Conjugated Linoleic Acid Reduces Body Fats and Cytokine Levels of Mice

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In order to discover the effect of CLA on the body fat size and serum cytokine levels, four groups of male mice were fed diets containing either 1% linoleic acid (LA) or conjugated linoleic acid (CLA) with or without 0.2% sesamin for 8 weeks. The weight gain and feed efficiency were significantly lower in the CLA groups. CLA significantly reduced relative weights (g/100 g body weight) of epididymal and perirenal adipose tissues, in particular the former. Concentrations of serum TNF-α and leptin were significantly reduced by dietary CLA. Sesamin did not show additional effects in all of these parameters. There was a positive correlation between cytokine production and body-fat reducing potential of CLA. These results indicated that mice appeared to be a hyperresponder to dietary CLA insofar as the reduction of body fat size is concerned.

Key words: conjugated linoleic acid; sesamin; adipose tissue; leptin; TNF-α

Conjugated linoleic acid (CLA) has been shown to reduce the body fat of experimental animals.1–3) However, the degree of response to this fatty acid differs among animal species and it appears to be moderate4,5) or practically negligible in humans.6,7) The mechanistic studies involved in the body-fat-reducing potential showed that in addition to the changes in enzyme activities responsible for both exogenous and endogenous fatty acid use, CLA activates liver peroxisome proliferator activating receptor-α.8) However, again there is a considerable difference depending on the animal species in the magnitude of response.8–10)

Leptin, a kind of adipocytokine exclusively produced in adipose tissue, plays a crucial role in the regulation of adipogenesis.11,12) In our preceding study, in which rats were fed 1% CLA,13) the serum level of leptin and TNF-α did not change even though there was a significant reduction of the weight of the adipose tissues. In contrast, when 2% CLA was fed to rats, the leptin level declined quickly but temporarily.14)

To confirm the relationship between adipogenesis and leptin production, the effects of CLA on these parameters were studied currently in mice, an animal model presumably sensitive to dietary CLA.1–3) Since hepatic fatty acid β-oxidation seems at least to be involved in the body fat-reducing potential of CLA, but its effect on this parameter is rather moderate.14) Thus, we expected the combined effect of CLA and sesamin. Sesamin is a potent stimulator of fatty acid β-oxidation in the liver of rats,15) and indeed augmented the body fat reducing activity of CLA in this animal species.13)

Three-week-old male Crj:CD-1 (ICR) mice were purchased from Japan Charles River Co., Tokyo and housed 5 mice in one stainless steel wire cage in an air-conditioned room (20 to 23°C, lights on 0800 to 2000). During a run-in period of 5 days, mice received a commercial powdered diet (Type NMF, Oriental Yeast Co., Tokyo). Thereafter, animals were randomly allocated to one of the four different diets, stratified for dietary fat and SES. Each group was composed of 20 animals. Experimental diets were prepared by adding lard and linoleic acid (LA, as safflower oil) or conjugated linoleic acid (CLA) to a commercial powdered diet (NMF) by the weight ratio of 80:20:1.0, respectively. Fatty acid compositions of LA and CLA are shown in Table 1. SES (the mixture of sesamin and episesamin, 53.6:43.7, w/w, Takemoto Oil Mill Co., Gamagouri, Aichi) was added at the 0.2% level. After the rats were fed the diets for 8 weeks, blood was withdrawn from their abdominal aortas under diethylether anesthesia. The
visceral tissues were excised immediately. This study was done in accordance with the Guidelines of Animal Experiments approved by the Prefectural University of Kumamoto, and Kyushu University School of Agriculture.

The concentration of serum TNF-α and leptin was measured using commercial mice ELISA kits (Yanaihara Institute Inc., Shizuoka).15)

The data were examined by 2-way ANOVA using Super ANOVA (Abacus Concepts Inc., Berkely, CA). If the effect of the diet was statistically significant \((p<0.05)\), the four treatments were compared pair-wise by Duncan’s new multiple range test.17) The results were shown as means ± SE.

