Glycerides Rich in Conjugated Linoleic Acid (CLA) Improve Blood Glucose Control in Diabetic C57BLKS-Lepr\(^{db}\)/lepr\(^{db}\) Mice

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Abstract: Although dietary conjugated linoleic acid (CLA) has been shown to have anti-diabetic activity in Zucker diabetic fatty \(fa/\bar{fa}\) rats, the long-term administration of glycerides rich in CLA (TG-CLA) had no effect on diabetic status in C57BLKS-Lepr\(^{db}\)/lepr\(^{db}\) (db/db) mice. In the present study, the effect of TG-CLA on blood glucose control in diabetic db/db mice was examined. Mice were fed a diet supplemented with either 1.2% safflower oil (control) or 1.2% TG-CLA. Oral glucose tolerance tests (OGTTs) were performed after 5 and 11 weeks of TG-CLA treatment. The body weight, fat pad weight and amounts of food consumed were lower in the TG-CLA fed mice than in the control. The administration of TG-CLA for 5 and 11 weeks suppressed the blood glucose increase after oral glucose ingestion. Furthermore, the insulin level during OGTTs had increased after 5 weeks of treatment, but it was suppressed after 11 weeks. These results that revealed beneficial effects of TG-CLA on blood glucose control in diabetic db/db mice, in addition to improved insulin sensitivity after a transient increase in insulin secretion, indicate the need for further study in human subjects.


Key words: conjugated linoleic acid, diabetes, oral glucose tolerance test, blood glucose, insulin, insulin sensitivity

1 Introduction

Conjugated linoleic acid (CLA), an octadecadienoic acid containing a conjugated double bond, has been reported to have anti-carcinogenic (1-3), anti-atherosclerotic (4-6), and anti-obese (7-9) activities. It was also shown that CLA improved glucose tolerance and insulin sensitivity in Zucker diabetic fatty \(fa/\bar{fa}\) (ZDF) rats (10), but AKR/J mice fed CLA had an increase in the plasma insulin level that suggested insulin resistance (11). In addition, we observed an increase in the fasting plasma glucose level in mice fed CLA (12). Plasma insulin levels also tended to increase in swine and man (13, 14). Furthermore, a recent study revealed that CLA given to C57BL/6J mice induced lipodystrophy and insulin resistance (15). In contrast, long-term administration of glycerides rich in CLA (TG-CLA) had no effect on diabetic status in C57BLKS-Lepr\(^{db}\)/lepr\(^{db}\) (db/db) mice (submitted). Therefore, the effect of TG-CLA on blood glucose control in db/db mice is the concern of the present study.

2 Experimental

2.1 Preparation of Glycerides Containing Conjugated Linoleic Acid

CLA was purchased from Rinoru Oil Mills (Nagoya, Japan). TG-CLA was prepared by an enzymatic esterification as follows: Two hundred grams of CLA, 21.9 grams of glycerol and 30 grams of Lipozyme IM (Novo Nordisk, Denmark) were mixed in a one-liter vacuum flask. The mixture was allowed to react for 17 hr under constant evaporation. The reaction proceeded at 80°C and then at 87°C after 7 hr. Eighty grams of molecular sieves were added to the mixture at 9 and 15 hr. The reaction was stopped after 17 hr by suspending

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the resultant in hexane, and the suspension was filtered to remove the immobilized lipase and the molecular sieves. Hexane was then removed by evaporation. The TG-CLA thus obtained consisted of 87% triglyceride, 9% diglyceride and 4% free fatty acids. The fatty acid composition determined by gas chromatography was 71.7% conjugated linoleic acid (33.7% cis, trans/trans, cis-9, 11; 35.2% trans, cis-10, 12; 2.8% other isomers), 16.8% oleic acid, 2.7% linoleic acid and 8.8% other fatty acids.

