Accelerative Effect of Olive Oil on Liver Glycogen Synthesis in Rats Subjected to Water-immersion Restraint Stress

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The effects of dietary oils on stress-induced changes in the liver glycogen metabolism of male Wistar rats at 6 weeks of age were investigated. The rats were subjected to repetitive water-immersion restraint and fed with a 20% saturated fatty acid mixture (PSC), olive oil (OLI), safflower oil (SAF), or linseed oil (LIS) diet. Stress loading decreased the body weight gain, although the food intake was hardly changed, and the weights of the liver and spleen generally declined regardless of the elapsed time after stress loading and the type of dietary oil. The adrenal weight was generally enhanced by stress in all dietary groups, and particularly tended to be greater in the OLI and PSC groups than in the other two. The plasma corticosterone concentration increased immediately after stressing (Stress-1), but approached the level of the rats with no stress (No stress) 2 h after releasing the stress load (Stress-2) in all groups. The enhancement of corticosterone level in the Stress-1 animals was large in the PSC and OLI groups, and the decline of this level in the Stress-2 animals was small in the OLI group when compared with the other groups. Although the concentrations of total cholesterol (T-CHOL) and triacylglycerol (TG) in the plasma were decreased by stress loading in all groups, these concentrations in the PSC and OLI groups were nearly always higher than in the other groups. The liver serine dehydratase (SDH) activity enhanced by stress was high in the OLI group and tended to be high in the PSC group when compared with the other groups. The contents of liver glycogen were reduced in the Stress-1 animals and extremely elevated in the Stress-2 animals of all groups, and particularly in the OLI group, the reduction in the Stress-1 animals was smaller and the enhancement in the Stress-2 animals was greater than in the other groups. These results suggest that feeding oleic acid to rats exposed to water-immersion restraint further accelerated liver glycogen synthesis through the rise in liver SDH activity due to increased corticosterone secretion when compared with the effect from linoleic and ω-linolenic acids.

Key words: olive oil; adrenal corticosterone; water-immersion restraint; glycogen synthesis; serine dehydratase

The appearance of many metabolic changes has been reported as the response in rats exposed to single or repetitive stress.1) For example, a rise in corticosterone secretion from the adrenal gland and a decline in the blood glucose concentration have been observed in rats subjected to stress. In a recent report,10) olive oil (OLI) rich in oleic acid accelerated the increase in corticosterone level in the plasma of rats subjected to stress when compared with safflower and linseed oils. On the other hand, the activity of serine dehydratase (SDH), which is an enzyme involved in the synthesis of glucose from amino acids, has been reported to be decreased by insulin and increased by glucocorticoid which corresponds to adrenal corticosterone in rats.11,12) The administration of OLI can be conjectured to accelerate liver glycogen synthesis through the enhancement of adrenal corticosterone secretion. This study was conducted to elucidate the effects of different types of fatty acid on the stress-induced change in glycogen metabolism in the liver of rats experimentally stress loaded by water immersion of the animals restrained individually in cages.13

Materials and Methods

Materials. Casein, olive oil (OLI), safflower oil (SAF), linseed oil (LIS), tripalmitin and tristearin (Nakalai Tesque, Kyoto, Japan), sugar (Fuji Seito Co., Shimizu, Japan), and mineral and vitamin mixtures14) (AIN-76,15) Nihon Nosan Kogyo, Yokohama, Japan) were purchased from the respective companies. Corn starch (α-type; Fuji Seifun, Shimizu, Japan) and corn oil (Honen Corporation, Shimizu, Japan) were presented by respective companies. The fatty acid composition of each oil used is shown in

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Abbreviations: PSC, saturated fatty acid mixture; OLI, olive oil; SAF, safflower oil; LIS, linseed oil; T-CHOL, total cholesterol; TG, triacylglycerol; SDH, serine dehydratase
Table 1. Fatty Acid Composition of the Dietary Fats

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>PSCI</th>
<th>Olive oil (OL) (wt%)</th>
<th>Safflower oil (SAF)</th>
<th>Linseed oil (LIS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>65.2</td>
<td>8.1</td>
<td>6.9</td>
<td>5.6</td>
</tr>
<tr>
<td>18:0</td>
<td>16.4</td>
<td>2.4</td>
<td>2.5</td>
<td>3.4</td>
</tr>
<tr>
<td>18:1</td>
<td>6.9</td>
<td>75.1</td>
<td>12.9</td>
<td>19.3</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>10.0</td>
<td>12.7</td>
<td>76.6</td>
<td>15.8</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.3</td>
<td>0.6</td>
<td>0.3</td>
<td>55.5</td>
</tr>
<tr>
<td>Others</td>
<td>1.2</td>
<td>1.1</td>
<td>0.8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

1 Mixture of tripalmitin (64%), tristearin (16%), and corn oil (20%).

Table 1.

