Combined Effects of Dietary Protein Type and Fat Level on the Body Fat-Reducing Activity of Conjugated Linoleic Acid (CLA) in Rats

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The interaction of dietary protein type and fat level on the body fat-reducing activity of conjugated linoleic acid (CLA) was studied in male rats fed diets containing casein (CAS) or soy protein (SOY) as a protein source with low fat (LF, 6.0% soybean oil) or high fat (HF, 13.0% soybean oil) combinations for 4 weeks. CLA was added at the 1.0% level to all diets. The weight of perirenal adipose tissue tended to be lower in the SOY groups than in the corresponding CAS groups, and the difference between the LF diets was significant. The weight of epididymal adipose tissue showed a similar but insignificant trend. The weight of brown adipose tissue was heaviest on the SOY-HF diet and lowest on two CAS diets, the SOY-LF diet being intermediate. The concentration of serum leptin was lowest on the SOY-LF diet and was significantly lower than that of the corresponding CAS group, but this difference disappeared when the dietary fat level increased. The serum cholesterol-lowering activity of SOY in relation to CAS was reproduced even when CLA was given. Thus the body fat-reducing activity of CLA was most marked when rats were fed the SOY-LF diet. Although the CAS-HF diet increased body fat deposition, the magnitude of the reduction by lowering dietary fat level was more marked than in the case of SOY. These results indicate a complicated interaction of dietary manipulations with the body fat-reducing effect of CLA, but the combination of CLA with the SOY-LF diet appears to be an appropriate approach.

Key words: conjugated linoleic acid (CLA); dietary protein; casein; soy protein; dietary fat

Conjugated linoleic acid (CLA) has many known beneficial health effects such as anti-carcinogenic, anti-obesity, and anti-atherogenic activities,1–6 but the magnitude of response to CLA differs from animal species to species, and humans appear to be the least responsive animal.7,8 A line of human studies on the body fat-reducing activity of CLA showed equivocal results under a wide range of dose level,9–12 although one recent study showed staple body fat-reducing potential of CLA in the long term human trials.13 It is, therefore, reasonable to examine the effect of dietary components that enhance the activities of CLA. This approach might additionally help to lower possible unfavorable side effects of CLA14–16 by reducing dietary intake of it. If the reinforcing effects of various food ingredients on the physiological functions of CLA are defined, CLA will be more beneficial for humans.

In a preceding study on rats, we showed that the body fat-reducing activity of dietary CLA can be enhanced by combination with sesamin, a lignan occurring abundantly in sesame seeds.17 Although it is well-known that soybean protein in relation to casein reduces body fats, the beneficial interaction of this protein with CLA has not been well clarified in this animal model.18 Thus insight into the interaction of dietary protein sources opens a new approach to dietary reinforcement of the physiological functions of CLA. It is also interesting to define the effect of dietary fat level on CLA effects, because dietary fat level appears to influence the effect that CLA exerts on the diabetic state.19

In this context, we studied in more detail the modifying effects of dietary protein type and fat level on the body fat-reducing activity of CLA in rats. Thus in the present study growth parameters, weights of adipose tissues, concentrations of serum components related to lipid metabolism, and hepatic fatty acid β-oxidation activity were measured in rats fed 1% CLA diets containing either SOY or CAS with low (6%) or high (13%) levels of dietary fat (soybean oil).
Materials and Methods

