Combined effects of rosiglitazone and conjugated linoleic acid on adiposity, insulin sensitivity, and hepatic steatosis in high-fat-fed mice

Li-Fen Liu, Aparna Purushotham, Angela A. Wendel, and Martha A. Belury

Department of Human Nutrition, The Ohio State University, Columbus, Ohio

Submitted 9 November 2006; accepted in final form 16 February 2007

Liu L-F, Purushotham A, Wendel AA, Belury MA. Combined effects of rosiglitazone and conjugated linoleic acid on adiposity, insulin sensitivity, and hepatic steatosis in high-fat-fed mice. Am J Physiol Gastrointest Liver Physiol 292: G1671–G1682, 2007. First published February 22, 2007; doi:10.1152/ajpgi.00523.2006.—Dysfunctional cross talk between adipose tissue and liver tissue results in metabolic and inflammatory disorders. As an insulin sensitizer, rosiglitazone (Rosi) improves insulin resistance yet causes increased adipose mass and weight gain in mice and humans. Conjugated linoleic acid (CLA) reduces adipose mass and body weight gain but induces hepatic steatosis in mice. We examined the combined effects of Rosi and CLA on adiposity, insulin sensitivity, and hepatic steatosis in high-fat-fed male C57Bl/6 mice. CLA alone suppressed weight gain and adipose mass but caused hepatic steatosis. Addition of Rosi attenuated CLA-induced insulin resistance and dysregulation of adipocytokines. In adipose, CLA significantly suppressed lipoprotein lipase and fatty acid translocase (FAT/CD36) mRNA, suggesting inhibition of fatty acid uptake into adipose; addition of Rosi completely rescued this effect. In addition, CLA alone increased markers of macrophage infiltration, F4/80, and CD68 mRNA levels, without inducing TNF-α in epididymal adipose tissue. The ratio of Bax to Bcl2, a marker of apoptosis, was significantly increased in adipose of the CLA-alone group and was partially prevented by treatment of Rosi. Immunohistochemistry of F4/80 demonstrates a proinflammatory response induced by CLA in epididymal adipose. In the liver, CLA alone induced microsteatotic liver but surprisingly increased the ratio of very-low-density lipoprotein-triglyceride production without inducing inflammatory mediator-TNF-α and markers of macrophage infiltration. These changes were accompanied by significantly increased mRNA levels of stearoyl-CoA desaturase, FAT/CD36, and fatty acid synthase. The combined administration of CLA and Rosi reduced hepatic liver triglyceride content as well as lipogenic gene expression compared with CLA alone. In summary, dietary CLA prevented weight gain in Rosi-treated mice without attenuating the beneficial effects of Rosi on insulin sensitivity. Rosi ameliorated CLA-induced lipodystrophic disorders that occurred in parallel with rescued expression of adipocytokine and adipocytes-abundant genes.

The pathogenesis of nonalcoholic fatty liver disease (NAFLD) is complex and likely to include the dysregulation of metabolic pathways between adipose, muscle, and liver tissue (15). The accumulation of fat as triglyceride (TG) in the hepatocytes is the first essential step in the development of fatty liver disease (4). The mobilization and transport of free fatty acid flux from adipose to the liver may amplify hepatic lipogenesis, leading to hepatic steatosis (4). Adipose tissues play a crucial role in the fatty acid mobilization and fat storage. In addition to their role in energy storage, adipocytes also produce pro- and anti-inflammatory mediators such as chemocytokines, e.g., TNF-α and IL-6, and adipocytokines, e.g., adiponectin, leptin, and resistin. Dysregulation of adipocytokines in adipose tissue is likely to contribute to inflammatory-mediated metabolic syndrome such as NAFLD (19, 30).

Rosiglitazone (Rosi), a ligand for peroxisome proliferator-activated receptor-γ (PPARγ), is commonly used in the treatment of Type 2 diabetes mellitus (T2DM) (27). As an insulin sensitizer, Rosi improves hyperglycemia and insulin resistance through enhancing adipose and muscle insulin sensitivity in humans and animals (3, 27). A recent study in humans showed that increased hepatic insulin sensitivity with Rosi treatment is associated with a decrease in liver fat content (41). In addition, the decreased hepatic lipid accumulation was associated with increased adiponectin concentrations following Rosi treatment (41). Rosi improves insulin sensitivity; however, it is less effective in the management of obesity in T2DM (22, 32). In fact, Rosi promotes weight gain in animals and humans (7, 25).

