Leptin Prevents Insulin Resistance Induced by Conjugated Linoleic Acid in Obese Mice

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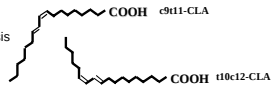
Abstract

Conjugated linoleic acid (CLA) reduces adipose mass and enhances insulin sensitivity in several animal models. Conversely, in some rodent models, CLA induces lipodystrophy, insulin resistance, and reduces adipokines. Leptin is an insulin sensitizing adipokine that may work by suppressing steatosis in liver and muscle, a condition that may contribute to insulin resistance. Therefore, we hypothesize that leptin prevents CLA-induced insulin resistance in obese mice by attenuating steatosis. In a 2x2 factorial design, 6-week old, male ob/ob mice were fed either a control diet or a diet supplemented with 1.5% mixed isomer CLA and received daily intraperitoneal injections of either PBS or 1.0 mg/kg leptin for 4 weeks. CLA and leptin alone or in combination decreased weight gain, which was reflected by a reduction of epididymal fat mass. At 2 and 4 weeks of feeding, leptin significantly attenuated CLA-induced increased fasting glucose, and at 4 weeks, leptin prevented CLA-induced insulin resistance. Although CLA alone significantly increased fasting insulin, leptin reduced fasting insulin levels in both diet groups. CLA significantly reduced serum adiponectin, regardless of leptin treatment. Liver and muscle triglycerides (TG) were not altered by CLA alone; however, leptin reduced liver and muscle TG in both diet groups. Fatty acid synthase mRNA, a marker of lipid synthesis was decreased by leptin, regardless of diet, but CPT-1, a marker of lipid oxidation, was not changed. These data suggest that restoration of insulin sensitivity by leptin may partially be attributed to the reduction of hepatic steatosis and by compensating for the reduction of adiponectin.

Introduction

Conjugated Linoleic Acid (CLA) (rev. in 1)

- Reduces adipose mass in a variety of species
- Controversial, species-specific effects on metabolism and insulin resistance
  - •In obese rats:
    - •Improves glucose and insulin tolerance
    - •Maintains or increases adipocytokines
    - •Loss of adipose perhaps not as severe as in mice
  - •In mice:
    - •Reduces adipocytokines
    - •Induces lipdystrophy and liver steatosis
    - •Induces insulin resistance



- Modulation of insulin sensitivity possibly dependent on maintenance of certain level of adipose and therefore adipocytokines

Leptin (rev. in 2)

- 16 kDa hormone produced by adipocytes
- Circulates at levels proportional to adipose tissue
- Appetite suppressant
  - •Metabolic effects largely independent of food intake
- Regulates energy homeostasis
- Leptin deficiency results in accumulation of lipid in tissues, such as adipose and liver
  - •Leads to disorders such as obesity, insulin resistance, and non-alcoholic fatty liver disease (NAFLD)

B6.V-Lep^{ob}/OlaHsd (ob/ob) mice

- Leptin (lep-/lep-) mutation
- Obese
- Hyperinsulinemic
- Insulin resistant
- Hyperlipemic
- Hepatic steatosis
- Adipocyte hyperplasia

Objective

Determine the individual and interactive effects of CLA and leptin on insulin resistance and lipid metabolism in ob/ob mice.

Materials & Methods

Experimental animals and diets. 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. Diets contained either 6.5% soybean oil (CON) or 5% soybean oil and 1.5% CLA mixed triglycerides (CLA). CLA mixed triglycerides (Tonalin TG 80, Cognis Corp., Cincinnati, OH) were ~80% CLA composed of 39.2% c9t11- and 38.5% t10c12-CLA isomers.

Experimental design. In a 2x2 factorial design, mice were fed either the CON or CLA diet and received daily injections intraperitoneally of either 1 mg/kg BW recombinant mouse leptin (R&D Systems, Minneapolis, MN) (CON + or CLA +) or a similar volume of the vehicle PBS (CON - or CLA -) for 4 weeks (n=8 mice per diet +/- leptin group). Mice were injected daily 2 hours before the dark cycle.

