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Conjugated linoleic acid does not reduce body fat but decreases hepatic steatosis in adult Wistar rats☆

Aparna Purushotham, Gayle E. Shrode, Angela A. Wendel, Li-Fen Liu, Martha A. Belury*

Department of Human Nutrition, The Ohio State University, Columbus, OH 43210, USA

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Abstract

The dietary fatty acid conjugated linoleic acid (CLA) reduces hepatic lipid accumulation in some rodent models for obesity and hepatic steatosis. However, these effects are variable and complex due to differences in isomer responses and degree and sensitivity to changes in adiposity. Here, we hypothesized that CLA decreases hepatic steatosis in a diet-induced model of obesity in rats which are resistant to the adipose-lowering effects of CLA. To investigate this, we fed male Wistar rats a high-fat (20%) diet for 4 weeks to induce obesity and hepatic steatosis followed by low-fat (6.5%) experimental diets containing either 6.5% soybean oil (CON) or 1.5% CLA triglyceride mix plus 5% soybean oil (CLA). Dietary CLA significantly lowered hepatic triglycerides without changing weight, adiposity or adipokines, and was associated with significantly lower hepatic fatty acid synthase and stearoyl CoA desaturase-1 (SCD-1) mRNA levels and SCD-1 index along with significantly lower sterol regulatory element binding protein-1 mRNA, a transcription factor that regulates lipogenesis. Furthermore, the lower lipogenesis was associated with significantly higher mRNA expression of lipid oxidation gene peroxisome proliferator-activated receptor-α and acetyl CoA oxidase in the livers of rats fed dietary CLA. The lipid-lowering effects of CLA in the liver were observed in the absence of changes in adipose tissue and body weight. Thus, we conclude that in the Wistar rat model, where adipose levels remain static after feeding dietary CLA, hepatic lipid accumulation is reduced and these effects are not due to an improvement in overall adiposity.

Keywords: Conjugated linoleic acid; Hepatic steatosis; Lipogenesis

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) occurs in up to 75% of people with obesity and diabetes mellitus [1]. NAFLD consists of a spectrum ranging from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH) [1]. Hepatic steatosis is the result of excess lipid stored in the liver and may lead to hepatic insulin resistance, glucose intolerance and diabetes [1,2]. Preventing fat accumulation in the livers of mice on a high-fat diet has been shown to prevent development of insulin resistance [2].

The group of fatty acids, conjugated linoleic acid (CLA), consists of positional and geometric isomers of octadecadienoate that are naturally found in foods such as meat, milk and other dairy products [3]. The isomers, *c*9*t*11 and *t*10*c*12, are available in commercial oils (e.g., Tonalin) for human consumption and are most widely studied. It is suggested that *c*9*t*11-CLA and *t*10*c*12-CLA may have differing effects on lipid metabolism in a variety of tissues including liver, muscle and adipose tissue [4,5]. Studies in rat models have shown that CLA attenuates development of hepatic steatosis [6–8], which improves glucose homeostasis and insulin-stimulated glucose uptake in the skeletal

Abbreviations: AOX, acetyl CoA oxidase; CLA, conjugated linoleic acid; CPT, carnitine palmitoyl CoA; FAS, fatty acid synthase; FBG, fasting blood glucose; NEFA, nonesterified fatty acid; NAFLD, nonalcoholic fatty liver disease; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl CoA desaturase; SREBP, sterol regulatory element binding protein; TG, triglyceride.

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* Corresponding author. Tel.: +1 614 292 1680; fax: +1 614 292 8880.
E-mail address: belury.1@osu.edu (M.A. Belury).

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muscle of the Zucker diabetic fatty (ZDF) rat model for type 2 diabetes [1,4].

The effects of CLA as both mixed isomers [6,8] and, recently, using individual isomers [9] on attenuation of NAFLD and insulin resistance have been demonstrated only in genetically obese rats. In these studies, CLA improves insulin resistance and lowers hepatic triglyceride (TG) levels in association with significant depletion of adipose tissue mass [6,9,10]. For instance, the *t*10*c*12 isomer decreases hepatic TG, but also decreases adiposity; in contrast, the *c*9*t*11 CLA isomer has no effect on either parameter [9]. Thus, it is unclear if the improvement in insulin sensitivity and hepatic triglycerides is a result of decreased adiposity. Therefore, we evaluated the effects of CLA in Wistar rats, a useful model to study hepatic steatosis [11,12] and a diet-induced obesity model which is less susceptible to changes in adiposity associated with dietary CLA [13].

