Investigation of the Anti-Obesity Action of Licorice Flavonoid Oil in Diet-Induced Obese Rats

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Licorice flavonoid oil (LFO), which contains hydrophobic flavonoids from Glycyrrhiza glabra LINNE, is a new ingredient for functional foods. In this study, we investigated the anti-obesity action of LFO in diet-induced obese rats. The addition of 2% LFO in a high-fat diet significantly decreased the weight of abdominal adipose tissue and the levels of hepatic and plasma triglycerides. We found that the enzymatic activities of acetyl-CoA carboxylase and fatty acid synthase, the rate-limiting enzymes in the fatty acid synthetic pathway, were significantly decreased by LFO, whereas the enzymatic activity of acyl-CoA dehydrogenase, the rate-limiting enzyme in the fatty acid oxidative pathway, was significantly increased. All our findings suggest that the anti-obesity action of LFO is controlled by regulation of the rate-limiting enzymes in the fatty acid synthetic and oxidative pathways in the liver.

Key words: insulin; fatty acid synthase (FAS); acyl-coenzyme A dehydrogenase (ACD); acetyl-coenzyme A carboxylase (ACC); cardiovascular disease

Metabolic syndrome is a complex disorder unifying dyslipidemia, insulin resistance, and hypertension.1,2) There is evidence that decreases in abdominal fat mass aids patients with metabolic syndrome.3) Hence new drugs and food ingredients that effectively suppress the accumulation of abdominal fat should be developed for pharmacotherapy or alimentotherapy for the syndrome.

Recently, much attention has been focused on plant flavonoids that might be beneficial in reducing the risk of obesity.4) For example, dietary catechins and anthocyanins significantly decrease the weight of abdominal adipose tissues.5,6) Investigation of the metabolic effects of plant flavonoids might lead to more effective strategies for the treatment of obesity.

Licorice, the root of the leguminous Glycyrrhiza plant species, has been used medicinally for the past 4,000 years.7,8) There are several species of licorice, including Glycyrrhiza uralensis FISCHER, G. glabra LINNE, and G. inflata BATALIN, and each of which contains species-specific flavonoids.9,10) Licorice flavonoid oil (LFO), which contains hydrophobic flavonoids from G. glabra LINNE, is a new ingredient for functional foods.9–12) We recently found that LFO decreases the size of hepatic lipid droplets, which are mainly composed of triglyceride (TG), and abdominal adipose tissue weight in diet-induced obese mice.10) It is well-known that TG is synthesized in the liver, secreted into the blood-stream, and transported to the peripheral organs, including the adipose tissues.13,14) The hepatic TG content is significantly correlated with plasma TG level and fat mass in humans.15) The hepatic TG availability is controlled by the balance between fatty acid synthesis and oxidation in the liver.16) Hence we hypothesized that LFO suppresses fatty acid synthesis and/or promotes fatty acid oxidation in the liver, which in turn results in decreases in the concentrations of hepatic and plasma TGs and the weight of abdominal fat.

In this study, we focused on the anti-obesity action of LFO in diet-induced obese rats as a model system because the fatty acid synthetic and oxidative pathways are well-established.17–19) Our results suggest that LFO suppresses abdominal fat accumulation by regulation of rate-limiting enzyme activities related to fatty acid synthesis and oxidation in the liver.

Materials and Methods

Preparation of licorice flavonoid oil (LFO). Licorice can cause the side effects such as hypermineralocorticid...

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Abbreviations: ACC, acetyl-coenzyme A carboxylase; ACD, acyl-coenzyme A dehydrogenase; FAS, fatty acid synthase; LFO, licorice flavonoid oil; LDL, low density lipoprotein; MCT, medium-chain triglycerides; PPAR-α, peroxisome proliferator-activated receptor-α; SREBP-1, sterol regulatory element-binding protein 1; TG, triglyceride; TNF-α, tumor necrosis factor-α; VLDL, very low density lipoprotein
Soybean oil was replaced with corn starch. The concentration of soybean oil and corn starch in the normal diet were 25.895% and 31.202%, respectively. Blood was collected from the carotid arteries. Plasma was separated immediately by centrifugation at 3,000 × g for 10 min at 4 °C, frozen with liquid nitrogen, and stored at −80 °C for plasma insulin analysis.

