGCGTAGGTAAT	TAATACGACTCACTATAGGAAGCTTA GGGTGGCAGGAAGGGAACAGAC
Uncoupling protein-2 (AF111999)	CTTACAGGCTAAGCTTCGGG CTGGTGGTGGTCGGAGATA	TAATACGACTCACTATAGGGGTTATAAGGAA TTCGGGGTGCAGGAAATGGGAAGAGAA

[a]Primers were designed using Lasergene PRIMERSELECT software (DNASTAR Inc., Madison, WI) based on previously sequenced fragments of target genes.
[b]Primers are listed from 5′ to 3′, where the nucleotide numbers indicate the primer location in the corresponding sequences of *Mus musculus* originally obtained from the GenBank/EMBL database.

samples and digested using the conditions suggested by the manufacturer. Protected fragments were electrophoresed on a 6% denaturing polyacrylamide gel, and the signals were analyzed on a Storm phosphoimager (Amersham Pharmacia; Buckinghamshire, England).

RNA preparation and RNA blot analysis. Total RNA was isolated from liver, retroperitoneal adipose, soleus muscle, and heart tissues using the Totally RNA kit (Ambion) according to the manufacturer's recommended procedures. Because the amount of retroperitoneal adipose tissue from the *t*10,*c*12-CLA and fenofibrate groups was limiting, we chose to pool tissue samples for molecular analysis. For RNA extraction from each tissue, a 30-mg tissue sample from each animal in the group was pooled (11/group in the control and fenofibrate groups, and 12/group in the *c*9,*t*11-CLA and *t*10,*c*12-CLA groups). Five to 15 µg of total RNA was electrophoresed on a one percent, 1.1 M formaldehyde agarose gel and then blotted onto a nylon membrane. Membranes were subsequently hybridized (ULTRAHyb; Ambion) overnight with ^{32}P-labeled cRNA probes prepared with the cRNA described above and the Strip-EZ RNA labeling kit (Ambion). After each hybridization, the RNA blots were stripped. Each blot was reprobed with a transcript-specific probe three to seven times. After all clone-specific hybridizations were completed, the blots were restripped and probed with a T7 primed ^{32}P-labeled 18S ribosomal RNA probe (Ambion).

After quantification with a phosphoimager, hybridization signals were corrected for background hybridization and total RNA loading. To correct for nonspecific background hybridization, the signal from a sample of the blot to which the probe did not specifically hybridize was subtracted from the signal generated by a specifically bound region of equal area. This adjustment prevented an underestimation of gene expression differences between samples when the transcript-specific signal was not high. Hybridization signals were analyzed and quantified by phosphoimagery, and target mRNA levels were calculated relative to the 18S ribosomal RNA levels. Each of the transcripts was analyzed by two or three RNA blots and by one to three ribonuclease protection assays; data shown are from representative gels.

Statistical analysis. The data on an individual mouse basis were subjected to one-way ANOVA between and within diets, using the SAS software (12). The one-tailed version of Dunnett's test was used to make comparisons of the test diet means with the control diet. Levene's test was used to examine the homogeneity of variance assumption.

RESULTS

CLA isomers and fenofibrate induce different changes in body composition. At the end of the study, mean ± SEM for body weights of the control, *c*9,*t*11-CLA, *t*10,*c*12-CLA, and fenofibrate groups were 25.4 ± 0.3, 26.7 ± 0.5, 23.2 ± 0.3, and 21.6 ± 0.2 g, respectively (Fig. 1). Body weight of the fenofibrate group was 10% less ($P < 0.005$) and that of the *t*10,*c*12-CLA groups was 6% less ($P < 0.02$) than that of the control group.