In an effort to understand the mechanism by which CLA modulates steatosis in rodent models, we and others have postulated that CLA isomers alter the progression of NAFLD, in part, by modulating the activity of peroxisome proliferator-activated receptors (PPARs) thereby regulating genetic markers of β-oxidation [14,15]. Furthermore, because the *t*10*c*12 CLA isomer was recently shown to decrease sterol regulatory element binding protein (SREBP-1) mRNA expression in Otsuka Long Evans Tokushima Fatty (OLETF) rats [9], we postulated that CLA lowers hepatic lipids by suppressing SREBP-1, a transcription factor from the basic helix loop helix leucine zipper family that stimulates lipid synthesis by regulating lipogenic genes such as fatty acid synthase (FAS) and stearoyl CoA desaturase (SCD-1) [16].

Therefore, we evaluated the effects of CLA in Wistar rats, a diet-induced obesity model that is less susceptible to changes in adiposity by dietary CLA [13]. The aims of the present study were to (1) determine the effect of feeding CLA on attenuation of hepatic steatosis in Wistar rats (fed a high-fat diet prior to CLA supplementation); and (2) explore the molecular mechanisms involved in the process.

2. Materials and methods

2.1. Materials

All the diet components were purchased from Bio-Serv (Frenchtown, NJ, USA). CLA mixed triglycerides were obtained from Cognis (Cincinnati, OH, USA). Insulin and leptin Multiplex ELISA kits were obtained from LINCO Research (St. Charles, MO, USA). The rat adiponectin ELISA kit was purchased from B-Bridge International, Inc. (Sunnyvale, CA, USA). The methyl ester standards, PUFA-3 and C:17 FA, were purchased from Matreya, Inc. (Pleasant Gap, PA, USA). All solvents and other chemicals were of the highest grade commercially available.

2.2. Animals

Four-week-old male Wistar rats were obtained from Charles River Laboratories, Inc. (Wilmington, MA, USA) and were housed two per cage at 22±0.5°C on a 12-h day/night cycle. Rats received standard rat chow for 2 weeks while adjusting to their new environment. All procedures were in accordance with institution guidelines and approved by the Institutional Animal Care and Use Committee of The Ohio State University.

2.3. Experimental diets

Because the objective of this study was to observe the impact of feeding a CLA-rich diet on hepatic steatosis, rats were fed ad libitum a high-energy diet, which has previously been shown to induce steatosis in Wistar rats [12] consisting of 20% fat (predominantly lard), 42% carbohydrates and 21% protein (by weight) for 4 weeks prior to assignment to the experimental diets to induce significant gains in hepatic lipids.

After 4 weeks on the high-energy diets, rats were randomized by body weight to one of the two isocaloric diets containing 6.5% fat. The diets contained either 6.5% soybean oil (CON diet) or 5% soybean oil with 1.5% CLA triglyceride mix (CLA diet) by weight (Table 1). CLA composition in our diet was a mixture of the two CLA isomers (39.2% *c*9*t*11 and 38.5% *t*10*c*12 CLA) which contributed approximately 0.5775% (weight of diet) of the adipose-lowering isomer, *t*10*c*12-CLA. All diets were modified forms of the AIN-93G diet [17]. Rats had free access to food and water. Food intake was measured every other day and body weights were measured weekly.

2.4. Fasting blood glucose

After 3 weeks on 6.5% experimental diets, rats were fasted for 15 h and blood was collected from a tail vein for analysis of fasting blood glucose using a One Touch Basic glucose analyzer (Lifescan, California). Insulin sensitivity

Table 1
Diet composition (in g/kg)

	High fat (20%)	Low fat (6.5%)	
		CON	CLA
Starch	333.39	402.49	402.49
Casein	234.10	200.00	200.00
Cystine	3.00	3.00	3.00
Vitamin mix	10.00	10.00	10.00
Choline bit	2.50	2.50	2.50
Salt mix	35.00	35.00	35.00
Lard	190.00	100.00	100.00
Sucrose	–	100.00	100.00
Lo Dex	132.00	132.00	132.00
Fiber	50.00	50.00	50.00
Tbhq	0.01	0.01	0.01
Soybean oil	10.00	65.00	50.00
CLA	–	–	15.00
Total	1000.00	1000.00	1000.00

index was calculated using the fasting serum insulin (FSI) and fasting blood glucose (FBG) with the quantitative insulin sensitivity check index (QUICKI), using the following formula: 1/[log(10) FSI+log(10) FBG] [18].

2.5. Necropsy

After 4 weeks on experimental diets, rats were euthanized (after 6 h of fasting) by CO_2, and liver, epididymal adipose, peri-renal and gastrocnemius muscle tissues were weighed, snap-frozen in liquid nitrogen and stored at −80°C for further analysis. Blood samples were collected in tubes, centrifuged to isolate serum and stored at −80°C for hormone and metabolite analyses.

2.6. Serum hormone and metabolite determination

Serum insulin, leptin and adiponectin levels were determined using ELISAs. Serum triglycerides and nonesterified fatty acids (NEFA) were measured using spectrophotometric assays purchased from Sigma (St. Louis, MO, USA) and Wako Chemicals (Richmond, VA, USA), respectively.

