Effects of Oolong Tea on Metabolism of Plasma Fat in Mice under Restraint Stress

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We investigated the effects of oolong tea on the basic metabolism of plasma lipids in mice under restraint stress. When a lipid emulsion (Intralipid 20%; a lipid emulsion containing 20% soybean oil) was injected intravenously into mice, the restraint stress prolonged the half-life ($T_{1/2}$) of elimination for plasma triglyceride (TG) from 28.7 to 55.5 min. The elimination rate per minute was 48.2% in stressed mice with the rate in starved control mice as 100%. Therefore, TG metabolism was disrupted by the stress, and the use of TG as an energy source decreased. We found that the metabolism of lipids significantly response to the restrained stress in the present study. Plasma TG was 515.9 ± 29.9 mg/dl 35 min after Intralipid administration in control stressed mice, 478.7 ± 26.7 mg/dl in the stressed group given caffeine 100 mg/kg of body weight, and 418.3 ± 18.4 mg/dl in the stressed group given 1000 mg/kg oolong tea, an improvement by 7.2% and 18.9%, respectively, with the value for the untreated control group. The intake of oolong tea alleviated the stress-induced decrease in the rate of blood lipid metabolism; this effect may have arisen from some non-specific stress-relieving property of the tea or from acceleration of lipid metabolism by properties of polyphenols, etc. in tea. Oolong tea had anti-stress effects on plasma TG metabolism, and the effects did not depend on caffeine.

Key words: oolong tea; energy metabolism; restraint stress; Intralipid; triglyceride

Tea is the most popular beverage in the world. Most of green tea and oolong tea are consumed in Japan and China, and most of black tea is consumed in America and Europe. All varieties of tea are prepared from one species of plant, *Camellia sinensis* L. Tea has been used as a daily beverage and known to have beneficial effects on health for thousands of years in China. In the *Compendium of Materia Medica*, a book of traditional Chinese medicine, the Ming Dynasty medical scientist Li Shizhen (1518–1593) reviewed the health benefits of tea, and suggested that it is useful for the prevention of various diseases related to stress. Recently, it has been widely reported that oolong tea has desirable effects on health such as antioxidative effects, antiobesity action, and acceleration of lipid metabolism. However, the effects of oolong tea on stress have not been reported. The purpose of this study was to examine the effects of oolong tea on the metabolism of TG in plasma of mice under restraint stress.

Stress is involved in various diseases, and there are many stressors in our environment. There have been a number of recent studies about how signs and symptoms of stress arise. A response to stress is transmitted to the organs through the autonomic nervous system and hormones. Stress directly affects the secretion of hormones, suppress the immune system and sometimes causes acute organ dysfunction. For example, some stresses reduce the production of insulin in animals and impair glucose metabolism. When less glucose is used as energy, not only fatigue but also physiological disorders may appear. Many animal models have been used to investigate the effects of stress on the nervous and hormone systems. However, there are no reports about TG metabolism after the restraint stress. A lipid emulsion clearance test with Intralipid, an artificial lipid-emulsion used to supply energy and essential fatty acids clinically, is available. Intralipid also has been used as a tracer for blood TG. We used a lipid loading test to investigate how blood TG metabolism responds to stress. For, we are able to use blood TG metabolism as an index for anti-stress effects of caffeine and oolong tea.

Oolong tea was provided by Fujian Tea Import & Export Co., Ltd. Dried leaves of tea which were 2.3% caffeine, were extracted with 20 volumes of

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Abbreviations: $T_{1/2}$, half-life; TG, triglyceride
water with boiling at 90°C for 15 min. After filtration, the extract was freeze-dried and weighed. The weight of the residue used here is expressed as the weight of the dried leaves before extraction. The oolong tea extract and the caffeine (Nacalai Tesque Inc., Kyoto) were dissolved in distilled water before use. Intralipid (Intralipid 20%; a lipid emulsion including 20% soybean oil, 1.2% lecithin, and 2.2% glycerol) was purchased from Pharmacia AB (Stockholm, Sweden). It was diluted with PBS to a 10% soybean oil solution immediately before use. Female ICR mice of 7 weeks old were purchased from SLC Japan Inc. (Shimizu, Japan). In a preliminary study, we found that the metabolic response of female mice was more stable than that of male mice. The animals were kept in a specific-pathogen-free animal room at 23 ± 1°C with a 12-h light-dark cycle (lights on from 0600 to 1800 h) and were fed standard laboratory chow (CE-2; Clea Japan, Inc.) and tap water. The animals were kept for 1 week before the experiment. The effects of restraint on TG metabolism were investigated as follows. First, mice were divided into three groups. In the untreated control group, mice were given no treatment. In the starved group, mice were deprived of food for 20 h. In restrained and starved group, mice were confined to an oval metal restraint cage for 20 h while being starved before the assay. Intralipid was injected through the tail vein at the dose of 0.1 ml/10 g of body weight. In the restrained and starved group, mice were placed in restraint cages and starved for 20 h before the assay.

