Conjugated linoleic acid increases Ucp1 and activated AMPK in adipose tissue

Angela A. Wendel, Aparna Purushotham, Li-Fen Liu, and Martha A. Belury.
Dept. Human Nutrition, The Ohio State University, Columbus, OH 43210

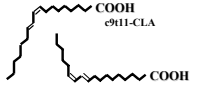
Abstract

Conjugated linoleic acid (CLA) is a group of polyunsaturated fatty acid isomers found naturally in ruminant products such as milk and beef. CLA reduces body weight and adipose mass in a variety of species. However, the mechanism by which CLA depletes adipose mass is unclear. Therefore, the objective of this study is to determine how CLA reduces adipose mass. 6-week old, male ob/ob mice were fed either a control diet (CON) or a diet supplemented with 1.5% mixed isomer CLA (CLA) for 4 weeks. CLA significantly reduced body weight (28.98 g vs. 42.20 g) and epididymal adipose mass (1.71 g vs. 2.96 g) compared to CON. While CLA generally induces hepatic steatosis in mice, which may account for some of the fat mobilization, here, neither liver nor muscle triglycerides were affected by CLA. Similarly, no markers of lipid oxidation, such as *Cpt-1, Ppara*, or *Acox1* mRNA, were altered by CLA in the liver or muscle. Notably, these markers of lipid oxidation were not altered or reduced by CLA in adipose tissue. CLA did, however, increase activation of AMP-kinase (AMPK), an energy sensor that, when activated, increases β-oxidation. Interestingly, mRNA expression of *Ucp1*, a mitochondrial protein involved in energy dissipation, was increased ~109-fold by CLA. Concomitantly, CLA increased mRNA expression of *Pgc1a*, a cofactor important in the upregulation of *Ucp1* and mitochondrial biogenesis, nearly 2.5-fold. These data suggest that CLA may reduce adipose mass by increasing β-oxidation through increased activation of AMPK and increased energy dissipation by *Ucp1* in adipose.

Introduction

Conjugated linoleic acid (CLA) (rev. in 1)

- Positional and stereo-isomers of octadecadienoic acid (18:2)
- Found naturally in foods derived from ruminants
- Reduces adipose mass in a variety of species
  - mediated by the *t10c12* isomer
- Commercially marketed as a weight loss supplement (eg. Tonalin™)



- Mechanism by which CLA reduces adipose mass is not completely understood

Uncoupling protein 1 (Ucp1) (rev. in 2)

- Uncouples mitochondria creating an energy leak that "pulls" fatty acids through the mitochondria
- Peroxisome proliferator-activated receptor gamma coativator 1alpha (PGC-1alpha) increases Ucp1 transcription

AMP-activated protein kinase (AMPK) (rev. in 3)

- Increases PGC1a and Ucp1 expression
  - Increased energy expenditure
- Increases fatty acid oxidation:
  - P-AMPK:
  - Phosphorylates acetyl-coA carboxylase (ACC) inactivating the enzyme
    - ACC: rate limiting step in malonyl-CoA synthesis
  - Decreased malonyl-CoA relieves inhibition of Cpt1
    - Cpt1: rate limiting step in B-oxidation
  - Increased B-oxidation

Objective

Determine mechanisms by which CLA reduces adipose mass in mice

Methods

Experimental design. 6-week old, male B6.V-Lep*ob*/OlaHsd (ob/ob) mice were obtained through Harlan (Indianapolis, IN) and housed 4/cage at 22ºC ± 0.5oC on a 12-hour light/dark cycle. Mice were maintained on isocaloric, modified AIN-93G diets (Bio-Serv, Frenchtown, NJ) containing 6.5% fat. Mice received diets that contained either 6.5% soybean oil (CON; n=8) or 5% soybean oil and 1.5% CLA mixed triglycerides (CLA; n=8). CLA mixed triglycerides (Tonalin TG 80, Cognis Corp., Cincinnati, OH) were ~80% CLA composed of 39.2% c9t11- and 38.5% t10c12-CLA isomers. At 4 weeks, after an overnight (12 hr) fast, mice were anesthetized with isoflurane and blood was collected via cardiac puncture. Tissues were quickly harvested, weighed, snap-frozen with liquid nitrogen, and stored at -80ºC until analyses. All procedures were in accordance with institution guidelines and approved by the Institutional Animal Care and Use Committee of The Ohio State University.

Analysis of triglycerides and free fatty acids. Serum free fatty acids were determined using a colorimetric kit (NEFA C, Wako Chemicals, Richmond, VA). Lipids were extracted from tissues with 2:1 (v/v) chloroform and methanol. Final extracts were solublized in 3:1:1 (v/v/v) tert-butanol, methanol, Tritton X-100 (4). Tissue lipid extracts and serum were analyzed for triglycerides or glycerol by colorimetric enzymatic hydrolysis (Triglyceride, Free-Glycerol reagents, Sigma, St. Louis, MO).

Real-time RT-PCR. RNA was extracted from epididymal adipose tissue using the RNeasy® Lipid Tissue Mini kit (Qiagen, Valencia, CA) and from liver using Trizol (Invitrogen, Carlsbad, CA) according to manufacturers' protocols. RNA was reverse transcribed with High Capacity cDNA Archive Kit (ABI, Foster City, CA) according to directions and then amplified by real-time PCR using pre-designed and validated primers (FAM probes) under universal cycling conditions defined by ABI (TaqMan Gene Expression Assays, ABI, Foster City, CA). Target gene expression was normalized to the endogenous control 18s (VIC probe) amplified in the same reaction and expressed as $2^{-\Delta\Delta ct}$ relative to the CON group (5).