2.7. Tissue TG analysis

TG levels from liver and muscle were quantitatively measured with an enzymatic colorimetric kit (Sigma). In short, tissue sections were homogenized and lysed in RIPA buffer (Santa Cruz Biochemicals, CA, USA). Protein was determined using BCA assay (Pierce Biotechnology, Inc., Illinois, USA). TG were extracted with 2:1 (v/v) chloroform/methanol using the Folch method [19] and dissolved in isopropanol. TG levels were measured using the glycerol determination procedure (Sigma). Values were normalized to tissue protein content.

2.8. Real-time reverse transcriptase–polymerase chain reaction

Liver tissue was homogenized in Trizol reagent (Sigma) and mRNA was isolated using manufacturer's protocol. RNA was quantified by spectrophotometry and diluted in RNase-free water. RNA integrity was assessed by electrophoresis using agarose gel and ethidium bromide staining. The first transcripts were reverse transcribed using reverse transcriptase (Invitrogen) and cDNA was amplified using real-time polymerase chain reaction (PCR) with TaqMan gene expression assays (Applied Biosystems, California, USA). In short, 10 ng of the reverse transcription reaction was amplified in a total reaction volume of 25 μl using predesigned, validated, FAM-labeled primers designed for stearoyl CoA desaturase (SCD-1; NM_139192.1), fatty acid synthase (FAS; NM_017332.1), liver fatty acid binding protein (L-FABP; NM_012556.1), acetyl CoA oxidase (AOX; NM_017340.1), sterol regulatory element binding protein (SREBP-1; AF286469), carnitine palmitoyl CoA transferase (CPT I; NM_031559.1 and CPT II; NM_012930.1), phosphoenolpyruvate carboxykinase (PEPCK; NM_198780.3) and glucose-6-phosphatase (G-6-Pase; NM_176077.3) using universal cycling conditions (Applied Biosystems). Target gene expression was normalized to Vic-labeled18 s, which was used as an endogenous control in the same reaction as the target gene.

2.9. Liver fatty acid composition

Lipids were extracted from livers using the Folch method [19]. Briefly, liver tissue was homogenized in methanol containing BHT, and lipids were extracted in chloroform/methanol (2:1, v/v). Heptadecanoic acid was used as an internal control. Extracted fatty acids from each rat were dissolved in methyl *tert*-butyl ether (MTBE)/acetic acid (100:0.2, v/v) and further separated into neutral and phospholipids using a silica Sep-Pak column procedure, as described previously [20]. Fatty acids were methylated by incubating with tetramethylguanidine in a 100°C water bath and centrifuging in 0.88% KCl/hexane (1:2) at 1000×*g* for 10 min. Fatty acid methyl esters were analyzed by gas chromatography (HP 5890 equipped with FID and 30-m Omegawax capillary column, Supelco Chromatography Products). Fatty acids were identified using authentic standards (Matreya), quantified by determining areas under identified peaks (ChemStation Software; Packard Instrument Company, Meriden, CT, USA) and adjusted to the internal control. SCD-1 activity index was calculated from product-to-precursor ratios as follows: palmioleate to palmitate (16:1n-7/16:0) and oleate to stearate (18:1n-9/18:0) [21].

2.10. Statistical analysis

All data are presented as mean±S.E. Data were analyzed using MINITAB (version 14, State College, PA, USA). Differences between means were analyzed using two-

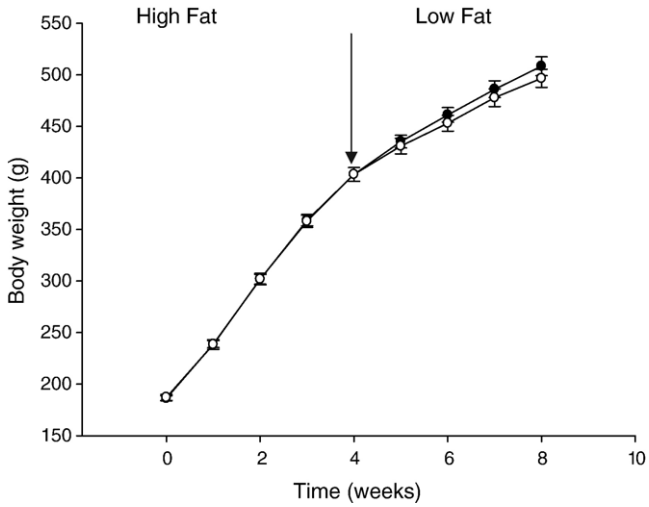

Fig. 1. Body weight over time in Wistar rats. Rats were fed a high-fat diet (20% fat by weight) for 4 weeks followed by switching to low-fat (6.5% fat) experimental diets for additional 4 weeks. The diets contained either 6.5% soybean oil (CON diet, closed circles) or 5% soybean oil with 1.5% CLA triglyceride mix (CLA diet, open circles). Values represent mean±S.E. of eight rats per group.

sample Student's t test. Differences were considered significant at $P<.05$.

