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Functional Comparison of Esterified and Free Forms of Conjugated Linoleic Acid in High-Fat-Diet-Induced Obese C57BL/6J Mice

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This study investigated the effects of dietary conjugated linoleic acid (CLA), in the form of free fatty acid (FFA-CLA) or triacylglycerol (TG-CLA), on serum and liver lipid composition and gene expression associated with lipogenesis and β-oxidation in high-fat-diet (HFD)-induced obese C57BL/6J mice. Animals were fed a control diet, HFD, HFD supplemented with 2% FFA-CLA, or HFD supplemented with 2% TG-CLA for 8 weeks. Supplementation with both forms of CLA significantly reduced the weights of whole body and adipose tissue and was positively associated with significant liver enlargement. Both forms of CLA significantly decreased serum TG concentration, but had no effect on total cholesterol levels, which were increased in mice fed HFD. There was a prominent increase in serum alanine aminotransferase (ALT) levels in mice that received either form of CLA. TG accumulation and lipogenic gene expression, including the expression of genes for fatty acid synthase (FAS), acetyl-coenzyme A carboxylase (ACC), and malic enzyme, were significantly lower in the livers of mice that received TG-CLA as compared to FFA-CLA. The gene expressions of sterol regulatory element binding protein-1c (SREBP-1c) in both liver and adipose tissue were suppressed in mice that were fed either form of CLA as compared to the mice fed HFD alone, whereas there were no increases in the levels of expression of β-oxidation-related genes. These findings demonstrated that free and esterified forms of CLA have differing effects on liver and adipose tissue lipogenesis.

KEYWORDS: Conjugated linoleic acids; lipogenesis; β-oxidation; liver enlargement; gene expression

INTRODUCTION

Conjugated linoleic acid (CLA) is a family of positional and geometric isomers of linoleic acid (LA) and is mainly found in foods such as ruminant meat, dairy products, and milk (*1*,*2*). Animal studies have elucidated a wide range of physiological activities of CLA, including antioxidative, anticarcinogenic, antiatherosclerotic, antiadipogenic, antidiabetogenic, and immune modulatory activities (*3*−*5*). It has also been demonstrated that the fat-lowering effects of CLA are mainly ascribed to the *trans*-10, *cis*-12 isomer (*6*, *7*). Adipose tissue reduction in mice fed a large dose of CLA is sometimes accompanied by significant liver enlargement (*6*,*8*). This may depend on several factors, including the inhibition of lipid uptake by adipose tissue and the accompanying hepatic accumulation of fat, altered secretion of liver lipoprotein, and reduced hepatic fatty acid oxidation (*9*).

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Most animal studies on the action of CLA were performed with CLA in the form of free fatty acid (FFA), despite the fact that CLA is mainly present in food as a triacylglyceride (TG) form. Previous studies comparing different forms of dietary CLA indicate similar effects on hamster plasma and liver TG (*10*) and on body composition and energy balance in mice (*11*). However, there have been no comparative studies on the effects of TG-CLA and FFA-CLA on lipogenic gene expression associated with liver enlargement. Rahman et al. (*12*) reported that TG-CLA was more effective than FFA-CLA in increasing the β-oxidation of fatty acids in muscle and liver of OLETF rats. Furthermore, it was shown that supplementation with a CLA byproduct, which was composed mainly of the esterified form of CLA, reduced lipogenic gene expressions in the liver of broilers compared with FFA-CLA (*13*).

Several genes for enzymes and signal mediators involved in the metabolism of lipid and glucose are responsive to long-term feeding with a high-fat diet (HFD) (*14*, *15*). Kim et al. (*16*) reported that lipogenic genes were down-regulated and genes implicated in the uptake and oxidation of fatty acids and

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Table 1. Composition of Experimental Diets (Grams per Kilogram)

ingredient	control	high-fat diet	HFD + 2% FFA-CLA	HFD + 2% TG-CLA
casein	200.0	200.0	200.0	200.0
sucrose	100.0	172.8	172.8	172.8
dextrose	132.0	100.0	100.0	100.0
corn starch	397.98	214.68	214.68	214.68
cellulose	50.0	50.0	50.0	50.0
soybean oil	70.0	25.0	5.0	5.0
lard	0	177.5	177.5	177.5
L-cystine	3.0	3.0	3.0	3.0
choline bitartrate	2.0	2.0	2.0	2.0
tert-butylhydroquinone	0.02	0.02	0.02	0.02
mineral mix[a]	35.0	45.0	45.0	45.0
vitamin mix[a]	10.0	10.0	10.0	10.0
CLA[b]	0	0	20	20
kcal/g	4	4.6	4.6	4.6
protein, % kcal	20	18	18	18
carbohydrate, % kcal	65	43	43	43
fat, % kcal	15	39	39	39