Western blot analysis. Adipose tissue was homogenized in 3 volumes of ice cold lysis buffer (20 mM Trizma base, 50mM NaCl, 250 mM sucrose, 50 mM NaF, 5 mM Na4P2O7 10H2O, 1% Triton-X100, and protease inhibitors). Protein concentrations were measured by the BCA method (Pierce, Rockford, IL). Protein (40 µg) was mixed and boiled for 5 min with 4X loading buffer (125 mM Tris HCl at pH 6.8, 50% glycerol, 4% SDS, 0.02% Bromophenol Blue) and β-mercapatoethanol and subjected to SDS-PAGE using 10% gels for 1 h. Protein was then transferred to nitrocellulose membranes for 1 h on ice. Membranes were probed for P-AMPK (Cell Signaling Technology, Inc., Danvers, MA) according to manufacturer's protocol. Membranes were incubated in SuperSignal West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL) for 5 min. Densities of bands were detected and measured by Kodak ImageStation 2000RT using 1D Kodak software. Membranes were stripped with RestoreTM Western Blot Stripping Buffer (Pierce, Rockford, IL) for 30 min at RT and reprobed with total-AMPK (Cell Signaling).

Statistical analyses. Data are expressed as means ± standard error (SE). Effects of diet were analyzed by 2-sample t-test (Minitab v.14, State College, PA). Differences of P<0.05 were considered significant.

Results

CLA reduced body and adipose weights, but did not divert lipid to other tissues

Table 1. Effects of CLA on body and tissue weights and tissue triglycerides.

	CON	CLA
Final body weight (g)	42.20 ± 1.36	28.98 ± 1.00*
Adipose (g)	2.96 ± 0.17	1.71 ± 0.14*
Liver (g)	2.76 ± 0.18	3.18 ± 0.28
Liver triglycerides (mg TG/ g tissue)	172.91 ± 18.64	173.31 ± 11.2
Gastrocnemius muscle (g)	81.40 ± 7.90	69.08 ± 8.60
Muscle triglycerides (mg TG/ g tissue)	52.52 ± 7.11	39.4 ± 5.20

* Values represent means ± SE with significant differences from CON (p<0.05) denoted by *

CLA did not increase markers of lipolysis

Table 2. Effects of CLA on markers of lipolysis.

	CON	CLA
Serum triglyceride (mg/dl)	88.13 ± 11.71	131.42 ± 16.86
Serum NEFA (mEq/L)	0.90 ± 0.07	1.03 ± 0.11
Serum glycerol (mg/dl)	56.96 ± 9.74	36.86 ± 4.48
Adipose HSL mRNA expression	1.07 ± 0.11	0.60 ± 0.03*
Adipose ATGL mRNA expression	1.05 ± 0.11	1.03 ± 0.06

* Values represent means ± SE with significant differences from CON (p<0.05) denoted by *

CLA increased FA transport into adipose, but did not alter mRNA markers of oxidation or lipogenesis

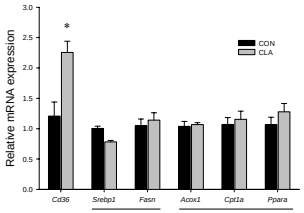


Fig. 1. CLA does not alter mRNA markers of oxidation or lipogenesis in adipose. Mice were fed either control (CON) or CLA-supplemented (CLA) diets. After 4 weeks, mRNA was measured from adipose of fasted mice by real time RT-PCR. Values represent means ± SE with significant differences from CON (p<0.05) denoted by *

CLA increased Ucp1 and PGC1α mRNA expression in adipose

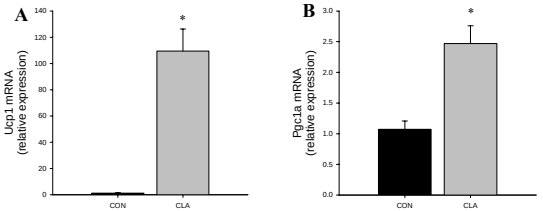


Fig. 2. CLA increased Ucp1 and PGC1α expression in adipose. Mice were fed either control (CON) or CLA-supplemented (CLA) diets. After 4 weeks, **A.** Ucp1 or **B.** Pgc1α mRNA was measured from adipose of fasted mice by real time RT-PCR. Values represent means ± SE with significant differences from CON (p<0.05) denoted by *

CLA increased activated AMPK in adipose

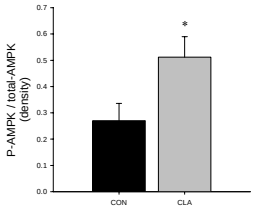


Fig. 3. CLA increases activated (phosporylated) AMPK in adipose. Mice were fed either control (CON) or CLA-supplemented (CLA) diets. After 4 weeks, P-AMPK and total-AMPK were measured from adipose of fasted mice by Western blot analysis. Values represent means ± SE with significant differences from CON (p<0.05) denoted by *

Conclusions

CLA may reduce adipose mass through increased energy dissipation (Ucp1 & Pgc1α) and increased activation of AMPK, which would relieve inhibition of Cpt1, thus increasing B-oxidation in adipose.

Research to determine the mechanism by which CLA increases Ucp1, Pgc1α, and P-AMPK are currently being investigated.

References

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