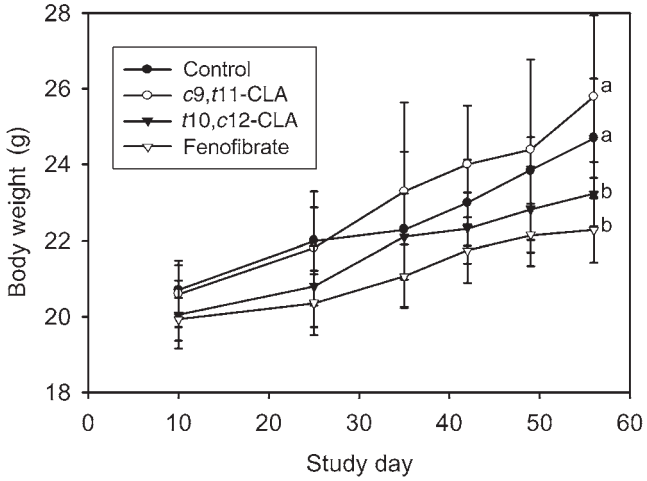

FIG. 1. Effect of CLA isomers and fenofibrate on body weights. Data are the mean ± SEM for 11 or 12 animals in each group. Means with different letters are significantly different ($P < 0.02$) using ANOVA.

Mean ± SEM for the liver weights in the control, *c*9,*t*11-CLA, *t*10,*c*12-CLA, and fenofibrate groups were 1.28 ± 0.03, 1.47 ± 0.06, 2.54 ± 0.07, and 2.52 ± 0.08 g, respectively (Fig. 2A). Mean liver weights in the fenofibrate and *t*10,*c*12-CLA groups were almost twice that of the mean liver weight in the control group ($P < 0.001$). Mean retroperitoneal adipose tissue weights in the control, *c*9,*t*11-CLA, *t*10,*c*12-CLA, and fenofibrate groups were 0.49 ± 0.04, 0.48 ± 0.05, 0.20 ± 0.01, and 0.26 ± 0.02 g, respectively (Fig. 2B). Thus, the retroperitoneal adipose tissue weights in the fenofibrate and *t*10,*c*12-CLA groups were about half of the corresponding value in the control group ($P = 0.001$). Body weight ($P = 0.18$), liver weight ($P = 0.11$), and adipose tissue weight ($P = 0.89$) for the *c*9,*t*11-CLA group did not differ from the corresponding values in the control group. Weights of hearts, soleus muscles, and spleens did not differ significantly among the four groups (data not shown).

Although the liver weights were increased in both the fenofibrate and *t*10,*c*12-CLA groups, this increase seemed to involve different mechanisms. In the *t*10,*c*12-CLA group, the liver lipids were fivefold greater than in the control group (mean ± SEM, 775 ± 119 vs. 147 ± 18 mg); liver lipids in the fenofibrate (234 ± 13 mg) and in the *c*9,*t*11-CLA groups (175 ± 13 mg) did not differ significantly from those in the control group (Fig. 3A). Lipids constituted 12% of the liver wet weights in the control and *c*9,*t*11-CLA groups; 30% in the