[a] Mineral mixture and vitamin mixture were based on the AIN-93G formulation (*28*). [b] FFA-CLA contained 4.80% C16:0, 1.50% C18:0, 13.40% C18:1, 1.00% C18:2, 38.20% *c*9,*t*11 CLA isomer, 39.00% *t*10,*c*12 CLA isomer, 0.90% *c*9,*c*11/*c*10, *c*12 CLA isomer, and 1.20% *t*9,*t*11/*t*10,*t*12 CLA isomer. TG-CLA contained 5.75% C16:0, 2.28% C18:0, 14.50% C18:1, 0.78% C18:2, 37.33% *c*9,*t*11 CLA isomer, 37.13% *t*10,*c*12 CLA isomer, 0.93% *c*9,*c*11/*c*10,*c*12 CLA isomer, and 1.31% *t*9,*t*11/*t*10,*t*12 CLA isomer.

ketogenesis were up-regulated in mice fed HFD and that these changes in gene expression might be crucial factors in the regulation of the physiological status of the animals, including fatty liver and increased fat accumulation. In the current study, we investigated the effect of two different forms (TG and free form) of CLA on serum and hepatic lipid composition and the expression of genes implicated in lipogenesis and mitochondrial β-oxidation of fatty acids in the liver and adipose tissue of C57BL/6J mice fed HFD. The same concentration of CLA was used as in our previous study, as this concentration showed decreases in lipogenic gene expressions by esterified form CLA in the liver of broilers (*13*).

MATERIALS AND METHODS

Animals and Diet. Male C57BL/6J 4-week-old mice (Daehan Biolink Co. Ltd., Eumseong, Korea) were housed in standard cages (33 × 23 × 12 cm, 5 mice/cage) under controlled temperature (22 ± 0.5 °C), humidity (50%), and light (light from 0900 to 2100 h) conditions. After a 1 week adaptation period, 10 mice each were assigned to one of the following four dietary groups: standard diet (control group), HFD (HFD group), HFD supplemented with 2% TG-CLA (HFD + TG-CLA group), or HFD supplemented with 2% FFA-CLA (HFD + FFA-CLA group). Food was supplied ad libitum, and the composition of each diet, stored frozen until use, is presented in **Table 1**. CLA was purchased from HK Biotech (Jinju, Korea). Animals were maintained on the feeding program for 8 weeks, and individual body weight and food intake per cage were recorded every week throughout the experimental period. The care and treatment of the animals conformed to the guidelines of Korea University (Seoul, Korea) for the ethical treatment of laboratory animals. At the end of the experimental period, mice were fasted overnight, anesthetized with ethyl ether, and then blood was collected from the vena cava. Liver and inguinal adipose tissue were dissected, weighed, and then immediately frozen in liquid nitrogen. Samples were stored at −80 °C until use.

Assay of Lipid Content. For the determination of serum lipid content, blood was subjected to centrifugation, and then serum levels of TG, total cholesterol, aspartic aminotransferase (AST), and alanine aminotransferase (ALT) were measured using a PRIME automatic photometer (BPC Biosed, Rome, Italy) and commercially available assay kits (Asan Pharmaceutical, Seoul, Korea). For the determination of hepatic lipid content, total lipid was extracted from mice liver using hexane and isopropanol (3:2 v/v). The extracts were dried under nitrogen flow and then resuspended in a suitable volume of 0.1% Triton X-100 before assays for TG and total cholesterol were carried out using commercially available kits.

Table 2. Primer Sequences for Real-Time PCR Analysis

gene[a]	primer sequence (5′→ 3′)	product size (bp)	annealing temp (°C)
SREBP-1c	sense: GGCACTAAGTGCCCTCAACCT antisense: GCCACATAGATCTCTGCCAGTGT	78	63
FAS	sense: CCTGGATAGCATTCCGAACCT antisense: AGCACATCTCGAAGGCTACACA	122	63
ACC	sense: CCCAGCAGAATAAAGCTACTTTGG antisense: TCCTTTTGTGCAACTAGGAACGT	70	63
malic enzyme	sense: GTCGTGCATCTCTCACAGAAG antisense: TGAGGGCAGTTGGTTTTATCTTT	102	63
CPT1	sense: CTCCGCCTGAGCCATGAAG antisense: CACCAGTGATGATGCCATTCT	100	63
UCP2	sense: CCTCAGCCCTCGATCAACTC antisense: CAGTACACCGCAGTGTGTCAT	101	63
ACO	sense: GGCCAACTATGGTGGACATCA antisense: ACCAATCTGGCTGCACGAA	73	60
β-actin	sense: AGTGTGACGTTGACATCCGTA antisense: GCCAGAGCAGTAATCTCCTTCT	112	63

[a] SREBP-1c, sterol regulatory element binding protein 1c; FAS, fatty acid synthase; ACC, acetyl-coenzyme A carboxylase; CPT1, carnitine palmitoyltransferase 1; UCP2, uncoupling protein 2; ACO, acetyl-coenzyme A oxidase.

