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 adiponectin. Leptin is an insulin sensitizing adipokine that may work by suppressing adipokines 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, 8-week old, male ob/ob mice were fed either a control diet or a diet supplemented with 1.5% mixed isomeric CLA and received daily intraperitoneal injections of either PBS or 0.5 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.

Materials & Methods

Experimental animals and diets: 8-week old B6.V-Lep+/−Ob/+ (ob/ob) mice were obtained through Harlan (Indianapolis, IN) and housed 4/cage at 22 oC ± 0.5 oC on a 12-hour light/dark cycle. Mice were fed the following diets for the duration of the experiment: control (CON) or CLA-supplemented (CLA) diets; leptin (+) or vehicle (-) by IP injection daily. After 4 weeks, serum leptin was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Linco Research, Inc., St. Charles, MO) according to manufacturer’s directions.

Analysis of triglycerides and free fatty acids: Serum samples were allowed to clot overnight at 4 oC and were centrifuged at 1500 rpm for 15 minutes. A colorimetric kit (NEFA C, Wako Chemicals, Richmond, VA) was used to determine serum FFA content as described by the manufacturer. Samples were analyzed in triplicate.

Summary

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 adiponectin. Leptin is an insulin sensitizing adipokine that may work by suppressing adipokines 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, 8-week old, male ob/ob mice were fed either a control diet or a diet supplemented with 1.5% mixed isomeric CLA and received daily intraperitoneal injections of either PBS or 0.5 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. 1)**

Reduces adipose mass in a variety of species
- Controversial, species-specific effects on metabolism and insulin resistance

Leptin

- 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)

Leptin

**Conclusions**

- Reduces adipokines
- Increases lipolysis and liver steatosis
- Induces insulin resistance
- Modulation of insulin sensitivity possibly dependent on maintenance of certain level of adipokine and therefore adipokine activity

**Materials & Methods**

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**Results**

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

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>CON + CLA</th>
<th>CLA</th>
<th>CLA + leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body weight (g)</td>
<td>24.00 ± 0.97</td>
<td>23.79 ± 0.97</td>
<td>20.00 ± 0.97</td>
<td>20.79 ± 0.97</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>4.72 ± 0.17</td>
<td>4.92 ± 0.17</td>
<td>3.72 ± 0.17</td>
<td>4.92 ± 0.17</td>
</tr>
<tr>
<td>Epididymal adipose (g)</td>
<td>2.93 ± 0.13</td>
<td>2.93 ± 0.13</td>
<td>2.93 ± 0.13</td>
<td>2.93 ± 0.13</td>
</tr>
<tr>
<td>Gastrocnemius muscle (g)</td>
<td>8.14 ± 0.88</td>
<td>8.14 ± 0.88</td>
<td>8.14 ± 0.88</td>
<td>8.14 ± 0.88</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>CON + CLA</th>
<th>CLA</th>
<th>CLA + leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>115.12 ± 10.38</td>
<td>117.00 ± 10.38</td>
<td>101.25 ± 10.38</td>
<td>107.18 ± 10.38</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>130.25 ± 10.38</td>
<td>106.20 ± 10.38</td>
<td>186.19 ± 10.38</td>
<td>102.50 ± 10.38</td>
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<tr>
<td>NEFA (mM)</td>
<td>128.37 ± 10.38</td>
<td>89.30 ± 10.38</td>
<td>159.83 ± 10.38</td>
<td>98.38 ± 10.38</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dl)</td>
<td>306.86 ± 15.99</td>
<td>660.0 ± 486.37</td>
<td>5456.0 ± 15.99</td>
<td>14525.8 ± 486.37</td>
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</tbody>
</table>

**References**