3. Results

3.1. Body weights, food intake and organ weights

After feeding a high-fat (20% fat) diet for 4 weeks, rats were fed experimental diets containing 6.5% soybean oil (CON) or 5% soybean oil plus 1.5% CLA for 4 additional weeks. Switching to low-fat diets did not result in weight loss in Wistar rats (Fig. 1). Diet containing CLA did not change either body weights or food intake significantly compared to diet without CLA (CON) (Table 2). In addition, there were no significant differences in the weights of liver, epididymal or peri-renal adipose tissue between the two diet groups (Table 2) after 4 weeks on the experimental diets.

3.2. Plasma concentration of metabolites

Because dietary CLA modulates metabolism which may be responsive to insulin and adipokines [6,22,23], we measured leptin and adiponectin levels post-necropsy (rats were fasted for 6 h) after 4 weeks on the experimental diets. Additionally, we measured fasting glucose levels after an overnight fast following 3 weeks of feeding experimental diets. Although rats fed neither CON nor CLA diet developed hyperglycemia in this study, feeding dietary CLA decreased fasting glucose levels significantly compared to rats fed the CON diet (Table 2). The lower fasting glucose levels were not accompanied by changes in circulating concentrations of insulin or leptin (Table 2). While CLA did not increase adiponectin levels significantly compared to rats fed the CON diet (Table 2), there was a higher trend of this insulin-sensitizing adipokine in the CLA group ($P=.058$). We measured insulin sensitivity in Wistar rats using the QUICKI index. Insulin sensitivity was not different between rats fed CON and CLA diet (Table 2).

Table 2
Body weights and serum metabolites

	CON	CLA	P value
Initial body weight (g)	430.62±4.25	430.85±7.70	.979
Final body weight (g)	508.35±9.18	496.57±8.69	.360
Change in weight (g)	70.23±3.08	65.71±2.74	.283
Food intake (g/rat per day)	25.05±0.13	23.95±0.87	.301
Liver weight (g)[a]	16.71±0.63	16.59±0.56	.885
Epididymal adipose tissue (g)[a]	7.79±0.59	7.33±1.01	.699
Peri-renal adipose tissue (g)[a]	2.25±0.35	1.91±0.34	.501
Fasting glucose (mmol/L)	3.6±0.1	3.3±0.1*	.037
Adiponectin (ng/ml)[a]	8777.40±666.22	10,536.80±205.63	.058
Leptin (pg/ml)	1153.38±91.86	1312.77±253.47	.571
Insulin(pg/ml)	515.28±92.86	406.77±88.57	.422
QUICKI[a]	0.22±0.01	0.23±0.01	.495
Liver TG (mg TG/mg protein)[a]	0.97±0.11	0.77±0.06*	.035

Values represent mean±S.E. of 16 rats per group.
[a] $n=8$ rats per group.
* Significantly different from CON group, $P<.05$.

3.3. Liver triglycerides

Dietary CLA significantly decreased TG accumulation in the liver by approximately 20% (Table 2) compared to rats fed the CON diet. However, muscle and serum TG levels did not differ significantly between the two groups (data not shown). Because adipose tissue can contribute to a significant amount of lipid influx to the liver by lipolysis from adipose tissue, we measured serum nonesterified fatty acid (NEFA) concentrations. Serum NEFA concentrations were unchanged by feeding the diet containing CLA for 4 weeks (data not shown) which corroborate with the lack of change in adipose tissue mass in Wistar rats (Table 2).

3.4. Liver mRNA expression

Next, to understand the mechanism by which dietary CLA decreased hepatic TG levels in the absence of a reduction in body weight, we measured the mRNA levels of genes that modulate lipid metabolism in the liver using real-time PCR. Dietary CLA significantly increased the mRNA levels of both PPAR-α and AOX. The mRNA level of L-FABP was higher in the CLA-fed rats but not significantly different compared to rats fed the CON diet (Fig. 2A–C). mRNA levels of CPT-I and CPT-II, key enzymes in mitochondrial β-oxidation, did not change with diet containing CLA (data not shown). Dietary CLA decreased SCD-1 transcript levels by 80% compared to rats fed CON diet (Fig. 2D) and significantly decreased FAS expression (53%) (Fig. 2E). Associated with lower expression of lipogenic genes FAS and SCD-1, mRNA levels of SREBP-1 were significantly decreased in rats fed the CLA diet (Fig. 2F).