2.2 Animals and Diets

Male db/db mice were obtained from the Institute for Animal Reproduction (Ibaraki, Japan) at 7 weeks of age. Two or three mice were housed in plastic cages and placed in an air conditioned room (24±1°C; relative humidity 60±5%) with a fixed day-night rhythm (lights on from 8:00 a.m. for 12 hr). The mice were first maintained ad libitum on a commercial MF diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and water. At 8 weeks of age, the mice were placed on a diet either with or without TG-CLA. Experimental diets were prepared by adding 1.2% safflower oil (control) or 1.2% TG-CLA (TG-CLA) to the MF diet (Table 1). The body weight and food intake were measured every week. Food intake per mouse was calculated from the values measured per cage. Half of the animals in each group were used for OGTTs after 5 weeks of treatment, and the rest were examined after 11 weeks. One week after OGTTs, the mice were fasted for 4 hr and sacrificed. Blood was collected via heart puncture, and the liver, the abdominal fat and the epididymal fat were dissected and weighed.

2.3 Oral Glucose Tolerance Test (OGTT)

After an 18 to 20 hr fast, the animals were bled from the retro-orbital sinus for the baseline (time 0) measurement of blood glucose. Then they were orally given D-glucose (2 g/kg body wt) dissolved in distilled water through a feeding needle. Blood was drawn from the retro-orbital sinus 30, 60, 90 and 120 min after the glucose administration. The area under the blood glucose curve (AUC) was calculated after subtracting the baseline value from each glucose measurement.

2.4 Analytical Procedure

Plasma glucose, plasma triglycerides and other clinical chemical variables were determined with commercial enzymatic assay kits as follows.

Glucose: Glucose CII-Test WAKO (Wako Pure Chemicals Industries, Ltd., Osaka, Japan)
Triglyceride: Triglyceride G-Test WAKO
Free fatty acids: NEFA C-Test WAKO
Blood urea nitrogen: BUN B-Test WAKO
GOT/GPT: Transaminase CII-Test WAKO
Total cholesterol: Determiner TC555 (KYOWA MEDEX Co., Ltd., Tokyo, Japan)

β-Hydroxybutyrate was determined by an enzymatic method described previously (12). The plasma concentrations of insulin were measured with an enzyme immuno assay kit (Morinaga Institute of Biological Science, Yokohama, Japan).

2.5 Statistical Analysis

Statistical comparisons of the data from two experimental groups were made by unpaired Student's t test. Differences in blood glucose and insulin during OGTTs were evaluated by the repeated-measures analysis of variance (ANOVA). Statistical significance was defined as P<0.05. Values are the mean±SD.

3 Results

3.1 Body Weight and Food Intake

The body weight of mice treated with TG-CLA was significantly lower than that of the control (Fig.1). A significant decrease in food intake was also observed in the TG-CLA group during the first 5 weeks (Fig.2).

3.2 Oral Glucose Tolerance Test

Although the fasting blood glucose level (time 0) was not influenced by TG-CLA, the increase in blood glucose after glucose loading was suppressed in the TG-CLA fed mice in comparison with the control mice (P<0.01; Fig.3A, P<0.05; Fig.3B). AUC was significantly reduced by TG-CLA administration (Fig.3C). Furthermore, TG-CLA affected the insulin response

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Fatty Acid Composition of Diets [%]</th>
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<tbody>
<tr>
<td></td>
<td>Fatty acid</td>
</tr>
<tr>
<td>16:0</td>
<td>13.0</td>
</tr>
<tr>
<td>18:0</td>
<td>1.6</td>
</tr>
<tr>
<td>18:1</td>
<td>25.2</td>
</tr>
<tr>
<td>18:2(Linoleic acid)</td>
<td>53.9</td>
</tr>
<tr>
<td>18:2(Conjugated linoleic acid)</td>
<td>0.7</td>
</tr>
<tr>
<td>c, t/t, c-9,11</td>
<td>(0.2)</td>
</tr>
<tr>
<td>t, c-10,12</td>
<td>(0)</td>
</tr>
<tr>
<td>other CLA isomers</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Other fatty acids</td>
<td>5.6</td>
</tr>
</tbody>
</table>
3.4 Blood Analysis