Animals and diets. Four-week-old male Sprague-Dawley rats were purchased from Seac (Fukuoka, Japan) and housed individually in stainless steel cages in an air-conditioned (22 to 23 °C), light-controlled room (lights on from 0800 to 2000). During a run-in period of one week, commercial pellets (Type NMF, Oriental Yeast, Tokyo) and water were given freely. Thereafter, rats were randomly allocated to one of the four different diets (eight or nine rats per group), stratified for source of dietary proteins and contents of dietary fat. Experimental diets were prepared as powders according to the AIN-93G formula; they contained 1.0% CLA (Rinoru Oil Mill, Tokyo) and water were given freely. Thereafter, rats were conditioned (22 to 23 °C) and 1.6% t,t-isomers was used. The dietary protein sources were casein (CAS, Seac) or soybean protein (SOY, Fujipro R, Fuji Oil, Osaka, Japan) at the 20% level. The dietary fat source was soybean oil (Fuji Oil) at the 6.0 or 13.0% level. Diets and water were freely given for 28 d. At the end of the feeding period, blood was withdrawn from the abdominal aorta under diethyl ether anesthesia and serum was harvested. The visceral tissues were excised immediately, rinsed, and weighed. This study was done in accordance with the Guidelines for Animal Experiments approved by the Prefectural University of Kumamoto.

Analysis. The concentrations of serum total and HDL-cholesterol, triglyceride, glucose, insulin, and total protein, and the activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured using a Fuji DRI-CHEM 5500 automatic analyzer (Fuji Film Medical, Tokyo). The concentrations of TNF-α and serum leptin were measured with commercial rat ELISA kits (Yanaihara Institute, Shizuoka, Japan). The concentration of serum adiponectin was measured with a mouse/rat ELISA kit purchased from Otsuka Pharmaceutical (Tokyo). The activity of carnitine palmitoyltransferase in liver mitochondria was measured by the method of Bieber et al.21

Statistics. Results were presented as means ± SE. Data were analyzed by two-way ANOVA to test statistical differences of the means among groups, followed by the Tukey-Kramer test to identify significant differences using a Stat View software system (version 5.0, SAS Institute Inc., Cary, NC). Differences were considered significant at p < 0.05.

Results

Effects on growth parameters and tissue weights

As shown in Table 2, food intake was significantly higher in the CAS groups than in the SOY groups, but there was no significant effect of dietary fat level on this parameter. Body weight gain was significantly higher in the CAS groups and increased with increasing dietary fat

| Table 2. Effects of Dietary Protein Type and Fat Level on Growth Parameters |
|-----------------------------|------------------|------------------|------------------|
|                            | CAS-LF           | CAS-HF           | SOY-LF           | SOY-HF           |
| Body weight                |                  |                  |                  |                  |
| Initial (g)                | 144 ± 3          | 144 ± 3          | 145 ± 4          | 142 ± 4          |
| Gain (g/28d)               | 239 ± 13         | 269 ± 8          | 199 ± 10         | 225 ± 7          |
| Food intake (g/d)          | 23.0 ± 1.0       | 23.5 ± 0.7       | 22.0 ± 0.9       | 20.7 ± 0.5       |
| Food efficiency (g gain/g intake) | 0.37 ± 0.01**   | 0.41 ± 0.01*     | 0.33 ± 0.02**    | 0.39 ± 0.01*     |
| Tissue weight (g/100 g bw) |                  |                  |                  |                  |
| Liver                      | 4.37 ± 0.10      | 4.36 ± 0.14      | 4.32 ± 0.08      | 4.55 ± 0.09      |
| Kidney                     | 0.73 ± 0.01      | 0.72 ± 0.02      | 0.74 ± 0.01      | 0.78 ± 0.02      |
| Heart                      | 0.30 ± 0.01      | 0.28 ± 0.01      | 0.31 ± 0.01      | 0.29 ± 0.01      |
| Lung                       | 0.41 ± 0.02      | 0.37 ± 0.01      | 0.34 ± 0.02      | 0.41 ± 0.03      |
| Spleen                     | 0.23 ± 0.01      | 0.23 ± 0.03      | 0.23 ± 0.01      | 0.21 ± 0.01      |

Protein | Fat | Protein X Fat
---|---|---
ns | ns | ns
p < 0.01 | p < 0.01 | ns
p < 0.05 | ns | ns
p < 0.01 | p < 0.0001 | ns
ns | ns | ns
ns | ns | ns
ns | p < 0.05 | ns
ns | ns | p < 0.05

Means ± SE of eight or nine rats in each group. Values with the same superscript letter (A, B, a, or b) or symbol (*) are significantly different at p < 0.05.
levels in the both protein groups. Consequently, food efficiency was significantly higher in rats fed CAS or HF diets as than in those fed SOY or LF diets respectively.