3.5. Hepatic SCD-1 index

To understand the liver-specific effects of dietary CLA, we analyzed the liver fatty acid composition in different lipid fractions using gas chromatography. Levels of both *c*9*t*11 and *t*10*c*12 isomers were significantly higher in liver neutral and phospholipid fractions of rats fed CLA compared to rats fed the CON diet (Table 3). Because mRNA levels of SCD-1 were dramatically decreased by dietary CLA, we examined the index of SCD-1 activity. The SCD-1 index was quantified by determining the amount of conversion of saturated (18:0 and 16:0) fatty acids to monounsaturated (18:1 and 16:1) fatty acids. The ratios of palmitoleic acid to palmitic acid (16:1/16:0) and oleic acid to stearic acid (18:1/18:0) serve as surrogate markers for SCD-1 activity as these fatty acids are the predominant substrates for SCD-1 enzyme [24]. Liver

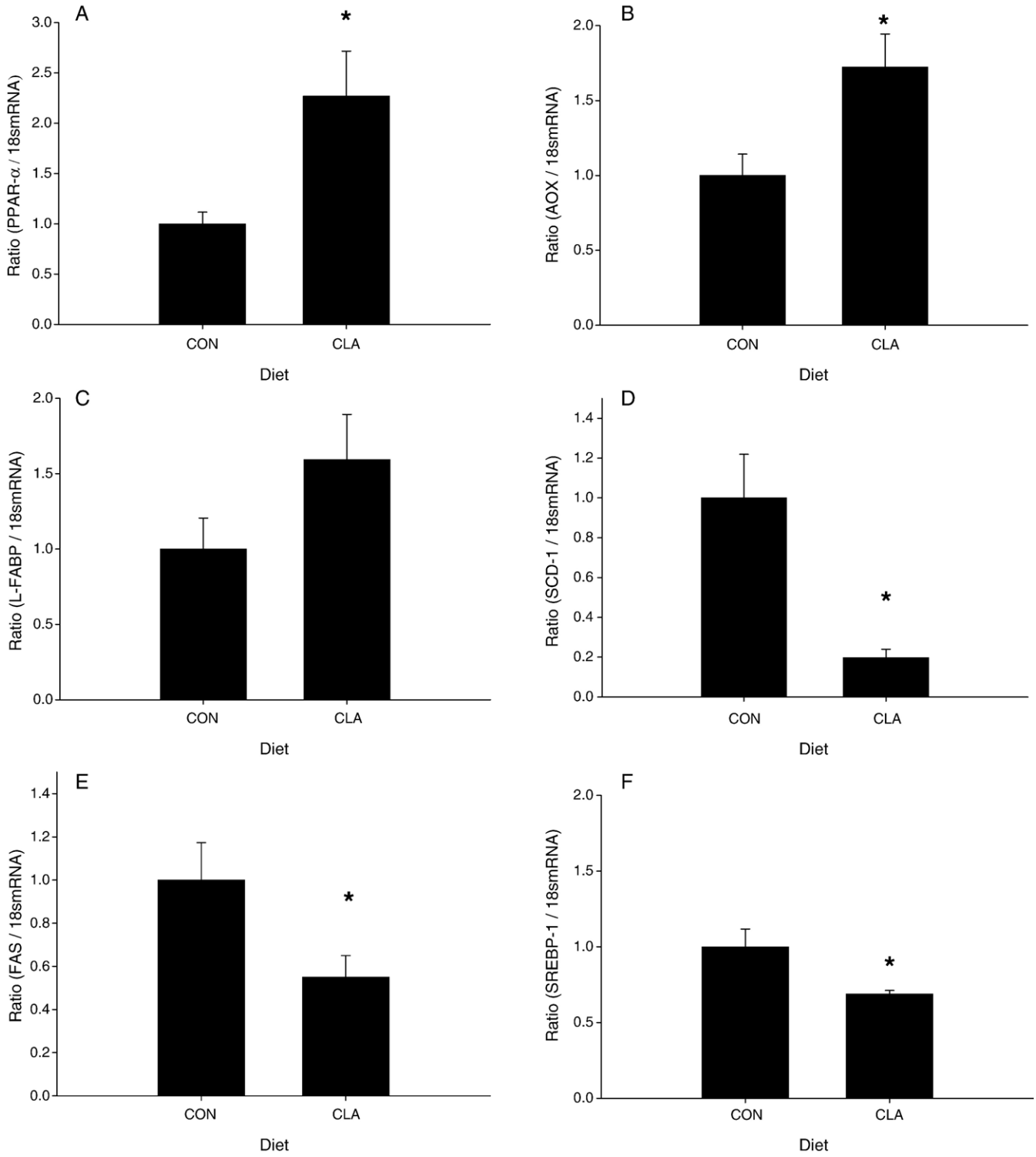

Fig. 2. Effect of CLA on hepatic mRNA expression of genes involved in lipid metabolism. Rats were fed a high-fat diet (20% fat by weight) for 4 weeks followed by switching to low-fat (6.5% fat) experimental diets for additional 4 weeks. The diets contained either 6.5% soybean oil (CON diet) or 5% soybean oil with 1.5% CLA triglyceride mix (CLA diet). (A) PPAR-α mRNA; (B) AOX mRNA; (C) L-FABP mRNA; (D) SCD-1 mRNA; (E) FAS mRNA; (F) SREBP-1 mRNA. Values represent mean±S.E. of eight rats per group; * represents significant differences between groups, $P<.05$.