As shown in Table 2, feeding CLA, as compared to LA, resulted in a significant reduction of weight gain, although the food consumption was comparable. Therefore, food efficiency was significantly lower in mice fed CLA. Dietary CLA signifi cantly increased relative weight of liver, heart, lung, spleen, and kidney. Although no direct evidence is available, there is a possibility that the increases in weight of those tissues are attributed to those of protein contents.18) SES neither affected these parameters, nor increased liver weight further as in the case of rats.13)

The weight of adipose tissues was decreased markedly by dietary CLA in relation to LA (Fig. 1). The magnitude of decrease was more marked in epididymal than in perirenal adipose tissues. Thus, the tissue weight decreased to approximately one-fifth and one-third, respectively. SES did not show any effect on adipose tissue weights irrespective of dietary fat sources. In rats, SES augmented the reduction by CLA of the weight of perirenal adipose tissues.13)

Figure 2 shows the effects of dietary manipulations on concentrations of serum cytokines. Serum leptin and TNF-α levels were decreased significantly by dietary CLA. The magnitude of the reduction tended to be greater for leptin than for TNF-α. Again there was no effect of dietary SES on the cytokine levels, although serum leptin tended to be lower when mice were fed CLA together with SES than in those fed CLA alone. In rats, the effect of CLA on serum leptin level was not necessarily definite.13,15) Overall, the combined effect of sesamin was not seen in this study, in contrast to our observation in rats,13) probably due to the hyperresponsive nature of mice to dietary CLA in relation to rats.

As shown in Fig. 3, there was a linear correlation between epididymal adipose tissue weight and serum cytokine concentration. A similar response was observed even when the comparisons were made between perirenal adipose tissue weight and leptin, although the relationship was to some extent moderate. The same response pattern was observed when comparison was made between TNF-α and adipose tissues.

Adipocytes secrete various cytokines (adipokines).11,12) The reduction of serum levels of leptin and TNF-α by dietary CLA was attributed to the reduction of adipose tissue weight, presumably not the cause for the weight reduction. A positive corre-

### Table 1. Fatty Acid Composition of Dietary Fat

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>LA (Weight%)</th>
<th>CLA (Weight%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>6.7</td>
<td>6.9</td>
</tr>
<tr>
<td>18:0</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>18:1</td>
<td>15.1</td>
<td>15.3</td>
</tr>
<tr>
<td>18:2</td>
<td>74.1</td>
<td>0.7</td>
</tr>
<tr>
<td>CLA</td>
<td>—</td>
<td>74.1</td>
</tr>
<tr>
<td>18:3</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Others</td>
<td>0.6</td>
<td>1.2</td>
</tr>
</tbody>
</table>

LA, safflower oil; CLA, conjugated linoleic acid (9c, 11r, 34.1%); 10r, 12c, 35.9%; 9c, 11c, 10c, 12c, 2.5%; and 9t, 11r, 10r, 12r, 1.6%).