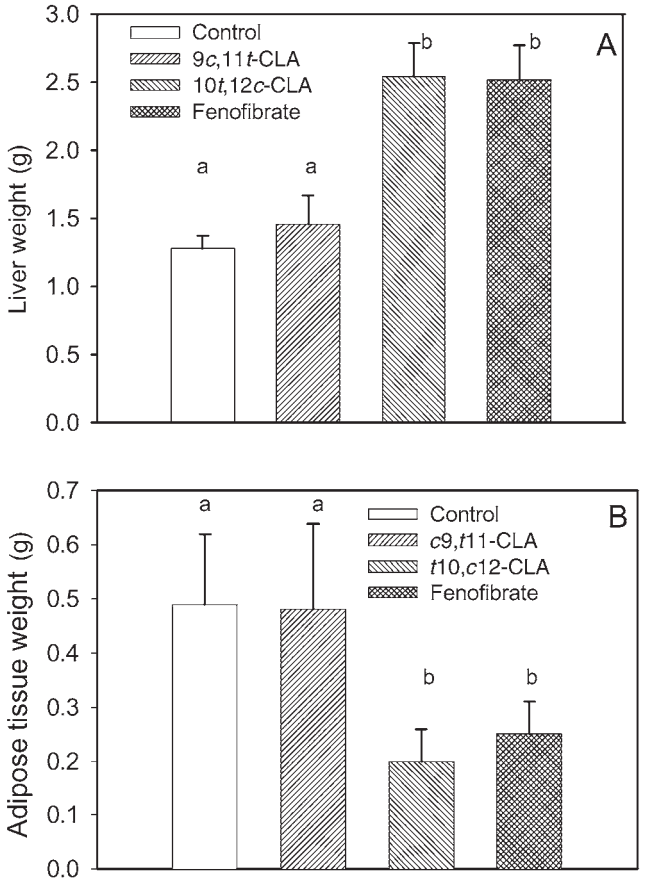

FIG. 2. Modulation of liver (A) and retroperitoneal adipose tissue (B) weights by dietary CLA isomers and fenofibrate. Data are the mean ± SEM for 11 or 12 animals in each group. Bars with different letters are significantly different ($P < 0.001$) using ANOVA.

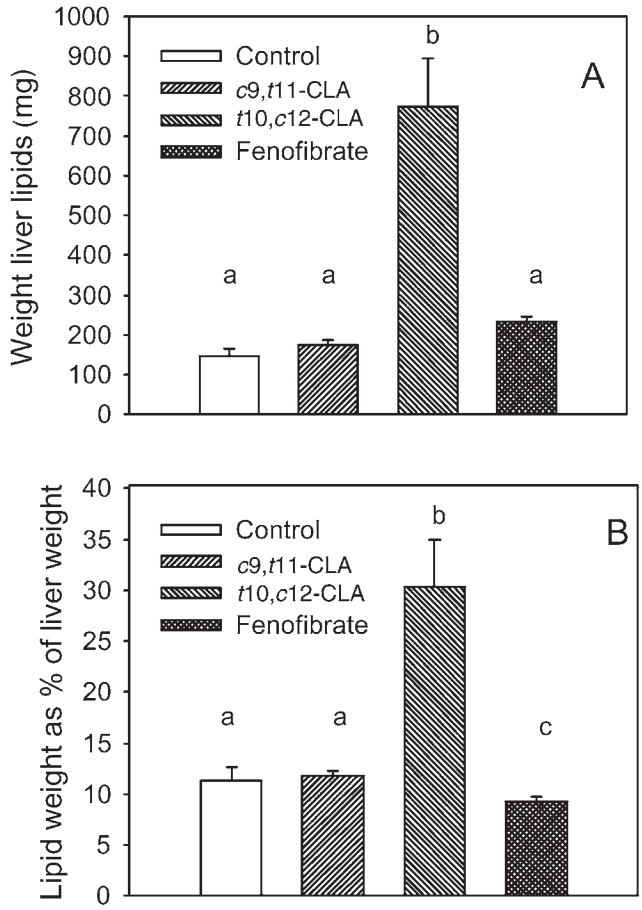

FIG. 3. Effect of CLA isomers and fenofibrate on liver lipids, as mg/liver (A) and as percentage of liver weight (B). Data are the mean ± SEM for 11 or 12 animals in each group. Bars with different letters are significantly different at $P < 0.0001$ (a vs. b) and $P < 0.0003$ (a vs. c) using ANOVA.

TABLE 2
Effect of feeding CLA Isomers and Fenofibrate on Serum TG; Total, LDL, and HDL Cholesterol (mg/dL)[a]

Diet	TG	Cholesterol	LDL	HDL
Control	19.6 ± 2.5	68.0 ± 7.4	7.8 ± 0.9	55.0 ± 6.0
*c*9,*t*11-CLA	20.3 ± 1.7	66.8 ± 4.8	8.3 ± 0.6	52.9 ± 4.2
*t*10,*c*12-CLA	17.8 ± 1.8	82.8 ± 5.0	9.9 ± 0.8	66.1 ± 3.7
Fenofibrate	15.8 ± 3.1	71.3 ± 4.2	10.1 ± 0.7	58.3 ± 3.1

[a]Mean value ± SEM, n = 10–12/group. Values within columns were not significantly different at $P \leq 0.05$ using ANOVA.