The dietary fatty acid t10c12-conjugated linoleic acid (t10c12-CLA) reduces adiposity and suppresses weight gain in animals and humans (13, 28, 33, 42). However, t10c12-CLA induces insulin resistance and hepatic steatosis in mice (9, 12, 34). Supplementation with 0.5% purified t10c12 isomer or 1.5% conjugated linoleic acid (CLA) commercial mixture induces severe liver steatosis and lipodystrophy in mice (32). Development of steatosis in the liver is associated with an increase in lipogenesis, mainly TG synthesis and the TG-enriched very-low-density lipoprotein (VLDL) synthesis (12, 23, 31). Previously, Ide (16) showed that an increase in lipogenesis triggered by hyperinsulinemia is primarily responsible for CLA-induced accumulation of liver TG. Furthermore, it has been demonstrated that CLA induces hyperinsulinemia by a marked reduction of adipocytokines (42). Recently, Poirier and colleagues (35) reported that insulin resistance caused by supplementation of t10c12-CLA in mice is associated with the induction of macrophage infiltration and inflammation in white adipose tissue. Rosi improves insulin resistance yet causes increased adipose mass and weight gain in mice and humans, whereas CLA reduces adipose mass and body weight gain but induces insulin resistance and hepatic steatosis in mice. Complementary use of therapeutic agents such as Rosi and other antidiabetic agent has resulted in improvements in both insulin resistance and adiposity in obese, diabetic mice (43). We hypothesized that supplementation with CLA prevents weight gain in Rosi-treated mice, whereas Rosi attenuates lipodystrophic disorder in CLA-fed mice. Therefore, this study examined the combined effects of CLA and Rosi on adiposity, hepatic steatosis, and insulin sensitivity. Here we...
show that lipodystrophy induced by CLA occurred in parallel with hepatic microsteatosis, dysregulated adipocytokine concentrations, and macrophage infiltration without evidence of inflammation in adipose. The combination of CLA with Rosi (CLA/Rosi) significantly prevented weight gain and improved hepatic steatosis and insulin resistance in association with rescued serum adiponectin concentrations.

**RESEARCH DESIGN AND METHODS**

**Experimental animals and diets.** Four-week-old male C57Bl/6 mice were obtained from Harlan (Indianapolis, IN) and housed 4–5 per cage at 22 ± 0.5°C on a 12:12 h day-night cycle. Mice received standard mouse chow for 2 wk while adjusting to their new environment and then were randomly assigned to four groups (n = 10 per diet +/− Rosi group) and a pair-fed group. Mice were maintained on a high-fat diet containing 23.6% total fat by weight (44% by energy from lard, Research Diets, Metuchen, NJ) fasted 3 wk before receiving experimental diets and treatments. Experimental diets contained 22.1% lard and were supplemented with either 1.5% soybean oil (Con) or 1.5% CLA (Tonalin TG 80, Cognis, Cincinnati, OH) (40). The CLA diet we used was Tonalin TG 80. Body fat loss has been demonstrated previously with Tonalin TG 80 in mice (18). The composition of CLA TGs was 38.5% 10c,12-CLA with remaining oil composed of other CLA isomers and unconjugated linoleic acid. In a 2 × 2 factorial design, mice were used either the Con or CLA diet and received intraperitoneal injections of either 10 mg/kg body wt Rosi daily (Cayman Chemical, Ann Arbor, MI) (Con/Rosi, CLA/Rosi) or a similar volume of the vehicle (PBS) (Con/PBS, CLA/PBS) for 6 wk (n = 10 mice per diet +/− Rosi group). An additional group of mice were pair-fed to the CLA/PBS group because CLA may affect food intake (PF/PBS). Body weight and food intake were measured every other day. After 3 wk on the high-fat diet and 6 wk on experimental diets, animals were anesthetized by isoflurane and blood was collected by heart puncture. Liver and epididymal and inguinal fat pads were isolated, weighed, and immediately snap-frozen in liquid nitrogen. Serum samples were stored −80°C for analyses. All procedures were in accordance with institution guidelines and approved by the Institutional Animal Care and Use Committee of The Ohio State University.

**Insulin tolerance test.** After a 12 h, overnight fast, mice were injected with 0.5 U/kg body wt insulin (Humulin R, Eli Lilly, Indianapolis, IN). Tail vein blood was used to measure glucose immediately before injection (time 0) and at 15, 30, 45, 60, 90, and 120 min following the injection. The insulin tolerance test (ITT) data were quantified as areas under the curve by the trapezoidal method.

**Metabolite measurements.** Fasted (12 h) serum was obtained via retro-orbital bleed in each animal at baseline and by cardiac puncture at the end of the study. Fasted serum insulin, leptin, and adiponectin levels were determined with commercial ELISA kits (Linco Research, St. Charles, MO). Fasted glucose levels were obtained by tail bleeding via a blood glucose monitoring system (One Touch, Lifescan). Serum-free fatty acid (NEFA C, Wako Chemicals, Richmond, VA), TG concentration (Triglyceride Reagent and Free Glycerol Reagent, Sigma, St. Louis, MO), and total cholesterol (Standbio, Boerne, Texas) were determined by enzymatic, colorimetric assays.