SCD-1 indices for the different fatty acids in the two pools of fatty acids are shown in Fig. 3. In comparison with rats fed CON diet, rats that were fed the diet containing CLA had significantly lower SCD-1 indices in both lipid fractions for the two fatty acids. Supplementation with CLA resulted in approximately 40% and 26% reduction in the SCD-1 index for neutral and phospholipid fractions, respectively. This suggests that CLA lowered liver TG not

Table 3
Liver fatty acid composition

Fatty acid (%)	Liver NL			Liver PL		
	CON	CLA	*P*	CON	CLA	*P*
16:0	26.38±0.78	26.54±0.63	.872	17.95±0.54	16.63±0.19	.061
16:1	6.50±0.74	3.91±0.43	.013*	1.37±0.16	0.80±0.07	.009*
18:0	2.33±0.19	3.15±0.19	.011*	20.32±0.83	24.54±0.58	.002*
18:1 n-9	27.65±0.76	26.58±1.11	.445	3.64±0.11	3.26±0.17	.098
18:1 n-7	5.47±0.47	4.62±0.37	.182	4.85±0.38	3.36±0.11	.004*
18:2n-6	26.45±1.25	24.35±1.64	.332	13.45±0.48	11.59±0.71	.056
c9t11 CLA	1.82±1.04	6.54±1.86	.000*	0.13±0.01	0.44±0.04	.000*
t10c12 CLA	0.00±0.00	1.04±0.05	.000*	0.00±0.00	0.30±0.03	.000*
20:4	2.68±0.22	2.53±0.37	.719	29.79±0.39	29.63±0.39	.780
22:6	0.74±0.21	0.75±0.23	.968	8.51±0.34	9.46±0.39	.096

Values represent mean±S.E. of eight rats per group.
* Significantly different from CON group, $P<.05$.

only by decreasing mRNA levels but also by decreasing activity as suggested by the SCD-1 indices.

4. Discussion

The impact of dietary CLA on hepatic steatosis and insulin sensitivity is controversial due to differences in species, isomer responses and degree and sensitivity to changes in adiposity [6,9,16,25]. Here, we investigated the effects of dietary CLA on attenuation of hepatic steatosis in male Wistar rats, a rodent model for hepatic steatosis that is less responsive to changes in adiposity induced by CLA [13]. Rats were fed a high-fat diet for 4 weeks prior to CLA supplementation (as part of a low-fat diet) to induce hepatic

