Dietary Conjugated Linoleic Acid (CLA) Attenuates Hepatic Steatosis by Modifying Stearoyl-CoA Desaturase (SCD-1) mRNA and Activity in High-Fat-Fed Rats

Aparna Purushotham, Gayle Shrode, Angela Wendel, Li Fan Liu, Martha Belury

Dept Human Nutrition, The Ohio State University, 1787 Neil Ave, Columbus, OH 43210

Abstract

Non alcoholic hepatic steatosis is associated with obesity and may lead to insulin resistance. Isomers of CLA are naturally occurring dietary compounds with health promoting effects. The role of CLA in attenuating hepatic steatosis and hyperglycemia is complex. Here, we investigated the extent that CLA protects male Wistar rats from developing hepatic steatosis. Rats were fed a 20% fat diet for 4 weeks then changed to either control (CON) diet or CLA diet containing 6.5% soybean oil or 5% soybean oil plus 1.5% CLA respectively. After 4 weeks, CLA diet did not change food intake or body weight however fasting blood glucose (FBG) and hepatic triglycerides (TG) were significantly reduced compared to CON group. Reduced TG levels were associated with significantly lower SCD-1 mRNA levels measured by real time PCR and SCD-1 activity which was determined by gas chromatography. As the rate-limiting enzyme in the cellular biosynthesis of oleate and palmitoleate, SCD-1 activity is indicative of synthesis of components of TG, phospholipids and cholesterol. The effects of CLA on SCD-1 were independent of the adipocytokine, leptin, which was unchanged in rats fed CLA. Because leptin is known to suppress SCD-1, these data suggest CLA acts in part as a leptin mimetic. The mRNA levels of gluconeogenic enzymes were unchanged in the CLA fed group. Thus, the lower hepatic lipid accumulation may be modulated by SCD-1 independent of changes on adipose or leptin levels. We anticipate these hepatic effects explain the improvement of glucose levels in Wistar rats fed CLA. This work was supported by the USDA..

Introduction

Non Alcoholic Fatty Liver Disease (NAFLD)

- Occurs in 75% people with obesity and diabetes mellitus (1)
- Characterized by elevated free fatty acids in blood and blood glucose
- Risk factor for development of type 2 diabetes (2)

Mechanism of hepatic triglyceride accumulation

- Increased influx of lipid into liver
- Increased lipogenesis mediated by sterol regulatory element binding protein (SREBP-1) (3)
- Decreased expression of lipid oxidation genes (3)

CLA and lipid metabolism

- Naturally occurring fatty acid found in ruminant sources e.g.. Meat, milk and dairy products
- Increases lipid oxidation by binding to and activating PPAR-α (4)
- Available commercially in an oil form (Tonalin™) and marketed mainly as a weight loss supplement
- Decreases hyperglycemia and hepatic steatosis in genetically obese rat models (Zucker fa/fa and Zucker diabetic) (5)

Controversy

- CLA increases hepatic steatosis in mice by rapid mobilization of adipose tissue and depletion of adipokines
- Effects of CLA to improve insulin sensitivity and hepatic steatosis in rats have been shown using overly obese models where greater adiposity may be protective against depletion of adipokines
- Improvements in hepatic steatosis and insulin resistance by CLA may be secondary to decrease in adiposity

Objective

To evaluate the effects of CLA on development of hepatic steatosis in a diet-induced obesity model using Wistar rats.

Materials and Methods

•Animals and experimental design: Four week old male Wistar rats were obtained from Charles River Laboratories, Inc. (Wilmington, MA) and were housed 2/cage at 22 ºC +/- 0.5 ºC on a 12hr day/night cycle. Rats received standard rat chow for two weeks while adjusting to their new environment. Six week old rats were fed *ad libitum* a high energy diet consisting of 20% fat (predominantly lard), 42% carbohydrates and 21% protein (by weight) for four weeks prior to assignment to the experimental diets to induce significant gains in body weight and fat mass. Wistar rats are a useful model to study diet-induced hepatic steatosis without overt obesity.

After four 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. CLA composition in our diet was a 50:50 mixture of the two CLA isomers (39.2% c9t11 and 38.5% t10c12 CLA) All diets were modified forms of the AIN-93G diet. All rats had free access to food and water. Food intake was measured every other day and body weights were measured weekly.