**Histology of adipose and liver tissue.** After animals were killed, epididymal adipose tissues were fixed in 4% formaldehyde with glutaraldehyde, while liver tissues were fixed in 4% formaldehyde. Histology of liver and adipose section was performed by Histoscientific Research Laboratories (Mt. Jackson, VA). Sections of liver and adipose tissue were embedded in paraffin and stained with hematoxylin and eosin to detect steatosis and changes in morphology.

In vivo VLDL-TG production assay. After 6 wk on experimental diets and treatments, mice were fasted for 4 h and injected with tyloxapol (Triton-1339; Sigma) an inhibitor of lipoprotein lipase (LPL), inhibiting TG uptake by adipose tissue. As described in Ref. 8, mice were injected via tail vein with 500 mg/kg tyloxapol dissolved in PBS at a concentration of 0.1 g/ml. Blood samples were obtained by tail bleeding at 0, 45, and 90 min following injection. Serum TGs were determined by the enzymatic, colorimetric assays (Triglycercide Reagent and Free Glycerol Reagent, Sigma).

**Liver TG analysis.** In brief, ~100 mg of tissue were homogenized and lysed in tissue lysis buffer. Lipids were extracted with 2:1 (vol/vol) chloroform-methanol and final extracts were dissolved in 3:1:1 (vol/vol/vol) tert-butanol, methanol. Triton X-100 and TG concentrations were measured by enzymatic colorimetric assay. Values were normalized to per gram tissue.

**Immunohistochemistry.** Sections (4 μM) of epididymal adipose tissue were mounted on glass slides, stained with 3,3-diaminobenzidine tetrahydrochloride (Dojindo, Kumamoto, Japan), and counterstained with hematoxylin and processed for immunohistochemical detection of F4/80 according to standard immunoperoxidase procedure. The mouse monoclonal F4/80 antibody was purchased from Serotec (MCA 97R, Raleigh, NC).

**RT-PCR analysis.** Total RNA was extracted from liver tissues with Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. Total RNA from epididymal adipose was isolated by use of an RNeasy Lipid Tissue kit (Qiagen, Valencia, CA). Expressions of mRNA levels were analyzed by quantitative real-time PCR (Prism 7300 sequence detection system, Applied Biosystems, Foster City, CA). Total RNA was reversed transcribed with random hexamers by using MultiScribe reverse transcriptase (Applied Biosystems). After cDNA synthesis, real-time PCR analysis was performed with predesigned primers and probes supplied by Applied Biosystems (TaqMan Gene Expression Assays). Target gene expression was expressed as 2^−ΔΔCt by the comparative Ct method and normalized to the expression of 18S ribosomal RNA, then to Con/PBS group.

**Western blot analysis detection of Bax and Bcl2 protein.** Protein (30 μg) from epididymal adipose lysate was separated on 15% polyacrylamide gel then transferred onto nitrocellulose membranes. Bax, Bcl2, and β-actin levels were determined by blotting with anti-Bax, anti-Bcl2, and anti-β-actin (Cell Signaling Technology, Danvers, MA) primary antibodies. Bands were visualized by chemiluminescence and bands were quantified by use of Kodak Image Station 2000RT (Eastman Kodak, Rochester, NY). Immunoblots were repeated twice (n = 8 mice/group) with one representative set presented.

**Statistical analysis.** Interactions of diet (Con and CLA) and treatment (PBS and Rosi) were analyzed by two-way ANOVA using the general linear model procedure (MINITAB version 14, PA) and Tukey’s method for post hoc analyses. Comparisons of effects of vehicle-treated groups (Con/PBS, CLA/PBS, and pair-fed) were analyzed by one-way ANOVA (MINITAB). Differences of VLDL-TG production rate among groups and times were analyzed by repeated-measures ANOVA (SYSTAT version 11, Systat Software, CA). Pearson’s rank correlation coefficients were used to calculate correlation coefficients between serum adiponectin, insulin, and liver TG content. All data are presented as least square means ± SE. Differences were considered significant at P < 0.05.

**RESULTS**

Effects of CLA, Rosi, and combination on weight gain and insulin sensitivity. We studied the combined effects of CLA and Rosi on insulin resistance and adiposity in high-fat fed C57Bl/6 mice, a diet-induced obesity model. CLA alone (CLA/PBS) and combined with Rosi (CLA/Rosi) significantly prevented weight gain in growing mice (Fig. 1A). However, during the first week after diets were initiated, Rosi significantly increased weight gain in both Con/Rosi and CLA/Rosi diet groups compared with vehicle-treated groups (Fig. 1A).