RNA Extraction and Real-time PCR. Liver and adipose tissue were homogenized in 1 mL of TRIzol reagent, and then RNA was isolated according to the TRIzol protocol. RNA was quantified by spectroscopy (NanoDrop, Wilmington, DE). From each sample, 1 μg of total RNA was reverse transcribed to cDNA with primers of random and oligo(dt) using a high-capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA) in a total volume of 20 μL, as described in the high-capacity RNA-to-cDNA kit protocol. cDNAs were used in real-time PCR with primers for the following target genes: sterol regulatory element binding protein-1c (SREBP-1c; GenBank accession no. NM_011480), fatty acid synthase (FAS; GenBank accession no. AK080374), acetyl-coenzyme A carboxylase (ACC; GenBank accession no. NM_133360), malic enzyme (GenBank accession no. M29546), carnitine palmitoyltransferase 1 (CPT1; GenBank accession no. NM_013495), uncoupling protein 2 (UCP2; GenBank accession no. NM_011672), and acetyl-coenzyme A oxidase (ACO; GenBank accession no. NM_017340). The sequences of the primers are provided in **Table 2**. The amplification was performed in a total volume of 20 μL, which included 2 μL of cDNA, 1 μL of each primer (10 pmol/ μL), 10 μL of Power SYBR Green Master Mix (Applied Biosystems), and 6 μL of distilled water. Reactions were carried out in an ABI 7500 system using the following thermal cycling parameters: 50 °C for 2 min, 94 °C for 2 min, and then 45 cycles of 94 °C for 30 s, the appropriate annealing temperature for 30 s, and 72 °C for 1 min. All samples were also examined in parallel for β-actin, and relative quantities of each gene were presented in terms of $2^{-\Delta\Delta Ct}$, calculated using the ΔCt (Ct value of target gene − Ct value of ss-actin) and ΔΔCt values (ΔCt value of tested sample − ΔCt value of control sample).

Statistical Analysis. Statistical analysis was carried out using ANOVA, with mean separations performed by the Duncan's multiple-range test using the general linear model procedure in the SAS statistical software package (SAS Institute Inc., Cary, NC). The variation between samples was expressed as the pooled standard error of the mean (SEM) or mean ± SEM, when applicable.

RESULTS

Effect of Dietary Supplementation of CLA on Animal Growth and Tissue Weight. Prior to the start of the feeding program, the body weights of 4-week-old mice (20.38 ± 0.13 g) were measured, and then the mice were randomized into four different groups, 10 per each group. Calorie intake during the experimental period showed no significant difference among the different dietary groups (from 525.0 ± 4.3 to 539.0 ± 4.7 kcal/5 mouse/week, $P > 0.05$). The body weight of the HFD group was increased from 1 week of feeding, whereas the body weight of mice fed HFD + FFA-CLA or HFD + TG-CLA were substantially lower than those of the HFD group throughout the experimental periods (**Figure 1**).

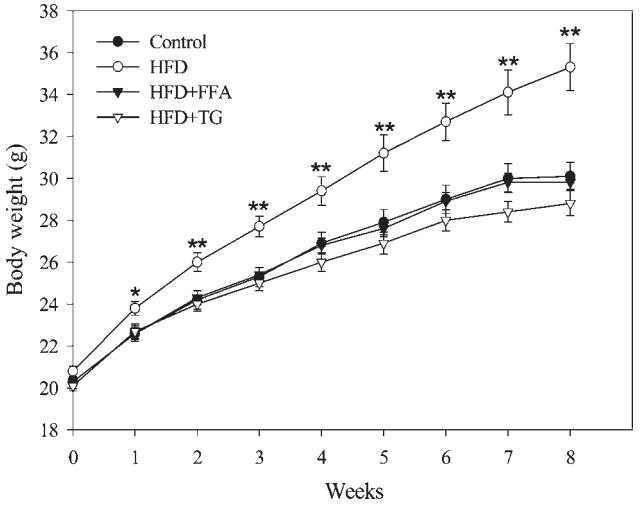

Figure 1. Effect of dietary supplementation of CLA on body weight. HFD, high-fat diet; HFD + FFA, high-fat diet + 2% FFA-CLA; HFD + TG, high-fat diet + 2% TG-CLA. Data represent the mean ± SEM of 10 animals. *, $P < 0.05$, and **, $P < 0.01$, as compared to control, HFD + FFA, and HFD + TG.