### Table 2. Effects of CLA and SES on Growth Parameters and Tissue Weights of Mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dietary group</th>
<th>LA</th>
<th>LA + SES</th>
<th>CLA</th>
<th>CLA + SES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td>29.2 ± 0.5</td>
<td>29.3 ± 0.4</td>
<td>29.5 ± 0.5</td>
<td>29.4 ± 0.4</td>
</tr>
<tr>
<td>Initial</td>
<td></td>
<td>46.5 ± 1.2a</td>
<td>47.1 ± 1.1a</td>
<td>44.1 ± 0.8a</td>
<td>43.4 ± 0.6a</td>
</tr>
<tr>
<td>Final</td>
<td></td>
<td>17.3 ± 1.0b</td>
<td>17.8 ± 0.9b</td>
<td>14.7 ± 0.6b</td>
<td>14.0 ± 0.4b</td>
</tr>
<tr>
<td>Gain (/80 days)</td>
<td></td>
<td>3.95 ± 0.03a</td>
<td>4.02 ± 0.05a</td>
<td>4.42 ± 0.05b</td>
<td>3.98 ± 0.02b</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td></td>
<td>0.080 ± 0.004a</td>
<td>0.081 ± 0.004a</td>
<td>0.063 ± 0.002b</td>
<td>0.064 ± 0.002b</td>
</tr>
<tr>
<td>Food efficiency</td>
<td></td>
<td>4.47 ± 0.17a</td>
<td>4.64 ± 0.18a</td>
<td>5.56 ± 0.15b</td>
<td>5.54 ± 0.17b</td>
</tr>
<tr>
<td>Tissue weight (g/100 g body weight)</td>
<td></td>
<td>1.29 ± 0.04a</td>
<td>1.31 ± 0.04a</td>
<td>1.51 ± 0.04b</td>
<td>1.50 ± 0.04b</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>0.39 ± 0.02a</td>
<td>0.39 ± 0.01a</td>
<td>0.47 ± 0.01b</td>
<td>0.46 ± 0.01b</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>0.44 ± 0.01a</td>
<td>0.43 ± 0.01a</td>
<td>0.49 ± 0.01b</td>
<td>0.51 ± 0.00b</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>0.23 ± 0.01a</td>
<td>0.23 ± 0.01a</td>
<td>0.30 ± 0.01a</td>
<td>0.30 ± 0.02b</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td>1.00 ± 0.04</td>
<td>0.99 ± 0.04</td>
<td>1.06 ± 0.03</td>
<td>1.06 ± 0.03</td>
</tr>
</tbody>
</table>

Means ± SE of 18 to 20 mice. Values not sharing a common superscript letter are significantly different at \(p<0.05\).
Fig. 1. Interaction of CLA and SES on Weights of White Adipose Tissues of Mice. Values are means ± SE of 20 mice per group. Values with different letters are significantly different at \( p < 0.05 \).

Fig. 2. Interaction of CLA and SES Levels of Serum Cytokines of Mice. Values are means ± SE of 20 mice per group. Values with different letters are significantly different at \( p < 0.05 \).

Fig. 3. Relationship between Weights of Perirenal Adipose Tissue and Serum Leptin Levels of Mice. Values are means ± SE of 18–19 mice per group. Values with different letters are significantly different at \( p < 0.05 \).

The relation between serum leptin and body mass index has been reported.\(^{17,18}\) The simultaneous decrease in serum leptin and TNF-\(\alpha\) suggests that CLA down-regulates the expression of these adipocytokines.

TNF-\(\alpha\) and leptin were diverse actions on the fatty acid metabolism of adipocytes and are likely to behave as adipostats.\(^{21-23}\) Turek \textit{et al}.\(^{20}\) have reported that CLA reduces basal levels of TNF-\(\alpha\) produc-
tion by resident peritoneal macrophages in rats given soybean oil for 6 weeks. Leptin, a protein hormone produced almost exclusively by adipocytes, reflects in most cases the body fat content, i.e., its serum concentrations are elevated in obese people in comparison with lean people, and there is a strong positive correlation between leptin and body fat content.\textsuperscript{24,25)}

The change in serum levels of cytokines may be concerned in the metabolism of blood glucose. In this context, CLA prevented the rise of blood sugar and improved glucose tolerance.\textsuperscript{26)} Therefore, this study supports the view that mice may be a more appropriate animal model than rats to study the body fat reducing potential of CLA, although the effect may be modified by the gender and strain of mice.\textsuperscript{27)} Medina et al.\textsuperscript{23)} however reported that 64 days of CLA supplementation in women produced a transient decrease in leptin levels and CLA did not affect this parameter in a manner that promoted decreases of adiposity.

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References