---

**AJP-Gastrointest Liver Physiol** • VOL 292 • JUNE 2007 • www.ajpgi.org
Beginning on day 21, supplementation of CLA significantly suppressed weight gain in the CLA/PBS and CLA/Rosi groups ($P < 0.05$). The suppressive effect of CLA on weight gain lasted for the remainder of the study. Because CLA has been reported to reduce food intake in rodents (46), a group of mice was pair-fed the Con diet to the CLA/PA group. Body weight gain of pair-fed mice was not significantly different from Con/PBS or CLA/PBS groups (data not shown). Despite the changes in weight gain in the CLA/PBS and CLA/Rosi group, the average food intake was not significantly different among any group, including pair-fed (data not shown). To measure insulin sensitivity in C57BL/6 mice, an ITT was performed in mice after 6 wk (Fig. 1B). Glucose levels during ITT were significantly increased in the CLA/PBS group compared with Con/PBS, Con/Rosi, and CLA/Rosi groups. CLA alone (CLA/ PBS) significantly increased area under the curve, indicating worsened insulin sensitivity. Treatment of Rosi in CLA-fed mice (CLA/Rosi) improved insulin sensitivity comparable to Con/Rosi, and CLA/Rosi groups. The CLA/Rosi group also significantly decreased fasting insulin to control levels compared with CLA/PBS and CLA/Rosi groups (Table 1).

### Table 1. Effects of dietary CLA, rosiglitazone, and combination on tissue weight and serum metabolites

<table>
<thead>
<tr>
<th>Tissue weight</th>
<th>Con/PBS</th>
<th>Con/Rosi</th>
<th>CLA/PBS</th>
<th>CLA/Rosi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver, g</td>
<td>1.05±0.1</td>
<td>1.14±0.1</td>
<td>1.76±0.1</td>
<td>1.43±0.1</td>
</tr>
<tr>
<td>Epididymal adipose, g</td>
<td>1.24±0.1</td>
<td>1.4±0.2</td>
<td>0.26±0.1</td>
<td>0.56±0.1</td>
</tr>
<tr>
<td>Inguinal adipose, g</td>
<td>0.59±0.1</td>
<td>0.67±0.1</td>
<td>0.12±0.1</td>
<td>0.47±0.1</td>
</tr>
</tbody>
</table>

Serum metabolites

<table>
<thead>
<tr>
<th>Glucose, mmol/l</th>
<th>4.78±0.25</th>
<th>4.84±0.24</th>
<th>5.97±0.41</th>
<th>5.20±0.41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride, mg/dl</td>
<td>44.8±4.2</td>
<td>30.3±3.3</td>
<td>19.9±3.5b</td>
<td>27.1±3.3a</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>150.7±10.3</td>
<td>150.5±11.5</td>
<td>232.9±18.6b</td>
<td>201±15.2</td>
</tr>
<tr>
<td>Nonesterified fatty acid, mmol/l</td>
<td>0.59±0.1</td>
<td>0.50±0.06</td>
<td>0.44±0.04*</td>
<td>0.50±0.03</td>
</tr>
</tbody>
</table>

Data are least square means ± SE of 6–10 mice per treatment. Tissue weights and serum metabolites were determined after 6 wk of dietary conjugated linoleic acid (CLA) and rosiglitazone treatments. Serum metabolites were determined at fasted state. Con/PBS, Control diet with PBS treatment; Con/Rosi, Control diet with Rosi treatment; CLA/PBS, CLA diet with PBS treatment; CLA/Rosi, CLA diet with Rosi treatment. *$P < 0.05$ vs. Con/PBS, b$P < 0.01$ vs. Con/PBS, c$P < 0.05$ vs. Con/Rosi, d$P < 0.01$ vs. Con/Rosi, e$P < 0.01$ vs. CLA/PBS.
C L A/ R o s i g l i t a z o n e a t t e n u a t e s C L A - I N D U C E D I N S U L I N R E S I S T A N C E I N M I C E

Table 2. Effects of dietary CLA, rosiglitazone, and combination on fasting insulin and adipocytokines

<table>
<thead>
<tr>
<th></th>
<th>Con/PBS</th>
<th>Con/Rosi</th>
<th>CLA/PBS</th>
<th>CLA/Rosi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial insulin, pg/ml</td>
<td>302.8 ± 13.5</td>
<td>305.8 ± 30.7</td>
<td>248.6 ± 23.1</td>
<td>261.3 ± 37.9</td>
</tr>
<tr>
<td>Final insulin, pg/ml</td>
<td>851 ± 176</td>
<td>434 ± 45</td>
<td>2,638 ± 323</td>
<td>1,684 ± 170</td>
</tr>
<tr>
<td>Leptin, pg/ml</td>
<td>7,241 ± 1,738</td>
<td>7,347 ± 322</td>
<td>1,069 ± 322</td>
<td>2,648 ± 1779</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>13.6 ± 2.7</td>
<td>24.2 ± 4.7</td>
<td>2.1 ± 0.33</td>
<td>9.5 ± 0.55</td>
</tr>
</tbody>
</table>