No demonstrable differences due to dietary protein type were observed in the relative weights (g/100 g body weight) of liver, kidney, heart, lung, or spleen, but there was a significant effect of dietary fat level on the relative weight of heart, being lower with HF diets. The HF diet decreased lung weight in the CAS group, while it increased that in the SOY group. Thus the response of lung weight to dietary fat depended on the type of dietary protein.

In rats fed LF diets, the weight of perirenal adipose tissue was significantly lower in the SOY group than in the CAS group, as shown in Fig. 1. This effect tended to be attenuated when the HF diets were fed, due to the increasing weight in the CAS group. A similar but lesser difference was also observed in the weight of epididymal adipose tissue. In this case, the weight tended to increase when the dietary fat level increased in both protein groups. The weight of brown adipose tissue increased significantly on the HF diet when rats were fed SOY diets. No such trend was observed in the CAS groups, and the weight remained unchanged even on the HF diet.

Effects on serum components

As shown in Table 3, the concentration of serum total cholesterol was significantly higher in rats fed CAS than in those fed SOY, while there was no remarkable effect

Fig. 1. Interaction of Dietary CLA, Protein, and Fat on Adipose Tissue Weights in Rats.

Values are means ± SE for eight or nine rats in each group. There are significant differences between the same letters or symbols (p < 0.05). The results of two-way ANOVA indicated a significant protein effect (p < 0.01) on the weight of perirenal and brown adipose tissue.

Table 3. Effects of Dietary Protein Type and Fat Level on Serum Components of Rats

<table>
<thead>
<tr>
<th></th>
<th>CAS-LF</th>
<th>CAS-HF</th>
<th>SOY-LF</th>
<th>SOY-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
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<td>Protein</td>
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<tr>
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<td>Fat</td>
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<td>X</td>
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<td>X</td>
</tr>
<tr>
<td>Protein Fat X</td>
<td>Protein Fat X</td>
<td>Protein Fat X</td>
<td>Protein Fat X</td>
<td>Protein Fat X</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS-LF</th>
<th>CAS-HF</th>
<th>SOY-LF</th>
<th>SOY-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>101 ± 4**</td>
<td>97 ± 6**</td>
<td>72 ± 2*</td>
<td>69 ± 3**</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>49.1 ± 5.0</td>
<td>55.9 ± 4.0*</td>
<td>37.1 ± 4.6</td>
<td>34.2 ± 1.6*</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>52.3 ± 4.0*</td>
<td>39.8 ± 3.6</td>
<td>35.2 ± 3.6*</td>
<td>34.9 ± 2.4</td>
</tr>
<tr>
<td>HDL-cholesterol/</td>
<td>0.48 ± 0.04</td>
<td>0.58 ± 0.03</td>
<td>0.51 ± 0.06</td>
<td>0.50 ± 0.02</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>242 ± 27</td>
<td>172 ± 29</td>
<td>206 ± 19</td>
<td>195 ± 26</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>206 ± 8</td>
<td>189 ± 4</td>
<td>194 ± 13</td>
<td>192 ± 3</td>
</tr>
<tr>
<td>Insulin (ng/dL)</td>
<td>187 ± 58</td>
<td>176 ± 27</td>
<td>144 ± 29</td>
<td>151 ± 29</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>5.49 ± 0.12</td>
<td>5.29 ± 0.04</td>
<td>5.59 ± 0.09</td>
<td>5.56 ± 1.11</td>
</tr>
<tr>
<td>GOT (IU)</td>
<td>65.0 ± 2.7</td>
<td>64.2 ± 2.6</td>
<td>60.9 ± 2.6</td>
<td>64.1 ± 1.7</td>
</tr>
<tr>
<td>GPT (IU)</td>
<td>10.4 ± 0.84</td>
<td>7.38 ± 0.80</td>
<td>9.38 ± 1.25</td>
<td>7.89 ± 0.98</td>
</tr>
</tbody>
</table>