A significant increase in the liver weight and a marked decrease (about 8-fold) in inguinal adipose tissue weight were found in mice fed HFD with both types of CLA, but there were no significant differences between mice that received FFA-CLA and TG-CLA (**Table 3**). After the 8 week feeding program, mice fed HFD supplemented with FFA-CLA or TG-CLA exhibited 3.3- and 3.1-fold decreases, respectively, in inguinal adipose tissue weight as compared to mice in the control group. The inguinal adipose tissue weight of mice fed HFD without CLA supplementation was significantly increased, whereas the liver weight of the HFD group was unaffected.

Effect of Dietary Supplementation of CLA on Serum and Liver Content. The concentration of serum TG was significantly lower and the level of serum cholesterol was significantly higher in the HFD group as compared to control group (**Table 4**). Supplementation with HFD + CLA significantly decreased serum TG concentration, but had no effect on serum cholesterol levels as compared to HFD. The level of serum AST was significantly lower in the HFD group compared to the control group, and mice fed HFD supplemented with both types of CLA exhibited increased serum ALT levels. There were no significant differences in any of the serum parameters between mice that received FFA-CLA and those that received TG-CLA.

TG levels in the liver were markedly increased in mice that were fed HFD + FFA-CLA. However, hepatic TG levels in mice fed HFD + TG-CLA were not significantly different compared to the CLA-free control groups. Hepatic cholesterol levels were increased in mice fed HFD, but there were no differences among other groups.

Expression of Lipogenic and Fat Oxidative Genes in the Liver. The mRNA levels of lipogenic and oxidative enzymes in the liver were analyzed using real-time PCR (**Figure 2**). There was no evidence of increased hepatic lipogenic gene expression in mice fed HFD supplemented with CLA as compared to the control or HFD group. Supplementation of the HFD with TG-CLA significantly decreased the mRNA levels of FAS, ACC, and malic enzyme as compared to HFD + FFA-CLA ($P < 0.05$). There was an overall increase in the mRNA expression of enzymes related to β-oxidation in all treatment groups. The gene expression of UCP2

Table 3. Effect of Dietary Supplementation of CLA on the Weight of Liver and Inguinal Fat in Mice

	dietary group[a]			
organ	control	HFD	HFD + FFA	HFD + TG
liver, g/100 g of body wt	3.84 ± 0.05b	3.79 ± 0.06b	5.97 ± 0.21a	5.60 ± 0.19a
inguinal fat, g/100 g of body wt	2.17 ± 0.08b	5.38 ± 0.18a	0.65 ± 0.05c	0.71 ± 0.06c

[a] HFD, high-fat diet; HFD + FFA, high-fat diet + 2% FFA-CLA; HFD + TG, high-fat diet + 2% TG-CLA. Values represent the mean ± SEM. Means with different letters within the same row are significantly different ($P < 0.05$, $n = 10$).

Table 4. Effect of Dietary Supplementation of CLA on Biochemical Parameters in Serum and Liver in Mice

	dietary group[a]			
	CON	HFD	HFD + FFA	HFD + TG
serum[b]				
triglyceride, mg/dL	90.87 ± 4.62a	73.86 ± 6.34b	46.48 ± 2.72c	44.55 ± 3.21c
cholesterol, mg/dL	136.96 ± 1.57b	173.43 ± 4.39a	167.44 ± 6.05a	165.58 ± 4.77a
AST,[c] U/L	57.90 ± 2.78a	47.49 ± 1.73b	49.14 ± 2.99ab	50.88 ± 3.24ab
ALT,[c] U/L	16.85 ± 1.14b	14.13 ± 1.12b	23.57 ± 2.34a	23.98 ± 2.82a
liver[b]				
triglyceride, mg/g of liver	6.22 ± 0.29b	6.34 ± 0.48b	10.59 ± 0.89a	7.82 ± 1.17b
cholesterol, mg/g of liver	0.27 ± 0.04b	1.18 ± 0.25a	0.27 ± 0.05b	0.45 ± 0.09b

[a] HFD, high-fat diet; HFD + FFA, high-fat diet + 2% FFA-CLA; HFD + TG, high-fat diet + 2% TG-CLA. Values represent the means ± SEM. Means with different letters within the same row are significantly different ($P < 0.05$, $n = 10$). [b] On the final day of the experiment, blood and livers were collected after 12 h of food deprivation. [c] AST, aspartic aminotransferase; ALT, alanine aminotransferase.