Data are least square means ± SE of 8–10 mice per treatment. Initial insulin and final insulin levels were measured at week 0 and week 6, respectively. Serum adipocytokines were determined at fasted state after 6 wk of dietary and Rosiglitazone treatments. *P < 0.01 vs. Con/PBS, bP < 0.05 vs. Con/Rosi, cP < 0.01 vs. Con/Rosi, dP < 0.05 vs. CLA/PBS, eP < 0.01 vs. CLA/PBS. leptin and adiponectin in pair-fed group (PF/PBS) were significantly higher than CLA/PBS but not significantly different from Con/PBS (data not shown). Fasting glucose was not changed by either diet (Con and CLA) or treatment (Rosi and PBS) (Table 1). The CLA/PBS alone had significantly lowered serum TGs but had higher serum total cholesterol concentrations compared with Con/PBS; Rosi treatment had no significant effect on any parameters (Table 1).

Effects of CLA, Rosi, and combination on liver fat content and VLDL-TG production rate. Histological sections of liver tissue from Con/PBS group showed predominantly large lipid-filled vacuoles (macrovesicular steatosis) (Fig. 2A). Liver sections from CLA/PBS group revealed a heterogeneous mixture of large lipid-filled vacuoles and small lipid droplets (microvesicular steatosis) (Fig. 2B). The Con/Rosi group exhibited visibly less lipid-filled vacuoles and were distinguishable from Con/PBS and CLA/PBS groups (Fig. 2C). CLA/Rosi showed less macro- and microvesicular steatosis compared with CLA/PBS (Fig. 2D). Histological observations were similar to quantification of hepatic TG levels: CLA/PBS significantly increased TGs 0.8-fold higher than Con/PBS, whereas Con/Rosi significantly decreased liver TGs by 1.1-fold lower than Con/PBS (Fig. 3A). The combination of Rosi treatment and dietary CLA (CLA/Rosi) had significantly decreased liver TGs by 0.45-fold compared with the CLA/PBS group (Fig. 3A) but was significantly higher than Con/Rosi and not different from Con/PBS. We next examined the VLDL-TG production rate to investigate whether CLA affects hepatic lipid production and transport into peripheral tissues. Figure 3B shows the plasma TG concentration after injection of Triton WR-1339, an inhibitor of LPL, which inhibits TG uptake by adipose tissue. VLDL-TG production rates were calculated from slopes of curves (Fig. 3C). CLA/PBS significantly increased rate of VLDL-TG production (~7.0-fold) relative to Con/PBS group (Fig. 3C). The Con/Rosi group also had a higher VLDL-TG production rate than the Con/PBS group, but the increase was not significant. The VLDL-TG production rate of the CLA/Rosi group was not significant different from the Con/Rosi group.

Effects of CLA, Rosi, and combination on adipocytes and mRNA expression in adipose tissue. Histological images of epididymal adipose tissue revealed differences in the morphology of adipocytes among groups (Fig. 4). Adipocytes in the Con/Rosi group appeared to be uniform in size (Fig. 4C), whereas adipocytes of the CLA/PBS were irregularly shaped and varied in size (Fig. 4B). The size of adipocytes in CLA/PBS mice appeared smaller than Con/PBS and Con/Rosi (Fig. 4A and C), whereas CLA/Rosi (Fig. 4D) showed increase in larger adipocytes compared with CLA/PBS group. It appeared that adipocyte hypertrophy occurred in epididymal adipose
Effects of dietary CLA, Rosi, and combination hepatic lipid accumulation and very-low-density lipoprotein (VLDL)-triglyceride (TG) production. A: liver TG content. B: time curves of serum TG concentration from livers in mice fed with or without CLA diets treated with or without Rosi (10 mg·kg⁻¹·day⁻¹) for 6 wk. C: VLDL-TG production rate expressed as milligrams per deciliter per minute, calculated from the serum TG-vs.-time curve. Data are means ± SE of 3–5 mice per treatment. *P < 0.05 vs. Con/PBS, $P < 0.05$ vs. Con/Rosi, ##P < 0.01 vs. Con/PBS.

Fig. 3. Effects of dietary CLA, Rosi, and combination hepatic lipid accumulation and very-low-density lipoprotein (VLDL)-triglyceride (TG) production.