Means ± SE of eight or nine rats in each group. Values with the same symbol are significantly different at p < 0.05.
of dietary fat level. The decrease in total cholesterol was attributed to a decrease in LDL-cholesterol, and the HDL-/total-cholesterol ratio remained unchanged. The HF diet tended to increase the concentration of serum HDL-cholesterol in the CAS group, while no such change was observed in the SOY group. The triglyceride level tended to decrease when the HF diet was given, irrespective of the source of dietary proteins. No significant difference was observed in the concentration of serum glucose and total protein, but they tended to be lower on the HF diet in the CAS group. The concentration of serum insulin was 15 to 20% lower on the SOY diets than on the CAS diets, although this difference is not significant. GOT activity was affected neither by the protein type nor by the fat level, but GPT activity was lower with increasing dietary fat levels in both protein groups. The range of the changes, however, was small.

**Effects on serum cytokine levels**

As Fig. 2 shows, there was no effect of dietary protein type or fat level on the concentration of serum TNF-α. However, the concentration of serum leptin was significantly higher in the CAS group when fed the LF diet. After the HF diet, the concentration in the SOY group significantly increased to a level similar to that of the corresponding CAS group. Consequently, the protein effect disappeared when the HF diet was fed. The concentration of serum adiponectin was significantly lower in rats fed the HF diet when the protein source was CAS, and the level observed on the CAS-HF diet was comparable with that observed on the two SOY diets. No such difference was seen when the protein source was SOY.

**Effects on hepatic fatty acid β-oxidation**

Figure 3 summarizes the effects of dietary protein and fat on the rate of fatty acid β-oxidation in the liver.
measured as the activity of mitochondrial carnitine palmitoyltransferase (CPT), one of the key enzymes in mitochondrial fatty acid β-oxidation. The activity tended to be higher on the HF diets in both protein groups, but no significant effect of dietary manipulation was observed.

**Discussion**

It appears likely that control groups free of CLA are necessary in the present type of study, but we did not settle such groups here, since we repeatedly confirmed the body fat-reducing potential of CLA in a series of feeding studies with rats[^17,18] under dietary regimens similar to that of the present study, 1% CLA in the diets. But due to this situation, we confined the explanation of the observed results to the effects of dietary manipulations alone.

The results of the present study indicate that dietary protein source in combination with CLA differently modifies lipid metabolism in rats. SOY in relation to CAS has been known not only to reduce serum cholesterol but also to stimulate hepatic fatty acid β-oxidation in several animal species, including humans.^[22-24] Therefore, the use of SOY as a dietary protein source is expected to enhance the body fat-reducing potential of CLA. In previous studies, we found in rats fed CLA that SOY as compared to CAS reduced the weights of white adipose tissues, while it increased the weight of brown adipose tissue.^[17,18] The present study confirmed these observations. In order further to reinforce the body fat-reducing potential of CLA by an appropriate dietary manipulation, here we examined the effect of dietary fat levels using soybean oil. The weight of white adipose tissue tended to increase when rats were fed the HF diets. Hence it is suggested that the body fat-reducing activity of CLA is attenuated when dietary fat levels increase irrespective of the protein source, although the weight of brown adipose tissue increased when rats were fed the HF diet in combination with SOY, but not CAS. These results indicate that when we hope for a body fat-reducing activity from CLA, the level of dietary fat must be selected appropriately. This might be particularly important when saturated fats are fed, because they tend to increase body fat or the concentration of serum cholesterol more than do polyunsaturated fats.