Seven-week-old female ICR mice were placed in the restraint cages for 20 h. The results are means of five mice at each time. Intralipid was diluted with PBS to the concentration of 10% soybean oil, and immediately injected into the vein in a volume of 0.1 ml/10 g of body weight. In the restrained and starved group, mice were placed in restraint cages and starved for 20 h before the assay.

Table 1. Effects of Restraint on TG Metabolism in Plasma from ICR Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>T1/2 (min)</th>
<th>Elimination rate (% per minute)</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>38.2</td>
<td>1.31%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Starved control</td>
<td>28.7</td>
<td>1.74%</td>
<td>51.7%</td>
</tr>
<tr>
<td>Restrained + starved</td>
<td>55.5</td>
<td>0.90%</td>
<td></td>
</tr>
</tbody>
</table>

Seven-week-old female ICR mice were placed in the restraint cages for 20 h. The results are means of five mice at each time. Intralipid was diluted with PBS to the concentration of 10% soybean oil, and immediately injected into the vein in a volume of 0.1 ml/10 g of body weight. The T1/2 was calculated from the changes with time in the plasma TG concentration, and the elimination rate was calculated as T1/2. % of control was percentage of the elimination rate for the starved mice. The mean plasma TG levels (±SE) of these normal mice were 68.8 ± 10.5 mg/dl.

Figure 1 shows changes in the TG elimination rate in plasma after the injection of Intralipid. The plasma TG levels were higher in mice under restraint stress. The half-life of the elimination rate, T1/2, for plasma TG was calculated from the slope of a line of regression for concentration versus time from the changes with time after the injection of Intralipid. The data were analyzed statistically with Student’s t-test. Differences were considered to be significant when p value was less than 0.01. The care and treatment of the animals conformed the guidelines established by the Japanese Society of Nutrition and Food Science (Law No. 105 and Notification No. 6 of the Japanese government).

Figure 1 shows changes in the TG elimination rate in plasma after the injection of Intralipid. The plasma TG levels were higher in mice under restraint stress. The half-life of the elimination rate, T1/2, for Intralipid was 38.2 min and the elimination rate was 1.31% per minute in the untreated control mice (Table 1). In the mice starved for 20 h, the T1/2 of TG was 28.7 min, which was 75.1% of that for the untreated control mice. The elimination rate was higher at 1.74% per minute. The T1/2 of Intralipid in mice

Fig. 1. Changes with Time in the Rate of TG Elimination from Plasma after Intralipid Injection.

Seven-week-old female ICR mice were placed in the restraint cages for 20 h. The results are means of five mice at each time. Intralipid was dilu ted with PBS to the concentration of 10% soybean oil, and immediately injected into the vein in a volume of 0.1 ml/10 g of body weight. In the restrained and starved group, mice were placed in restraint cages and starved for 20 h before the assay.

Water with boiling at 90°C for 15 min. After filtration, the extract was freeze-dried and weighed. The weight of the residue used here is expressed as the weight of the dried leaves before extraction. The oolong tea extract and the caffeine (Nacalai Tesque Inc., Kyoto) were dissolved in distilled water before use. Intralipid (Intralipid 20%; a lipid emulsion including 20% soybean oil, 1.2% lecithin, and 2.2% glycerol) was purchased from Pharmacia AB (Stockholm, Sweden). It was diluted with PBS to a 10% soybean oil solution immediately before use. Female ICR mice of 7 weeks old were purchased from SLC Japan Inc. (Shimizu, Japan). In a preliminary study, we found that the metabolic response of female mice was more stable than that of male mice. The animals were kept in a specific-pathogen-free animal room at 23 ± 1°C with a 12-h light-dark cycle (lights on from 0600 to 1800 h) and were fed standard laboratory chow (CE-2; Clea Japan, Inc.) and tap water. The animals were kept for 1 week before the experiment. The effects of restraint on TG metabolism were investigated as follows. First, mice were divided into three groups. In the untreated control group, mice were given no treatment. In the starved group, mice were deprived of food for 20 h. In restrained and starved group, mice were confined to an oval metal restraint cage for 20 h while being starved before the assay. Intralipid was injected through the tail vein at the dose of 0.1 ml/10 g of body weight. In the examination of TG metabolism, the animals conformed the guidelines established by the Japanese Society of Nutrition and Food Science (Law No. 105 and Notification No. 6 of the Japanese government).
Effect of Oolong Tea on Fat Metabolism

Table 2. Effects of Oolong Tea on TG Metabolism in Plasma Obtained from ICR Mice Loaded with Restraint Stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>TG (mg/dl)</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starved</td>
<td>376.2 ± 15.7</td>
<td></td>
</tr>
<tr>
<td>Restrained + starved</td>
<td>515.9 ± 29.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Restrained + starved +</td>
<td>515.9 ± 29.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Caffeine (100 mg/kg)</td>
<td>478.7 ± 26.7</td>
<td>92.7</td>
</tr>
<tr>
<td>Caffeine (50 mg/kg)</td>
<td>484.0 ± 19.2</td>
<td>93.8</td>
</tr>
<tr>
<td>Caffeine (25 mg/kg)</td>
<td>517.1 ± 31.2</td>
<td>100.2</td>
</tr>
<tr>
<td>Oolong tea (1000 mg/kg)</td>
<td>418.3 ± 18.4</td>
<td>81.0</td>
</tr>
<tr>
<td>Oolong tea (500 mg/kg)</td>
<td>432.7 ± 26.3</td>
<td>83.8</td>
</tr>
<tr>
<td>Oolong tea (250 mg/kg)</td>
<td>501.4 ± 20.9</td>
<td>97.1</td>
</tr>
</tbody>
</table>