Animals and diets. Male rats of the Wistar strain (Japan SLC, Hamamatsu, Japan) about 120 g in body wt. and 6 weeks of age were individually housed in suspended stainless-steel wire cages and fed in a temperature- (23±1°C) and humidity-controlled (50±3%) room having a 12-h light (06:00–18:00) and dark cycle. They had free access to food and water. The animals were fed with a basal diet according to the dietary recommendations of the American Institute of Nutrition, except for the addition of a vitamin C & K mixture (2,500 mg of L-ascorbic acid and 22.5 mg of menadione per 100 g), to accustom themselves to the surroundings for 4 to 5 d. The basal diet contained (in weight %) casein (20.0), DL-methionine (0.3), mineral mixture (3.5), vitamin mixture (1.3), vitamin C & K mixture (0.2), choline chloride (0.2), corn oil (5.0), sucrose (19.9), α-corn starch (44.9), and cellulose (5.0). Butylated hydroxytoluene was supplemented to the diets at 0.02% by weight as an antioxidant.

The rats, now weighing about 150 g, were randomly divided into 4 groups of 6 animals each. They were given the test diets containing 20% of a mixture of tripalmitin (64%), tristearin (16%), and corn oil (20%) (PSC), OLI, SAF, or LIS for 7 d, and then fed with the same diets while being subjected to a water-immersion restraint every other day (a total of 4 times) for 7 d. The stress was basically applied according to the method of Takagi and Okabe. A stress cage which is divided into ten compartments each containing a rat was immersed in a water bath (25°C) up to the necks of rats for 2 h. The test diets were prepared by increasing the content of oils in the basal diet at the expense of sucrose and starch, while keeping the starch/sucrose ratio at 9:4. At the end of the feeding period, immediately after subjecting to water-immersion restraint stress for 2 h (Stress-1), 2 h after releasing from the stress (Stress-2), and 4 h after depriving unstressed rats from food (No stress) to keep in step with the stressed rats, the animals were killed by decapitation, and blood samples were collected into polyethylene tubes with heparin. The liver, spleen, and adrenal were instantly removed, rinsed in physiological saline, and weighed. The plasma was separated by centrifuging at 900×g for 10 min. All samples were stored at −80°C until being analyzed. The animal experiment plan was approved by the Experimental Animal Care Committee of the Faculty of Agriculture at Shizuoka University.

Analytical procedure. The concentrations of total cholesterol (T-CHOL) and triacylglycerol (TG) in the plasma were determined by enzymatic kits (Cholesterol C-test and Triglyceride G-test, Wako Pure Chemical Industries, Osaka, Japan). The liver glycogen content and SDH activity were respectively measured by the method of Bruss and Black and according to the earlier report. The corticosterone concentration was assayed according to the method of Spencer et al.

Statistical analysis. Each data value is presented as the means±SEM and was subjected to a one-way analysis of variance (ANOVA), before inspecting all differences among means with Duncan's multiple range test at p<0.05. The correlation coefficient (r) was determined by a least significant difference analysis.

Results

Body weight gain, food intake, and organ weight

The changes in body weight gain, food intake, and organ weight of the rats during the experimental period are shown in Table 2. In all the rats subjected to the stress, the body weight gain was reduced, although the food intake scarcely changed. The weight gain of those animals fed on the PSC diet was lowest regardless of the high level of food intake when compared with the other diets in the No stress, Stress-1, and Stress-2 animals. The liver weight tended to be lower and the spleen weight significantly declined in the dietary groups with the stress load. The adrenal weight was generally enhanced by stress loading, and particularly more so by feeding with the PSC and OLI diets than by feeding with the SAF and LIS diets.