*t*10,*c*12-CLA group, and only 9% in the fenofibrate group (Fig. 3B, $P < 0.0003$ for both groups compared to the control group). Concentrations of serum TG, cholesterol, LDL, and HDL did not differ between the control and the three experimental groups (Table 2).

CLA isomers and fenofibrate result in differential steady-state transcript levels of mRNA for proteins associated with lipid metabolism and energy homeostasis. After 8 wk, the level of the apoC-3 transcript in liver was consistently reduced to an average of 47% of control in the animals fed fenofibrate. No differences in apoC-3 mRNA were detected for both the CLA isomers. No differences in the liver mRNA levels for the apoA-1 were observed between the four groups. Levels of the ACO transcript from liver and heart of animals fed the fenofibrate diet were increased (Figs. 4,5). The average increases of these transcripts were ninefold (SD = 1.4) and sixfold (SD = 0.4) over the control, respectively. ACO was also increased in the liver of animals fed *c*9,*t*11-CLA and *t*10,*c*12-CLA. In these tissues the transcript was increased 1.3-fold (SD = 0.2) and 1.2-fold (SD = 0.1) over the control value (Fig. 4). CYP4A1 mRNA was elevated in liver and adipose tissue when animals were fed fenofibrate (Figs. 4,6). The average increases in these tissues were eight (SD = 9.1) and 2.6 (SD = 0.9) times the control value, respectively. LPL was expressed fivefold more in the livers of mice fed the fenofibrate diet than that in the livers of mice fed control diet (Fig. 4). The CLA isomers had no effect on LPL mRNA levels. Interestingly, the PPAR-α transcript was increased over the control value in the *c*9,*t*11-CLA and fenofibrate conditions but suppressed in the *t*10,*c*12-CLA condition. The average differences were 1.6-fold (SD = 0.6), 2-fold (SD = 0.7), and 0.7-fold (SD = 0.2), respectively. The transcript level of UCP-2 in heart and soleus muscle (Figs. 5,7) and liver and adipose (data not shown) was not affected by any treatment.

Transcript level for leptin in the retroperitoneal adipose tissue of the mice fed *t*10,*c*12-CLA was reduced to 18% (SD = 0.06) of that in the control group (Fig. 6). Furthermore, adiponectin transcript levels were reduced in retroperitoneal adipose tissue, soleus muscle, and hearts of animals fed the *t*10,*c12*-CLA isomer when compared to the corresponding expression in the control group (Figs. 5–7). These reductions were 11 (SD = 0.07), 27 (SD = 0.07), and 38% (SD = 0.17) of the control values, respectively. In the control group, adiponectin mRNA levels in the heart and soleus muscle were 15 and 12% of the levels found in retroperitoneal adipose tissue (data not shown). *c*9,*t*11-CLA and fenofibrate did not alter the expression of adiponectin in these three tissues. In liver, the adiponectin transcript level was too low to be accurately assessed (data not shown).