•Necropsy: After four weeks on high fat diet and four weeks on experimental diets, animals were euthanized (after 6hr of fasting) by CO_2 and blood, liver, epididymal adipose, and gastrocnemius muscle tissues were weighed, snap-frozen in liquid nitrogen, and stored at -80ºC for further analysis. Serum adiponectin, leptin and insulin were determined using ELISA (Linco Research, St.Charles, MO)

•Tissue TG analysis: TG from liver and muscle were quantitatively determined using free glycerol determination kit (Sigma). In short, approximately 25mg tissue was homogenized and lysed in RIPA buffer (Santa Cruz Biochemicals). Protein was determined using BCA assay (Pierce). TG were extracted with 2:1(v:v) chloroform:methanol, dissolved in isopropanol using Folch method (6). Values were normalized to tissue protein content.

•Liver fatty acid composition: Lipids were extracted from livers using the Folch method (6). Briefly, 200 mg of 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 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 (7). Fatty acids were methylated by incubation with tetramethylguanidine and 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 Inc, Pleasant Gap, PA), quantified by determining areas under identified peaks (ChemStation Software; Packard Instrument Company, Meriden, CT), and adjusted to the internal control.

•Real-time RT PCR: 10-15mg liver tissue was homogenized in Trizol reagent (Sigma) and mRNA was isolated using manufacturer's protocol. The first transcripts were reverse transcribed using reverse transcriptase (Invitrogen) and cDNA were amplified using real-time PCR with TaqMan gene expression assays (Applied Biosystems, CA). In short, 10ng of the reverse transcription reaction was amplified in a total reaction volume of 25ul using primers designed for SCD-1, fatty acid synthase (FAS) liver fatty acid binding protein (L-FABP), acetyl CoA oxidase (AOX) and SREBP-1 using universal cycling conditions (Applied Biosystems, CA). Target gene expression was normalized to18s, which was used as an endogenous control.

•Statistical analysis: All data are presented as mean ± SE. Data were analyzed using MINITAB (version 14, PA). Differences between means were analyzed using Student's Student's *t* test.

Results

CLA Does Not Suppress Weight, Adiposity or Adipokines in Wistar Rats

Table 1 Body weights, organ weights and food intake

Table 1.	CON	CLA
Initial body weight (g)	430.6 ± 4.4	430.9 ± 7.7
Final body weight (g)	500.8 ± 5.7	496.6 ± 8.7
Change in weight (g)	70.2 ± 3.1	65.7 ± 3.1
Food intake (g/rat/day)	25.05 ± 0.13	23.95 ± 0.87
Liver weight (g)**	16.71 ± 0.63	16.59 ± 0.56
Epididymal adipose tissue (g)**	7.79 ± 0.59	7.33 ± 1.0

Table 2 Serum metabolites

Table 2	CON	CLA
Fasting Glucose (mg/dl)	64.89 ± 1.39	60.14 ± 1.68[a]
Leptin (pg/ml)**	1153 ± 92	1313 ± 253
Insulin (pg/ml)**	515 ± 93	406.77 ± 89
Adiponectin (ng/ml)**	8777.4 ± 666.22	10536.8 ± 205.63
NEFA**	0.712 ± 0.104	0.7 ± 0.102

Values are mean ± SE. [a] Significantly different from CON animals, p<0.05. Values represent mean ± SE of 16 rats per group. ** represent n=8rats per group

CLA Decreases Hepatic Triglycerides

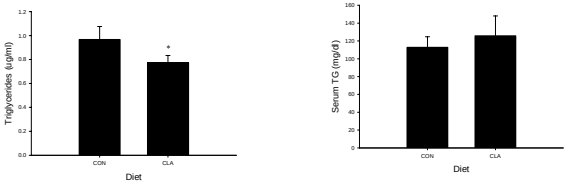

Figure 1: CLA lowers hepatic but not serum TG levels. Sections of liver were used for tissue TG analysis and lipids were extracted using 2:1 chloroform methanol as described in the methods section. Extracted TG were analyzed using TG reagent kit. TG were normalized to the total protein content. ***A:*** Hepatic TG accumulation ***B:*** Serum TG levels. Values represent mean ±SE of 8 rats per group. * represent significant differences between groups, p<0.05.