T-CHOL, TG, and corticosterone in the plasma

The concentrations of T-CHOL, TG, and corticosterone in the plasma of rats subjected to the stress or not during feeding the test diets are presented in Table 3. In the No stress animals, the level of plasma T-CHOL was inclined to be higher in the rats fed on the PSC and OLI diets than in those given the SAF and LIS diets. The plasma T-CHOL level inclined to be highest in the OLI group, followed in order by the PSC, SAF, and LIS groups for the Stress-1 and Stress-2 animals, between which little difference was apparent. The plasma TG concentration tended to be highest in the order of the No stress,
Table 2. Body Weight Gain, Food Intake, and Organ Weights of Rats Subjected to Water Immersion-restraint Stress (2 h) Every Other Day during the Period of Feeding the Test Diets for a Further 7 d after Giving the Diets for 7 d

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Weight gain (g/d)</th>
<th>Food intake (g/d)</th>
<th>Liver weight (g/100 g body wt.)</th>
<th>Spleen weight (mg)</th>
<th>Adrenal weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSC No stress</td>
<td>1.77 ± 0.08b,2</td>
<td>7.16 ± 0.14a</td>
<td>3.83 ± 0.16cd</td>
<td>449 ± 84</td>
<td>16.4 ± 0.4bcd</td>
</tr>
<tr>
<td>Stress-1</td>
<td>1.12 ± 0.12c</td>
<td>6.93 ± 0.18ab</td>
<td>3.58 ± 0.06de</td>
<td>337 ± 39</td>
<td>19.4 ± 0.6bc</td>
</tr>
<tr>
<td>Stress-2</td>
<td>1.15 ± 0.06c</td>
<td>7.01 ± 0.13a</td>
<td>3.46 ± 0.08b</td>
<td>336 ± 39</td>
<td>20.0 ± 0.7a</td>
</tr>
<tr>
<td>OLI No stress</td>
<td>2.24 ± 0.06c</td>
<td>6.38 ± 0.18bc</td>
<td>4.08 ± 0.10cf</td>
<td>467 ± 10c</td>
<td>15.4 ± 0.5cd</td>
</tr>
<tr>
<td>Stress-1</td>
<td>1.49 ± 0.12c</td>
<td>6.22 ± 0.16d</td>
<td>3.59 ± 0.04de</td>
<td>354 ± 9</td>
<td>20.0 ± 1.5a</td>
</tr>
<tr>
<td>Stress-2</td>
<td>1.65 ± 0.09d</td>
<td>6.32 ± 0.12d</td>
<td>3.80 ± 0.04df</td>
<td>367 ± 9</td>
<td>18.5 ± 1.4abc</td>
</tr>
<tr>
<td>SAF No stress</td>
<td>2.30 ± 0.11d</td>
<td>6.25 ± 0.23d</td>
<td>3.74 ± 0.12def</td>
<td>471 ± 9</td>
<td>13.3 ± 0.7cd</td>
</tr>
<tr>
<td>Stress-1</td>
<td>1.61 ± 0.07d</td>
<td>6.34 ± 0.15d</td>
<td>3.32 ± 0.04e</td>
<td>353 ± 4</td>
<td>17.9 ± 0.7abc</td>
</tr>
<tr>
<td>Stress-2</td>
<td>1.61 ± 0.05d</td>
<td>6.53 ± 0.15d</td>
<td>3.57 ± 0.10ef</td>
<td>344 ± 6</td>
<td>17.4 ± 0.5abc</td>
</tr>
<tr>
<td>LIS No stress</td>
<td>2.32 ± 0.16d</td>
<td>6.44 ± 0.30d</td>
<td>4.24 ± 0.09f</td>
<td>479 ± 11</td>
<td>15.8 ± 0.8dcd</td>
</tr>
<tr>
<td>Stress-1</td>
<td>1.62 ± 0.03d</td>
<td>6.34 ± 0.16d</td>
<td>3.55 ± 0.11d</td>
<td>365 ± 11</td>
<td>17.7 ± 1.0abc</td>
</tr>
<tr>
<td>Stress-2</td>
<td>1.61 ± 0.13d</td>
<td>6.22 ± 0.19d</td>
<td>3.94 ± 0.03d</td>
<td>367 ± 10</td>
<td>17.6 ± 0.8abc</td>
</tr>
</tbody>
</table>

1 Rats with no stress were killed after 4 h of food deprivation, and rats with stress were killed immediately (Stress-1) or 2 h (Stress-2) after stress loading for 2 h at the end of the feeding period. PSC, a mixture of tripalmitin (64%), tristearin (16%), and corn oil (20%); OLI, olive oil; SAF, safflower oil; LIS, linseed oil
2 Each value is the mean ± SEM for 6 rats. Values in a column not sharing the same superscript letter are significantly different at P < 0.05 by Duncan’s multiple-range test.

Stress-1, and Stress-2 animals with the OLI, PSC, SAF, and LIS diets. The plasma corticosterone level in the Stress-1 animals was extremely enhanced by feeding any of the diets, the level in the rats fed on the PSC and OLI diets being generally higher than that of the animals supplied with the SAF and LIS diets. In the other groups, except for the OLI group, these levels in the Stress-2 animals were lower and came near the level in the No stress animals. The levels of the OLI group tended to be higher than those of the other groups.