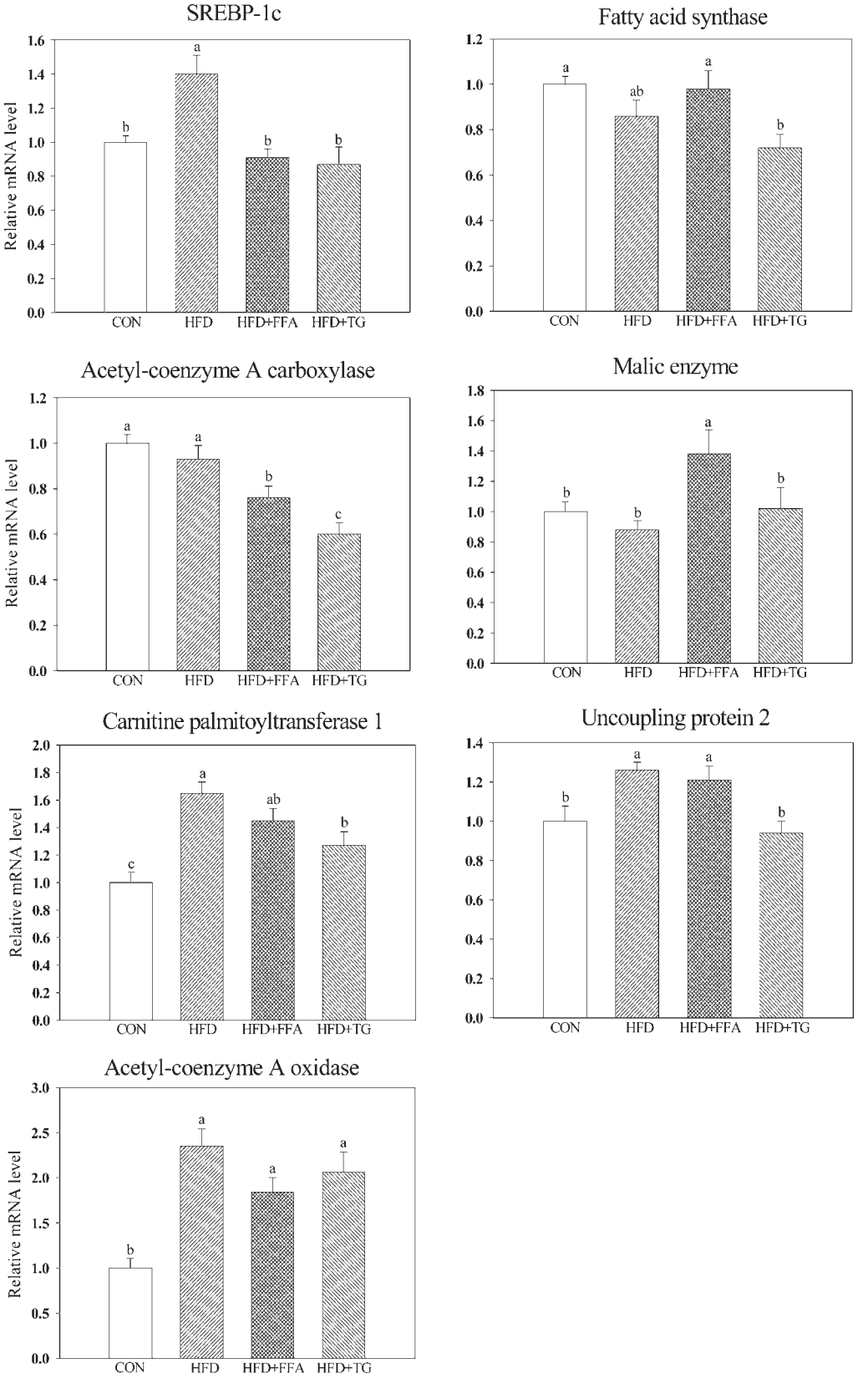

Figure 2. mRNA levels of lipogenic and oxidative enzymes in the liver of mice fed HFD supplemented with FFA-CLA and TG-CLA. HFD, high-fat diet; HFD + FFA, high-fat diet + 2% FFA-CLA; HFD + TG, high-fat diet + 2% TG-CLA. Values represent the mean ± SEM for eight or nine mice. Means with different letters are significantly different ($P < 0.05$). Relative quantities of each gene are represented as $2^{-\Delta\Delta Ct}$, calculated using the ΔCt (Ct value of target gene − Ct value of β-actin) and ΔΔCt values (ΔCt value of tested sample − ΔCt value of control sample). The value of the control group was set as 1.

was significantly decreased in mice fed HFD + TG-CLA as compared to FFA-CLA ($P < 0.05$), but there was no difference in the expression of CPT1 and ACO.