CLA inhibits gene products indicative of lipogenesis

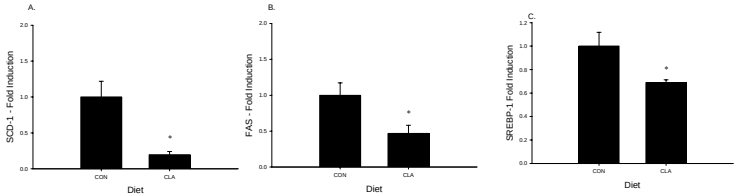

Figure 2: CLA feeding decreased mRNA expression of lipogenic genes stearoyl Co A desaturase (SCD-1) and fatty acid synthase (FAS) in the liver by modulating sterol regulatory element binding protein (SREBP-1) expression. Gene expression was measured using real time PCR and data were normalized to 18s which was used as an endogenous control. ***A:*** SCD-1 mRNA levels were decreased in the liver by approximately 80% in CLA fed rats; ***B:*** FAS mRNA was approximately 50% lower with CLA feeding; ***C:*** SREBP-1 mRNA levels were significantly lower with CLA feeding. Data are expressed as fold change, * represent significant differences between groups, p<0.05. Values represent mean ± SE of 8 rats per group.

CLA Increases Gene Products Indicative of Lipid Oxidation

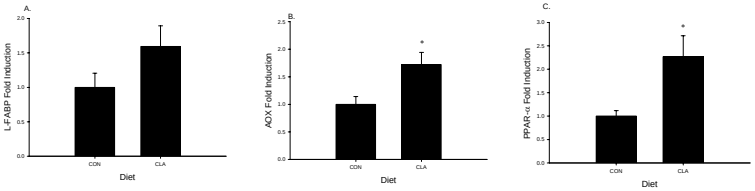

Figure 3: mRNA expression of lipid oxidation genes from CON and CLA fed rat livers. CLA Feeding significantly increased PPAR-α mRNA and its downstream responsive gene acetyl Co A oxidase (AOX). ***A:*** liver fatty acid binding protein (L-FABP) mRNA expression; ***B:*** AOX mRNA expression; ***C***: PPAR- α mRNA expression. Data are expressed as fold change, * represent significant differences between groups, p<0.05. Values represent mean ± SE of 8 rats per group.

CLA Decreases Hepatic SCD-1 Activity

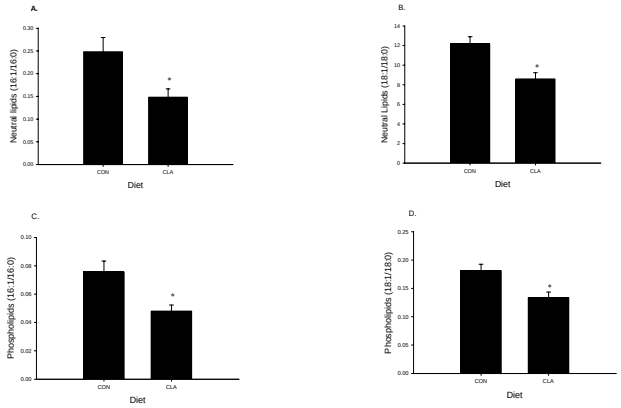

Figure 4: Liver SCD-1 activity was measured by measuring the SCD-1 index in neutral and phospholipid fractions using gas chromatography. SCD-1 index was calculated for the two predominant substrates palmitate and stearate. ***A-B***: Neutral lipids fraction. ***C-D:*** Phospholipid fraction. Significant differences are indicated by *, p<0.05. Values represent mean ± SE of 8 rats per group.

Summary

- CLA decreases hepatic TG significantly in Wistar rats, a diet induced model to study hepatic steatosis
- The lower hepatic TG by CLA may be attributed to lower expression of lipogenic genes, FAS and SCD-1 mRNA regulated by SREBP-1, a key transcription factor that modulates hepatic lipogenesis.
- The lower lipogenesis by CLA was associated with increased expression of lipid oxidation genes AOX and PPAR-α
- The effects of CLA on lower hepatic lipids were not associated with lower adiposity or weight in Wistar rats suggesting that effects of CLA are not due to an overall improvement in adiposity.
- We conclude that when CLA does not induce fat-lean partitioning, CLA reduces hepatic TG accumulation in the presence of adequate adiposity and adipokines.

References

1. Angulo, P. (2002) *N. Engl. J. Med.* **346,** 1221–1231
2. Samuel, V.T. et al. (2004) *J. Biol. Chem.* **279**:32345-32353
3. Sampath, H., Natmbi, J.M . (2006) *Curr Opin Clin Nutr Metab Care.* **9**, (2):84-8
4. Moya-Camarena, S. Y., et. al. (1999) *J Lipid Res.*. **40,** 1426–1433.
5. Koji Nagao et al. (2005) *J. Nutr*. **135**, 9-13
6. Folch J. et al. (1957) *J Biol Chem* **226**, 497-509
7. Hamilton JG and Comai K. (1988) *Lipids* **23**, 1146-1149.