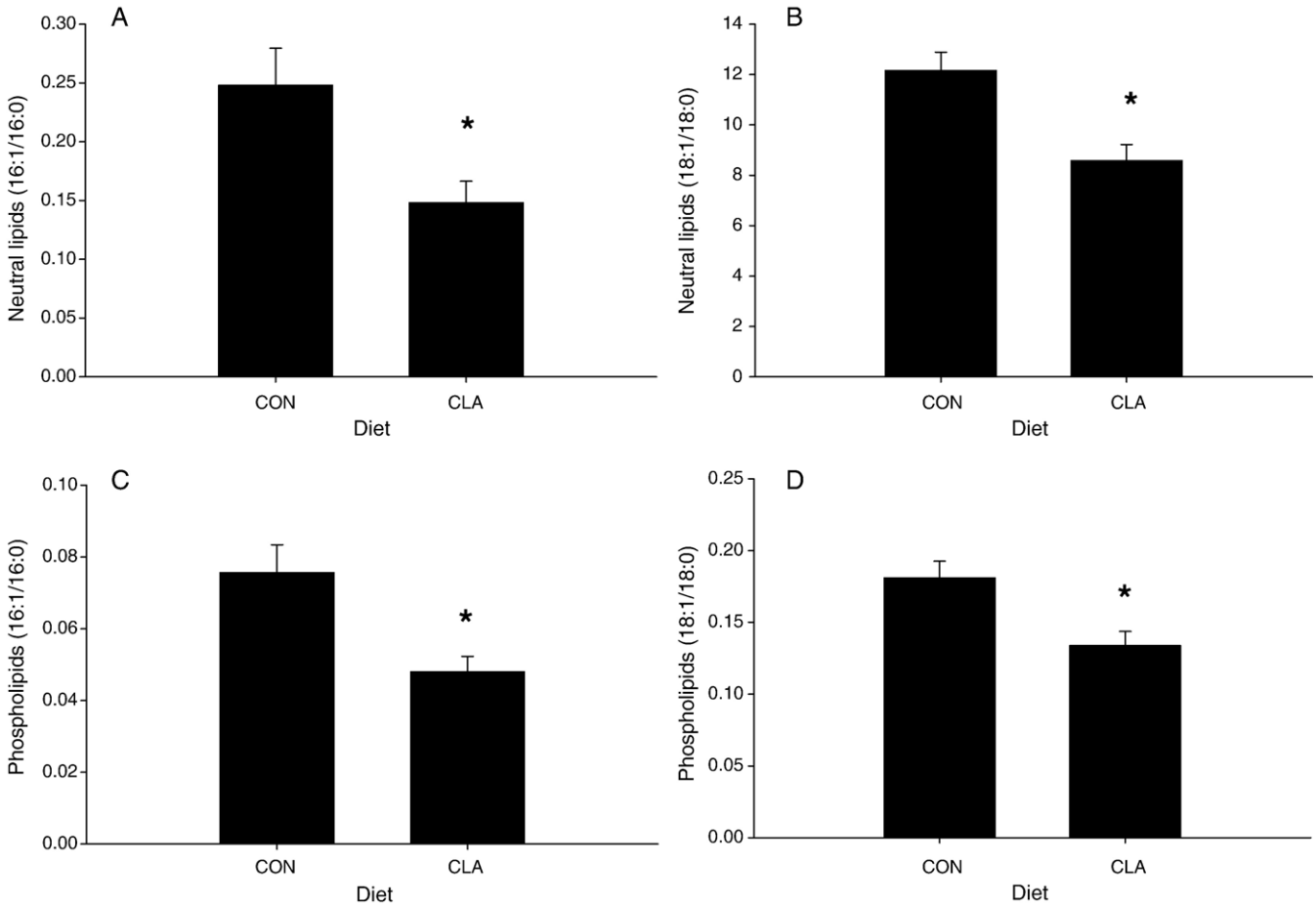

Fig. 3. Effect of CLA on hepatic SCD-1 index. Liver SCD-1 index was measured by measuring the SCD-1 index in neutral lipid and phospholipid fractions using gas chromatography. SCD-1 index was calculated for the two predominant substrates, palmitate and stearate. (A–B) Neutral lipid fraction. (C–D) Phospholipid fraction. Values represent mean±S.E. of eight rats per group; * represents significant differences between groups, $P<.05$.

steatosis [11,12]. While rats did not lose body weight when switched to low-fat experimental diets, feeding CLA as part of a low-fat diet to adult Wistar rats for 4 weeks significantly decreased hepatic lipid accumulation compared to rats fed low-fat diet without CLA (CON diet). Furthermore, these changes in hepatic TG were observed in the absence of changes in body weight, epididymal and peri-renal adipose tissue or food intake. These results may be in accordance with a previous study in Wistar rats where CLA supplementation increased adipose tissue turnover without altering adipose mass [13]. While rats fed the CON diet developed hepatic steatosis (5.7%), which is defined as accumulation of lipids >5% of liver weight [26], the livers of rats fed dietary CLA were not steatotic (4.6%). Our results using this diet-induced model of fatty liver are in accordance with other studies showing similar effects in the livers of genetically obese rat models [6,7,9].

In contrast to studies showing changes in serum lipid concentrations [10,16] and muscle in ZDF rats [10], we did not observe any significant differences in muscle and serum TG or NEFA with feeding CLA to Wistar rats. The absence of changes in serum TG and NEFA in the present study indicates that the reduction of hepatic steatosis by dietary CLA could be due to the action of CLA to modulate lipid metabolism in the liver. Prior studies using various rat models have shown that CLA supplementation is associated with alterations in more than a particular fat depot [9,10,27]. In the present study, the reduction in hepatic TG by CLA occurred in the absence of changes in weight and at least two depots of adipose mass: epididymal and peri-renal adipose mass. These results suggest that, in the present study, the attenuation of liver TG was not a consequence of overall improvement in adiposity.

CLA regulates lipid metabolism in various tissues by modulating lipid oxidation [6,14,28], lipolysis [4,6] and de novo lipogenesis [22,29,30]. We measured PPAR-α responsive genes in the liver (e.g., L-FABP, CPT-1 and AOX-1) as a possible mechanism contributing to lower lipids in the livers of rats fed the CLA diet. In Wistar rats, CLA significantly increased the mRNA levels of PPAR-α and AOX, suggesting increased peroxisomal oxidation. These results are in accordance with data suggesting that reduced expression of PPAR-α and AOX, a key enzyme which regulates peroxisomal β-oxidation, is associated with development of NAFLD in OLETF rats [31]. Furthermore, our data showing unchanged mRNA levels of CPT-I and CPT-II, key enzymes involved in mitochondrial β-oxidation, support findings by others using human fibroblasts, which demonstrate that NAFLD is not associated with reduced mitochondrial β-oxidation [32]. In contrast to these findings, other studies have shown that CLA feeding is associated with unchanged mRNA levels but higher CPT-I activity in obese rats along with improvement in hepatic steatosis [6,9]. The higher expression of lipid oxidation genes in the livers of rats fed dietary CLA was accompanied by significantly lower lipogenic gene FAS mRNA, similar to findings in a recent study conducted in the OLETF rat model using 1% *t*10*c*12 CLA isomer [9]. In the present study, mixed isomers of CLA (providing approximately 0.6% of the *t*10*c*12 CLA isomer) had similar effects on lowering hepatic TG in adult Wistar rats.