Expression of Lipogenic and Fat Oxidative Genes in Inguinal Adipose Tissue. The expression of genes involved in the regulation of lipogenesis and β-oxidation was examined in inguinal adipose

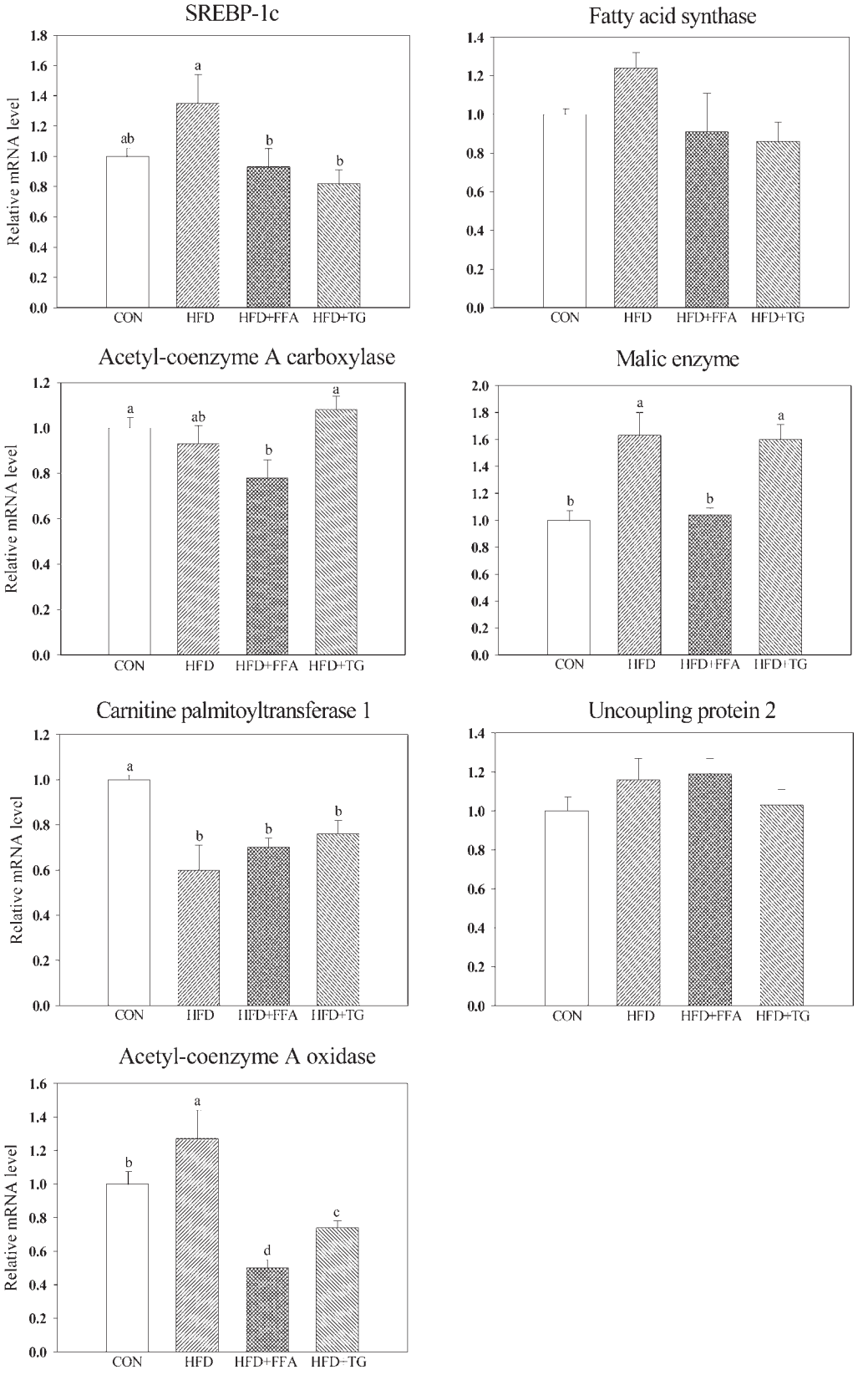

Figure 3. mRNA levels of adipose lipogenic and oxidative enzymes in mice fed HFD supplemented with FFA-CLA and TG-CLA. HFD, high-fat diet; HFD + FFA, high-fat diet + 2% FFA-CLA; HFD + TG, high-fat diet + 2% TG-CLA. Values represent the mean ± SEM for eight or nine mice. Means with different letterss are significantly different ($P < 0.05$). Relative quantities of each gene are represented as $2^{-\Delta\Delta Ct}$, calculated using the ΔCt (Ct value of target gene − Ct value of β-actin) and ΔΔCt values (ΔCt value of tested sample − ΔCt value of control sample). The value of the control group was set as 1.

tissue (**Figure 3**). With the exception of malic enzyme, the mRNA levels of lipogenic enzymes were not influenced by feeding with HFD. Supplementation of the HFD with CLA significantly suppressed the gene expression of SREBP-1c in adipose tissue as compared to the supplementation of HFD alone ($P < 0.05$), but had no significant effect on the mRNA levels of FAS and

ACC. There was a significant decrease in the mRNA levels of ACC and malic enzyme in mice fed HFD + FFA-CLA as compared to HFD + TG-CLA. In addition, dietary supplementation with CLA had no effect on the mRNA levels of CPT1 and UCP2. The mRNA levels of ACO were lower in mice fed HFD supplemented with CLA as compared to HFD alone.

DISCUSSION

It was previously shown that CLA reduces body fat mass and enhances hepatic lipogenesis in mice (*17*). The effects of CLA are mediated through a variety of metabolic pathways, but the regulation of expression of enzymes involved in lipogenesis and β-oxidation has been suggested as the most important. It has been generally believed that different forms of CLA do not differently affect body composition and liver TG concentration (*10*, *11*). However, to date, it is unclear whether esterification of CLA can differently affect the expression of genes involved in fatty acid metabolism. In the current study, we evaluated the effects of two different forms of CLA on the expression of genes associated with body fat accumulation and liver enlargement in mice fed HFD.