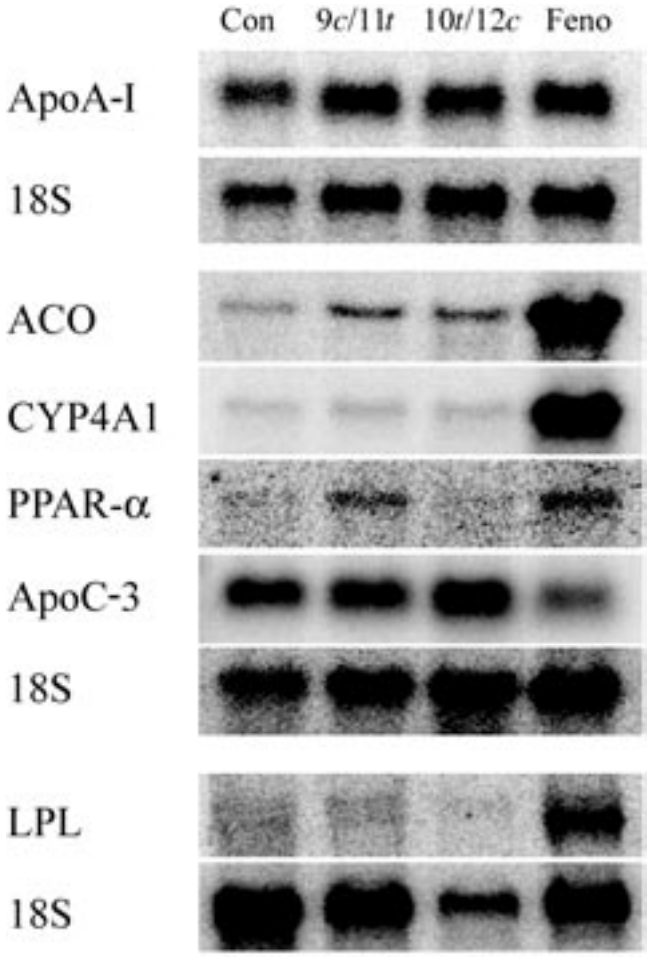

FIG. 4. Representative RNA blot analyses of lipid-metabolizing genes in liver from mice fed control (Con), 0.5% *c*9,*t*11-CLA (9*c*/11*t*), 0.5% *t*10,*c*12-CLA (10*t*/12*c*), or 0.2% fenofibrate (Feno) diets for 8 wk. The 18S loading control for individual blots is shown below the associated experimental panels above it. ApoA-1, apolipoprotein A-1; ACO, acyl-CoA oxidase; CYP4A1, cytochrome P450A1; PPAR-α, peroxisome proliferator-activated receptor α; ApoC-3, apolipoprotein C-3; LPL, lipoprotein lipase.

DISCUSSION

We compared the effects of feeding two purified isomers of CLA (*c*9,*t*11-CLA, and *t*10,*c*12-CLA) on body weights, liver and retroperitoneal adipose tissue lipids, and steady-state transcription of lipid-metabolizing enzymes that may mediate effects of CLA. Comparisons were made to the corresponding values found in mice fed a control diet and also in animals fed a known lipid-lowering agent, fenofibrate. Our results show that the two CLA isomers differed in their effects on body and organ weight as well as liver lipids. The *t*10,*c*12-CLA reduced the body and retroperitoneal adipose tissue weights significantly, compared with those found in the

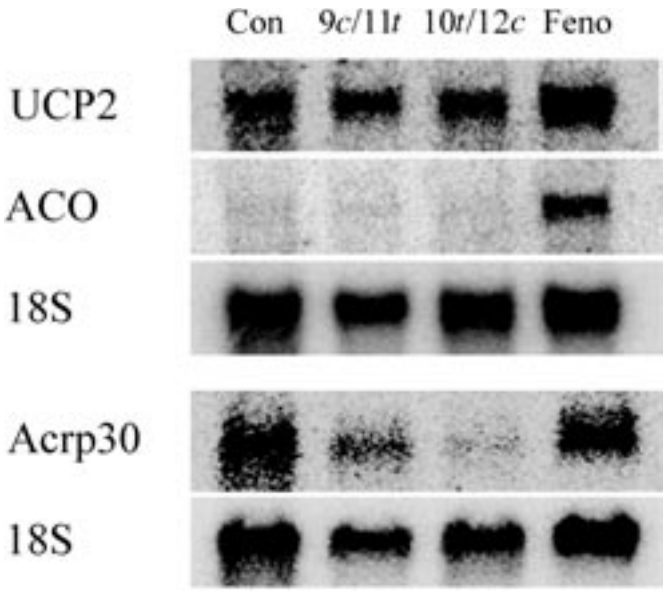

FIG. 5. Representative RNA blot analysis in heart from mice fed control, CLA, or fenofibrate diets. UCP2, mitochondrial uncoupling proteins; Acrp 30, complement-related protein 30 (= adiponectin); for other abbreviations see Figure 4. The 18S loading control for individual blots is shown below the associated experimental plots.