Effects of CLA, Rosi, and combination on hepatic mRNA expression. Because hepatic TG content and the production rate of VLDL-TG were increased by CLA supplementation and Rosi treatment in high-fat-fed mice, we determined mRNA levels of genes involved in hepatic lipogenesis and fatty acid transport in liver tissues. The CLA/PBS group showed significant increase in mRNA levels of stearoyl-CoA desaturase (SCD-1) (~4.5-fold) and fatty acid synthase (FAS) (~2.5-fold) compared with Con/PBS group (Fig. 7). Rosi treatment (Con/Rosi and CLA/Rosi) had no significant effects on SCD-1 but significantly decreased FAS mRNA expression when combined with CLA (CLA/Rosi) (Fig. 7). Similarly, CLA/PBS group had significantly higher mRNA levels of fatty acid translocase FAT/CD36 (~11-fold) in liver compared with both control-fed groups. The CLA/Rosi significantly decreased gene expression of FAT/CD36 in the liver compared with CLA/PBS (~6-fold) to a similar level of Con/Rosi but remained significantly higher than Con/PBS. CLA, regardless of Rosi treatment, significantly increased mRNA levels of apolipoprotein CII (ApoCII), which is involved in the modulation of LPL activity. CLA/PBS also significantly increased AdipoR2 mRNA levels but not adiponectin mRNA compared with the CLA/PBS group. Rosi had no effect on lepton mRNA expression on either Con/Rosi or CLA/Rosi group (Fig. 5).

Effects of CLA and Rosi on markers of inflammation and macrophage infiltration in adipose. TNF-α is a major inflammatory mediator of macrophages in adipocytes. Therefore we hypothesized that CLA induces TNF-α, which leads to macrophage infiltration apoptosis of adipocytes. However, expression of TNF-α mRNA was not altered by either the CLA diet or Rosi treatment (Fig. 6). mRNA levels of F4/80 and CD68 were used as two markers of macrophage infiltration and were significantly increased (nearly 3.0-fold) by CLA alone compared with all other groups (Fig. 6A). Administration of Rosi treatment did not reverse this effect (Fig. 6A). Consistent with the induction of in the gene expression of F4/80 and CD68 in CLA-fed groups, immunohistochemistry using the macrophage marker F4/80 revealed marked macrophage infiltration into adipose (Fig. 6, B and C). In addition, the ratio of Bcl2, repressor of apoptosis, and Bax, promoter of apoptosis (Bax-to-Bcl2), was significantly greater in the CLA/PBS group whereas treatment with Rosi partially prevented this effect (CLA/Rosi vs. CLA/PBS groups) (Fig. 4).

AJP-Gastrointest Liver Physiol • VOL 292 • JUNE 2007 • www.ajpgi.org

G1675

ROSIGLITAZONE ATTENUATES CLA-INDUCED INSULIN RESISTANCE IN MICE
DISCUSSION

Findings in this study agree with previous reports showing that supplementation with t10c12-CLA (0.6%) decreases fat mass that accompanies the development of hepatic steatosis in mice (16, 39, 42). Body fat loss has been demonstrated previously with Tonalin TG 80 in mice (18). We believe that the cause of lipodystrophy and insulin resistance by CLA in the present study is due to a transient induction of inflammatory cytokines causing activation of macrophages and apoptosis in adipose tissue. Following the changes in adipose tissue, subsequent metabolic disorders include hyperinsulinemia, reduction of adiponectin, and dysregulation of gene expression in fatty acid transport, uptake, and efflux in adipose and liver tissues. Recruitment of macrophages into adipose tissue in obese mice and humans (44) triggers an inflammatory response and causes insulin resistance (38, 45). Our findings are consistent with several studies showing that the initiation of apoptosis and macrophage recruitment participates in insulin resistance induced by CLA (35, 42).

To our knowledge, this is the first paper to show the simultaneous interaction of Rosi and CLA on adipose and liver. Here, we show that supplementation with CLA significantly prevents weight gain in Rosi-treated mice without inducing insulin resistance. Neither CLA nor Rosi treatment influenced food intake, suggesting that the effects of treatments on weight gain were independent of energy intake. Previously, it was demonstrated that supplementation with CLA provided either as mixed isomer or as t10c12-CLA isomer, dramatically decreased adipose mass but caused insulin resistance accompanied by hepatic steatosis in mice (11, 33, 34). In agreement with previous reports, we show that CLA alone (CLA/PBS) significantly decreased fat mass but caused insulin resistance.

Fig. 4. Effects of dietary CLA, Rosi, and combination on adipocyte morphology and apoptosis. A–D: H&E-stained adipose sections from representative mice. E: Western blot analysis of Bax and Bcl2 protein in adipose from C57BL/6 male mice fed with (open bars) or without (solid bars) CLA diet treated with or without Rosi for 6 wk. Images are \( \times 40 \) magnifications. Data are expressed as relative to control-fed mice (\( n = 8 \) mice per treatment group). *\( P < 0.05 \) vs. Con/PBS, #\( P < 0.05 \) vs. CLA/PBS.
Addition of Rosi to the CLA group attenuated CLA-induced insulin sensitivity and lipodystrophy but did not abrogate CLA-mediated fat mass loss. Not surprisingly, the attenuation of insulin resistance and hepatic steatosis with CLA/Rosi was associated with the restored adipocytokine levels. Tsuboyama-Kasaoka and others (42) demonstrated that administration of leptin in CLA-fed mice reduced hyperinsulinemia. However, leptin-deficiency caused by CLA is not likely the sole cause of insulin resistance (34). Lipodystrophy induced by HX531, an inhibitor of PPARγ/H9253, was completely reversed by administration of adiponectin in mice (49). In this study, the reduction in adipose mass and serum leptin and adiponectin levels by CLA was reversed by administering the PPARγ/H9253 agonist Rosi. Our findings that Rosi-induced increases of adipokines reversed some of CLA’s effects suggest that both leptin and adiponectin deficiency contribute to lipodystrophy associated disorders.