In the present study, mice fed HFD supplemented with CLA exhibited a > 3-fold decrease in inguinal fat weight as compared to control mice. Kim et al. (*16*) reported that long-term feeding of HFD for 12 weeks induced the formation of fatty liver, with increased fat accumulation in the liver in C57BL/6J mice, but in the current study, a HFD with a similar level of fat was applied for 8 weeks, and this effect was not shown. Both forms of CLA significantly increased serum ALT levels, whereas there was no increase in serum ALT level in HFD mice at the end of the feeding study. Serum ALT level in mice fed HFD with TG-CLA was increased without increases of hepatic lipid level. These results indicate that increased serum AST and ALT in mice fed CLA-supplemented HFD is not due to hepatic lipid accumulation. It has been suggested by Hamura et al. (*18*) that changes in energy partitioning between glucose and fatty acids through dietary supplementation with CLA account for increased serum AST and ALT levels. Our results suggest that ALT is more responsive to CLA-induced changes in the cellular microenvironment of the liver than AST. It is worth noting that hepatic TG accumulation in mice that received TG-CLA was significantly lower than the mice that received FFA-CLA, which is inconsistent with a previous study in which different forms of CLA did not differently affect hepatic TG levels in hamsters after an 8 week feeding trial (*10*). Moreover, hepatic cholesterol levels were reduced by feeding both types of CLA, which is inconsistent with the results of previous studies in which CLA was ineffective in lowering cholesterol in the mouse liver (*9*, *17*). Discrepancies between the current data and the previous study might be due to differences in the type of experimental animals used, the amount and type of CLA, and other dietary factors. We found that TG-CLA also significantly reduced the gene expression of lipogenic enzymes such as FAS, ACC, and malic enzyme in the liver as compared to FFA-CLA. These results suggest that the TG form of CLA is more effective in lowering hepatic TG levels than the CLA in the free form. There were no differences in the liver size or liver weight between the two CLA groups. Macarulla et al. (*19*) reported that dietary CLA increased the number of cells in the liver and that this might contribute to increased liver weights. Additional studies are needed to distinguish the action mechanisms of different forms of CLA on liver enlargement, cell number, and phospholipid distribution, as well as TG levels in the liver.