The analysis of blood after 4 hr fasting was done at 6 and 12 weeks of TG-CLA treatment (Table 3). At 6 weeks of treatment, the serum fatty acids and GPT level of the TG-CLA fed mice were significantly higher than those of the control mice. At 12 weeks of treatment, the serum fatty acids and the insulin level of the TG-CLA fed mice were lower than those of the control.

4 Discussion

The administration of TG-CLA to diabetic db/db mice resulted in a reduction in body weight and abdominal fat pad weight. Food intake was also reduced in the TG-CLA fed mice, which had not been observed in our previous study (submitted). The effect of CLA on appetite is unclear, but some reports showed a tendency for food intake to be reduced by feeding CLA (7, 16). It is not clear whether the reduced food intake observed in the present study is the main reason for the body fat loss caused by TG-CLA in db/db mice, but it can be assumed that the reduced food intake involves in body fat reduction to a lesser extent, since a reduction in body fat independent of energy intake was reported (11).

Hepatic hypertrophy was observed in 14-week-old mice fed TG-CLA. The GPT/GOT ratio, a marker of fatty liver, in 14-week-old mice differed significantly (0.41 ± 0.21 and 0.84 ± 0.27 in the control and TG-CLA fed mice, respectively; *P < 0.01). These observations raise the possibility of hepatic fatty accumulation caused by dietary TG-CLA, but such a difference had disappeared in 20-week-old mice (0.70 ± 0.17 and 0.77 ± 0.20 in the control and TG-CLA fed mice, respectively). So far the hepatomegaly and hepatic steatosis caused by dietary CLA has been reported in AKR/J mice and C57BL/6J mice (11, 15). These phenomena were explained by the enhanced lipogenesis in the liver via an action of TNF-α induced by CLA in the adipose tissue (15). In the present study, TG-CLA might act through the same pathway to increase some impairment of the liver. In our previous study, histopathological observations in db/db mice fed TG-CLA for 6 months revealed no abnormality in the liver in spite of an increase in GPT (submitted). CLA is assumed to act through the same mechanism as peroxisome proliferators (PPs) to which species-specific responses have been recognized (17). The sensitivity to CLA is much greater in mice than in
Fig. 3  Effect of TG-CLA on OGGT.
A: Blood glucose values during OGGT at 5-weeks administration. B: Blood glucose values during OGGT at 11-weeks administration. C: Area under the OGGT's curve (AUC) from 0 to 120 min after glucose administration. *$P<0.05$ and **$P<0.01$ vs. control mice.

Fig. 4  Effect of TG-CLA on Insulin Secretion.
Circulating insulin concentrations during OGGT at 5-weeks administration (A) and 11-weeks administration (B). *$p<0.05$ and **$p<0.01$ vs. control mice.
Table 2  Tissue Weight of db/db Mice.

<table>
<thead>
<tr>
<th>Administration period Group</th>
<th>6 wks</th>
<th>12 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cont</td>
<td>TG-CLA</td>
</tr>
<tr>
<td>Body weight (g)†</td>
<td>51.1 ± 7.4</td>
<td>37.9 ± 2.1**</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>3.0 ± 0.8</td>
<td>3.8 ± 0.9*</td>
</tr>
<tr>
<td>Epididymal fat pad (g)</td>
<td>2.7 ± 0.5</td>
<td>1.8 ± 0.3**</td>
</tr>
<tr>
<td>Abdominal fat pad (g)</td>
<td>3.7 ± 1.0</td>
<td>1.9 ± 0.2**</td>
</tr>
</tbody>
</table>

†: Body weight after 4h fasting. *P<0.05, **P<0.01, and ***P<0.001 vs. control mice.