Recently, we have shown that removing CLA from the diet rescued leptin and adiponectin levels and attenuated insulin resistance induced by dietary CLA in mice (30, 36). Although it is clear that the dramatic decrease in adiponectin concentration is important to the development of hepatic steatosis and insulin resistance induced by CLA, the mechanisms by which supplementation of CLA decreases adiponectin have not been understood. In the present study, in addition to the reduction of serum adiponectin concentration, the mRNA levels of PPARγ alone with mRNA levels of AdipoR-2 and adiponectin in adipose tissue were significantly reduced by CLA. A functional PPAR-response element has been identified in the adiponectin promoter region, and PPARγ is required for adiponectin promoter activity (17). Evidence from in vivo and in vitro studies has shown that CLA (predominantly t10c12-CLA) decreased PPARγ expression in adipocytes and inhibited differentiation of preadipocytes to mature adipocytes (5, 20). It is possible that a potential mechanism for decreased serum adiponectin and mRNA in adipose tissue is through the diminishment of PPARγ levels.

In adipose, Rosi improves adipocyte function and insulin resistance associated with activation of PPARγ. PPARγ and its target genes are involved with fatty acid uptake, adipocyte differentiation, and secretion of adiponectin from adipose (1, 2). In this study, we showed that the CLA/PBS group had significantly reduced mRNA levels of PPARγ and its target genes including LPL (TG hydrolysis) and FAT/CD36 (fatty acid transport) compared with Con/Rosi. Other studies have shown that CLA decreases PPARγ expression and its target genes (6). In this study, we show that feeding with CLA for 6 wk caused a significant reduction in mRNA levels of LPL, suggesting that fatty acid uptake in adipose was suppressed in the CLA/PBS group. Pariza and colleagues (48)

![Graphs showing gene expression changes](image-url)
ROSIGLITAZONE ATTENUATES CLA-INDUCED INSULIN RESISTANCE IN MICE

A

<table>
<thead>
<tr>
<th></th>
<th>CLA</th>
<th>-</th>
<th>+</th>
<th></th>
<th>CLA</th>
<th>-</th>
<th>+</th>
<th></th>
<th>CLA</th>
<th>-</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>F4/80</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>*</td>
<td></td>
<td>1</td>
<td>2</td>
<td>*</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>CD68</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>*</td>
<td></td>
<td>1</td>
<td>2</td>
<td>*</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

B

Epididymal WAT X20

CON/PBS

CON/ROSI

CLA/PBS

CLA/ROSI

C

Epididymal WAT X40

CON/PBS

CON/ROSI

CLA/PBS

CLA/ROSI
reported that short-term feeding of CLA (1 wk) caused significant suppression of LPL activity before changes in adipose occurred. It is possible that the early events that occur in adipocytes in response to CLA such as the reduction of LPL and adiponectin may contribute the development of hepatic steatosis and insulin resistance.

Previously, it was demonstrated that supplementation of CLA-mediated lipodystrophy depends on the t10c12-CLA iso-
mer (26). The CLA diet we used was Tonalin TG 80. Body fat loss has been demonstrated previously with Tonalin TG 80 in mice (18). Our findings agree with previous reports showing that supplementation with t10c12-CLA suppressed PPARγ-mediated adipogenesis, suggesting that the effects we observed are due to the t10c12-CLA isomer, which comprises ~0.6 wt% of our diets. In addition to PPARγ-mediated mechanisms in adipose function, Rosi improves metabolic syndrome through an anti-inflammatory effect of adiponectin (19). However, in this study, insulin resistance occurred with evidence of macrophage infiltration and apoptosis but without involvement of inflammation. Rosi attenuated the suppression of adipose abundant genes by CLA and prevented apoptosis (Bax/Bcl2) induced by CLA; however, Rosi had no effect on either markers of macrophage infiltration (F4/80 and CD68 mRNA) or inflammation.

The induction of inflammatory response and macrophage recruitment in adipose tissue preceded or coincided with the onset of hyperinsulinemia (47). In the present study, we cannot conclude the sequence of events (macrophage recruitment, inflammation, and insulin resistance). It is possible that the inability of Rosi to attenuate inflammatory effects of CLA could be attributed to the increase in adipogenesis by Rosi accompanied by an increase in production of cytokines by Rosi. In addition, a recent study indicated that Rosi had no effect on the expression of TNF-α and macrophage recruitment in obese mice (43).