Seven-week-old female ICR mice were placed in the restraint cages for 20 h. The results are means of five mice at each time. Intralipid was diluted with PBS to the concentration of 10% soybean oil, and immediately injected into the vein in a volume of 0.1 ml/10 g of body weight. The TG concentration in plasma was measured 35 min after Intralipid was administered. % of control was percentage of value of the starved and restrained mice. Oolong tea, caffeine or water (stress control mice) was given orally at 0.1 ml/10 g, 35 min before restraint began, which also was 35 min before the administration of Intralipid. The mean plasma TG levels (± SE) of these normal mice were 68.8 ± 10.5 mg/dl. Significantly different from restrained and starved control mice at *p<0.01 by Student’s t-test.

restrained and starved was prolonged to 55.5 min (193.4% of the value for the starved group) and the elimination rate was decreased to 0.90% per minute (51.7% of the value for the starved group).

The mean baseline value of the plasma TG concentration in the starved mice was 376.2 ± 15.7 mg/dl (± SE) at 35 min after the administration of Intralipid (Table 2). When mice were restrained for 20 h, the concentration was 515.9 ± 29.9 mg/dl (p = 0.0014) compared with the starved mice. After restrained and starved mice were given 1000 mg/kg oolong tea (containing 23 mg of caffeine), the concentration of TG in plasma was 418.3 ± 18.4 mg/dl, so plasma TG metabolism in these mice under restraint stress was improved by the tea. The effects were dose-dependent. The effects of caffeine alone (at the dose of 25 mg/kg) were significantly less than the same amount of caffeine in oolong tea in this group.

The plasma TG elimination rate was lower in mice restrained and starved than in the starved mice. Our results showed that this rate of TG elimination was affected by the stress and may play an important role in physiological changes caused by stress. We do not know the mechanism by which stress slows the elimination of plasma TG, but stress limits the supply of energy to organs, which causes poor utilization of biological energy sources, and leads to fatigue and various physiological disorders. Therefore, the elevated levels of plasma TG in the restrained and starved mice may reflect disrupted energy metabolism, and the elimination rate of plasma TG may be a marker of stress.

The anti-stress effects of tea have been recognized since ancient times. Maruyama et al. reported that the ingestion of tea was related with working hours, and this correlation seems to be due to anti-stress effects. In our study, the half-life of plasma TG elimination was 35 min in the restrained and starved mice. Oolong tea lowered the concentration of plasma TG in the mice which were restrained and starved compared with other also restrained and starved but give no tea. In general, such anti-stress effects are thought to be due to the action of caffeine, one of the bioactive components of oolong tea. Bianchi reported that caffeine suppresses adenosine receptors and increases the efflux of calcium ions from nerve terminals. Caffeine promotes energy metabolism by modifying catecholamine release through the stimulation of calcium channels, and thus caffeine may attenuate stress. Arciero et al. found that caffeine ingestion elevates the metabolic rate and fatty acid availability through lipolysis in fat cells and the release of catecholamines. The effects of caffeine have been attributed to the adenylcyclase-cAMP phosphodiesterase cycle. cAMP-dependent protein kinase A in turn activates hormone-sensitive lipase, and this activated enzyme catalyzes the hydrolysis of TG in fat cells. However, in our study, oolong tea ingestion reduced the plasma TG level compared with the stressed mice which were given no tea or caffeine in the same way. The effect may be due to polyphenols or other active components of oolong tea.

Stress probably lowers insulin secretion because of functional damage of the pancreas associated with peroxidation related to the stress. Generally, physical or mental stress increases the oxygen concentration in organs, followed by the generation of active oxygen molecules and free radicals. These radicals react with the lipids in the cell membrane. This process causes a chain reaction of lipid peroxide generation in the membrane and seriously damages the cell. The effects of oolong tea on insulin secretion are not understood in detail, but Serafini et al. found that tea ingestion inhibits lipid peroxide production in humans, and Lin et al. found the same effects in rats. Tea also reduces DNA damage caused by oxidative agents in vitro. Our results suggested that the effects of oolong tea on TG metabolism arise from the anti-stress and antioxidant effects of a variety of polyphenols and saponins including theasaponin E1 and theasaponin E2, which are antioxidants. These compositions might contribute to the protection against disorders by stress.

Illness caused by stress has been recognized since ancient times. Moreover, energy metabolism is important in various stress reactions. We found that oolong tea significantly promoted energy metabolism. Our results suggested that oolong tea may be useful for the prevention of many diseases related to stress without adversely affecting appetite or physical fitness.
References