**Experiment 1.** The rats were randomly divided into four groups and fed a high-fat diet or a high-fat diet containing LFO at 0.5, 1.0, or 2.0% (Table 1) for 21 d. When the diet was supplemented with LFO, the LFO was added at the expense of the oil mixture, which contained 90% MCT and 10% soybean oil. Consequently, the MCT concentration in each diet was 1.8%. Plasma was separated and frozen as described above for plasma TG analysis. The liver was excised, weighed, frozen immediately with liquid nitrogen, and stored at −80 °C for lipid analysis.

**Experiment 2.** The rats were randomly divided into two groups and fed a high-fat diet or a high-fat diet containing LFO at 2.0% (Table 1) for 21 d. Plasma was separated and frozen as described above for plasma lipids, insulin, and glucose analysis. The liver was excised and weighed. A portion of the fresh liver was frozen immediately in liquid nitrogen and stored at −80 °C for lipid analysis. Another portion of the fresh liver was used to prepare mitochondria and cytosol fractions. The liver was homogenized in 9 volumes of 10 mM phosphate buffer (pH 7.0), 0.3 M sucrose, and 1 mM EDTA. The homogenate was centrifuged at 750 × g for 5 min at 4 °C, and the supernatant was recentrifuged at 10,000 × g for 10 min at 4 °C. The resulting pellet (hepatic mitochondria fraction) was frozen with liquid nitrogen and stored at −80 °C for enzymatic analysis. The supernatant was recentlyfuged at 105,000 × g for 60 min at 4 °C. The resulting supernatant (hepatic cytosol fraction) was frozen with

### Table 1. Compositions of Experimental Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>LFO (%)</th>
<th>MCT (%)</th>
<th>Glucose (%)</th>
<th>Cellulose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-fat diet</td>
<td>0.000</td>
<td>0.500</td>
<td>1.000</td>
<td>5.000</td>
</tr>
<tr>
<td>High-fat diet + LFO</td>
<td>1.000</td>
<td>2.000</td>
<td>1.000</td>
<td>5.000</td>
</tr>
<tr>
<td>High-fat diet + LFO</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>5.000</td>
</tr>
</tbody>
</table>

1. Licorice flavonoid oil containing MCT at 90% (w/w).
2. Medium-chain triglyceride (Riken Vitamin, Tokyo).
3. AIN-93 vitamin mixture (Japan SLC, Shizuoka, Japan).
4. AIN-93 mineral mixture (Japan SLC).
liquid nitrogen, and stored at −80 °C for enzymatic analysis.

**Analytical methods.**

*Plasma and hepatic lipid analyses.* The total lipid of each liver was extracted from the frozen liver with chloroform-methanol (2:1, vol/vol). The lipid extract was dried, dissolved in 2-propanol, and used in the analyses of TG, cholesterol, and phospholipids. Very low density lipoprotein (VLDL) was isolated from the frozen plasma by the method of Cham.²⁴ Plasma, VLDL, and hepatic TG levels were determined enzymatically using commercial kits (Triglyceride E-test Wako, Wako Pure Chemical Industries, Osaka, Japan). Plasma and hepatic total cholesterol levels were determined enzymatically using commercial kits (Cholesterol ELISA kit (Rat Insulin ELISA Kit, Shibayagi, Gunma, Japan). The plasma insulin concentration was determined using a commercial kit (Gluca ELISA kit (Rat Insulin ELISA Kit, Shibayagi, Gunma, Japan). The plasma insulin concentration was determined using a commercial kit (Gluca ELISA kit (Rat Insulin ELISA Kit, Shibayagi, Gunma, Japan).

*Enzymatic analyses.* The acetyl-CoA carboxylase (ACC) activity in the hepatic cytosol fractions was determined by H¹⁴CO₃⁻ fixation assay.²⁶ The fatty acid synthase (FAS) activity in hepatic cytosol fractions was determined by the spectrophotometric method.²³ The acyl-CoA dehydrogenase (ACD) activity in hepatic mitochondria fractions was determined by the method of Mason et al.²⁷ The protein concentrations in the mitochondria and cytosol fractions were determined by the method of Lowry et al.²⁸

*Plasma insulin and glucose analyses.* The plasma insulin concentration was determined using a commercial ELISA kit (Rat Insulin ELISA Kit, Shibayagi, Gunma, Japan). The plasma glucose concentration was determined enzymatically using a commercial kit (Glucose CII-test Wako, Wako Pure Chemical Industries).