control group, but it caused a twofold increase in liver weights, which was largely due to an increase in liver lipids. The *c*9,*t*11-CLA did not alter any of these response variables. Our results regarding the effects of *t*10,*c*12-CLA on liver lipids are consistent with those previously reported with a mixture of CLA isomers (8,9). The increase in liver lipids in these studies was most likely due to *t*10,*c*12-CLA, because in

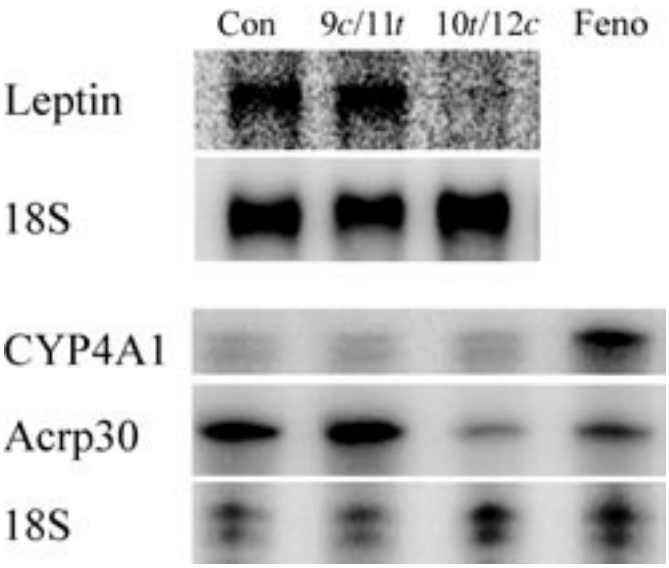

FIG. 6. Representative RNA blots for leptin and ribonuclease protection assay (RPA) for CYP4A1 and adiponectin (Acrp30) in adipose tissues from mice fed control, CLA, or fenofibrate diets for 8 wk. The 18S loading control for each experiment is shown below the associated experimental panel above it. The doublet observed in the 18S loading control for the RPA experiment is due to small differences in probe length due to radiolysis of this small probe. For abbreviations see Figures 4 and 5.

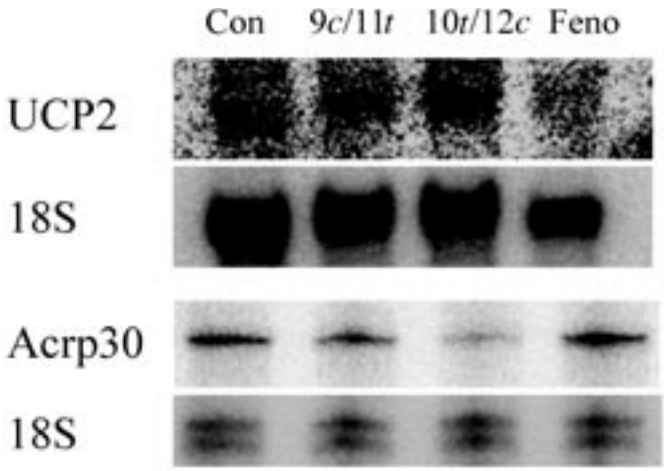

FIG. 7. Representative RNA blots of UCP2 and RPA (Acrp30) for muscle tissues from mice fed control, CLA-, or fenofibrate-containing diets. The 18S loading control for each experiment is shown below the experimental panel. The doublet observed in the 18S loading control for the RPA experiment is due to small differences in probe length due to radiolysis of this small probe. For abbreviations see Figures 4–6.

our current study *c*9,*t*11-CLA did not cause an increase in liver lipids. Our results regarding the effects of *t*10,*c*12-CLA on mice liver lipids differ from those reported with hamster liver lipids, where it did not alter the concentration of liver lipids (6). We are not certain of the reasons for this discrepancy, but hamster has been reported to more resistant to CLA than other species (13,14).