Fasting glucose and insulin tolerance test. Glucose levels were measured after a 12 hr fast at baseline and at 2 and 4 weeks of experimental treatments via tail blood using a One Touch Basic glucometer (Lifescan, Milpitas, CA). An insulin tolerance test was conducted after 4 weeks of dietary and leptin intervention after an overnight (12 hr) fast. Mice were injected intraperitoneally with 1.5 U/kg BW insulin (Humulin® R, Eli Lilly and Co., Indianapolis, IN). Tail vein blood was used to measure glucose prior to injection (time 0) and at 15, 30, 45, 60, 90, and 120 minutes following the injection. Area under the curve (AUC) was calculated as the net area contained between individual baselines (set by the glucose value at time 0) and curves using the trapezoidal rule[4].

Insulin and Adiponectin. Fasted serum levels of insulin and adiponectin were determined by ELISA (Linco Research, Inc., St. Charles, MO) according to manufacturer's directions.

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 using the Folch[3] method. Extracts were solublized in 3:1:1 (v/v/v) tert-butanol, methanol, Trition X-100. Serum and tissue lipid extracts were analyzed for triglycerides by colorimetric enzymatic hydrolysis (Triglyceride, GPO-Trinder, reagents, Sigma, St. Louis, MO).

RT-PCR. RNA was isolated from tissue with Trizol (Invitrogen, Carlsbad, CA) and reverse transcribed with High Capacity cDNA Archive Kit (ABI, Foster City, CA) according to directions. cDNA was amplified by real-time PCR in a total reaction volume of 25 µl with TaqMan Gene Expression Assays (ABI) using pre-designed and validated primers (FAM probes) from ABI under universal cycling conditions defined by ABI. Target gene expression was normalized to the endogenous control 18s (VIC probe) amplified in the same reaction.

Statistical analysis. Data are expressed as least square mean (LSM) ± standard error (SE). Interactions of diet (CON or CLA) and treatment (leptin or vehicle) were analyzed by two-way ANOVA using the MIXED procedure of Statistical Analysis System (SAS v9.1; Cary, NC). Fasting glucoses and glucose curves were analyzed as repeated measures. Differences of P<0.05 were considered significant.

Results

Table 1. Effects of CLA and Leptin on Body Weights and Tissue Sizes

	CON -	CON +	CLA -	CLA +
Final body weight (g)	42.20 ± 0.97^{c}	34.79 ± 0.97^{b}	28.98 ± 0.97^{a}	28.41 ± 0.97^{a}
Liver (g)	2.763 ± 0.173^{b}	1.288 ± 0.173^{a}	3.175 ± 0.173^{b}	1.563 ± 0.173^{a}
Epididymal adipose (g)	2.963 ± 0.136^{c}	2.163 ± 0.136^{b}	1.713 ± 0.136^{a}	1.363 ± 0.136^{a}
Gastrocnemius muscle (g)	81.4 ± 8.88^{a}	112.09 ± 8.88^{b}	69.075 ± 8.88^{a}	101.25 ± 8.88^{b}

* Values represent LSM ± SE with significant differences (p<0.05) denoted by different superscripts within each row

Insulin Tolerance

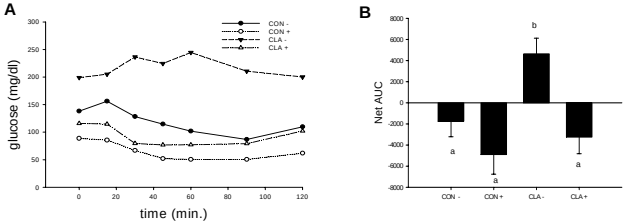


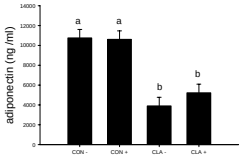
Fig. 1. Leptin prevents CLA-induced insulin resistance. Mice were fed either control (CON) or CLA-supplemented (CLA) diets and received either leptin (+) or vehicle (-) by IP injection daily. After 4 weeks, mice received 1.5 U/kg insulin by IP injection after an overnight fast (12 hr). Blood glucose was measured at intervals indicated over 2 hrs. **A.** Glucose response to insulin tolerance test over time. Values represent LSM of n=8 mice. Statistical differences at each time point were omitted for clarity. **B.** Net area contained within curve of insulin tolerance test. Area was calculated as the net area contained between individual baselines (set by the glucose value at time 0) and curves using the trapezoidal rule. Values represent LSM ± SE with significant differences (p<0.05) denoted by different superscripts.