**Statistical methods.** The data from experiment 1 were analyzed by the Tukey-Kramer test. The data from the other experiments were analyzed by Student’s t test. All statistical analyses were performed using a commercial package (StatView version 5, SAS Institute, Cary, NC, 1998).

**Results**

**Preliminary experiment**

The rats fed the high-fat diet showed significantly (p < 0.05) greater body weight gain (116%) and development of abdominal adipose tissue (124%) than those fed the normal diet (data not shown), indicating that the high-fat diet induced obesity. The plasma insulin concentration of the high-fat diet group was significantly (p < 0.01) higher than that of the normal diet group (193%, data not shown). Based on these results, the high-fat diet (Table 1) was used in experiments 1 and 2.

**Experiment 1**

The results are shown in Table 2. The addition of 2.0% LFO in the high-fat diet significantly (p < 0.05) decreased the weight of abdominal adipose tissue (71.0%), which was composed of epididymal, perirenal, and mesenteric adipose tissues. Each of these three adipose tissue weights was significantly (p < 0.05) decreased in the high-fat diet + 2.0% LFO group (60.4%, 66.2% and 89.5% respectively). The addition of 2.0% LFO to the high-fat diet significantly (p < 0.05) decreased the hepatic TG concentration (71.6%). The addition of 1.0 and 2.0% LFO to the high-fat diet significantly (p < 0.05) decreased the plasma TG concentrations (45.6% and 40.9% respectively). There were no significant differences in final body weight, body weight gain, or feed intake among groups. Since the addition of 2.0% LFO to the high-fat diet decreased abdominal adipose tissue weight and the levels of hepatic and plasma TGs, the high-fat diet and the high-fat diet containing 2.0% LFO (Table 1) were used in experiment 2.

**Experiment 2**

The effects of dietary LFO on body weight, feed intake, abdominal adipose tissue weight, and hepatic and plasma lipid components are shown in Table 3. The addition of 2.0% LFO to the high-fat diet significantly (p < 0.01) decreased the abdominal adipose tissue weight (71.5%). The epididymal, perirenal, and mesen-

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**Table 2.** Effects of Dietary LFO on Body Weight, Feed Intake, Hepatic, and Plasma Triglyceride Concentrations, and Abdominal Adipose Tissue Weight in Diet-Induced Obese Rats

<table>
<thead>
<tr>
<th></th>
<th>High-fat diet</th>
<th>High-fat diet + LFO 0.5%</th>
<th>High-fat diet + LFO 1.0%</th>
<th>High-fat diet + LFO 2.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial body weight (g)</strong></td>
<td>181 ± 1.5</td>
<td>184 ± 2.9</td>
<td>182 ± 1.7</td>
<td>181 ± 2.0</td>
</tr>
<tr>
<td><strong>Final body weight (g)</strong></td>
<td>354 ± 7.0</td>
<td>354 ± 5.2</td>
<td>356 ± 5.4</td>
<td>352 ± 5.1</td>
</tr>
<tr>
<td><strong>Body weight gain (g)</strong></td>
<td>173 ± 6.0</td>
<td>171 ± 2.7</td>
<td>174 ± 4.8</td>
<td>171 ± 6.5</td>
</tr>
<tr>
<td><strong>Feed intake (g/rat/21 d)</strong></td>
<td>384 ± 10.8</td>
<td>382 ± 11.1</td>
<td>377 ± 10.6</td>
<td>357 ± 10.5</td>
</tr>
<tr>
<td><strong>Hepatic triglyceride (mg/g of liver)</strong></td>
<td>103 ± 9.3</td>
<td>108 ± 21.4</td>
<td>94.1 ± 14.4</td>
<td>73.7 ± 9.1*</td>
</tr>
<tr>
<td><strong>Plasma triglyceride (mg/dl)</strong></td>
<td>67.9 ± 8.52</td>
<td>42.3 ± 11.35</td>
<td>30.9 ± 5.01*</td>
<td>27.8 ± 1.22*</td>
</tr>
<tr>
<td><strong>Abdominal adipose tissue weight (g)</strong></td>
<td>18.2 ± 1.04</td>
<td>18.1 ± 0.59</td>
<td>17.5 ± 1.31</td>
<td>13.0 ± 1.35*</td>
</tr>
<tr>
<td><strong>Epididymal adipose tissue weight (g)</strong></td>
<td>6.49 ± 0.31</td>
<td>6.18 ± 0.44</td>
<td>5.24 ± 0.31</td>
<td>3.92 ± 0.38*</td>
</tr>
<tr>
<td><strong>Perirenal adipose tissue weight (g)</strong></td>
<td>6.33 ± 0.46</td>
<td>6.22 ± 0.51</td>
<td>6.33 ± 0.63</td>
<td>4.19 ± 0.85*</td>
</tr>
<tr>
<td><strong>Mesenteric adipose tissue weight (g)</strong></td>
<td>5.41 ± 0.21</td>
<td>5.69 ± 0.20</td>
<td>5.94 ± 0.56</td>
<td>4.84 ± 0.21*</td>
</tr>
</tbody>
</table>