But the HF diet, as compared to the LF diet, reduced the concentrations of serum total and LDL-cholesterol and triglyceride when rats were fed CAS, but not SOY. Dietary fat level did not influence serum lipids when the protein source was SOY. As a result, serum concentrations of these lipids in the CAS group decreased to those experienced in the SOY groups. A similar response pattern was also observed as to the concentration of serum glucose. These results indicate that the selection of an appropriate dietary fat level that satisfies both serum lipid and body fat levels might be difficult when the dietary protein source is CAS. In this context, SOY appeared to be a more appropriate protein source as far as these two parameters are concerned.

The concentration of serum TNF-α was influenced neither by dietary protein type nor by fat level. In contrast, the concentration of serum leptin tended to be lower in the SOY groups than in the CAS groups, and it increased with increasing dietary fat levels. TNF-α and leptin as adipocytokines characteristically modify fatty acid metabolism, and act as adipostats.^[25-27] These cytokines differently influence glucose metabolism and hence body fat accumulation in complicated ways through their effects on insulin action.^[28-30] The present study showed that serum leptin concentrations increased with increasing dietary fat levels or body fat, but not body weight gain. Thus size of body fat cells is an important determinant of serum leptin concentration rather than body weight gain.^[31] TNF-α might not work as a regulator in this respect, since its serum concentration, did not change with dietary manipulations in the present study. The concentration of serum adiponectin, a specific adipocytokine, was significantly higher in the CAS-LF group than in the CAS-HF group, but there was no dietary fat level-dependent increase in this cytokine level in the SOY groups. It has been shown that adiponectin reduces atherosclerosis by activating the insulin effect,^[32,33] and increases fatty acid β-oxidation and energy expenditure through improvement in insulin resistance.^[34] Since the concentration of serum adiponectin decreased only when the diet was changed from LF to HF in the CAS groups, it appears likely that dietary protein source is one of the dietary regulators for the production of this cytokine.

The activity of liver CPT showed an increasing trend toward the HF diet in both protein groups, while dietary protein source did not influence it. With rats fed a diet free from CLA, it has been reported that the hepatic CPT-I mRNA level increases with high fat diets in rats,[^35] while in ponies high fat diets lowered the activity of CPT-I, which is sensitive to inhibition by malonyl-CoA, although hepatic mitochondrial activity remained uninfluenced.^[36] The increase in CPT activity on the HF diet observed in the present study might be attributed to an increase in CPT-I activity. These observations indicate the possibility that the hepatic activity of fatty acid β-oxidation was reinforced by the HF diet containing CLA, but this stimulation is perhaps not modified by the type of dietary protein.

The body fat-reducing effect of CLA was reinforced in the SOY-LF combination than in other dietary combinations. In contrast, dietary fat level influenced size of body fat cells to a lesser extent when the dietary protein was CAS. This was attributed mainly to the observation that SOY itself reduces body fat more than CAS does. In any case, these observations indicate the possibility that CLA is useful to prevent obesity in people of developed countries where high intake of animal foods is prevalent. Partial replacement of dietary...
protein from animal to plant origins might not be difficult. But when rats were fed SOY in combination with HF, the serum leptin concentration increased to a level comparable to that observed in rats fed the CAS-HF diet. Also, the concentration of serum adiponectin was maintained at a low level irrespective of the dietary fat level, although the concentration of serum total-cholesterol decreased and the weight of brown adipose tissue increased in rats fed the CAS diets. Thus dietary fat level interacted in a complicated way with the body fat-reducing effect of CLA.

In conclusion, dietary manipulations markedly modify the effect that CLA exerts on lipid metabolism, and hence size of body fat cells. Consequently, consideration appears to be necessary for an appropriate combination of CLA and dietary components, dietary protein type, and fat level in this case. For populations consuming diets that are abundant in energy and animal foods, diets containing more plant protein and less fat are appropriate if one is to expect the health benefits of CLA.

References
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