One mechanism by which CLA modulates lipid synthesis and accumulation is by decreasing SCD-1 mRNA and activity [29,33,34]. SCD-1 catalyzes the rate-limiting step in the cellular biosynthesis of monounsaturated fatty acids, primarily oleate (18:1) and palmitoleate (16:1) from stearic (18:0) and palmitic (16:0) acids. Oleate and palmitoleate can be incorporated into and stored as TG in the liver [35]. Additionally, inhibition of SCD-1 is associated with increased β-oxidation [36], as demonstrated in SCD-1 knockout mice, which are protected against diet- and leptin deficiency-induced adiposity and hepatic steatosis. In the present study, CLA significantly decreased both mRNA expression and index of hepatic SCD-1 compared to CON animals. Our results are in accordance with studies showing similar effects of CLA on SCD-1 [29,34]. However, the effect of CLA on TG accumulation was not determined in these prior studies. While the effects of CLA on adiposity have been shown to be independent of SCD-1, the *t*10*c*12 CLA isomer failed to promote hepatic steatosis in SCD-1 knockout mice [37], suggesting that some of the effects of CLA on hepatic lipid metabolism are modulated by SCD-1.

In the present study, the hepatic lipid-lowering effect of CLA may be attributed at least in part to suppression of SCD-1 and FAS. The precise mechanism by which CLA regulates SCD-1 is still unknown. However, it has been postulated that CLA inhibits SCD-1 by binding to the active site or to an unidentified allosteric site on the SCD-1 enzyme [33]. Lipogenic genes such as FAS are regulated by SREBP-1. In addition, PUFAs have been shown to modulate SCD-1 by direct inhibition of SREBP-1 transcription by disrupting liver X receptor action [38]. In the present study, we saw a significant suppression of SREBP-1 mRNA by CLA, which may be responsible for the decrease in lipogenic genes observed in this study. We further show that the effects of CLA on SCD-1 in this study were independent of the adipokine leptin, a known SCD-1 suppressor, which was unchanged in rats fed CLA.

Hepatic steatosis is closely associated with decreased sensitivity of the liver to insulin, which may lead to hyperglycemia and hyperinsulinaemia. In the present study, rats fed neither CON nor CLA diet developed significant hyperglycemia. In fact, rats fed a CLA-enriched diet had significantly lower fasting glucose levels but was not accompanied with changes in mRNA levels of glucose metabolizing genes (e.g., PEPCK, G-6-Pase; data not shown). We did not see a change in insulin sensitivity calculated using QUICKI either. We chose to study the effects of CLA in the Wistar rat model, which is known to be sensitive to hepatic steatosis, but is not a typical model to study hyperglycemic conditions such as diabetes and metabolic syndrome.

Hormones secreted from the adipose tissue, such as leptin, adiponectin and tumor necrosis factor-α (TNF-α), have a profound impact on steatosis and insulin sensitivity [22,39–42]. Higher circulating concentrations of adiponectin are inversely associated with insulin resistance and hepatic steatosis by increasing fatty acid oxidation along with reduction of hepatic gluconeogenesis and increased glucose uptake by the skeletal muscle [43]. Previously, it has been shown that CLA feeding attenuates hepatic steatosis and improves hyperglycemia in ZDF rats in association with lower TNF-α mRNA levels and increased adiponectin levels [6]. Studies that report induction of insulin resistance and NAFLD by CLA have shown that CLA causes a sharp decline in circulating levels of adiponectin even before depletion of adipose tissue occurs [23]. In the present study, we saw a modest but not significant increase in serum adiponectin levels with CLA supplementation.

In conclusion, our data suggest that CLA lowers hepatic lipids in adult Wistar rats despite no changes in body weight and epididymal or peri-renal adipose tissue. Therefore, it appears that in Wistar rats, which are induced to be obese by diet (and not genetic manipulation), the effect of CLA on lowering hepatic triglycerides was not likely a result of an overall improvement in adiposity. Furthermore, the effects of CLA in adult Wistar rats occur when adipokines (e.g., leptin and adiponectin) are maintained, suggesting that, in the presence of sufficient adipose and/or adipokines, CLA attenuated hepatic steatosis by modulating hepatic genes involved in lipogenesis and lipid oxidation. The varied effects of CLA on hepatic steatosis and insulin sensitivity in numerous animal models for obesity may depend on differences in fat-to-lean partitioning between adipose and liver tissues.

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