Table 2. Effects of CLA and Leptin on Fasting Insulin, Glucose, NEFA, and Serum Triglyceride Levels

	CON -	CON +	CLA -	CLA +
Fasting glucose (mg/dl)*				
baseline	115.12 ± 10.38	117.00 ± 10.38	101.25 ± 10.38	107.38 ± 10.38
2 weeks	130.25 ± 10.38	106.25 ± 10.38	161.59 ± 12.10*	102.50 ± 10.38
4 weeks	122.87 ± 10.38	89.50 ± 10.38	153.63 ± 10.38*	98.38 ± 10.38
Fasting insulin (pg/ml)**	3066.6 ± 519.9^{b}	660.2 ± 486.3^{a}	5455.8 ± 519.9^{c}	1452.5 ± 486.3^{a}
Serum NEFA (mEq/L)**	0.9029 ± 0.094	0.8325 ± 0.0875	1.0263 ± 0.0875	0.8250 ± 0.0875
Serum triglyceride (mg/dl)**	88.13 ± 14.42^{a}	122.94 ± 12.49^{ab}	131.45 ± 14.42^{b}	103.23 ± 14.42^{ab}

* Values represent LSM ± SE with significant differences (p<0.05) from baseline denoted by #; baseline values among diet/treatment groups were not significantly different.
** Values represent LSM ± SE with significant differences (p<0.05) denoted by different superscripts within each row.

Adiponectin



Tissue Triglycerides

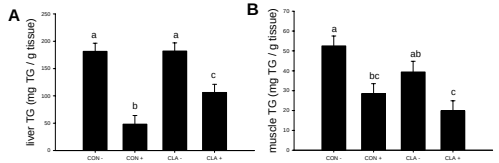


Fig. 2. CLA decreases serum adiponectin. Mice were fed either control (CON) or CLA-supplemented (CLA) diets and received either leptin (+) or vehicle (-) by IP injection daily. After 4 weeks, serum adiponectin was measured by an ELISA from fasted mice. Values represent LSM ± SE of n=8 mice with significant differences (p<0.05) denoted by different superscripts.

Fig. 3. Leptin decreases liver and muscle lipid accumulation. Mice were fed either control (CON) or CLA-supplemented (CLA) diets and received either leptin (+) or vehicle (-) by IP injection daily. After 4 weeks, fasted mice were sacrificed. Triglycerides were measured from lipid extracts of tissues **A.** Triglyceride concentration (mg TG/g tissue) of liver. Values represent LSM ± SE of n=8 mice with significant differences (p<0.05) denoted by different superscripts. **B.** Triglyceride concentration (mg TG/g tissue) of muscle. Values represent LSM ± SE of n=8 mice with significant differences (p<0.05) denoted by different superscripts.

Hepatic Markers of Lipid Synthesis and Oxidation

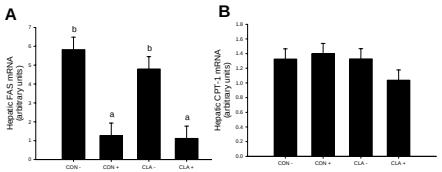


Fig. 4. Leptin decreases fatty acid synthase (FAS), but does not change carnitine palmitoyl transferase-1 (CPT-1) hepatic mRNA expression. Mice were fed either control (CON) or CLA-supplemented (CLA) diets and received either leptin (+) or vehicle (-) by IP injection daily. After 4 weeks, **A.** FAS and **B.** CPT-1 mRNA were measured from livers of fasted mice by RT-PCR. Values represent LSM ± SE of n=8 mice with significant differences (p<0.05) denoted by different superscripts.

Summary

In ob/ob mice:

- •CLA worsened insulin resistance; leptin prevented CLA-induced insulin resistance,
- •CLA increased fasting glucose and insulin, and serum triglycerides; leptin prevented these increases
- •CLA decreased serum adiponectin concentration; administration of leptin had no effect on adiponectin
- •CLA did not increase hepatic steatosis compared to control diet alone
- •Leptin decreased hepatic and muscle triglyceride concentrations; leptin decreased hepatic lipid synthesis, but did not affect hepatic lipid oxidation

These results show that despite ob/ob mice being overtly obese, CLA significantly reduces adipose mass and adiponectin and significantly worsens insulin resistance. Administration of leptin to CLA-fed mice does not alter adipose mass or adiponectin, but prevents insulin resistance. The effects of leptin on insulin resistance in CLA-fed mice may be modulated through the reduction of hepatic steatosis and by compensating for the reduction of adiponectin.

References

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