Values are means ± SEM for five rats per group. *p < 0.05 vs. high-fat diet group

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teric adipose tissue weights were significantly \( (p < 0.05) \) decreased in the high-fat diet + 2.0% LFO group (69.0%, 68.3% and 77.7% respectively). LFO significantly \( (p < 0.01) \) decreased TG and cholesterol concentrations in the liver (47.8% and 53.0% respectively). LFO significantly \( (p < 0.01) \) decreased plasma TG and VLDL-TG concentrations (50.9% and 48.3% respectively). LFO significantly decreased the plasma insulin \( (58.6 \pm 7.3\% ) \) and glucose \( (4.17 \pm 0.25\% ) \) in abdominal adipose tissue weight. Also, it is possible to prevent or ameliorate obesity and hyperlipidemia. LFO significantly decreased abdominal adipose tissue weight and effectively decreased TG levels in the liver and plasma, and VLDL (Table 3). As mentioned in the introduction, TGs are synthesized in the liver, secreted into the bloodstream mainly as VLDL, and transported to the peripheral organs, including adipose tissues.\(^{13,14}\) VLDL formation is highly dependent on TG availability in the liver.\(^{16}\) Hepatic TG availability is altered by the balance between fatty acid synthesis and oxidation in the liver.\(^{16}\) Hence we examined the enzymatic activities of the rate-limiting enzymes related to fatty acid synthesis and oxidation in the liver, and found that the enzymatic activities of ACC and FAS, the rate-limiting enzymes of the fatty acid synthetic pathway, were significantly decreased by LFO, whereas the enzymatic activity of ACD, the rate-limiting enzyme of the fatty acid oxidative pathway, was significantly increased (Figs. 1 and 2). These findings suggest that LFO decreases hepatic and plasma TG levels by regulation of rate-limiting enzymes involved in fatty acid synthesis and oxidation in the liver, which in turn results in decreases in abdominal adipose tissue weight. Also, it is possible

Table 3. Effects of Dietary LFO on Body Weight, Feed Intake, Hepatic, and Plasma Lipid Concentrations, and Abdominal Adipose Tissue Weight in Diet-Induced Obese Rats

<table>
<thead>
<tr>
<th></th>
<th>High-fat diet</th>
<th>High-fat diet + 2% LFO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial body weight (g)</strong></td>
<td>189 ± 2.4</td>
<td>189 ± 2.5</td>
</tr>
<tr>
<td><strong>Final body weight (g)</strong></td>
<td>352 ± 5.0</td>
<td>352 ± 8.1</td>
</tr>
<tr>
<td><strong>Body weight gain (g)</strong></td>
<td>163 ± 4.1</td>
<td>162 ± 8.3</td>
</tr>
<tr>
<td><strong>Feed intake (g/rat/24 d)</strong></td>
<td>401 ± 6.2</td>
<td>390 ± 6.8</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/g of liver)</td>
<td>123 ± 14.2</td>
<td>58.6 ± 7.3**</td>
</tr>
<tr>
<td>Total cholesterol (mg/g of liver)</td>
<td>7.87 ± 0.68</td>
<td>4.17 ± 0.25**</td>
</tr>
<tr>
<td>Phospholipids (mg/g of liver)</td>
<td>24.8 ± 0.74</td>
<td>25.4 ± 0.52</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>82.2 ± 8.58</td>
<td>41.8 ± 7.38**</td>
</tr>
<tr>
<td>VLDL-triglyceride (mg/dl)</td>
<td>69.5 ± 9.42</td>
<td>33.6 ± 5.33**</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>66.9 ± 6.12</td>
<td>53.1 ± 5.06</td>
</tr>
<tr>
<td>Phospholipids (mg/dl)</td>
<td>74.2 ± 9.40</td>
<td>83.7 ± 9.25</td>
</tr>
<tr>
<td><strong>Abdominal adipose tissue weight (g)</strong></td>
<td>17.0 ± 0.64</td>
<td>12.2 ± 1.32**</td>
</tr>
<tr>
<td>Epidydymal adipose tissue weight (g)</td>
<td>5.38 ± 0.10</td>
<td>3.71 ± 0.38</td>
</tr>
<tr>
<td>Perirenal adipose tissue weight (g)</td>
<td>6.35 ± 0.44</td>
<td>4.34 ± 0.65*</td>
</tr>
<tr>
<td>Mesenteric adipose tissue weight (g)</td>
<td>5.29 ± 0.28</td>
<td>4.11 ± 0.36*</td>
</tr>
</tbody>
</table>