Previously, it was shown that dietary CLA profoundly increases the gene expression of enzymes involved in lipogenesis and β-oxidation in the liver (*17*, *20*). In the current study, we could not observe an increase in lipogenic gene expression in the livers of mice fed CLA. This might be due to a regulatory feedback mechanism in response to long-term HFD feeding. Indeed, Gregoire et al. (*21*) found that a one day feeding of HFD greatly enhanced the mRNA levels of lipogenic enzymes such as *Fasn* and glycerol-3-phosphate acyltransferase in the mouse liver and that mRNA levels returned to baseline within 11 days. Similarly, Kim et al. (*16*) suggested that hepatic TG accumulation associated with HFD drives the down-regulation of lipogenic gene expression through a feedback mechanism in obese C57BL/6J mice. Thus, the increased expression of hepatic lipogenic genes induced by dietary CLA could be offset by long-term feeding with HFD. Comparisons between the results reported here and those of previous studies may also be complicated by differences in sampling time relative to feeding status, because lipogenesis also varies according to nutrient levels, and this should be accounted for in future studies. One of the most interesting results of the current study was that the mRNA levels of FAS, ACC, and malic enzyme, which play key roles in fatty acid biosynthesis, were significantly suppressed in the liver by TG-CLA compared to FFA-CLA. Whereas it is difficult at this stage to clearly interpret these results, it would be worthwhile exploring whether differences in the absorption of the TG form and FFA form of CLA contribute to changes in lipogenic gene expression. The TG form is more favored in the formation of mixed micelles composed of FFA and 2-monoglycerol, resulting in good chylomicrometer formation in the intestinal mucosa. Thus, the esterified fats are believed to be efficient vehicles for the absorption of unsaturated fatty acids (*22*, *23*), and TG is regarded as the most suitable form for incorporation into food materials (*24*). In our previous studies, supplementation with a CLA byproduct, which was composed mainly of the esterified form of CLA, increased the total CLA levels more efficiently in egg yolks (*25*) and decreased lipogenic gene expressions in the liver of broilers (*13*) compared to FFA-CLA. Therefore, further studies are needed to elucidate the physiological mechanisms of CLA incorporation into tissues and its effects on lipogenesis. There was an overall increase in the mRNA expression of the hepatic enzymes involved in β-oxidation in all treatment groups, but these changes might not necessarily be accompanied by parallel changes in the actual rate of hepatic fatty acid oxidation, due to the strong inhibitory action of malonyl-CoA, a product of ACC, on CPT1 (*17*).

One mechanism by which CLA reduces body fat is increasing energy expenditure. This was observed within 1 week of CLA supplementation and was sustained for the following 6 weeks (*26*). However, increased gene expression related to β-oxidation did not appear to be essential for the increased energy expenditure. DeLany and West (*26*) showed that there was no significant effect of CLA supplementation on the gene expression of uncoupling protein in skeletal muscle, epididymal adipose tissue, or kidney in mice, despite enhanced energy expenditure in these animals that resulted in a considerable reduction of body fat mass. Similarly, Ribot et al. (*27*) reported that the induction of UCP1 or UCP2 in white adipose tissue and brown adipose tissue is not likely to be responsible for the fat-reducing ability of CLA. Rather, increased expression of CPT1 in skeletal muscle might explain the effects of dietary CLA in lowering adiposity. Our results are in agreement with these previous studies and indicate that dietary CLA significantly reduces the body weight and adipose tissue of mice, without an accompanying increase in the expression of genes for oxidative enzymes in adipose tissue. Alternatively, our results suggest that in adipose tissue, decreased mRNA levels of SREBP-1c, a transcription factor that stimulates the expression of mammalian lipogenic genes, underlie the decreased accumulation of body fat in CLA-supplemented mice. With the exception of

ACO, the expression of oxidative genes was similar in adipose tissue of mice fed both types of CLA.

In summary, dietary supplementation with CLA in the form of FFA or TG strongly decreased body fat accumulation and induced significant liver enlargement in HFD-induced obese C57BL/6J mice. TG-CLA significantly decreased TG accumulation and lipogenic gene expression in the liver as compared to FFA-CLA. The current findings will introduce a way of efficient dietary CLA supplementation with a mechanism to reduce adiposity and body weight without apparent adverse effects on the liver.

ABBREVIATIONS USED

ACC, acetyl-coenzyme A carboxylase; ACO, acetyl-coenzyme A oxidase; ALT, alanine aminotransferase; AST, aspartic aminotransferase; CLA, conjugated linoleic acid; CPT1, carnitine palmitoyltransferase 1; FAS, fatty acid synthase; FFA, free fatty acid; HFD, high-fat diet, LA, linoleic acid; SREBP-1c, sterol regulatory element binding protein-1c; TG, triglyceride; UCP2, uncoupling protein 2.

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