In the liver, mechanisms by which CLA induces lipid accumulation are not known. Several studies have reported an increase in both liver fatty acid synthesis and oxidation in rodents supplemented with mix isomer of CLA (20, 29). Other studies in mice also reported that supplementation with t10c12-CLA reduces adipose mass but does not significantly increase fat accumulation in liver (10, 11). In the present study, supplementation with CLA induces a typical lipodystrophic syndrome, a condition that occurs after a dramatic loss of fat mass leading to hyperinsulinemia, insulin resistance, and liver steatosis. That CLA induces lipodystrophic syndrome in mouse has been well documented in several studies using a mouse model (33). In the present study, hepatic steatosis induced by CLA is associated with the elevated of SCD-1 and the increased VLDL-TG production. An increase in SCD-1 activity provides monounsaturated fatty acids for the assembly (14) of VLDL particles, thereby increasing production of VLDL from the liver (14, 24). Overexpression of SCD-1 in leptin deficient ob/ob mice results in hepatic overproduction of TGs and increased TGs storage in the liver (8, 24). However, an increase in VLDL-TG production rate did not improve fat accumulation in the liver. In this study, the CLA/PBS group significantly induced mRNA levels of PPARγ and SREBP-1c responsive genes: SCD-1 and FAS. Furthermore, the CLA/PBS group had significantly increased hepatic lipid storage without altering genes involved in fatty acid oxidation (PPARα, CPT1a) in the liver (Fig. 4). These data along with previous reports (9, 24) suggest that CLA-induced hepatic lipid accumulation is not due to impaired fatty acid oxidation in mice. It is possible that livers of CLA/PBS mice remained steatotic despite the increase in VLDL-TG production rate, owing to decreased fatty acid uptake by adipose tissue by suppressing LPL and CD36 in adipose along with possibly increased fatty acid reuptake by the liver as the mRNA levels of fatty acid transporter FAT/CD36 were significantly elevated in these mice. When combined with Rosi treatment, the CLA/Rosi group showed significant improvement in hepatic lipid accumulation that was associated a decrease in mRNA levels of SCD-1 and FAS. Although the dysregulated gene expression in hepatic lipid metabolism could not explain the potential mechanisms for causing hepatic lipid accumulation, these dramatic changes in lipogenic gene expression reflect the fat redistribution observed between adipose and liver tissues.

We proposed that the modulation of levels of serum adiponectin and adiponectin gene expression in the liver and adipose tissues could be the potential mechanism by which Rosi improved insulin sensitivity and hepatic steatosis in mice fed with CLA. Recently, a study in humans showed that the increase in plasma adiponectin concentration following Rosi treatment is inversely associated hepatic lipid accumulation in humans (41). Among circulating adipocytokines (TNF-α, adiponectin, resistin, and leptin), only lower circulating adiponectin levels were significantly correlated with liver steatosis in nonalcoholic steatohepatitis (30). Additionally, reduced hepatic expression of adiponectin and AdipoR-2 was reported in subjects with NAFLD (21). In this study, hepatic expression of adiponectin-receptor II was significantly reduced in the CLA/
PBS group. Consistent with these findings in humans, here we show that the serum adiponectin concentration was inversely correlated with liver TG content (data not shown; \( r = -0.708, P < 0.001 \)), and the serum insulin concentration was positively correlated with liver TG content (data not shown; \( r = 0.644, P < 0.001 \)) in mice. TNF-\( \alpha \) concentration (data not shown) in the liver and mRNA levels were not altered among diet and treatment groups in this study. Although there are multiple mechanisms involved in the development of liver steatosis (37), the significant reduction of adiponectin caused by CLA/PBS could be the potential mechanism that leads to the development of hepatic steatosis in the present study. It is likely that Rosi improves liver steatosis in the CLA/Rosi group, in part, by increasing serum adiponectin concentrations and upregulating adiponectin receptor-2, thereby reducing hyperinsulinemia and hepatic lipogenesis.

In summary, CLA seemed to induce apoptosis and macrophage infiltration, which was associated with the redistribution of lipid from adipose to liver tissue leading to increased hepatic steatosis and worsened insulin resistance. Administration of Rosi improved hyperinsulinemia and hypothaipidocytinemia by enhancing production of adipocytokines and adipocyte function, thereby improving hepatic steatosis without affecting markers of inflammation and macrophage activation in CLA-fed mice.

ACKNOWLEDGMENTS

We thank Dr. Parthasarathy and members of the Belury laboratory for feedback regarding this manuscript.

GRANTS

This work was supported by funds from the Carol S. Kennedy professorship, the Ohio Agriculture Research and Development Center (OARD) investigator award and graduate research award, and the Hazel Williams Lapp Fellowship.

REFERENCES