Values are means ± SEM for eight rats per group. \(* p < 0.05\), \(** p < 0.01\)

Discussion

In this study, we found that LFO significantly decreased both abdominal adipose tissue weight and the plasma TG concentration (Tables 2 and 3). In addition, the plasma total cholesterol concentration tended to decrease in the high-fat diet + 2% LFO group (Table 3). Hence LFO might serve as a drug or food ingredient to prevent or ameliorate obesity and hyperlipidemia.

LFO significantly decreased abdominal adipose tissue weight and effectively decreased TG levels in the liver and plasma, and VLDL (Table 3). As mentioned in the introduction, TGs are synthesized in the liver, secreted
that inhibition of dietary fat absorption is involved in the anti-obesity action of LFO, but the addition of a very low level (0.2%) of licorice ethanolic extract to the high-fat diets (more than 27% fat), apparently reduced the abdominal adipose tissue weight by approximately 30% (Table 3). Such a large decrease in abdominal adipose tissue under the high-fat diet is difficult to explain by inhibition of dietary fat absorption. As shown in Figs. 1 and 2, we found that LFO apparently decreases the rate-limiting enzymes of the fatty acid synthetic pathway and increases the rate-limiting enzyme of the fatty acid oxidative pathway in the liver. Thus it appears that the anti-obesity effect of LFO is due mainly to regulation of lipid metabolism in the liver.

We also found that LFO significantly reduces the plasma insulin concentration in diet-induced obese rats (Fig. 3). Insulin is known to upregulate sterol regulatory element-binding protein 1 (SREBP-1) mRNA levels, and Steineger et al. have reported that insulin strongly downregulates peroxisome proliferator-activated receptor-α (PPAR-α) mRNA levels in both normal rat hepatocytes and hepatoma cells in vitro. SREBP-1 and PPAR-α are transcription factors for fatty acid synthetic and fatty acid oxidative enzymes respectively. Thus it appears that the decrease in insulin concentration due to LFO observed in the current study might downregulate SREBP-1 and upregulate PPAR-α.

Several adipokines, such as tumor necrosis factor-α (TNF-α) and resistin, induce insulin resistance and raise blood glucose levels. There is evidence that plasma TNF-α and resistin concentrations positively correlate with body fat mass. In this study, dietary LFO significantly decreased plasma glucose and insulin concentrations and abdominal adipose tissue weight (Fig. 3 and Table 3). Thus it is possible that the decrease in the plasma concentration of TNF-α and/or resistin due to anti-obesity action of LFO reduced the plasma glucose concentration, which in turn resulted in a low plasma insulin concentration in the rats fed the high-fat diet containing 2.0% LFO (Fig. 3). Further studies are required to address this possibility.

In conclusion, we examined the effects of dietary LFO on abdominal fat accumulation and plasma and hepatic TG metabolism in diet-induced obese rats. Our findings indicate that LFO significantly decreases the weight of abdominal adipose tissue and the levels of plasma and hepatic TG, and suggest that the anti-obesity action of LFO can be controlled by decreasing the rate-limiting enzymes of the fatty acid synthetic pathway and increasing the rate-limiting enzyme of the fatty acid oxidative pathway in the liver. Thus the natural ingredient LFO is a promising candidate for a drug or food ingredient to prevent or ameliorate obesity, the most prevalent manifestation of metabolic syndrome.
Acknowledgments
We thank Dr. Takao Sakai (Department of Biomedical Engineering, The Cleveland Clinic, Cleveland, Ohio) for helpful discussion. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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