Liver glycogen
The glycogen content in the liver of the rats fed on the test diets with or without stress loading is shown in Fig. 1. In the Stress-1 animals, the liver glycogen content was substantially lower in the rats supplied with diets other than the OLI diet, by which the reduction in glycogen content was small. In the Stress-2 animals, the content was much higher in all groups, and particularly in the OLI group.

Liver SDH activity
The activity of liver SDH in the animals given the diets with or without stress exposure is presented in Fig. 2. The liver SDH activity of the Stress-1 rats fed on any of the diets was increased, and particularly the increase with the OLI diet was generally greater than that of the other groups. In the Stress-2 animals, the activity was lower than that of the No stress animals in any group, among which little difference was apparent.

Correlation between the corticosterone and SDH levels
As shown in Fig. 3, a positive correlation was observed between the plasma corticosterone concentration and the glycogen content (r = 0.82, p < 0.05) or SDH activity (r = 0.92, p < 0.05) in the liver, and between the liver glycogen level and SDH activity (r = 0.89, p < 0.05) in the Stress-1 animals.
Discussion

As shown in Table 2, the body weight gain, food intake, and weights of the liver, spleen, and adrenal were similar to those in the earlier reports.\(^{10,18-20}\) Although the water immersion of restrained rats in the present study was originally designed for inducing experimental ulcers in animals,\(^{13,20}\) ulcers were not visibly found, as was the case in the earlier work.\(^{10}\) This seems to have been due to the shorter stress-loading time than that used in previous studies.\(^{13,21}\) The plasma T-CHOL and TG levels in the No stress and Stress animals (Table 3) were inclined to be higher in the PSC and OLI groups than in the other two, which is similar to the results in earlier reports.\(^{10,22-24}\) The inclination toward a rise in the plasma T-CHOL and TG concentrations in the PSC group might have been due to enhancement of the oleic acid level by the metabolic change to oleic acid from saturated fatty acids.\(^{23}\) The increased concentration of plasma T-CHOL was reconfirmed to be closely related to the stress-induced increase in plasma corticosterone level as described in our recent report.\(^{10}\)
The increase in level of plasma corticosterone (Table 3) in all groups of the Stress-1 animals, especially in the OLI group, was lower in all the groups of the Stress-2 animals, the OLI group tending to show the smallest decline among the groups. The effect of PSC on the plasma corticosterone concentration to match that of OLI might also have been caused by the increased metabolic conversion to oleic acid from saturated fatty acids.

The liver glycogen content (Fig. 1) nearly always declined in the Stress-1 animals, being accompanied by a rise in the plasma corticosterone concentration (Table 3) and liver SDH activity (Fig. 2), but was extremely elevated in the Stress-2 animals. This enhancement in the Stress-2 animals would have been caused by the reduction in energy consumption due to the release from stress. In particular, feeding the OLI diet to the rats caused less reduction of the liver glycogen content in the Stress-1 animals and greater enhancement of this content in the Stress-2 animals than by the supply of the diets abundantly containing linoleic or α-linolenic acid (Fig. 2), although the SDH activity in the Stress-2 animals was hardly any different among the dietary groups. This result seems to have been caused by the similar decline in plasma corticosterone concentration regardless of the type of fatty acid. These findings suggest that stress loading needs a large quantity of glucose as an energy source, which is mainly supplied by liver glycogen synthesized from amino acids, with oleic acid particularly accelerating glycogen synthesis in the liver. The content of liver glycogen in the PSC group next highest to that of the OLI group might also have been due to the increased metabolic change by oleic acid from saturated fatty acids as already described.

The activity of liver SDH (Fig. 2) was generally higher in all groups of the Stress-1 animals, the enhancement in the OLI group being higher than that in the other groups, but was lower in all groups of the Stress-2 animals. A positive correlation between the liver SDH activity and plasma corticosterone level was observed in the rats subjected to stress (Fig. 3). It seems from these results that the activity of liver SDH was controlled by corticosterone secretion, as has been proposed in previous studies. The SDH activity in the liver was also positively related to the content of liver glycogen in the Stress-1 animals (Fig. 3). These foregoing results support the notion that oleic acid accelerates further glycogen synthesis from enhanced liver SDH activity by higher corticosterone secretion than that with other fatty acids.

In conclusion, feeding oleic acid to rats subjected to water-immersion restraint stress further elevated liver glycogen synthesis through the rise in liver SDH activity by increased concentration of plasma corticosterone when compared with the effect from linoleic and α-linolenic acids.

References

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