Table 3  Blood Profile of db/db Mice.

<table>
<thead>
<tr>
<th>Administration period Group</th>
<th>6 wks</th>
<th>12 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cont n=7</td>
<td>TG-CLA n=7</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>836.6 ± 244.8</td>
<td>811.6 ± 109.5</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>12.2 ± 11.0</td>
<td>12.6 ± 10.2</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>233.9 ± 58.9</td>
<td>209.6 ± 70.4</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>147.7 ± 39.7</td>
<td>184.6 ± 51.6</td>
</tr>
<tr>
<td>Free fatty acids (mEq/L)</td>
<td>1.2 ± 0.3</td>
<td>1.7 ± 0.2**</td>
</tr>
<tr>
<td>β-Hydroxybutyrate (mg/dL)</td>
<td>10.2 ± 6.9</td>
<td>19.0 ± 13.8</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>18.2 ± 6.3</td>
<td>15.6 ± 2.1</td>
</tr>
<tr>
<td>GPT (karmen)</td>
<td>53.5 ± 23.9</td>
<td>129.8 ± 71.1**</td>
</tr>
<tr>
<td>GOT (karmen)</td>
<td>149.0 ± 89.4</td>
<td>155.5 ± 54.0</td>
</tr>
</tbody>
</table>

*P<0.05 and **P<0.01 vs. control.

Rats as to the induction of PPAR-responsive genes in liver and hepatic lipid accumulation (17). Toxicological evaluation in rats supported the potential determination for the GRAS (generally recognized as safe: a criterion defined by FDA) status of CLA (18). In addition, hepatic hypertrophy was not observed in BALB/c mice, but it was observed in C57BL/6J mice (unpublished data). Furthermore, in a human study, CLA has been reported not only to have no effect on GPT but also to be a safe substance with regard to the other safety parameters (19). These findings support the idea that dietary CLA is a safe nutritional substance.

The results of OGTTs revealed that the dietary TG-CLA improved blood glucose control in the diabetic db/db mice. The reduced insulin levels of mice fed TG-CLA for 11 weeks suggested an improvement in insulin sensitivity, though a transient rise in insulin levels was observed after 5 weeks of TG-CLA treatment. A rise in the insulin level was also observed in nondiabetic AKR/J mice (11) for which it was suggested that the mild chronic lipolytic state induced by CLA could lead to a mild insulin resistance. In the present study, an increase in serum free fatty acids was observed in TG-CLA fed mice at 6 weeks treatment. On the contrary, a decrease in serum free fatty acids was observed in TG-CLA fed mice at 12 weeks treatment. It was assumed that the transient increase in the insulin level was due to the promotion of a lipolytic state, and once circulating fatty acids were lost as a consequence of body fat reduction, an improvement of insulin sensitivity took place. Consistent with this hypothesis, the anti-diabetic effect of CLA reported in the ZDF rats was also accompanied by a reduction in fatty acids (10). On the other hand, the anti-diabetic effect of CLA in the ZDF rats was suggested to be due, in part, to activation of PPARγ in adipocytes (10) so that PPAR ligand activity might be involved in the anti-diabetic effect of TG-CLA in the db/db mice.

Tsuboyama et al. observed impaired glucose tolerance and insulin resistance in C57BL/6 mice fed CLA (15). The increase in TNF-α in adipose tissue caused by the CLA supplementation was thought to induce insulin
resistance. In addition, it was also suggested that a decrease in GLUT4 in adipose tissue and leptin deficiency contributed to insulin resistance. In their study, the mice fed CLA presented with lipodystrophy. The excessive fat loss induced by CLA supplementation might be responsible for the insulin resistance. Further studies on this are needed.

In conclusion, an appropriate TG-CLA intake is assumed to have beneficial effects on diabetic obesity. Although there is a possibility of transient insulin resistance, the continuation of treatment with TG-CLA is expected to improve insulin sensitivity.

References