The changes in body, liver, and adipose tissue weights caused by *t*10,*c*12-CLA were similar to those caused by fenofibrate, a known PPARα agonist; however, different mechanisms may have mediated the effects of these two agents. Reduction in adipose tissue lipids in the *t*10,*c*12-CLA group was at the cost of storing lipids in the liver (five times more than in the control group), while the concentration of liver lipids in the fenofibrate group did not differ from those in the control group. Furthermore, *t*10,*c*12-CLA decreased the transcription of PPARα, whereas fenofibrate increased it. These results suggest that PPARα did not mediate the effects of *t*10,*c*12-CLA on body composition. This interpretation is consistent with the results from a study with the PPARα knock-out mice, where the effects of a mixture of CLA isomers on body and organ weights were similar to those found in the wild type (15).

Decreased transcription of leptin and of adiponectin in adipose tissue in the *t*10,*c*12-CLA group may have mediated its effects on liver and adipose tissue lipids. Leptin administration in animals has been reported to favor lipolysis and inhibit lipogenesis (16); the converse would be anticipated with a reduction in leptin levels. Adiponectin reduces hepatic glucose production (17); therefore, a reduction in adiponectin could increase hepatic glucose production that may be used for FA synthesis. Thus, reductions in leptin and adiponectin transcripts may account for the effects of *t*10,*c*12-CLA on liver and adipose lipids. These findings need to be corroborated by

investigating the changes in the proteins for these hormones. A reduction in the transport of lipids from liver to storage depots also could contribute to increased liver lipids. A reduction in adiposity is usually accompanied by a reduction in plasma leptin and increase in plasma adiponectin levels (18). However, in our study the mRNA for these molecules were reduced in mice fed *t*10,*c*12-CLA. These results suggest that adiponectin levels may be regulated by mechanisms not directly coupled to adiposity. This hypothesis can be supported by the work of Matsuzawa and colleagues (19) who have shown that although hypoadiponectemia is correlated with development of atherosclerosis, this effect is independent of well-known cardiovascular disease risk factors, such as body mass index.

Neither of the CLA isomers altered the mRNA for CYP4A1, LPL, or apoC-3. This is in contrast to a report indicating that *t*10,*c*12-CLA feeding decreased expression and activity of LPL in adipose and mammary lipids in dairy cows (20). Our results indicate that these genes may not be involved in mediating the effects of *t*10,*c*12-CLA on lipid metabolism. However, each of these PPARα-mediated transcripts was altered after fenofibrate treatment. These mRNA alterations are similar to what others have found with fibrate feeding (21,22). Both CLA isomers increased the transcripts for ACO, as previously reported by others feeding a mixture of CLA isomers (15). Since the ACO transcription was increased by both CLA isomers, it could not be responsible for the effects of *t*10,*c*12-CLA on liver and adipose tissue lipids.

The effects of *c*9,*t*11-CLA, and *t*10,*c*12-CLA on body composition differ from their effects on immune cell functions as previously reported (4). Both isomers increased secretion of inflammatory cytokines tumor necrosis factor α and interleukin 6 (IL-6) and decreased secretion of the T helper 2 cytokine IL-4; many other indices of immune status were not affected by either isomer. Comparison of the effects of CLA isomers on immune cell functions and body composition suggests that separate mechanisms may mediate the effects of CLA on immune cell functions and body composition.

In conclusion, results of our study show that the two isomers of CLA differed in their effects on body composition: *c*9,*t*11-CLA did not alter any of the response variables tested, whereas *t*10,*c*12-CLA reduced body and retroperitoneal adipose tissue weights, and increased liver weight and lipids. These effects of *t*10,*c*12-CLA were most likely the result of decreased transcription of leptin and adiponectin. Reduction of adipose tissue fat by *t*10,*c*12-CLA in mice and hamsters may be viewed as a beneficial effect; however, such benefits to humans are questionable. Supplementing diets of human volunteers with this isomer (3.4 g/d for 12 wk) did not change body weight or fat mass; however, it increased insulin resistance, fasting glucose, serum VLDL and C reactive protein, and lipid peroxidation, and decreased serum HDL and leptin (23–26). These metabolic changes indicate increased risk for diabetes and cardiovascular disease. It is therefore important to establish the risk/benefit ratio of this isomer before human use can be recommended.

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